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ISOLATION AND IDENTIFICATION OF CELLULOSE PRODUCING BACTERIA FROM TROPICAL FRUITS IN THAILAND AND PROPERTIES OF BACTERIAL CELLULOSE PAPER

อมรรัตน์ สุวรรณโพธิ์ศรี
AMORNRAT SUWANPOSRI

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ
คณะวิทยาศาสตร์
สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง
พ.ศ. 2557
KMITL-2014-SC-D-020-012

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AMORNRAT SUWANPOSRI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENT FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN BIOTECHNOLOGY
FACULTY OF SCIENCE
KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG
2014
KMITL-2014-SC-D-020-012

Faculty of Science
King Mongkut's Institute of Technology Ladkrabang
Thesis certification

……………………………

Thesis title Isolation and Identification of Cellulose Producing Bacteria from Tropical Fruits in Thailand and Properties of Bacterial Cellulose Paper

Student Amornrat Suwanposri

Student ID 49067251

Degree Doctor of Philosophy

Program Biotechnology

Thesis Advisor Assoc. Prof. Duangjai Ochaikul

Thesis Co-advisor Dr. Pattaraporn Rattanawaree

Examiners	Signatures
Assoc. Prof. Dr. Nuanpan Na Ranong	N. Naranong
Assoc. Prof. Duangjai Ochaikul	Duangjai Ochaikul
Dr. Pattaraporn Rattanawaree	Pattaraporn R.
Asst. Prof. Dr. Saranya Phunpruch	Saranya Phunpruch
Asst. Prof. Dr. Charoen Charoenchai	C-Chai

Examination Date 7th May 2014 Time 1.00-4.00 p.m.

Place Chulabhornwalailak building 4 floor room 439

Dusanee

(Assoc. Prof. Dr. Dusanee Thanaboripat)
Dean
Date 16 May 2014

หัวข้อวิทยานิพนธ์	การคัดแยกและการจัดจำแนกแบคทีเรียซึ่งผลิตเซลลูโลสจากผลไม้เขตร้อนในประเทศไทยและคุณสมบัติของกระดาษที่ผลิตจากแบคทีเรียลเซลลูโลส
นักศึกษา	นางสาวอมรรัตน์ สุวรรณโพธิ์ศรี
รหัสประจำตัว	49067251
ปริญญา	ปรัชญาดุษฎีบัณฑิต
สาขาวิชา	เทคโนโลยีชีวภาพ
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อาจารย์ที่ปรึกษาวิทยานิพนธ์	รศ. ดวงใจ โอชัยกุล
อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม	ดร. ภัทรพร รัตนวารี

บทคัดย่อ

การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อเพิ่มประสิทธิภาพในการผลิตเซลลูโลสจากแบคทีเรียและลดต้นทุนการผลิต โดยการคัดแยกเชื้อแบคทีเรียที่มีประสิทธิภาพสูงในการผลิตเซลลูโลสโดยใช้วัสดุเหลือทิ้งทางการเกษตรเป็นสารตั้งต้น ได้เก็บรวบรวมผลไม้ที่เน่าเสียจำนวน 48 ชนิด จากส่วนต่างๆของประเทศไทยและนำมาใช้เป็นแหล่งในการคัดแยกเชื้อแบคทีเรียที่ผลิตเซลลูโลส สามารถแยกเชื้อแบคทีเรียที่ผลิตเซลลูโลสจำนวน 204 สายพันธุ์ จากผลไม้จำนวน 16 ชนิด และคัดเลือกไอโซเลทตัวแทนจากผลไม้แต่ละชนิดโดยคัดเลือกจากปริมาณเซลลูโลสที่ผลิตได้และนำไปตรวจพิสูจน์เอกลักษณ์ จากการศึกษาลักษณะทางสัณฐานวิทยา ลักษณะทางกายภาพ ลักษณะทางเคมี และการวิเคราะห์ลำดับเบสของยีน 16S rRNA พบว่าไอโซเลทตัวแทนจำนวน 29 ไอโซเลท เป็นแบคทีเรียในจีนัส *Komagataeibacter* และแบ่งเป็น 7 กลุ่ม ดังนี้ กลุ่มที่ 1 *K. rhaeticus* จำนวน 1 ไอโซเลท กลุ่มที่ 2 *K. oboediens* จำนวน 5 ไอโซเลท กลุ่มที่ 3 *K. sucrofermentans* จำนวน 2 ไอโซเลท กลุ่มที่ 4 *K. swingsii* จำนวน 2 ไอโซเลท และกลุ่มที่ 5 *K. hansenii* จำนวน 5 ไอโซเลท ส่วนไอโซเลทที่เหลือจัดเป็นกลุ่ม 6a จำนวน 3 ไอโซเลท และกลุ่ม 6b จำนวน 9 ไอโซเลท ซึ่งเป็นกลุ่มที่ไม่สามารถพิสูจน์เอกลักษณ์ถึงระดับสปีชีส์ได้ จากการศึกษาความสามารถในการผลิตเซลลูโลสของไอโซเลทที่เป็นตัวแทนในสภาวะนิ่งเปรียบเทียบกับในสภาวะเขย่าโดยใช้อาหารสูตรมาตรฐาน Hestrin & Schramm (HS) เลี้ยงที่อุณหภูมิ 30 องศาเซลเซียส เป็นเวลา 7 วัน พบทุกไอโซเลทผลิตเซลลูโลสในสภาวะนิ่งได้สูงกว่าในสภาวะเขย่า โดยไอโซเลท PAP1 ซึ่งเป็นแบคทีเรียที่แยกได้จากมะละกอจัดอยู่ในกลุ่ม 6b และพิสูจน์เอกลักษณ์ได้เป็น *Komagataeibacter* sp. PAP1 เป็นแบคทีเรียที่มีประสิทธิภาพสูงสุดโดยสามารถผลิตเซลลูโลสได้ 1.15 กรัมต่อลิตร

เมื่อแทนที่น้ำตาลกลูโคสซึ่งเป็นแหล่งคาร์บอนในอาหารสูตรมาตรฐาน HS ด้วยน้ำตาลแมนนิทอลและเอทานอล พบว่า *Komagataeibacter* sp. PAP1 ผลิตเซลลูโลสในปริมาณสูงถึง 3.50 กรัมต่อลิตร และ 3.43 กรัมต่อลิตร ตามลำดับ แต่อย่างไรก็ตามเมื่อพิจารณาในด้านราคาจึงเลือกใช้เอทานอลเป็นแหล่งคาร์บอน เพื่อลดต้นทุนในการผลิตเซลลูโลสจากแบคทีเรียจึงนำน้ำทิ้งจากกระบวนการผลิตเต้าหู้หรือเวย์มาใช้เป็นสารตั้งต้นและหาสภาวะที่เหมาะสมของปัจจัยหลักในกระบวนการหมักจำนวน 3 ปัจจัย ได้แก่ พีเอชเริ่มต้นของอาหารเลี้ยงเชื้อ ความเข้มข้นของแหล่งคาร์บอน (ร้อยละ) และอุณหภูมิที่ใช้ในการหมัก (องศาเซลเซียส) วางแผนการทดลองโดยใช้การออกแบบส่วนประสมกลาง (central composite design) และวิเคราะห์ข้อมูลด้วย response surface methodology (RSM) พบว่าสภาวะที่เหมาะสมในการผลิตเซลลูโลสจากแบคทีเรียคือ พีเอช 6.21 เอทานอลความเข้มข้นร้อยละ 1.61 และหมักที่อุณภูมิ 28.4 องศาเซลเซียส ซึ่งให้ผลผลิตเซลลูโลสได้ 4.04 กรัมต่อลิตร มากกว่าการผลิตในอาหารสูตรมาตรฐาน HS ถึง 3.6 เท่า กระดาษจากเซลลูโลสที่ผลิตในอาหารสูตรเวย์มีค่าความแข็งแรงดึง (49.16 เมกกะพาสคาล) และมอดูลัสของยังค์ (2498.69 เมกกะพาสคาล) สูงกว่ากระดาษที่ได้จากเซลลูโลสที่ผลิตในอาหารสูตรมาตรฐาน HS สำหรับอัตราการซึมผ่านของไอน้ำ (1986 กรัมต่อตารางเมตรต่อวัน) และอัตราการซึมผ่านของก๊าซออกซิเจน (23.1 ลูกบาศก์เซนติเมตรต่อตารางเมตรต่อวัน) ของกระดาษจากเซลลูโลสที่ผลิตในอาหารสูตรเวย์มีค่าต่ำกว่ากระดาษที่ผลิตในอาหารสูตรมาตรฐาน HS แสดงให้เห็นว่ากระดาษจากเซลลูโลสที่ผลิตในอาหารสูตรเวย์สามารถป้องกันไอน้ำและก๊าซออกซิเจนได้ดีกว่ากระดาษที่ผลิตในอาหารสูตรมาตรฐาน HS นอกจากนี้กระดาษจากเซลลูโลสที่ผลิตในอาหารสูตรเวย์ยังมีความสามารถในการดูดซับน้ำที่สูงถึงร้อยละ 232.29 และไม่จำเป็นต้องใช้กระบวนการฟอกสีให้ขาว

คำสำคัญ : เซลลูโลส, เซลลูโลสจากแบคทีเรีย, การผลิตกระดาษ, *Komagataeibacter* sp.

Thesis title	Isolation and Identification of Cellulose Producing Bacteria from Tropical Fruits in Thailand and Properties of Bacterial Cellulose Paper
Student	Amornrat Suwanposri
Student ID	49067251
Degree	Doctor of Philosophy
Program	Biotechnology
Year	2014
Thesis Advisor	Assoc. Prof. Duangjai Ochaikul
Thesis Co-advisor	Dr. Pattaraporn Rattanawaree

ABSTRACT

The aims of the present study were to increase bacterial cellulose (BC) productivity and to reduce BC production cost by isolation of highly effective cellulose producing bacteria and using an agricultural waste as substrate. Forty-eight rotten tropical fruits were collected from different parts of Thailand and used as the isolation sources of cellulose producing bacteria. Two hundred and four strains of cellulose producing bacteria were isolated from 16 fruits, and the representative isolates were selected from each sources based on the amount of BC production. Twenty-nine representative isolates were identified by morphological, physiological and biochemical characteristics and 16 rRNA gene sequence analysis. The selected 29 isolates were grouped into seven subgroups within the genus *Komagataeibacter* and identified as *K. rhaeticus* (subgroup I, one isolate), *K. oboediens* (subgroup II, five isolates), *K. sucrofermentans* (subgroup III, two isolates), *K. swingsii* (subgroup IV, two isolates) and *K. hansenii* (subgroup V, seven isolates). The remaining isolates were grouped into subgroups VIa (three isolates) and VIb (nine isolates) and unidentified to species level. The BC production ability of all selected isolates cultivated in standard Hestrin and Schramm (HS) medium were compared under static and shaken condition at 30 °C for 7 days. All selected isolates gave higher BC production in static than those in shaken condition. The most effective cellulose producing bacterium with the maximum BC yield of 1.15 g/L was isolate PAP1 which was isolated from papaya and identified as *Komagataeibacter* sp. PAP1 (subgroup VIb). *Komagataeibacter* sp. PAP1 gave high BC yields of 3.50 g/L and 3.43 g/L when D-glucose was replaced by 2.0% mannitol and ethanol, respectively. However, by cost consideration ethanol was selected as carbon source for BC production by *Komagataeibacter* sp. PAP1. In order to reduce BC production cost, a by-product from tofu production soybean whey, was used as substrate. The three main fermentation factors, including initial pH of culture medium, concentration of carbon source and incubation temperature were chosen for optimization using central composite design of response surface methodology (RSM). The optimal conditions for BC production were: pH 6.21, 1.61% (v/v) ethanol concentration and 28.4 °C. Under the optimal condition, *Komagataeibacter* sp. PAP1 produced 4.04 g/L BC yield which was 3.6 times higher than that from standard HS medium. The BC paper produced from the optimized soybean whey-based medium (SBW paper) gave a higher tensile strength (49.16 MPa) and Young's modulus (2498.69 MPa) than that produced from standard HS medium (HS paper). The lower water vapor transmission rate (1986 $g/m^2/day$) and oxygen gas transmission rate (23.1 $cm^3/m^2/day$) in SBW paper indicated that it was more impermeable to water vapor and oxygen than to HS paper. The SBW paper showed a high absorption capacity of 232.29% without any requirement of an additional step of de-colorization.

Keywords : cellulose, bacterial cellulose, paper making, *Komagataeibacter* sp.

ACKNOWLEDGEMENTS

I would like to express the deepest appreciation to my thesis advisor, Assoc. Prof. Duangjai Ochaikul and my thesis co-advisor, Dr. Pattaraporn Ratanawaree for their invaluable help and constant throughout the course of this research. I am most grateful for their teaching and advice, not only the research methodologies but also many other methodologies in life. This thesis would not have been completed without all the support that I have always received from them.

I would like to special thanks for Prof. Dr. Yuzo Yamada for all comments, good suggestion and revision of my manuscripts for publication in journal. Also, I would like to special thanks for my committee members, Assoc. Prof. Dr. Nuanpan Na Ranong, Asst. Prof. Dr. Saranya Phunpruch and Asst. Prof. Dr. Charoen Charoenchai for their suggestions and all help.

I would like to special thanks for the scholarship from King Mongkut's Institute of Technology Ladkrabang and National Science and Technology Development Agency.

Finally, my graduation would not be achieved without best wishes from my parents, who help me for everything and financial support until this study has been completed. Last gratefully special thanks to my friends, Mr. Chalit Nopparat for teaching of response surface methodology, Miss Piroonporn Srimongkol for the beautiful picture of scanning electron micrographs, Miss Pharita Tongsuknok, Miss Jirarat Jiranuwatkul, Miss Varisara Lathiwongsakon and Miss Wannisa Pansuk for all their help and encouragement throughout the period of my research were acknowledged.

Amornrat Suwanposri

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CHAPTER 1

INTRODUCTION

1.1 Statement and significance of the problems

Bacterial cellulose or bio-cellulose (BC) is an exopolysaccharide naturally produced by some aerobic acetic acid bacteria in the genus *Komagataeibacter*, which reclassified from *Gluconacetobacter* by Yamada et al. (2012), including *K. xylinus, K. hansenii, K. nataicola*, etc. (Hestrin and Schramm, 1954; Park et al., 2003; Lisdiyanti et al., 2006). These cellulose producing bacteria are commonly found in natural sources such as flowers, sugar canes, nuts and some rotten fruits (Seto et al., 1997; Tsuchida and Yoshinaga, 1997; Park et al., 2003). The isolation of individual cellulose producing bacteria from other microorganisms usually requires specific growth media. The determination of rRNA gene is a convenient and reliable method, which have been used for species identification of cellulose producing bacteria (Park et al., 2003; Yang et al., 2013b).

Nowadays, BC is considered as an alternative biomaterial since it possesses many distinctive qualities to cellulose from the other sources including high purity, high mechanical strength, high degree of polymerization, fiber-binding ability, excellent biodegradability and biocompatibility (Shah and Brown, 2005; Shoda and Sugano, 2005; Chawla et al., 2009). These unique qualities of BC made it become an attractive recently subject of many studies concerning the development of alternative biological materials for the use in various modern industries. However, the huge expenses for BC culture medium, which take up to almost 30% of the total expenditure (Rivas et al., 2004), and low level of BC production are major obstacle in the development of BC for industrial application. Therefore, reducing the cost of BC production and maximizing BC yield by the use of wastes and by-products as culture medium, optimization of culture conditions and isolation of an efficient cellulose producer are desirable ways for solving these problems.

Soybean whey is a pale yellow liquid waste resulting from the protein coagulation of soybean milk during tofu production. Soybean whey is 79-80% of the total soybean milk and rich of protein, sugar, starch and fat (Fung et al., 2008; Singh and Banerjee, 2013). Many reports have shown that soybean whey can be used as low cost substrate for microbial fermentations (Fung et al., 2008; Monajjemi et al., 2012; Singh and Banerjee, 2013). However, there are very limited reports on the utilization of soybean whey as substrate for BC production (Lu et al., 2004; Photisarach, 2011).

Response surface methodology (RSM) is an efficient statistical technique commonly used to determine the optimal culture conditions for multiple independent variables with a minimum number of trials (Box and Hunter, 1957). This technique can minimize error in determining the effect of parameters, explain the relationship when interaction between different components take place and less-time consuming compared to the conventional one-variable-at-a-time method (Liong and Shah, 2005). RSM have been successfully applied in optimization of microbiological culture conditions, enzyme hydrolysis and parameters for food processes (Barrington and Kim, 2008; Fung et al., 2008; Pal and Khanum, 2010).

Thailand is a country with relatively high temperature and humidity, having various traditional fruits that might be a rich source of cellulose producing bacteria. This study was focused on the isolation and identification of an efficient cellulose producing bacterium isolated from tropical fruits in Thailand. With a using of soybean whey as low cost substrate to reduce the cost of BC production and optimizing the culture conditions using central composite design of RSM to increase the level of BC yield. BC pellicle synthesized in optimal condition was applied as raw material in the production of paper and the properties of produced BC paper were characterized.

1.2 Goal and objectives

The objectives of this research are as followed:

1.2.1 To isolate cellulose producing bacteria from tropical fruits in Thailand and identify them by using simple morphological characteristics, biochemical tests and 16S rRNA gene sequence analysis.

1.2.2 To study the BC production of the isolated strains and find the most effective cellulose producing isolate.

1.2.3 To determine the optimal condition for BC production by the most effective cellulose producing isolate using soybean whey as a raw material.

1.2.4 To produce paper from BC-synthesized in the optimal condition by the most effective cellulose producing isolate.

1.2.5 To study the properties of the produced BC paper.

1.3 Scope of the study

In this study, cellulose producing bacteria were isolated from tropical fruits in Thailand and identified using simple morphological characteristics, biochemical tests and 16S rRNA gene sequence analysis. The most effective cellulose producing isolate was selected and used for BC production. The optimal condition including initial pH of culture medium, concentration of carbon source and incubation temperature of BC production by the most effective cellulose producing isolate was determined using central composite design (CCD) of RSM. The BC-synthesized in the optimal condition was applied as raw material for production of BC paper. The properties of BC paper produced from BC-synthesized in the optimal condition (SBW paper) such as surface morphology, mechanical strength, water transmission rate, oxygen transmission rate, water absorption capacity and color appearance were characterized and compared with that obtained from HS medium.

1.4 Expected results

The expected results from this research are as followed:

1.4.1 The basic knowledge of morphological, biochemical and genotypic characteristics of cellulose producing isolates which are isolated from tropical fruits in Thailand.

1.4.2 The most effective cellulose producing isolate was obtained from tropical fruits in Thailand.

1.4.3 The optimal condition for BC production of the most effective cellulose producing isolate using soybean whey as major component in BC culture was found.

1.4.4 The BC paper can be produced in the optimal condition, and properties of the BC paper were obtained.

CHAPTER 2

LITERATURE REVIEW

2.1 Cellulose producing bacteria

Some genus of Gram-negative bacteria, including *Komagataeibacter* (formerly *Gluconacetobacter* reclassified by Yamada et al., 2012), *Achromobacter*, *Aerobacter, Agrobacterium*, *Azotobacter*, *Alcaligenes*, *Enterobacter*, *Escherichia*, *Pseudomonas*, *Rhizobium* and *Salmonella* (Deinema and Zevenhuizen, 1971; Ross et al., 1991; Jonas and Farah, 1998; Römling, 2002) are called cellulose producing bacteria due to their ability to produce solid extracellular cellulose. Bacterial-produced cellulose is generally called bacterial cellulose or bio-cellulose (BC), which usually exhibits specific bacteria-dependent characteristics as shown in Table 2.1.

Table 2.1 Cellulose characteristics based on various types of bacteria.

Type of bacteria	Cellulose characteristic
Komagataeibacter	Extracellular pellicle (Park et al., 2003; Castro et al., 2011)
Achromobacter	Cellulose fibrils (Deinema and Zevenhuizen, 1971)
Agrobacterium	Short fibrils (Matthysse et al., 2005)
Alcaligenes	Cellulose fibrils (Kaur et al., 2013)
Enterobacter	Extracellular pellicle (Hungund and Gupta, 2010)
Escherichia	Thin fibrils (Re et al., 2007; Gualdi et al., 2008)
Gluconobacter	Extracellular pellicle (Jia et al., 2004)
Pseudomonas	No distinct fibrils (Ude et al., 2006; Baum et al., 2009)
Rhizobium	Short fibrils (Napoli et al., 1975; Parthiban et al., 2011)

Gromet-Elhanan and Hestrin (1963) and others (Brown, 1986; Geyer et al., 1994; Yoshino, 1996; Park et al., 2003; Jung et al., 2005) suggested that the most effective cellulose producing bacteria are *K. xylinus*, *K. hansenii* and *Acetobacter pasteurianus*. *K. xylinus* is one of the most commonly studied sources of BC, because of its high productivity on a wide range of carbon sources such as glucose, fructose, sucrose, invert sugar, ethanol and glycerol as well as nitrogen sources such as yeast extract, peptone, corn steep liquor and ammonium sulphate in liquid medium (White and Brown, 1989; Jagannath et al., 2008; Nguyen et al., 2008a). Various strains of cellulose producing bacteria and their yields are presented in Table 2.2.

Table 2.2 Different strains of BC producers and their BC productivity.

BC producer	Carbon source	Culture time (day)	Yield (g/L)	Reference
Acetobacter sp. A9	Glucose	8	15.20	Son et al., 2001
Acetobacter sp. V6	Glucose	8	4.16	Jung et al., 2010
Acetobacter sp. 4B-2	Sucrose	8	11.98	Pourramezan et al., 2009
A. xylinum BPR2001	Maple syrup	21	1.51	Zeng et al., 2011
A. xylinum E25	Glucose	7	3.50	Krystynowicz et al., 2002
Gluconacetobacter sp. A06O2	Fructose	7	6.70	Karahan et al., 2011
Gluconacetobacter sp. F6	Glucose	6	4.50	Jahan et al., 2012
Gluconacetobacter sp. RKY5	Glycerol	6	4.59	Kim et al., 2006
G. hansenii PJK	Glucose	10	7.40	Ha et al., 2011
G. hansenii UAC09	Coffee cherry husk	14	6.24	Rani et al., 2011a
G. hansenii UAC09	Sugar cane	14	7.47	Rani et al., 2011b
G. intermedius Cls26	Glucose	8	2.10	Yang et al., 2013b
G. medellinensis	Glucose	8	3.0	Castro et al., 2012
G. sacchari	Glucose	4	2.70	Trovatti et al., 2011
G. xylinus ATCC 10245	Glucose	30	4.40	Keshk, 2006
G. xylinus ATCC 53524	Sucrose	4	3.83	Mikkelsen et al., 2009
G. xylinus CH001	Elephant grass	14	6.40	Yang et al., 2013a
G. xylinus IFO 13693	Glucose	30	7.90	Keshk, 2006
G. xylinus K3	Mannitol	7	0.82	Nguyen et al., 2008a

Process of extracellular cellulose synthesis has been a subject to many studies for over a decade. Two hypotheses describing the process of BC synthesis by these microorganisms are presumed. First, Schramm and Hestrin (1954) and Valla and Kjosbakken (1982) presumed that aerobic bacteria produce cellulose pellicle, which enable them to maintain their position near the air-liquid interface where bacterial cells are able to obtain oxygen. Another hypothesis presented by Williams and Cannon (1989) and Iguchi et al. (2000) presumed that bacterial cells produce cellulose in order to prevent themselves from ultraviolet radiation, foes and heavy metal ions. According to this hypothesis bacterial cells produce and extrude cellulose microfibrils through pores on their cell envelopes. The released microfibrils then weave into a net and enfold the bacterial cells as armor.

2.1.1 Sources of cellulose producing bacteria

Mostly cellulose producing microorganisms in nature are bacteria in genus of *Komagataeibacter*. Formerly, *Komagataeibacter* was some species in the genus *Acetobacter* which was transferred to the genus *Gluconacetobacter* by Yamada et al. (1997, 1998). Later, some species of *Gluconacetobacter* was transferred to the genus *Komagataeibacter* by Yamada et al. (2012, 2014). Generally, these cellulose producing bacteria can be found in various natural sources as shown in Table 2.3.

Table 2.3 Sources of cellulose producing bacteria.

Source	Reference
Rotten fruit	
- apple	Park et al., 2003; Jahan et al., 2012
- melon, cherry and grape	Seto et al., 1997
- mango, guava, pineapple and sapodilla	Lapuz et al., 1967
- orange	Yang et al., 2013b
Wine, fruit juice and beverage	Krieg and Holt, 1984; Rani et al., 2011b
Cane	Coronel and Joson, 1986
Flower and nut	Toyosaki et al., 1995a
Vinegar	Toyosaki et al., 1995a; Kim et al., 2006; Pourramezan et al., 2009; Karahan et al., 2011;

Table 2.3 Sources of cellulose producing bacteria (continued).

Source	Reference
	Castro et al., 2012
Activated sludge	Deinema and Zevenhuizen, 1971
Kombucha tea	Dutta and Gachhui, 2007; Trovatti et al., 2011

2.1.2 Structure and properties of BC

Cellulose is an organic compound with the molecular formula of $(C_6H_{10}O_5)_n$ as shown in Figure 2.1, which is a polysaccharide consisting of a straight chain of approximately one hundred to over ten thousand β(1→4) linked glucopyranose residues (Updegraff, 1969; Crawford, 1981). The multiple hydroxyl groups on one chain of glucose are linked with oxygen molecule on the same or another adjacent chain, by hydrogen bonds. The intra and intermolecular hydrogen bounds of the glucose chains result in a stronger reticulate structure of cellulose. In the process of BC synthesis, initially the nascent chains of BC are aggregated into approximate 1.5 nm (width) subfibrils (Kudlicka, 1989). Subsequently, the subfibrils assemble into 3-4 nm (thickness) × 70-133 nm (width) × 1-9 μm (length) ribbon-shaped microfibrils (Brown et al., 1976; Zaar, 1979; Yamanaka et al., 2000). Finally, the ribbon-shaped microfibrils form a reticulate structure on the surface of the liquid medium.

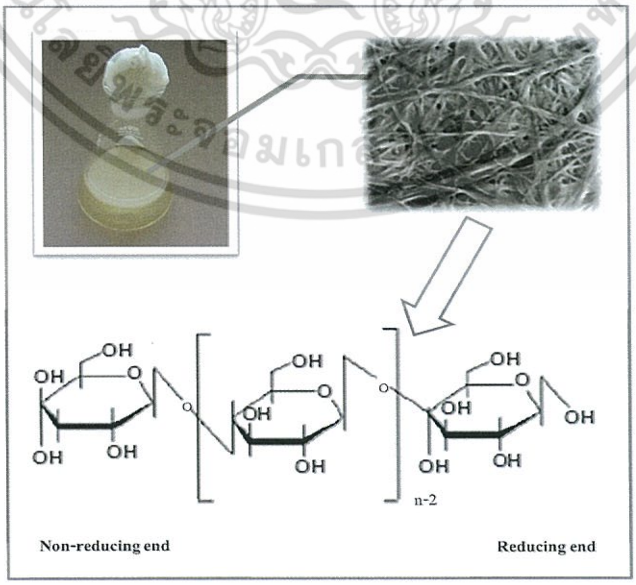


Figure 2.1 Structure of BC produces by cellulose producing bacteria.

In general, there are two crystalline forms of cellulose including cellulose I and cellulose II. Cellulose I is a metastable structure with parallel $\beta(1\rightarrow4)$ and uniaxially arranged of glucan chains. This form of cellulose has been found in cell wall of plants and produced by *K. xylinus* at static culture. In addition, cellulose I consists of 2 allomorphs which are cellulose I_α and cellulose I_β (Watanabe et al., 1998). The two structures are mostly found in algae, bacteria and plant cellulose (PC). However, BC contains more of these structures than in PC. On the other hand, cellulose II is constructed from antiparallel $\beta(1\rightarrow4)$ glucan chains randomly linked together and stabilized by a higher amount of hydrogen bonds, resulting in higher thermodynamic stability of the cellulose II. Naturally, cellulose II is produced by few organisms as some strains of algae, mold and bacteria (Jonas and Farah, 1998). The formation of cellulose I and cellulose II is displayed in Figure 2.2.

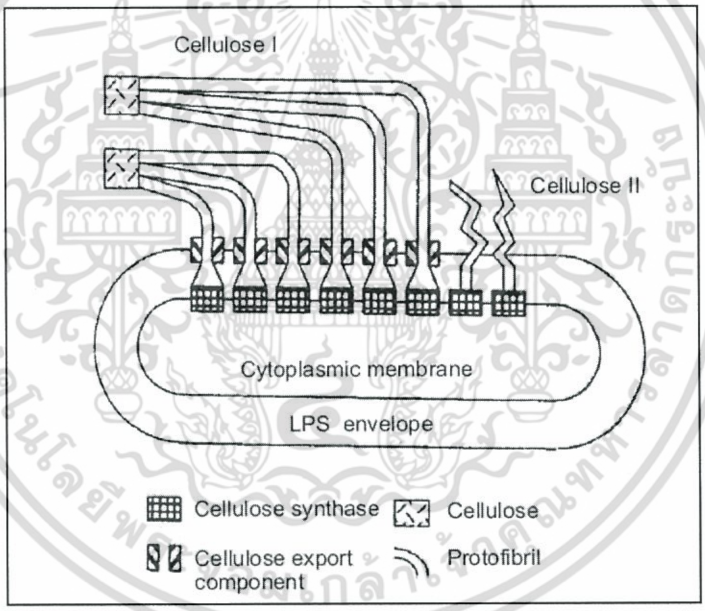


Figure 2.2 The formation of cellulose I and cellulose II by *K. xylinus* (Chawla et al., 2009).

Moreover, the characterization and structure of BC are also dependent on the culture conditions. In static condition, BC develops into a pellicle form at the surface of the liquid medium as shown in Figure 2.3. During BC cells development, submicrofibrils are continuously released from linearly ordered pores at the cell envelope. Later, subfibrils will aggregate into microfibrils and a disorganized form of parallel bonds.

Figure 2.3 BC pellicle produces in static culture.

On the other hand, in shaken condition, BC is produced in the form of fibrous and pellets which are spread throughout the liquid medium (Ross et al., 1991; Watanabe et al., 1998) as displayed in Figure 2.4. In this condition, BC microfibrils interconnect and form a grid-like pattern with perpendicular and parallel orientation (Watanabe et al., 1998). Interestingly, different arrangements of BC at these different conditions result in different structures and components of BC.

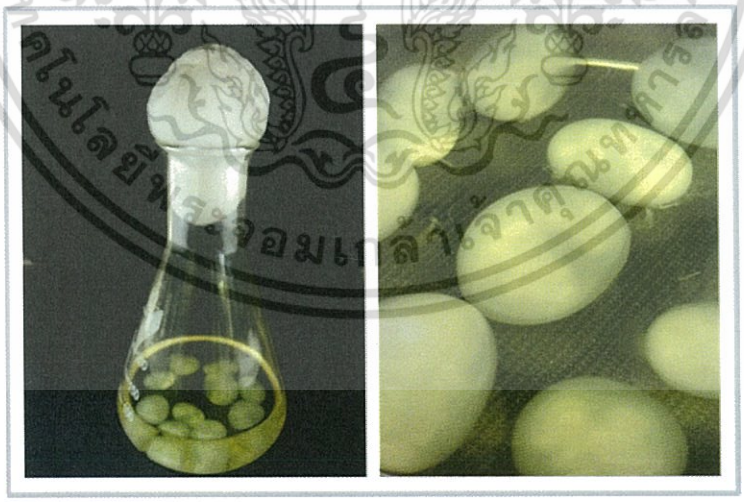


Figure 2.4 The formation of BC pellets in shaken culture.

In general, the molecular formulas of BC and PC are identical $(C_6H_{10}O_5)_n$, however the cellulose derived from plant and bacteria possess unique physical and chemical features

(Yoshinaga et al., 1997). BC is a highly uncontaminated substance without hemicellulose and other impurities, which usually contain in PC. In addition, BC subfibrils are about 100 times thinner than PC fibrils (Figure 2.5). BC shows a higher crystallinity index (above 60%) and a higher degree of polymerization (DP) approximately 2000-20000 (Jonas and Farah, 1998; Watanabe et al., 1998) whereas the DP of PC varies from 13000-14000 (Teeri, 1997).

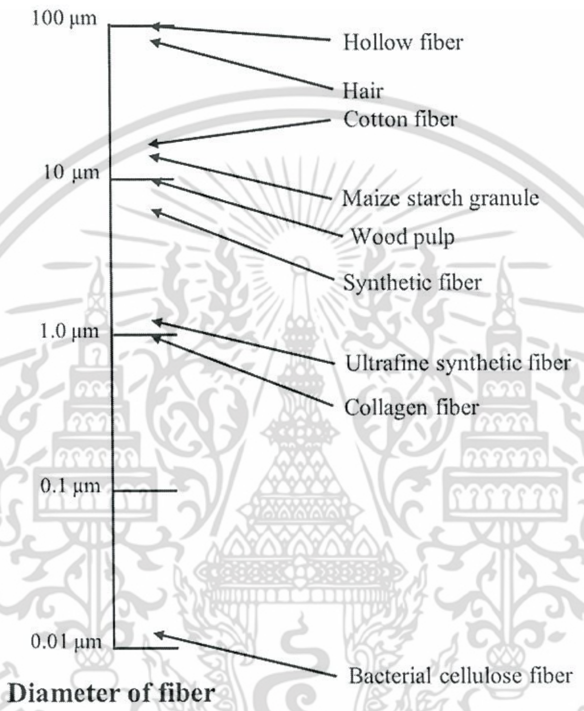


Figure 2.5 Comparison of BC fiber with fiber from other sources.

2.1.3 Biosynthesis pathway

Bacterial cellulose synthesis is a specifically regulated multi-step process involving a large number of both individual enzyme, and complex catalytic, and regulatory proteins of which supramolecular structure has not yet been well defined (Chawla et al., 2009). This multi-step process includes the production of uridine diphosphate glucose (UDPglc), which is a precursor of cellulose formation, followed by the polymerization of glucose into β (1→4) glucan chains and the aggregation of hundreds to even thousands of individual cellulose chains into a ribbon-like structure. Later, cellulose chains are secreted out from the cell and self-assembled into fibrils.

In *K. xylinus*, cellulose synthesis is linked to catabolic processes of oxidation and uses as much as 10% of energy derived from the total of catabolic reactions. Bacterial cellulose production does not interfere with other anabolic processes, such as protein synthesis. *K. xylinus* employs either pentose phosphate cycle or the Krebs cycle coupled with gluconeogenesis (Ross et al., 1991; Tonouchi et al., 1996).

Figure 2.6 displays the biosynthesis pathway of cellulose production (Chawla et al., 2009). Initially, glucose is imported into bacterial cell in the form of glucose-6-phosphate catalyzed by glucokinase in phosphorylation process. Then isomerization of this intermediate to glucose-1-phosphate is catalyzed by phosphoglucomutase and the conversion of the resulting metabolite to UDPglc by UDPglc pyrophosphorylase. Finally, UDPglc is synthesized to bacterial cellulose by cellulose synthase which is activated by cyclic diguanylic acid (C-di-GMP) (Figure 2.7). The C-di-GMP is produced from GTP (guanosine triphosphate) activated by diguanylate cyclase or it can be produced by GTP decomposition using phosphodiesterase A and B activators (Ross et al., 1991). The cellulose synthase or 1,4-β-D-glucosyltransferase; EC 2.4.4.12 plays an important role in cellulose biosynthesis and it is specific to UDPglc, which is the precursor of β-glucan chain and UDP synthesis. The synthesis of cellulose synthase is controlled by the operon, which contains four types of bacterial cellulose synthase (*bcs*) genes, including *bcsA*, *bcsB*, *bcsC* and *bscD* (Yoshinaga et al., 1997) as shown in Figure 2.8.

2.1.4 Effect of growth medium components

Generally, the growth medium contains carbon, nitrogen and other macronutrients, and micronutrients required for the growth of organism. Change of the medium components usually affects the growth and production of BC directly or indirectly. The production and extrusion of extracellular cellulose is usually most conspicuous when bacteria are supplied with plentiful carbon source and minimal nitrogen source (Ramana et al., 2000). Sometimes, amino acids and vitamins are applied to enhance cell growth and BC production. The effects of various growth medium components on BC production are described in the following sections.

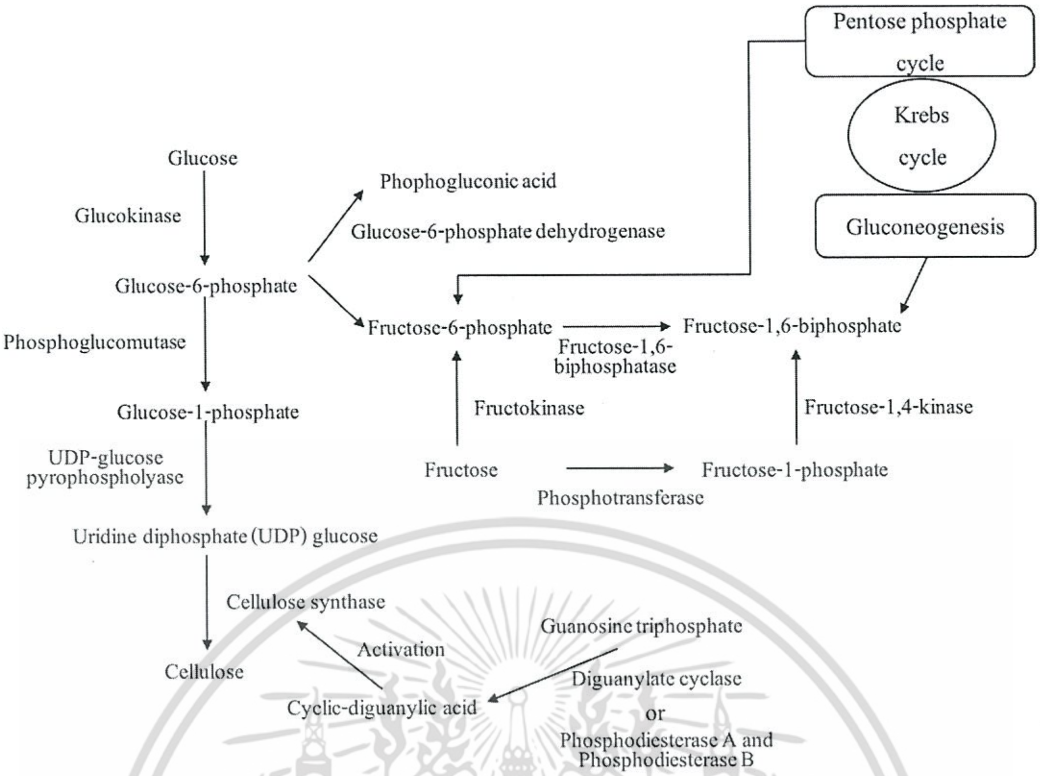


Figure 2.6 Pathway of cellulose synthesis in *Komagataeibacter xylinus*.

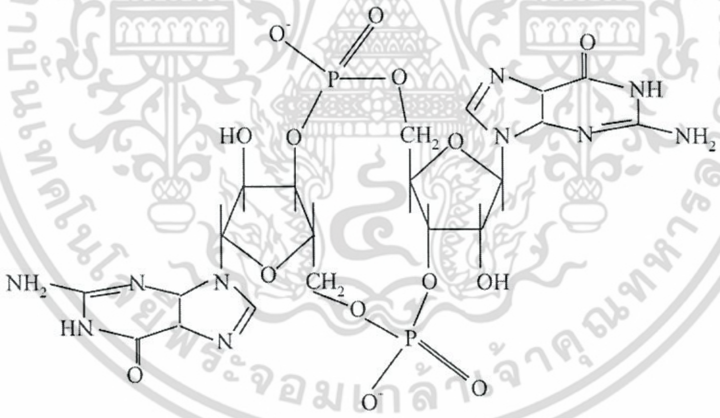


Figure 2.7 Structure of cyclic diguanylic acid.

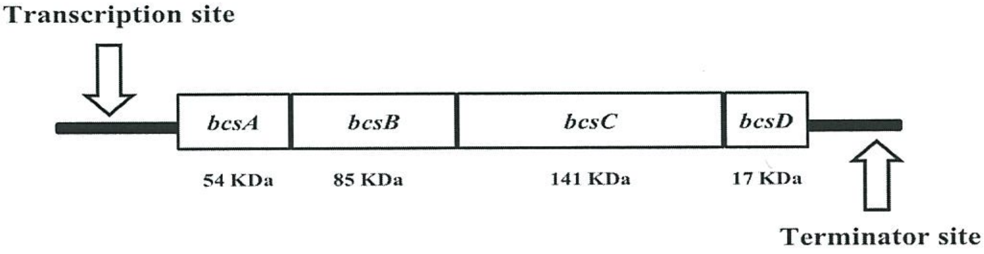


Figure 2.8 Structure of bacterial cellulose synthase (*bcs*) genes.

2.1.4.1 Carbon source

Carbon is an important element in the production of cell and energy. In general, aerobic bacteria use approximately 50-55% of carbon sources for cell synthesis, while anaerobic bacteria use only 10% of their carbon sources (Stanbury and Whitaker, 1984). Normally, pure sugars including glucose, sucrose, fructose, mannitol, sorbitol and maltose are used as common sources of carbon for the production of BC as well as other carbohydrates such as xylose, glycerol and starch. Many researchers evaluated and compared the efficiency of different carbon sources in the production of BC (Keshk and Sameshima, 2005; Surma-Ślusarska et al., 2008; Pourramezan et al., 2009).

1. Glucose

In the study of characteristics of BC obtained from *A. xylinum* culture for application in papermaking, Surma-Ślusarska et al. (2008) compared the efficiency of these carbon sources such as glucose, arabinose, mannose, galactose, xylose and mannitol in BC production of *A. xylinum* at 30 °C for 7 days under static condition. They found that bacteria grown on glucose produced the maximum yield of 25% compared to the others. Moreover, Keshk and Sameshima (2005) compared the application of different glucose concentrations at 0, 0.2, 0.5, 1.0, 1.5, 2.0 and 3.0%. They found that 1.0% and 1.5% glucose concentrations resulted in the highest yields, while 0.2% and 3% glucose concentrations gave the lowest BC yields. However, the use of glucose as a carbon source for BC production usually results in the formation of gluconic acid as by-product in the medium which naturally decreases the pH of the culture medium and eventually affects the production of BC. For solving this problem, Keshk and Sameshima (2006) studied the BC production and gluconic acid formation in the presence of 1.0% lignosulfonate. They found that BC production was increased, and gluconic acid formation was decreased when the medium was supplemented with 1.0% lignosulfonate. This was ascribed to the inhibition of gluconic acid formation in the presence of antioxidant and polyphenolic compounds in lignosulphonate.

2. Sucrose

Pourramezan et al. (2009) optimized culture conditions for BC production by *Acetobacter* sp. 4B-2. They found that the highest BC yield was obtained when 2.0% (w/v) of sucrose was used as carbon source, followed by glucose, xylose and lactose, respectively. Jagannath et al. (2008) studied the effect of pH, sucrose and ammonium sulphate concentrations on the production of BC (nata-de-coco) by *A. xylinum*. The results showed that this bacterium could effectively use sucrose as sole carbon source in coconut water medium and the BC synthesis was more dependent on pH than either sucrose or ammonium sulphate concentrations. The maximum BC thickness was attained at pH 4.0 with 10.0% sucrose and 0.5% ammonium sulphate concentrations.

3. Glycerol

Keshk and Sameshima (2005) evaluated 2.0% (w/v) of different carbon sources used in cellulose production by *G. xylinus* ATCC 10245 and found that glycerol was the most effective carbon source. The same result was found by Kim et al. (2006) who examined the optimized medium composition for BC production, and glycerol was found to be the most optimal carbon source for *Gluconacetobacter* sp. RKY5 isolated from persimmon vinegar. The maximum BC yield of 3.27 g/L was achieved at 14 g/L of glycerol. The results was supposed that *Gluconacetobacter* sp. RKY5 had another metabolic pathway to synthesize cellulose from a C3 component, while *Acetobacter* species produced cellulose by converting of glucose to uridine diphosphate glucose.

4. Mannitol

Mannitol has been used as a carbon source in many researches such as the characterization of cellulose production by *G. xylinus* strain isolated from a commercially available Kombucha culture by Nguyen et al. (2008a). The results indicated that mannitol was the most suitable carbon source for *G. xylinus*. The highest BC yield of approximately 2.64 g/L was obtained when this bacterium was cultivated with a combination of 20 g/L mannitol (carbon source) and 40 g/L corn steep liquor (nitrogen source) in HS medium. In addition, mannitol was found to be the optimal carbon source for BC synthesis by *Enterobacter amnigenus* GH-1 isolated from rotten apples with the BC yield of 2.70 g/L (Hungund and Gupta, 2010).

5. Xylose

D-xylose is another type of the most available sugars found in natural sources. However, it is not as easily metabolized by *Acetobacter* species as D-glucose. The formation of BC in this carbon source is also more difficult. Ishihara et al. (2002) utilized D-xylose as carbon source for the production of BC. They found that D-xylose was not metabolized by any bacterial strains, which generally exhibited high BC production in D-glucose medium. Therefore, it was concluded that the production of BC in D-xylose medium was relatively unsuccessful. On the other hand, when D-xylose is converted into D-xylulose, which can be employed in promoting the process of BC production as the metabolic product such as pyruvate, it will be used as substrate for BC production of *Acetobacter* species. In general, most bacteria employ the xylose-isomeration pathway for metabolism of D-xylose. When xylulose was used, *A. xylinum* IFO 15606 gave the maximum BC yield of 89 mg per 100 mL of medium.

6. Ethanol

Ethanol is an additional carbon source which also reduces non-cellulose producing cells of *G. hansenii* (Cel^-) PJK culturing under agitated culture conditions (Park et al., 2003). Ethanol supplementation of 1.0% (v/v) usually result in the increased of BC production by *G. hansenii* from 1.30 up to 2.31 g/L (Park et al., 2003). Likewise, Son et al. (2001) studied the effect of the addition of ethanol on BC production by *Acetobacter* sp. A9. They found that the addition of 1.4% (v/v) ethanol to the medium increased the BC production four times higher than the BC production without ethanol addition. Furthermore, addition of ethanol was also found to enhance the elimination of spontaneous mutations of non-cellulose producing cells.

7. Waste or by-product

Carbon sources such as glucose, sucrose, mannitol and glycerol are usually considered economically disadvantageous in the large commercial scale. These nutrients are so relatively expensive. Wastes or by-products such as molasses, coconut juice, soybean whey, etc. have been applied as alternative carbon sources for BC production in order to reduce the production cost.

- Sugar cane molasses

Keshk and Sameshima (2006) studied the BC production by six strains of *Acetobacter* using sugar cane molasses (MO) as sole carbon source in HS medium. The results showed that, the yields of BC among all strains cultured in MO medium was much higher than that from the original HS medium (glucose as sole carbon source). The results suggested that sugar cane molasses could be used as an efficient advantage economically substrate for BC production in large scale commercial.

- Konjac powder

Hong and Qiu (2008) discovered a new carbon source for BC synthesis of *A. aceti* subsp. *xylinus* ATCC 23770 by diluted acid hydrolysis of konjac powder. In addition the konjac powder was detoxified by different alkaline treatment methods to remove microbial growth inhibitors. The results indicated that detoxification with calcium hydroxide treatment worked better than that with sodium hydroxide. Based on the same concentration of carbon source, BC production using konjac hydrolysate was three times higher than that using glucose, six times higher than that using mannose, and five times higher than that using glucose-mannose mixture as carbon sources in static culture.

- Coconut water and pineapple water

Kongruang (2008) studied BC production by *A. xylinum* strains using agricultural waste products. The results showed that coconut juice seems to be a better substrate than pineapple juice and the productivity of BC on this medium is considerably high. The BC pellicles of all strains could be easily applied in several productions such as paper, textile, food industries, etc.

- Waste from beer culture broth

Ha et al. (2008) produced BC using the waste obtained from fermentation broth (WBFB) instead of a chemically defined medium. WBFB contained small amount of sulfur and more than 4.0% of ethanol. They found that, BC production under static condition was better than shaking condition. The BC yield when using WBFB was ten times higher than with HS chemically defined medium.

- Coffee cherry husk extract

Rani et al. (2011a) reported the use of coffee cherry husk (CCH) extract and corn steep liquor as low cost sources of carbon and nitrogen, respectively. CCH and corn steep liquor are by-products of coffee and starch production, respectively. The results showed that, after two weeks of cultivation the BC yield was 6.24 g/L.

- Residues from agro-industries

Carreira et al. (2011) evaluated the possibility of using residues from Portuguese agro-forest industries such as grape skin aqueous extract, cheese whey, crude glycerol and sulfite pulping liquor as raw material for BC production by *G. sacchari*. The results showed that these residues can be used as carbon substrates. However, dilution of the residues and nutritional supplementation are required.

- Waste fiber sludge

Cavka et al. (2013) utilized waste fiber sludge as a low cost and abundant raw material for the co-production of BC and enzymes. The results indicated that BC from fiber sludge hydrolysate showed a cellulose polymer displaying superior properties than from a glucose based reference medium.

- Soybean whey

Soybean whey is a liquid by-product resulting from pressing of coagulated soybean milk during tofu production. Soybean whey is approximately 79-80% of the total soybean milk (Fung et al., 2008). Production of one ton of tofu generates ten tons of soybean whey as by-product (Lu et al., 2004). Formation and characterization of soybean whey are shown in Figure 2.9 and Figure 2.10, respectively. Because of soybean whey contains only 1-3 % (w/v) solid, recovery of it is not now economically feasible and it is discarded. Since, soybean whey has a high biological oxygen demand (BOD) of 13730 ppm (Smith et al., 1962) and a high chemical oxygen demand (COD) of 25000 mg/L (Mitra et al., 2010), it presents a serious waste disposal problem. Although soybean whey is considered as waste but it is rich of nitrogen, starch, fat, phosphorus, phytate salts, trace elements and sugars (Smith et al., 1962; Mitra et al., 2010). Fung et al. (2008) reported that most of sugars in soybean whey composed of sucrose (11.3 g/L), stachyose (6.4 g/L), raffinose (1.6 g/L), glucose (1.2 g/L) and fructose (1.1 g/L). Due to high nutrients content, soybean whey have been applied as low cost substrate for microbial fermentation in the production of fungal mycelial protein, probiotic, food preservative and food additive (Falanghe et al., 1964; Fung et al., 2008; Mitra et al., 2010; Singh and Banerjee, 2013).

Recently, soybean whey has been initially studied as raw material for BC production. Lu et al. (2004) produced BC by *A. xylinum* C5 using soybean whey based medium. In their study, soybean whey was a byproduct of soybean protein, which composed of 1.5% solid with approximately 0.97% saccharides and 0.32% protein and pH 4.5. The results indicated that the bacteria could be grown and produced BC in soybean whey without addition of any carbon substrates. These revealed that soybean whey comprises of some oligosaccharides that could be utilized by *A. xylinum* C5, and the best culture medium was 7.0% (w/v) sucrose, 0.5% (w/v) ammonium sulphate, 0.2% (w/v) $MgSO_4.7H_2O$, and 0.1% (w/v) citrate. In this soybean whey medium, the bacteria produced the maximum BC yield with 5.89 g/L which was higher than 3.39 g/L of BC produced in normal medium.

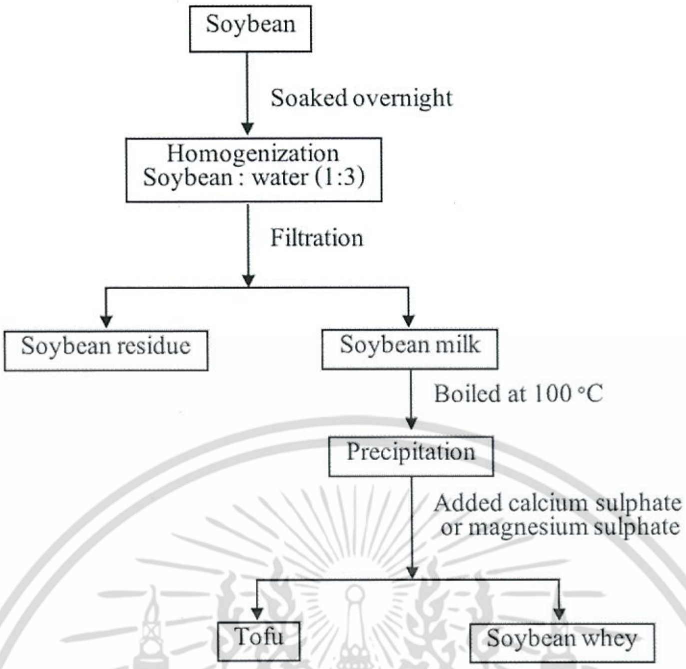


Figure 2.9 Process of tofu production.

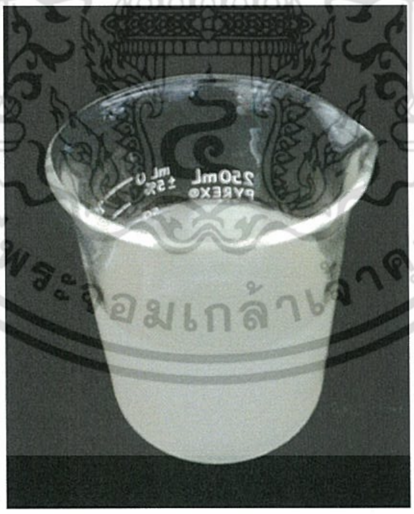


Figure 2.10 Soybean whey.

2.1.4.2 Nitrogen source

Nitrogen is a main component of proteins necessary in cell metabolism and contains 8-14% of dry cell mass of bacteria. The effect of various nitrogen sources on BC production has been studied. Matsuoka et al. (1996) reported that 0.15% (v/v) corn steep liquor had a stimulating effect on BC production when it was added to the medium containing 4.0% (w/v) fructose. This was attributed to the presence of lactase in corn steep liquor, which was absent from other nitrogen sources. Ramana et al. (2000) studied the effect of various carbon and nitrogen sources on cellulose synthesis by *A. xylinum.* The results showed that when casein hydrolysate and peptone were used as a nitrogen source, *A. xylinum* gave BC yield with 5.0 g/L and 4.8 g/L, respectively. Hungund and Gupta (2010) found that casein hydrolysate was the best nitrogen source for BC production by *Enterobacter amnigenus* GH-1. The highest BC yield of 2.9 g/L was obtained at 0.6% (w/v) casein hydrolysate.

2.1.4.3 Effect of precursors

The addition of precursor molecules is important in cellulose synthesis process as metabolic driving force. Many researchers have used amino acids as nitrogen source or as a stimulator for improving biopolymer yield. Masaoka et al. (1993) indicated that methionine was an important effect on BC production by *A. xylinum* ssp. *sucrofermentans*. In addition, they found that vitamins such as pyridoxine, nicotinic acid, *p*-aminobenzoic acid and biotin were also found to be important for cell growth and BC production. The pantothenate and riboflavin were found to have contradictory effects. Son et al. (2003) observed that nicotinamide was an important substance for BC synthesis. When nicotinamide was added in the range of 0.00001 to 0.00008%, the maximum BC production was at 0.00005%.

2.1.4.4 Effect of environmental factors

1. Temperature

Temperature is a crucial factor that affects both cell growth and BC synthesis. In general, the maximum BC production was observed from 25-30 °C although most researchers used 28-30 °C (Hestrin and Schramm, 1954; Gromet-Elhanan and Hestrin, 1963). In *Acetobacter* sp. 4B-2, the temperature of 30 °C was the optimal temperature for BC production with approximately 11.5 g/L BC yield (Pourramezan et al., 2009). Surma-Ślusarska et al. (2008) reported that when cultivation of *A. xylinum* at 30 °C the BC yields was approximately two times higher than the BC yield at 25 °C and 35 °C. Hungund and Gupta (2010) indicated that the optimal temperature for BC production by *Enterbacter amnigenus* GH-1 was found to be 28 °C.

2. pH

It is generally accepted that the optimal pH of the culture medium for BC production is in the range of 4.0-7.0 and the yields of cellulose decreasing below pH=4 (Masaoka et al., 1993). Most researchers used pH 5.0-6.0 in their research work (Hestrin and Schramm, 1954; Watanabe and Yamanaka, 1995). Pourramezan et al. (2009) studied the optimal culture conditions for BC synthesis by *Acetobacter* sp. 4B-2 and found that the optimal pH of BC production was 7.0. Likewise, Hungund and Gupta (2010) found that pH 6.5 was the optimal pH for BC production by *Enterbacter amnigenus* GH-1. Jagannath et al. (2008) found that pH 4.0 was the optimal pH for BC production by *A. xylinum*.

3. Dissolved oxygen

The dissolved oxygen in the culture medium is an important factor affecting cellulose production. Tantratian et al. (2005) found that too high dissolved oxygen in the medium increased the gluconic acid content and reduced the BC production when glucose was used as a carbon source. On the other hand, too low dissolved oxygen in the medium did not provide enough oxygen for the culture to grow and BC production was again reduced. In the fed-batch culture, the highest yield of BC was attained at 10.0% saturation of dissolved oxygen (Hwang et al., 1999).

2.1.5 Recovery and purification of BC

In common, the BC obtained after fermentation is not pure. It contains some impurities such as bacterial cells or medium components. Therefore, prior to further application BC will be purified in order to obtain completely pure cellulose. The processes of isolation and purification of BC and PC are indicated in Figure 2.11. In general, the process of BC purification in culture medium involves the treatment with alkali such as sodium hydroxide or potassium hydroxide, organic acid such as acetic acid or repeated washing of the mixtures with deionized water or hot tap water for a period of time. The above purification steps can be used alone or in combination with others (Chawla et al., 2009). Hungund and Gupta (2010) purified cellulose pellicle by boiling in 2.0% (w/v) sodium hydroxide solution for 30 minutes and thoroughly washing with distilled water. Keshk and Sameshima (2005) purified BC by washing with water, boiled with 1.0% (w/v) sodium hydroxide at 90 °C for 15 minutes, neutralized with 1.0% (v/v) acetic acid and washed again with distilled water. George et al. (2005) studied the characterization of chemically treated bacterial (*A. xylinum*) biopolymer. In their study, BC pellicle was treated with 0.2N alkali solutions including sodium hydroxide, potassium hydroxide (KOH), sodium carbonate (Na_2CO_3) and potassium carbonate (K_2CO_3) by boiling individually for 30 minutes and rinsing 4-5 times with distilled water until a neutral pH was obtained in the drained water. The biochemical analysis indicated that various alkali treatment methods were highly effective in removing proteins and nucleic acid from native BC membrane which eventually permits resulting in pure cellulose membrane.

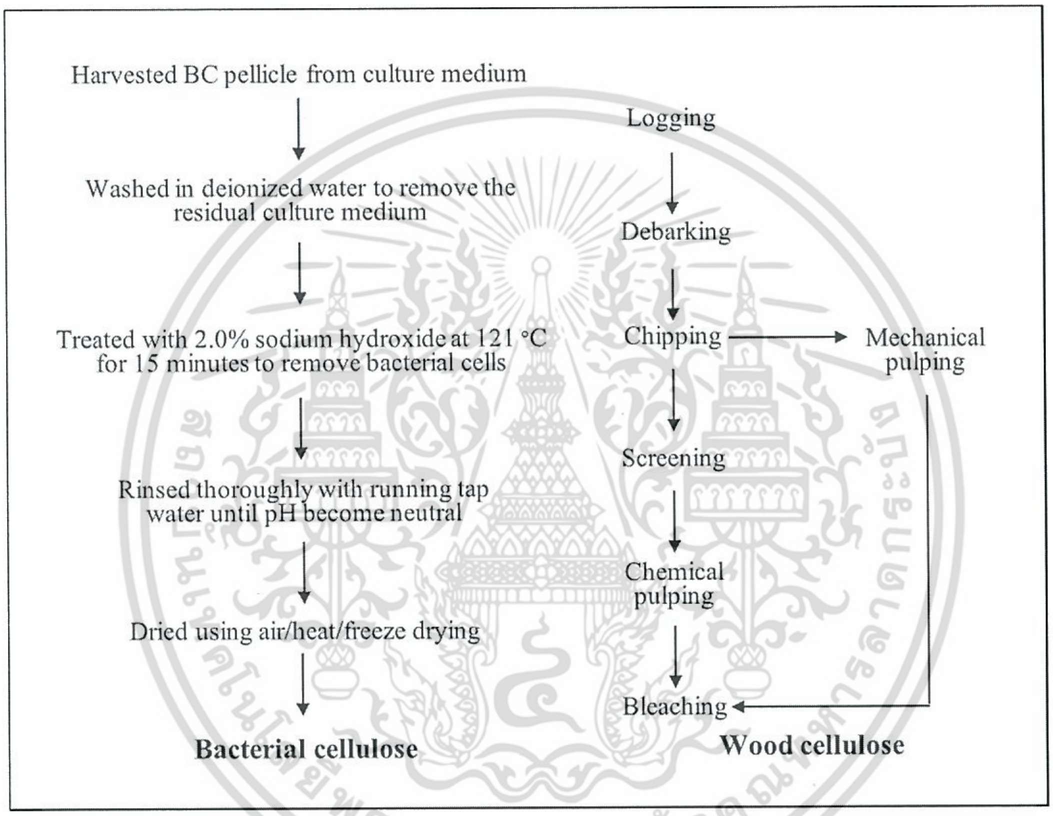


Figure 2.11 Purification processes of wood cellulose and bacterial cellulose.

2.1.6 Application of BC

For decades BC has been proved encompassing a number of unique properties, such as high purity, high mechanical strength, good shape retention, high water holding capacity, excellent biodegradability and biocompatibility (Chawla et al., 2009). From these properties, this natural biopolysaccharide has been applied in a wide range of applications including paper, textile, food, waste treatment, broadcasting, and pharmaceutics. The applications of BC are described in the following sections.

2.1.6.1 Paper production

Fragment BC is empirically accepted as a competent raw material in the production of flexure-durable papers and high filler-content papers, which are an ideal material for the production of banknote and Bible papers (Iguchi et al., 2000). In general, the production of banknote and Bible needs paper which is durable and highly retentive. The paper must have high water and oil resistant. Result from a study of Suwannapinunt et al. (2007) showed that BC paper displayed the tearing index value of 34.00 KN.m/Kg which was higher than plant cellulose paper. Characteristic of BC paper is shown in Figure 2.12.

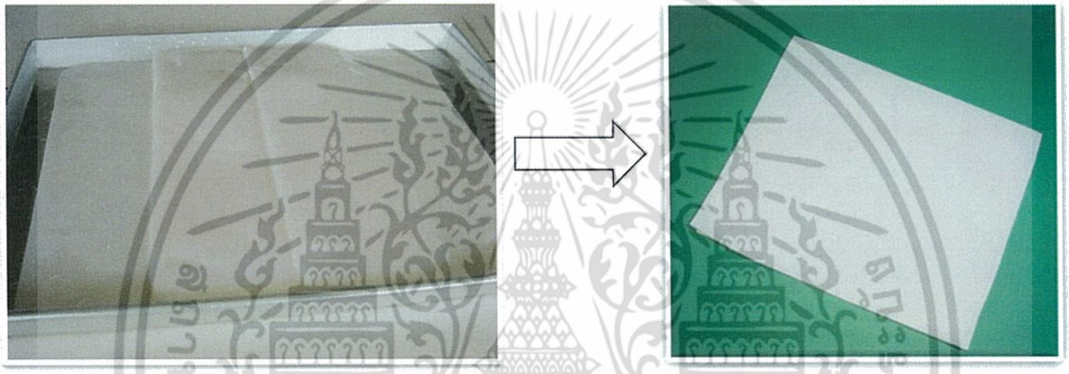


Figure 2.12 Paper production using bacterial cellulose as raw material.

2.1.6.2 Food production

It is well-known that BC has long been useful as the raw material for nata-de-coco, a popular dessert food of Philippines (Ng and Shyu, 2004). It is one cm. thick BC pellicle fermented by *A. xylinum* in coconut water, cut into cubes and immersed in sugar syrup (Figure 2.13). Now, nata-de-coco is manufactured in large quantities in Indonesia and also found in other countries such as some parts of Russia and Middle-Asia, where it is known in the name of Teekvass (Chen et al., 2010). In 1992, BC was developed into diet drinks in Japan in the name of Manchurian or Kombucha tea containing *Acetobacter* grown along with yeast in tea extract and sugar (Chawla et al., 2009). In addition, BC can be used as thickening and stabilizing agent in many other food productions.

Figure 2.13 Nata-de-coco.

2.1.6.3 Pharmaceutical and medical production

BC shows a highly porous property which allows potential transfer of antibiotics or other medicines into wound, while at the same time provides efficient physical barrier against any external infection. Due to this unique property, BC has been applied in the production of high-quality wound dressing, artificial blood vessel, bone tissue engineering, bone grafting, regenerative medicine such as guided tissue regeneration (GTR) or as a replacement for dura mater (a membrane that surrounds brain tissue) (Klemm et al., 2001; Svensson et al., 2005). The BioFill company investigated its unique properties for wound healing and produced two products Bioprocess® and Gengiflex®, which were dressings for extensive wounds. Likewise, Xylos Corp. (USA) developed Prima Cel™ from BC for clinical tests of ulcers and wounds treatment (Johnson and Neogi, 1989). The commercial wound dressing produces from BC are shown in Figure 2.14.

Figure 2.14 Wound dressing from bacterial cellulose (National Innovation Agency, 2014).

2.1.6.4 Other production

The unique dimension stability of BC gives rise to sound transducing membrane while maintains high sonic velocity over a wide frequency ranges, thus being the best material to meet the rigid requirements for optimal sound transduction. Sony Corporation (Japan) and Ajinomoto (Japan) developed the first audio speaker diaphragms using BC. However, this speaker membrane is not suitable for large scale commercial because of the high production cost (Iguchi et al., 1988).

In addition, BC pellicle in the form of dried sheet is also the best medium for displaying information and is far better than the assorted existing display technologies owing to its high reflectivity, contrast, low cost and flexibility. BC becomes more attentive as a substrate in the production of electronic paper displays and had the potential to be extended to various applications such as e-book tables, e-newspapers, dynamic wall papers, rewritable maps and learning tools (Shah and Brown, 2005).

Beside, BC indicates high tensile strength, biodegradability and potential as a good carrier (low oxygen transmission rate), these distinct qualities make BC more attentive for its application as packaging material in food packaging technology. For example, Nguyen et al. (2008b) produced nisin-containing BC film to inhibit *Listeria monocytogenes* on the surface of vacuum-packaged frankfurters. The results showed that BC films demonstrated high potential antimicrobial packaging film by reducing 3.3 log CFU/g of total aerobic bacteria after 14 days of storage.

2.2 Isolation and identification of cellulose producing microorganisms

2.2.1 Isolation of cellulose producing microorganisms

Mostly cellulose producing microorganisms in nature are bacteria in genus of *Komagataeibacter* particularly, *K. xylinus*, *K. hansenii* and *K. nataicola* (Park et al., 2003; Chawla et al., 2009). These cellulose producing bacteria are commonly found symbiosis with other microorganisms in natural sources such as flowers, sugar cane and some particular rotten fruits (Seto et al., 1997; Tsuchida and Yoshinaga, 1997; Park et al., 2003). For these reasons, isolation of cellulose producing bacteria from other microorganisms usually requires regular specific growth media.

2.2.1.1 Growth media for isolation of cellulose producing bacteria

In general, they are many types of growth media used in isolation and identification of cellulose producing microorganisms. One of the most accepted media is Hestrin & Schramm (HS) medium (2.0% (w/v) glucose, 0.5% (w/v) yeast extract, 0.5% (w/v) peptone, 0.27% (w/v) disodium hydrogen phosphate (Na_2HPO_4) and 0.115% (w/v) citric acid, pH 5.0 or 6.0) adding 0.2% (v/v) acetic acid, 0.5% (v/v) ethanol and 0.02% (w/v) cyclohexcimide (Toyosaki et al., 1995a; Park et al., 2003). Park et al. (2003) and Hungund and Gupta (2010) applied this medium for the isolation of cellulose producing bacteria from rotten apples.

2.2.2 Identification of cellulose producing microorganisms

From the above described, *Komagataeibacter* is an acetic acid bacteria in the family of *Acetobacteraceae*. Early identification systems for these bacteria were based on morphological and biochemical characteristics. Nowaday, polyphasic taxonomy analysis comprising of phenotypic methods, chemotaxonomical methods and genotypic method is generally applied in the identification of acetic acid bacteria (Cleenwerck and De Vos, 2008).

2.2.2.1 Phenotypic methods

Classical tests for phenotypic characterization of acetic acid bacteria are generally complex (analysis in tube and plate media), time-consuming and often not straightforward to construe. As a result, many researchers have replaced these tests either by miniaturized versions and or direct molecular DNA linked methods, especially in identification procedures. The general tests that are used to characterize acetic acid bacteria phenotypically compose of determination of colony and cell morphology, Gram staining and tests to determine catalase and oxidase activity. In common, acetic acid bacteria are Gram-negative, rod-shape, while there are also possibilities for the formation of or Gram variability and irregular cells. Acetic acid bacteria are catalase positive but oxidase negative (De Ley et al., 1984; Cleenwerck and De Vos, 2008). The other tests of phenotypic characteristics have been applied for *Komagataeibacter* sp. identification including; (1) acid production from ethanol by examining a clearing of the opacity in the medium around the bacterial growth (Frateur, 1950) or by observing a color change of the bromocresol purple in the medium from green to yellow (Carr, 1968), (2) oxidation of acetate and lactate to carbon dioxide and water (Asai et al., 1964), (3) growth in the presence of 0.35% acetic acid, pH 3.5 (Yamada et al., 2000), (4) growth on carbon sources (Gosselé et al. 1983), (5) formation of water soluble brown pigment (Swings, 1992), (6) acid production from sugars and sugar alcohols (Asai et al., 1964), (7) cellulose production determined by boiling pellicle in 5.0% sodium hydroxide for 2 hours, true cellulose is confirmed when the pellicle does not dissolve after boiling (Navarro et al., 1999) and (8) growth in the presence of 30% D-glucose (Cleenwerck et al., 2002).

2.2.2.2 Chemotaxonomical methods

The Chemotaxonomical methods applied in the characterization of acetic acid bacteria are quinone and fatty methyl ester (FAME) analyses. In general, isoprenoid quinones are extracted and purified according to method described by Yamada et al. (1969) and analysed using reverse-phase high performance liquid chromatography (HPLC) as explained by Komagata and Suzuki (1987). For FAME analysis, fatty acid profiles are extracted from cells grown on media suitable for acetic acid bacteria and analyzed according to the recommendations of the commercial identification system MIDI (Microbial Identification System, Inc., Delaware U.S.A.). The whole-cell fatty acid composition is determined by gas chromatography (Cleenwerck and De Vos, 2008).

2.2.2.3 Genotypic methods

1. DNA base composition analysis

The determination of the DNA base composition is one of the first genomic methods, and the G+C content is often considered as a part of the standard description of bacterial taxa. DNA molecules are composed of two long strands of polydeoxyribonucleotides that form a double helix. Purine and pyrimidine bases are connected by hydrogen bonds. Adenine of a strand pairs with thymine of an opposite strand, and cytosine pairs with guanine. The chemical analyses of DNAs demonstrate a variation in molar ratios of $(G+C)/(G+C+A+T) \times 100$ in DNA preparations attained from different groups of microorganisms. This is DNA base composition, and values are expressed as GC content or G+C content. The methods that have been used recently in the determination of DNA bases composition are explained by Mesbah et al. (1989), a direct method through which nucleotides or nucleosides can be derived from DNA by enzymatic actions and easily analyzed by HPLC or sometimes by the thermal denaturation method described by Marmur and Doty (1962). The latter method is an indirect method, which requires a rather expensive spectrophotometer equipped with a thermal control. The range of G+C content of acetic acid bacteria vary from 52 to 67 mol%.

2. DNA-DNA hybridization

When a double stranded DNA is heated, the two strands separate into single stranded molecules. Subsequently, the mixture is cooled slowly, re-association of the two complementary strands takes place and the original double complex is formed. If single stranded DNAs from different microorganisms are mixed and the nucleotide sequences are identical or nearly identical, DNA-DNA hybridization takes place. The relatedness of bacteria can be analyzed by measuring the degree of hybridization. DNA of a reference strain is labeled with radioisotope and its degree of hybridization with DNA of the tested strain is analyzed by measuring the radioactivity bound (Figure 2.15). Recently, fluorometric photobiotin labeling DNA hybridization is applied for the study of relatedness of bacteria and identification of bacterial strains (Cleenwerck and De Vos, 2008).

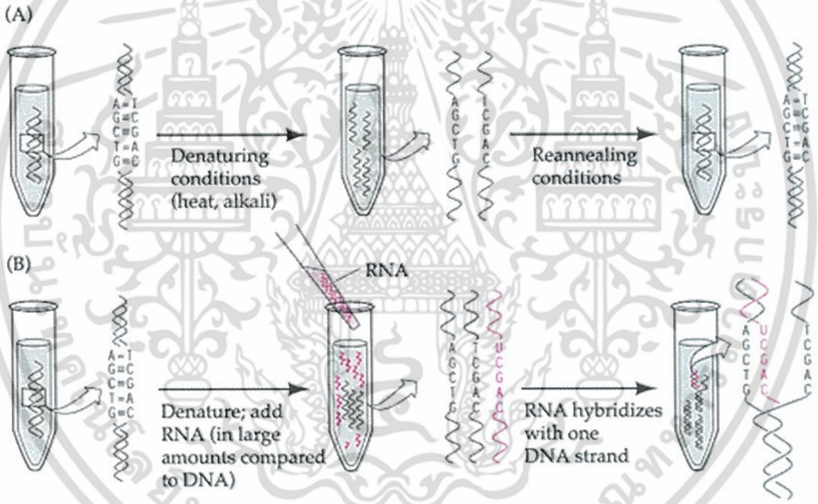


Figure 2.15 DNA-DNA hybridization (Gilbert, 2013).

3. 16S rRNA gene sequence analysis

There are three types of ribonucleic acid (RNA) in prokaryotes including the short-lived messenger (m)RNA, the stable form, transfer (t)RNA and ribosomal (r)RNA. The mRNA transmits information from the chromosome to the ribosome. The tRNA decodes the message and rRNA is a part of ribosome and read the message. The analysis of RNA for taxonomic purposes focuses on the three rRNAs including the 5S, 16S and 23S molecules. These molecules are valuable as indicators of relatedness for the following reasons (Priest and Austin, 1993):

(1) The rRNAs are essential elements in protein synthesis and present in all living organisms (exception of viruses).

(2) Because of the conserved functions of these molecules, they have changed very little during evolution. Therefore, rRNAs from even the most taxonomically distant organisms, which share virtually no DNA sequence homology, will have common rRNA sequence, thus relatedness can be assessed. Ribosomal RNA is probably unique amongst macromolecules in this respect.

(3) Some segments of rRNA evolve more rapidly than others and sequence variation occurs between closely related organisms allowing comparisons to be made at the species level.

However, there is a possibility of that the small size of the 5S rRNA molecule can detracts from its value in measuring relatedness between organisms, because it can undergo marked mutational change that would be obscured by the long stretches of conserved sequence present in the larger molecules. On the other hand, the larger size of 23S, the mutational change might not effect of the relatedness measurement but it larger size, greater level of base modification and lack of conserved primer sites complicate the procedure which results in higher experimental expense. Largely for this reason, the 16S molecule, which is rather easier to handle than the 23S, has been used extensively for comparative sequencing studies. Cleenwerck and De Vos (2008) mentioned that the complete 16S rRNA sequence analysis is more rapid and inexpensive, and is a direct method to accurately determine the phylogenetic position of an unknown organism. The 16S rRNA can now be sequenced using various approaches. Historically, cataloguing was replaced by reverse transcriptase sequencing in which the 16S rRNA from cells was applied as the template for dideoxy sequencing reactions. Rather than using DNA polymerase, reverse transcriptase (RNA dependent, DNA polymerase) was applied to generate the dideoxy-terminated, deoxyribooligonucleotides. These were then separated by gel electrophoresis as in standard DNA sequencing procedure (Priest and Austin, 1993). More recently, the polymerase chain reaction (PCR) has been used to generate amplified rRNA gene (Figure 2.16). Primers to the extremities of the gene are used to amplify the DNA. The amplified DNA can either be sequenced directly or cloned into a plasmid or phage vector prior to sequencing. This is now the method of choice for most rRNA gene sequencing since it provides a straightforward route to unambiguous DNA sequencing. Having generated the sequences, they are aligned so that nucleotide sites in correspondence are being compared. Simple comparisons of sequence positions will provide a phonetic estimate of relatedness, but most phylogenetic comparisons attempt to account for mutation rates including estimates of back mutations. As described above, UPGMA clustering will provide a phonetic classification, but again phylogenetic analyses must be used to indicate evolutionary pathways.

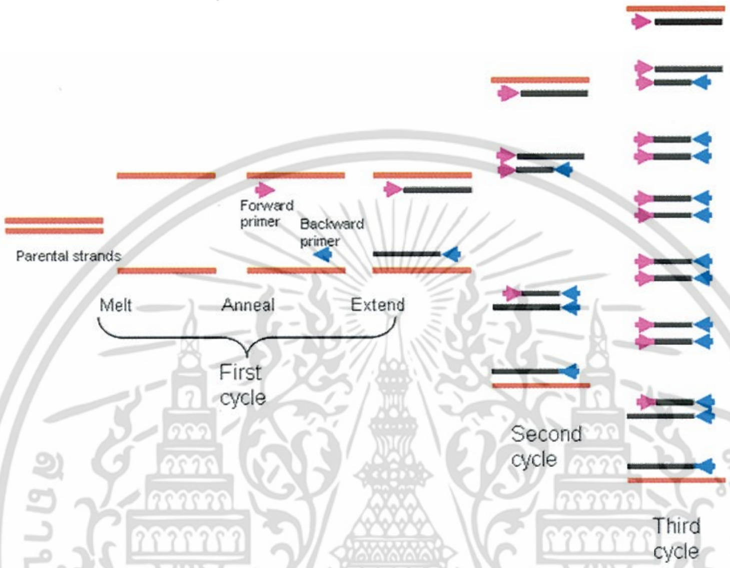


Figure 2.16 The polymerase chain reaction (Aqileware Pty Ltd., 2014).

In addition, the analysis of 16S rRNA gene has been applied for identification of cellulose producing bacteria such as *G. hansenii* from rotten apple (Park et al., 2003), *Gluconacetobacter* sp. RKY5 from persimmon vinegar (Kim et al., 2006), *Acetobacter* sp. 4B-2 from traditionally fermented vinegar in Iran (Pourramezan et al., 2009), *Gluconacetobacter* sp. F6 from rotten apple (Jahan et al., 2012) and *G. intermedius* Cls26 from rotten mandarin fruit (Yang et al., 2013b).

2.3 Optimization of culture conditions for BC production

In general, one-variable-at-a-time (OVAT) method has been used for optimization of culture condition for BC production. However, this method is considerably time-consuming and cost consuming, and usually leading to misinterpretation of results when interactions between different components are presented (Shoda and Sugano, 2005). Therefore, statistically experimental designs have been replaced the conventional method in order to minimize the error in determining the effects of parameters, and allow simultaneous, systematic and efficient variation of all parameters (Gawande and Patkar, 1999). These statistically experimental designs especially, response surface methodology (RSM) can be adopted for various optimization processes, such as comparative studies, screening experiments or determining optimal conditions (Shoda and Sugano, 2005).

2.3.1 Response surface methodology (RSM)

Response surface methodology (RSM) is a collection of mathematical and powerful statistical technique commonly used to determine the optimal conditions for multiple variables with a minimum number of experiments (Box and Hunter, 1957). RSM requires less experimental processes which result in time and cost reduction, and associated numerical noise. By carefully designed experiments usually optimize a response (output variable) which is influenced by several independent variables (input variables). An RSM experiment is a series of tests and called runs, in which changes are made in the input variables in order to identify the reasons for changes in the output response which can be displayed graphically, either in the three-dimensional space or as contour plots (Figure 2.17).

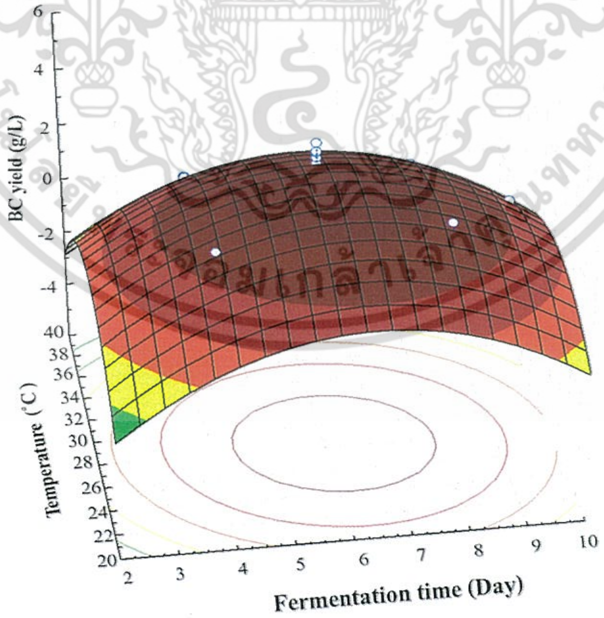


Figure 2.17 Three-dimensional response surface and contour plots.

2.3.2 Approximate model function

In general, the structure of the relationship between response and independent variable is unknown. Therefore, the first step of RSM is to find a suitable approximation to the true relationship and the most common forms are low-order polynomials (first or second-order).

2.3.3 Design of experiments

Design of experiments is a process in RSM which are originally developed for model fitting in physical experiments, but can also be applied in numerical experiments. These processes involve the selection of points where the response should be evaluated. In addition, central composite design (CCD) is mostly used as design of experimental for culture condition optimization in BC production (Jagannath et al., 2008; Rani et al., 2011a).

2.3.3.1 Central composite design

Central composite design (CCD) is an optimal design of experiment for BC culture condition while RSM which various independent variables but less experimental runs. In general, there are three parts including: (1) 2^k factorial in CRD, complete or partial RCBD, (2) 2k axial point or the point on coordinate axial and (3) central point when k is a number of independent variables and c is the number of experimental runs at the central points (Figure 2.18). Therefore, the total of experimental runs is calculated from equation 2.1 (Rigas et al., 2005).

$$n = 2^k+2k+c \tag{2.1}$$

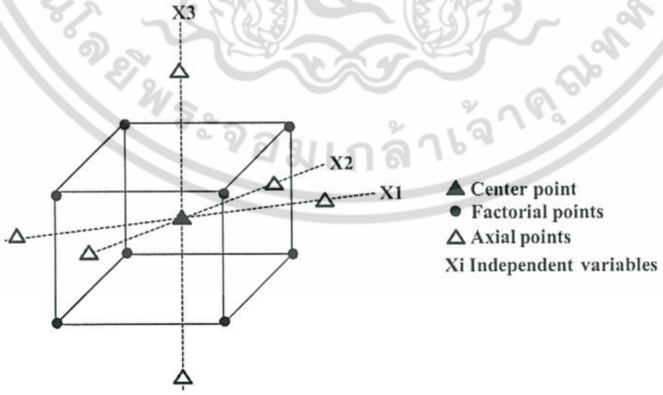


Figure 2.18 Central composite design.

There are many researches investigating culture condition optimization for BC production using RSM such as, Bacterial cellulose II optimization of cellulose production by

A. xylinum through response surface methodology was described by Embuscado et al. (1994). In their study, the effect of four independent variables including fructose and sucrose concentrations, pH and temperature were studied. The predicted BC yield from RSM was 13.2 g/L which is close to the experimental yield 12.7 g/L (in flask experiments). Likewise, the effect of pH, sucrose and ammonium sulphate concentrations on the production of BC (*nata-de-coco*) by *A. xylinum* was studied by Jagannath et al. (2008). In their study, CCD of RSM was used to predict the levels of three independent variables. A total of 20 experimental runs, with treatment combinations of each variable were performed in trays (LxBxH : 30×25×6 cm.). The three-dimensional response surfaces were generated to examine the effect of the factors and the optimal concentrations of each factor for maximum thickness (*nata*) and good quality of *nata*. The predicted results showed that the maximum thickness of nata was attained at pH 4.0 with 10.0% sucrose and 0.5% ammonium sulphate concentrations. RSM was also used for optimization of medium composition for BC production by *G. hansenii* UAC09 in the work of Rani et al. (2011a). The interactions between pH, corn steep liquor concentration, alcohol concentration, acetic acid concentration and water dilution rate ratio to coffee cherry husk extract were determined. The optimal conditions for maximum BC yield were pH 6.6, 10.0% of corn steep liquor, 0.5% of alcohol, 1.13% of acetic acid and 1:1 water to coffee cherry husk extract ratio.

2.4 Production of BC paper

Since, 1986 BC has been used as a subject for production of high quality film and paper by Japanese, German and American researchers. The aim of these researches was to develop a new raw material for film and paper production in order to reduce the uses of PC. BC is a product which produces by some strains in genus *Komagataeibacter* such as *K. xylinus*, *K. hansenii*, *K. nataicola*, etc. These bacteria produce approximately 12-70 glucose molecules and spun out of cell through linear ordered pores at cell envelope. The glucan chain spread in liquid medium and linked with another glucan chain by hydrogen bonds, followed by a ribbon-shaped microfibril formation. BC contains smaller of microfibrils (0.01 μm) than in wood and other plant (30 times). BC indicates higher holding capacity when comparing with PC and form a reticulate structure (Shoda and Sugano, 2005). Therefore, BC have been used as raw material in the production of acoustic diaphragm which a material for audio speakers industry (Iguchi et al., 1988).

Many researchers studied about BC papermaking such as Yamanaka et al. (1989) studied the structure and properties of BC paper produced by *A. aceti*. The BC pellicle produced in 5.0% (w/v) sucrose, 5.0% (w/v) yeast extract, 0.5% (w/v) ammonium sulphate, 0.3% (w/v) potassium dihydrogen phosphate (KH_2PO_4) and 0.005% (w/v) magnesium sulphate ($MgSO_4$), pH 5.0 under static condition at room temperature for 7 days, was used as raw material for paper making. The produced BC paper showed more than 15 GPa of Young's modulus, which was ascribed that cellulose molecules tightly assembled. As a result in BC paper showed a similar mechanical strength to synthesized polymer. Likewise, Suwannapinunt et al. (2007) studied effect of culture conditions on BC production by *A. xylinum* TISTR 976 and physical properties of BC parchment paper. In theirs study, BC was produced in coconut water based medium under three conditions including static tray, shaking flask and 5 liter stirring bioreactor with pH 4.5 at 30 °C for 6 days. Subsequently, BC produced from three conditions was harvested and immersed in 1.0% (w/v) ammonium hydroxide for 24 hours. Then BC was pressed with a ten tons press unit to squeeze out water and to get the BC wet sheets. The BC wet sheets were whitened by soaking in 1.5% (v/v) hydrogen peroxide, which was adjusted to pH 11.0 with 0.5% (w/v) sodium hydroxide, in a sealed plastic bag. Later, BC was shaken for 10 minutes and boiled in water for 30 minutes or until the BC color turned white. Next, white BC sheets were washed with tap water and pressed again. Finally, the wet white BC sheets were dried at 140 °C for 30 seconds and the dried white BC sheets were called BC paper. When BC paper was tested for physical properties, the results showed that BC paper derived from static condition demonstrated the highest wet strength retention (124.18%), castor oil penetration proof, and tearing index values (34.00 KN.m/Kg). They concluded that the static condition was suitable for BC production in order to make good physical properties BC paper.

Keshk (2006) examined the physical properties of BC sheets produced in presence of lignosulfonate. The results indicated that, addition of lignosulfonate to HS medium enhanced the mechanical strength and average viscosity value of BC sheets. The BC sheets gave higher average Young's modulus of 633 MPa compared with the absence of lignosulfonate (450 MPa). The average viscosity values of BC sheets was increased up to 76.98 cP compared with the absence of lignosulfonate (36.48 cP). These results indicated that BC sheet produced in the presence of lignosulfonate will have a high potential for industrial application.

Rani et al. (2011b) evaluated properties of BC film produced from BC synthesized in grape medium by *Gluconacetobacter* sp. The BC film showed higher tensile strength (41.15 MPa) compared with low-density polyethylene (LDPE) (16.08 to 17.17 MPa). Moreover, BC film indicated a high oxygen barrier (415.27 $cm^3/m^2/day$) which can be applied as low-oxygen sensitive packing materials.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Materials

3.1.1 Sources of bacterial isolation

Rotten tropical fruits were collected from the different parts of Thailand such as Bangkok, Chiangmai, Chanthaburi, Chumporn, Kamphaengphet, Nakhonphanom, Nakhon Ratchasima, Rayong and Sakholnakhon provines (Appendix A).

3.1.2 Soybean whey

Soybean whey was provided by small tofu processing factory in Ladkrabang district, Bangkok, Thailand and kept at -10 °C until further use.

3.1.3 Chemical reagents

3.1.3.1 Reagent for isolation of cellulose producing bacteria

1. Cycloheximide 95 % (Acros organics, USA.)

3.1.3.2 Reagents for morphological and biochemical characteristics

1. Acetic acid (CH_3COOH) (RCI Labscan, Thailand)
2. Acetone (CH_3COCH_3) (BDH, England)
3. Agar (Scharlau, Spain)
4. Ammonium hydroxide (NH_4OH) (J.T. Baker, USA)
5. Ammonium sulphate ($(NH_4)_2SO_4$) (Scharlau, Spain)
6. Bromocresol purple (BDH, England)
7. Bromothymol blue (Sigma, USA)
8. Calcium carbonate ($CaCO_3$) (Carlo Erba, France)
9. Crystal violet (Merck, Germany)
10. Citric acid ($C_6H_8O_7$) (Sigma, Germany)
11. D-arabinose ($C_5H_{10}O_5$) (Sigma, USA)
12. D-arabitol ($C_5H_{12}O_5$) (Sigma, USA)
13. D-fructose ($C_6H_{12}O_6$) (Sigma, USA)
14. D-galactose ($C_6H_{12}O_6$) (Merck, Germany)
15. D-glucose ($C_6H_{12}O_6$) (Sigma, USA)
16. D-lactose ($C_{12}H_{22}O_{11}$) (BDH, England)

17. D-maltose ($C_{12}H_{22}O_{11}$) (Merck, Germany)
18. D-mannitol ($C_6H_{14}O_6$) (Univar, Australia)
19. D-mannose ($C_6H_{12}O_6$) (Fluka, Germany)
20. D-melibiose ($C_{12}H_{22}O_{11}$) (Sigma, USA)
21. D-raffinose ($C_{18}H_{32}O_{16}$) (Merck, Germany)
22. D-sorbitol ($C_6H_{14}O_6$) (Univar, Australia)
23. D-sucrose ($C_{12}H_{22}O_{11}$) (Merck, Germany)
24. D-xylose ($C_5H_{10}O_5$) (Sigma, USA)
25. Disodium hydrogen phosphate (Na_2HPO_4) (BDH Prolabo, Australia)
26. Ethanol 95% (The Excise department, Thailand)
27. Glycerol ($C_3H_8O_3$) (Scharlau, Spain)
28. Gram's iodine (Sigma, USA)
29. Hydrochloric acid (HCl) (Scharlau, Spain)
30. Hydrogen peroxide (H_2O_2) (The Government Phamaceutical Organization, Thailand)
31. L-arabinose ($C_5H_{10}O_5$) (Sigma, USA)
32. L-rhamnose ($C_6H_{12}O_5$) (Sigma, USA)
33. L-sorbose ($C_6H_{12}O_6$) (Sigma, USA)
34. Methanol (CH_3OH) (J.T. Baker, USA)
35. Meso-erythritol ($C_4H_{10}O_4$) (Sigma, USA)
36. Peptone (Sigma, USA)
37. Safranin (Merck, Germany)
38. Sodium chloride (NaCl) (Merck, Germany)
39. Sodium hydroxide (NaOH) (Univar, Australia)
40. Yeast extract (Scharlau, Belguim)

3.1.3.3 Reagents for morphological and biochemical characteristics

1. Absolute ethanol (CH_2OH) (BDH, England)
2. Agarose (Bio Whittaker molecular Applications, USA)
3. Chloroform ($CHCl_3$) (Sigma, USA)
4. Deoxy nucleotide triphosphate (dNTP) (Fermentas Life sciences, Germany)
5. Ethidium bromide (Bio Basic, USA)
6. Ethylene diamine tetra-acetic acid (EDTA, $C_{10}H_{16}N_2O_8$) (Promega, USA)

7. Gene 1 Kb DNA Ladder (Fermentas Life sciences, Germany)
8. Isoamyl-alcohol ($C_5H_{12}O$) (Sigma, USA)
9. Lysozyme (Fluka, Belguim)
10. Magnesium chloride ($MgCl_2$) (Fermentas Life sciences, Germany)
11. Phenol (C_6H_6O) (Merck, Germany)
12. QIA quick PCR purification kit (Qiagen, Germany)
13. Sodium acetate ($C_2H_3NaO_2$) (Scharlau, Spain)
14. Sodium dodecyl sulphate ($NaC_{12}H_{25}SO_4$) (Merck, Germany)
15. *Taq* buffer (Fermentas Life sciences, Germany)
16. *Taq* DNA polymerase (Fermentas Life sciences, Germany)
17. Tris (hydroxymethyl) aminomethane hydrochloride ($C_3H_{11}NO_3$) (Scharlau, Spain)

3.1.3.4 Reagent for determination of Sugar

1. Anthrone reagent (Fluka, Germany)

3.1.4 Instruments

3.1.4.1 Analytical balances (BP 221S, Sartorius, Germany)

3.1.4.2 Autoclave (ES 315, Tomy SEIKO Co., LTD, Japan)

3.1.4.3 Biological safety cabinet (Astec/ABS 1200, Astec microflow, UK)

3.1.4.4 Centrifuge (Spectrafuge 16 M, Labnet, USA)

3.1.4.5 Colony counter (Model 13332600, Reichert Analytical Instruments Inc., USA)

3.1.4.6 Colorimeter (Minolta CR-300, Konica Minalta, USA)

3.1.4.7 DNA thermal cycler (ALD 1244, MJ Research, Mexico)

3.1.4.8 Electronic convection dryer (12 trays, Kluaynamthaitowop, Thailand)

3.1.4.9 Electronic digital caliper (BEC, Wenling Lifeng Precision Tool Co., Ltd., China)

3.1.4.10 Electronic digital micrometer (DMM02, Swastik Scientific Co., India)

3.1.4.11 Fine coater (JEC-12000, JEOL Ltd., Japan)

3.1.4.12 Glass wares

3.1.4.13 Hot air oven (UNE 600, Memmert, Germany)

3.1.4.14 Incubator (INB 500, Memmert, Germany)

3.1.4.15 Microscope (C011, Olympus optical Co., Ltd., Japan)

3.1.4.16 Nanodrop spectrophotometer (ND-1000, Thermo Fischer Scientific, USA)

3.1.4.17 Oxygen permeation tester (Illinois 8008, Systech Instruments Ltd., UK)

3.1.4.18 pH meter (Cyberscan pH 2000, Utech cybernetics, Singapore)

3.1.4.19 Pressing machine (Toyo, Sarawootmachinery, Co., Ltd., Thailand)

3.1.4.20 Scanning electron microscopy (JSM S410, JEOL Ltd., Japan)

3.1.4.21 Universal testing machine (TA plus, Lloyd Instruments Ltd., England)

3.1.4.22 UV-transilluminator (Syngene Gene Genius, Gel documentation, USA)

3.1.4.23 UV-visible spectrophotometer (UV 1601, Shimadzu corporation, Australia)

3.1.4.24 Vortex (Genie 2, Scientific Industries, USA)

3.1.4.25 Water vapor permeation tester (Lyssy L80-4000, Systech Instruments Ltd., UK)

3.2 Methods

3.2.1 Isolation of cellulose producing bacteria

Cellulose producing bacteria used in this study were isolated from rotten tropical fruits collected in Thailand using the modified method described by Park et al. (2003). Firstly, ten grams of each rotten fruit was transferred separately into 90 mL HS medium (Appendix B) (Hestrin and Schramm, 1954) adding 0.01% (w/v) cycloheximide, 0.2% (v/v) acetic acid and 0.5% (v/v) ethanol, in a 250-mL flask. The flasks with rotten fruit and liquid medium were then incubated statically at 30 °C for 7 days. After incubation, the culture broth of the flasks with white pellicle covering the surface of the liquid medium were serially diluted with 0.85% (w/v) sodium chloride, and 0.1 mL portions of the serial dilution were spread on GEY agar (Appendix B). The agar plates were incubated at 30 °C until the colonies were formed. The colonies with a clear zone around were selected and transferred to vials, which contained 5 mL of HS medium, and then incubated at 30 °C for 3-7 days. Subsequently, only the vials with white pellicle on the surface were collected for further purification by using cross streak plate technique. The pellicles produced were confirmed as cellulose by boiling with 0.5N sodium hydroxide for 15 minutes. The pellicle is resistant to this treatment and thus remaining material was accepted as cellulose (Son et al., 2002).

3.2.2 Selection of cellulose producing bacteria

The cellulose producing isolates with the highest and lowest yields were selected from each fruit on the basis of BC yield and BC appearances. A single colony of each BC-producing isolate was transferred into vials, which contained five mL of HS medium, and the vials were incubated statically at 30 °C for 7 days. The resulting pellicles were harvested and washed three times with distilled water. Subsequently, BC appearances were observed by naked eyes and the pellicles were purified with 2.0% (w/v) sodium hydroxide at 121 °C for 15 minutes to eliminate bacterial contaminant and other debris. Finally, the purified celluloses were dried in a hot air oven at 70 °C until a constant weight was obtained. The BC yield was expressed as gram dry weight of BC per volume of culture medium (g/L).

3.2.3 Identification of cellulose producing bacteria

After isolation and selection of the highest and the lowest yield cellulose producing bacteria isolated from each fruits, identification of the representative isolates was performed by studying their morphological, physiological and biochemical characteristics and 16S rRNA gene sequence analysis (Cleenwerck and De Vos, 2008).

3.2.3.1 Colony morphology

The colony characteristics including color, shape, elevation, surface texture and margin were studied after cultivation of representative isolates on HS medium at 30 °C for 48 hours (Cappuccino and Sherman, 2000).

3.2.3.2 Gram stain

The 24-48 hours old of each selected isolates were transferred separately into a drop of water on the slide and smeared by means of a circular motion. The smears were air-dried and then fixed with heat. Next, crystal violet was added into dried smears for 1 minute and washed with tap water. Then, Gram's iodine was added into the smears for 1 minute and washed with tap water. Later, the smears were discolored with 95% (v/v) ethyl alcohol drop by drop for 20 seconds and then washed with tap water. Finally, safranin was added into the smears for 45 seconds and washed with tap water. All slides were examined under 100× oil immersion lens and the results were record (Cappuccino and Sherman, 2000).

3.2.3.3 Catalase test

This test facilitated the detection of the catalase production. All selected isolates were grown on HS agar plates at 30 °C for 48 hours. Colonies on HS agar plates were flooded with three drops of 3.0% (v/v) hydrogen peroxide and the production of bubbles on colonies denoted a positive reaction (Cappuccino and Sherman, 2000).

3.2.3.4 Growth on pH 3.0-7.0

All selected isolates were tested in basal medium A (Appendix B) which pH adjusted to 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 by hydrochloric acid and sodium hydroxide. One loop of each selected isolates were suspended separately in 1 mL of 0.85% (w/v) sodium chloride and theirs suspensions were transferred into vials, which contained 5 mL of basal medium A at the different pH levels followed by incubation of culture vials at 30 °C for 7 days. BC formations on the surface of culture broth at different pH levels were recorded and BC thickness was measured with electronic digital caliper.

3.2.3.5 Growth on different media

Glucose, sucrose, methanol and sorbitol were used as carbon sources for the utilization of nutrients of selected isolates. One loop of each selected isolates was suspended separately in 1 mL of 0.85% (v/v) sodium chloride. The 100 μL of cell suspensions were transferred into vials, which contained 5 mL of the test media (Appendix B). After 7 days of incubation at 30 °C, BC production was recorded by measuring BC thickness (Gosselé et al., 1983).

3.2.3.6 Growth with or without 0.2% (v/v) acetic acid

The test of growth with or without acetic acid was determined according to a modified method of Entani et al. (1985) using HS medium by adding or omitting 0.2% (v/v) acetic acid (Appendix B). The 5 mL of culture broth was inoculated with 100 μL of bacterial cell suspended in 0.85% (v/v) sodium chloride. After 7 days of incubation at 30 °C, BC production was record.

3.2.3.7 Growth on 30% D-glucose

The selected isolates were streaked on the test medium (Appendix B) and incubated at 30 °C for 2-7 days (Gosselé et al., 1983).

3.2.3.8 Growth and acid production on different carbon sources

Twenty sugars, including D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, ethanol, glycerol, L-arabinose, L-rhamnose, L-sorbose and meso-erythritol were used as carbon sources for the study of bacterial growth and acid production. One loop of each selected isolates was suspended separately in 1 ml of 0.85% (v/v) sodium chloride. The 100 μL of cell suspensions were added into the test media, which contained 1.9 mL of basal medium B and 0.1 mL of 20% (w/v) various carbon sources, pH 6.8 (Appendix B). Acid production was recorded daily for up to 14 days. The positive results were shown by color change of the indicator for purple to yellow (Carr, 1968). BC production was recorded at the last day of incubation by measuring BC thickness.

3.2.3.9 16S rRNA gene sequence analysis

All the selected isolates were examined for 16S rRNA gene sequence analysis according to the method described by Yukphan et al. (2004).

1. DNA extraction

Chomosomal DNA was isolated from cell grown on HS agar plates at 30 °C for 24-48 hours. One loop of cells was suspended in 360 µL TE buffer, pH 8.0 (100 mM Tris and 30 mM EDTA, pH 8.0). The cell suspension was inoculated with 20 mg of lysozyme and incubated at 37°C for 2 hours and the lysis of cells were completed by adding 40 µL of 10% (w/v) sodium dodecyl sulphate and incubated at 55 °C for 30 minutes. The phenol extraction was then carried out by adding 400 µL of phenol:chloroform:isoamyl-alcohol (25:24:1). The upper layer of the mixture was collected after centrifugation at 14500 rpm for 15 minutes. Chromosomal DNA was precipitated by addition of 1/10 volume of 3M sodium acetate and 2 volume of cold absolute ethanol. DNA was pooled by glass rod and dried at room temperature. The dried DNA was dissolved with TE buffer to obtain a concentration of 250-400 ng/µL as a template DNA for polymerase chain reaction (PCR) (Yukphan et al., 2004).

2. Polymerase chain reaction (PCR)

The 16S rRNA gene fragments were PCR-amplified with two primers, 20F (5'-GAG TTT GAT CCT GGC TCA G-3'; positions 9-27) and 1500R (5'-GTT ACC TTG TTA CGA CTT-3'; positions 1509-1492) were used. The positions in the rRNA gene fragments were based on the *Escherichia coli* numbering system (accession number V00348; Brosius et al., 1981). The PCR reaction was performed in a total of 100 µL as shown in Table 3.1. The reaction conditions of PCR amplification were composed of an initial denaturation step at 94 °C for 3 minutes followed by 25 amplification cycles of a denaturation step at 94 °C for 1 minute, annealing step at 50 °C for 1 minute and extension step at 72 °C for 2 minutes. The final cycle was followed by an additional extension step at 72 °C for 3 minutes.

3. Analysis of PCR products by agarose gel electrophoresis

The PCR amplified products were analyzed by running 5 µL of reaction mixture on 0.8% (w/v) agarose gel. The mixture contained 2 µL of PCR products and 3 µL of loading dye. For DNA marker, 3 µL of it (size 1 KB) were mixed with the same volume of loading dye. The electric current of 100 v/cm was applied to the gel. After electrophoresis, the gel was stained in ethidium bromide solution for 5 minutes and washed with distilled water for 5 minutes. The gel was examined under UV-transilluminator to visualize the amplified 16S rDNA band.

Table 3.1 PCR reaction.

Component	Volume (μL)
10 μL 20F	4.0
10 μL 1500R	4.0
2 mM deoxynucleotide triphosphate	10.0
25 mM magnesium chloride	8.0
10× *Taq* buffer	10.0
Taq DNA polymerase	0.5
Nanopure water	59.5
DNA Template	4.0
Total volume	100.0

4. PCR products purification

The PCR products were purified using QIA quick purification kit. Briefly, 95 μL of PCR product and 500 μL of DF buffer were transferred into a 1.5 mL microcentrifuge tube, and mixed by vortex. The sample mixture was transferred into DF column which placed in a 2 mL collection tube and centrifuged at 8,000 rpm for 30 seconds. The flow-through was discarded and the DF column was placed back in the collection tube. The 600 μL of wash buffer were added into the center of DF column and centrifuged at 10000 rpm for 30 seconds. The flow-through was discarded, the DF column was placed back in the collection tube and centrifuged again for 2 minutes at 10000 rpm. Next, the dried DF column was transferred to a new 1.5 mL microcentrifuge tube and 30 μL of elution buffer were added into the center of the column for 2 minutes. The column was centrifuged for 2 minutes at 14500 rpm to elute the purified DNA.

5. DNA sequencing and construction of phylogenetic tree

The purified 16S rRNA genes from positions 9 to 1509, approximately 1,500 bases were sequenced by using four primers, 27F (5’-AGA GTT TGA TCM TGG CTC AG-3’; positions 27-46), 800R (5’-TAC CAG GGT ATC TAA TCC-3’; position 800-783), 518F (5’-CCA GCA GCC GCG GTA ATA CG-3’; position 518-537) and 1492R (5’-TAC GGY TAC CTT GTT ACG ACT T-3’; position 1492-1471). The phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) in the program MEGA (version 6.0; Tamura et al., 2007) after multiple alignments of the sequences obtained with CLUSTAL W (Thompson et al., 1994). The distance matrices for the aligned sequences were calculated by two-parameter method of Kimura (1980). The confidence values of branches of the phylogenetic tree were determined using the bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. A 16S rRNA gene sequence similarity between the type strains of cellulose producing bacteria and a selected isolate was calculated for 1271 base pairs.

3.2.4 Selection of the most effective cellulose producing isolate

One loop of all selected cellulose producing isolates were transferred separately to 100 mL of HS medium in 250-mL flasks and incubated statically at 30 °C for 48 hours as starter culture. The 90 mL HS medium in 250-mL flasks were inoculated with 10% of the starter culture and incubated statically at 30 °C for 7 days. The BC yields were observed according to above procedure (3.2.2).

3.2.5 Comparison of BC production in HS medium with different carbon sources by using the most effective cellulose producing isolate

The carbon sources, including galactose fructose, sucrose, lactose, glycerol, ethanol, mannitol and sorbitol were used to investigate the effect of different carbon sources on BC production by the most effective cellulose producing isolate. The original carbon source (2.0% (w/v) or (v/v) of glucose) in HS medium was replaced with these carbon sources. The 90 mL of HS medium were inoculated with 10% starter culture and incubated statically at 30 °C for 7 days. The BC yields were observed according to above procedure (3.2.2).

3.2.6 Optimization of culture condition for BC production by the most effective cellulose producing isolate

3.2.6.1 Analysis of soybean whey components

The component of soybean whey including, moisture, protein, fat, ash, total carbohydrate and the amount of lactose were analyzed using standard methods according to AOAC (2005) (Appendix C). The pH of soybean whey was measured using a pH meter.

3.2.6.2 Preparation of starter culture

Two loopfuls of 48 hours of the most effective BC isolate grown on HS agar were inoculated into 300 mL of sterilized soybean whey medium (Appendix B) in 500-mL flasks. These flasks were incubated statically at 30 °C for 48 hours and used as starter culture.

3.2.6.3 Production of BC using soybean whey-based medium

The soybean whey-based medium was prepared by adjusting the pH of filtrated soybean whey with glacial acetic acid and 6N sodium hydroxide. The adjusted soybean whey was autoclaved at 110 °C for 20 minutes and then supplemented with varying amounts of sterilized carbon source according to the experimental runs (Table 3.2). The BC production was performed in 250-mL Erlenmeyer flasks containing 90 mL of the soybean whey-based medium (Table 3.3). Each flask was inoculated with 10 mL of starter culture and incubation was done at different temperatures for 7 days under static condition. The BC yields were observed according to above procedure (3.2.2).

Table 3.2 The code levels and the actual values of the factors in the three independent variables and five levels of CCD.

Independent variable	Code	Actual factor level at code level of:				
		-α (-1.682)	-1	0	+1	+α (+1.682)
Initial pH of culture medium	X1	2.64	4.0	6.0	8.0	9.36
Concentration of carbon source (%)	X2	0	0.75	2.0	3.25	4.10
Incubation temperature (°C)	X3	21.6	25.0	30.0	35.0	38.4

Table 3.3 The treatment combinations of CCD.

Treatment	Code variable level		
	X1	X2	X3
1	-1	-1	-1
2	-1	-1	+1
3	-1	+1	-1
4	-1	+1	+1
5	+1	-1	-1
6	+1	-1	+1
7	+1	+1	-1
8	+1	+1	+1
9	-1.682	0	0
10	+1.682	0	0
11	0	-1.682	0
12	0	+1.682	0

Table 3.3 The treatment combinations of CCD (continued).

Treatment	Code variable level		
	X1	X2	X3
13	0	0	-1.682
14	0	0	+1.682
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

3.2.6.4 Experimental design and statistical analysis

The effect of three independent variables (k=3) including; initial pH of culture medium (X1), concentration of carbon source (%) (X2) and incubation temperature (°C) (X3) on BC production by the most effective BC isolate were studied using a CCD of RSM. Each independent variables were analyzed at five levels including the lower limit ($-\alpha$), low (-1), central (0), high (+1) and upper limit ($+\alpha$) as indicated in Table 3.2. The upper and the lower limits of each independent variable were set at a distance of 1.682 ($2^{k/4} = 1.682$) from the design center. For statistical analysis, the three independent variables were coded according to the following equation (Pal and Khanum, 2010):

$$X_i = (A_i - A_c)/\Delta A_i \qquad (3.1)$$

Where Xi is the independent variable code value; Ai is the independent variable actual value in the original unit; Ac is the independent variable actual value at the center point in the original unit; and ΔAi is the step change in the original unit.

In this study six replications were performed at the center point and the number of experimental runs was calculated from the following equation (Rigas et al., 2005):

$$N = 2^k + 2k + c \qquad (3.2)$$

Where N is the number of experimental run, k is the number of independent variables and c is the number of replication at the center point.

The response data (yields of BC) from each experimental runs were analyzed by multiple linear regressions using the PASW Statistics software (Version 18.0). Based on these results a regression model was constructed to predict the effect of the combined variables on BC production by the most effective BC isolate. The responses can be predicted by the following second-order polynomial regression equation (Pal and Khanum, 2010).

$$Y = b_0 + b_1X1 + b_2X2 + b_3X3 + b_{11}(X1)^2 + b_{22}(X2)^2 + b_{33}(X3)^2 + b_{12}(X1*X2) + b_{13}(X1*X3) + b_{23}(X2*X3) \quad (3.3)$$

Where Y is the predicted response (yield of BC, g/L), b_0 is the intercept term; b_1-b_3 are the linear coefficients; b_{11}, b_{22}, b_{33} are the quadratic coefficients; b_{12}, b_{13}, b_{23} are the cross product coefficients and X1, X2, X3 are initial pH of culture medium, concentration of carbon source (%) and incubation temperature (°C), respectively.

The quality of fit of the model was tested by an analysis of variance (ANOVA) and the coefficient of determination (R^2). A value of $R^2 > 0.75$ indicates aptness of the model (Pal and Khanum, 2010). The response surface plots were created to examine an optimal condition using the fitted second order polynomial equations obtained by holding one of the independent variables at a constant value and changing the levels of the other two variables (Malisorn and Suntornsuk, 2008).

3.2.7 Growth and BC production under optimal conditions

To verify the predicted responses, cell growth and BC production of the most effective cellulose producing isolate were studied in 250-mL Erlenmeyer flasks under optimal condition for 14 days. The viable cell counts were determined everyday using spread plate technique. The cell suspensions used for plating were attained by vigorously shaking the culture flasks to release attached cells from cellulose pellicles (Mikkelsen et al., 2009). Subsequently, these flasks were serially diluted with 0.1% (w/v) peptone, and 0.1 mL of each dilution was spread onto optimized soybean whey-based agar plates. Colonies were counted after 3-4 days of incubation using a colony counter. The supernatant was collected to examine residual sugar content by using anthrone method (Dreywood, 1946).

3.2.8 BC production by the most effective cellulose producing isolate in optimized soybean whey-based medium and standard HS medium

Ten mL of inoculum was transferred to 250-mL Erlenmeyer flasks containing 90 mL of optimized soybean whey-based medium or standard HS medium. These flasks were incubated statically for 7 days at the optimal temperature for the optimized soybean whey-based medium and at 30 °C for standard HS medium. After incubation, BC yields of both culture media were determined.

3.2.9 Preparation of BC paper

The BC pellicles produced in both media were harvested, washed with tap water three times and neutralized by immersed in 0.5% (v/v) ammonium hydroxide overnight. The BC pellicles were washed again with tap water three times and boiled for 30 minutes to remove excessive ammonium hydroxide. Then, the BC pellicles were rinsed thoroughly with running tap water until pH was neutral. Finally, the BC pellicles were squeezed to remove water using the pressing machine and dried in tray drier at 65 °C for 3 hours.

3.2.10 Study on the properties of the BC paper

3.2.10.1 Scanning electron microscopy (SEM)

The BC papers of BC produced in optimized soybean whey-based medium (SBW paper) and HS medium (HS paper) were cut and then coated with gold using Fine coater (JFC-12000, JEOL Ltd., Japan) for 15 minutes. The surface morphology of SBW paper and HS paper were characterized using a scanning electron microscopy (JSM S410, JEOL Ltd., Japan) at 10 KV with 5000× and 30000× magnification.

3.2.10.2 Mechanical properties

Tensile strength, Young's modulus and elongation at break of SBW paper and HS paper were carried out under Thai Industrial Standard, TIS. 1353 (1997) using a Universal Testing Machine at a test speed of 0.25 mm/ minute. For these measurements, rectangular specimens of both BC papers were cut with a gauge length of 30 mm. Three specimens were performed to present the average results.

3.2.10.3 Water vapor transmission rate

Water vapor transmission rate (WVTR) of SBW paper and HS paper with area of 50 cm^2 was analyzed using water vapor permeation tester. The test condition followed the procedure of ASTM E398-13 (2013). The WVTR determination was performed under 38 °C with 90% relative humidity. As water solubilized into the membrane and permeated through the sample, nitrogen gas swept and transported the transmitted water vapor molecules to a calibrated infrared sensor. The results were reported as a transmission rate.

3.2.10.4 Oxygen gas transmission rate

Oxygen gas transmission rate (O_2GTR) of SBW paper and HS paper was determined using oxygen permeation tester following the procedure of ASTM D 3985-05 (2010) using a coulometric sensor method at 23 °C. The O_2GTR was expressed as $cm^3/m^2/day$.

3.2.10.5 Water absorption capacity

The water absorption capacity (WAC) was performed according to the procedure of ASTM D570-98 (2010). Each dried BC papers were cut into 2.54 cm (width) × 7.62 cm (length) and then immersed in distilled water at 23 °C until equilibration. The swollen BC papers were removed from water and excess water at the surface of the BC paper was blotted out with Kimwipes paper. The weights of the swollen papers were measured, and the procedure was repeated until no further weight change was observed. The WAC was calculated with the following equation:

$$\text{WAC (\%)} = [(\text{wet weight-dry weight})/\text{dry weight}] \times 100) \qquad (3.4)$$

3.2.10.6 Color analysis

The color appearance of SBW paper and HS paper were examined using L*, a* and b* values of the CIELAB color system with a colorimeter, where L* is lightness, with a scale from 0(black) to 100(white); and a* and b* are the color directions: +a*, red direction; -a*, green direction; +b*, yellow direction; and –b*, blue direction.

3.2.11 Statistical analysis

Unless stated otherwise, all quantitative data is presented as means of triplicate with error represented by standard deviation. The statistical significance of the evaluated data was analyzed by one-way analysis of variance (ANOVA) using PASW Statistics software (version 18.0). In the comparison of BC yields between produced from optimized soybean whey-based medium and standard HS medium were analyzed by independent *t*-test. Differences among the mean values were tested by Duncan's test at 95% confidence level (p <0.05).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Isolation of cellulose producing bacteria

Since, many strains of cellulose producing bacteria such as *G. hansenii*, *Gluconacetobacter* sp. F6, *G. intermedius* Cls26, etc. were isolated from fruits (Park et al., 2003; Jahan et al., 2012; Yang et al., 2013b), and Thailand has various traditionally tropical fruits that might be a rich source of cellulose producing bacteria. Therefore, forty-eight rotten tropical fruits were collected from the different parts of Thailand such as Bangkok (24 samples), Chiang Mai (1 sample), Chanthaburi (2 samples), Chumporn (3 samples), Kamphaeng Phet (2 samples), Nakhon Phanom (8 samples), Nakhon Ratchasima (4 samples), Rayong (2 samples) and Sakholnakhon (2 samples) provinces and used for isolation of cellulose producing bacteria. From these fruits, 2,500 bacterial strains were isolated as cellulose producing bacterial candidates, which were then examined for cellulose production using a modified HS medium adding with acetic acid, ethanol and cycloheximide. These substances were used as an additive to enhance the growth of acetic acid bacteria, cellulose formation and antifungal, respectively (Toyosaki et al., 1995a). As a result, 204 isolates from only 16 rotten tropical fruits were confirmed as cellulose producing bacteria (Table 4.1). No cellulose producing isolates were found in other rotten tropical fruits. The most effective isolation source was governor's plum (*Flacourtia indica*) with 25 isolates, approximately 1.00% of the total 2,500 collected isolates and the least was lady finger's banana (*Musa acuminata*) with one isolate, approximately 0.04% of the total isolates.

Table 4.1 Cellulose producing strains isolated from tropical rotten fruits collected in Thailand.

Isolation source	Code	Cellulose producing isolate (%^{a})
Beleric myrobalan (*Terminalia bellerica*)	BEL	3 (0.12)
Fetid passionflower (*Passiflora foetida*)	FET	15 (0.60)
Governor's plum (*Flacourtia indica*)	GOV	25 (1.00)
Grape (*Vitis vinifera*)	GRA	11 (0.45)
Java plum (*Syzygium cumini*)	JAV	3 (0.12)
Lady's finger banana (*Musa acuminata)*	LAD	1 (0.04)

Table 4.1 Cellulose producing strains isolated from tropical rotten fruits collected in Thailand (continued).

Isolation source	Code	Cellulose producing isolate (%[a])
Lychee (*Litchi chinensis*)	LYC	15 (0.60)
Mamao (*Antidesma thwaiteaianum*)	MAM	4 (0.16)
Mangosteen (*Garcinia mangostana*)	MAG	23 (0.92)
Papaya (*Carica papaya*)	PAP	2 (0.08)
Rambutan (*Nephelium lappaceum*)	RAM	20 (0.80)
Sapodilla (*Manikara achras*)	SPO	23 (0.92)
Star fruit (*Averrhoa carambola*)	STA	21 (0.85)
Sugar apple (*Annona squamosa*)	SUG	20 (0.80)
Water melon (*Citrullus lanatus*)	WAT	15 (0.60)
Wild lemon (unknown species)	WIL	3 (0.12)
Total		204 (8.16)

[a] The numeral enclosed with parentheses indicate the rate of cellulose producing isolate in a total of 2,500 isolates

4.2 Selection of representative isolates

Cellulose production by all 204 isolated strains was studied in a vial containing 5 mL of standard HS medium under static condition. After 7 days of incubation at 30 °C, cellulose yields were examined. The cellulose yields produced by all isolates were reported in each isolation source.

4.2.1 Beleric myrobalan

Cellulose production by all three strains isolated from beleric myrobalan is displayed in Figure 4.1. Isolate BEL2 showed significantly ($p < 0.05$) highest cellulose yield of 0.69 g/L followed by BEL3 with the cellulose yield of 0.64 g/L. Isolate BEL1 showed significantly ($p < 0.05$) lowest cellulose yield of 0.57 g/L.

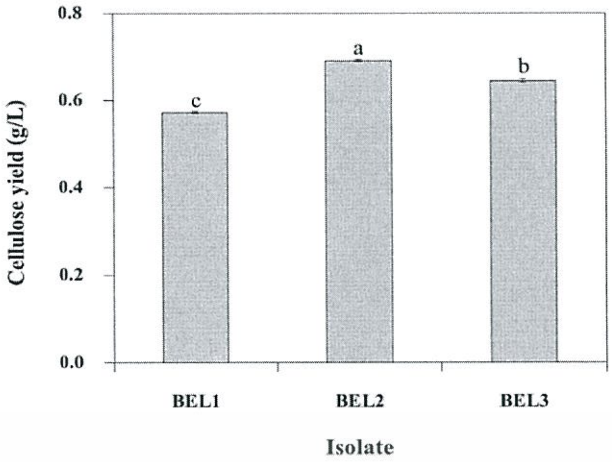


Figure 4.1 Cellulose production by three strains isolated from Beleric myrobalan. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.2 Fetid passionflower

Cellulose production by all 15 strains isolated from fetid passionflower is displayed in Figure 4.2. Isolate FET4 showed significantly ($p < 0.05$) highest cellulose yield of 0.58 g/L followed by FET2, FET6, FET9, FET7, FET1, FET12, FET11, FET13, FET3, FET15, FET5, FET14, and FET10 with the cellulose yields of 0.56, 0.56, 0.54, 0.53, 0.51, 0.51, 0.50, 0.49, 0.47, 0.46 and 0.44 g/L, respectively. Isolate FET8 showed significantly ($p < 0.05$) lowest cellulose yield of 0.40 g/L.

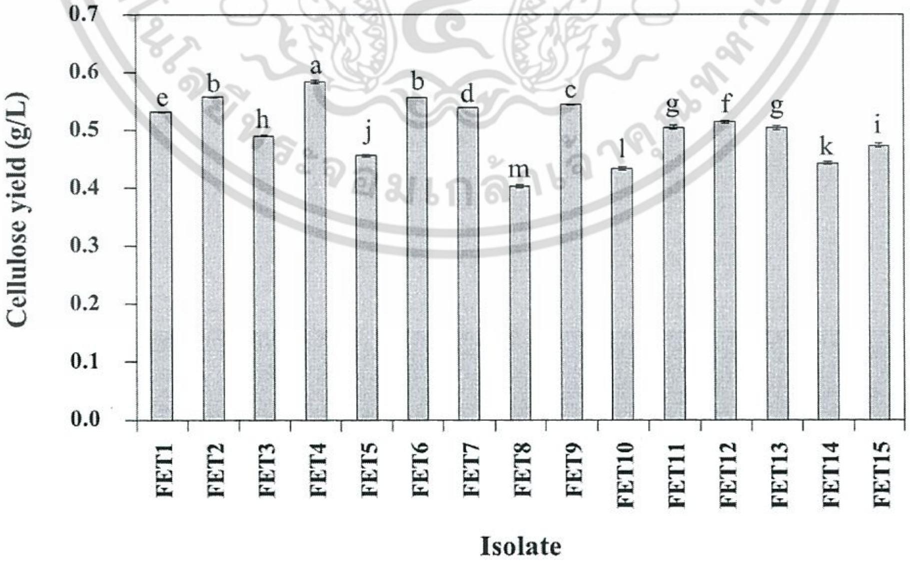


Figure 4.2 Cellulose production by 15 strains isolated from fetid passionflower. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.3 Governor's plum

Cellulose production by all 25 strains isolated from governor's plum is displayed in Figure 4.3. Isolate GOV15 showed significantly ($p < 0.05$) highest cellulose yield of 0.49 g/L followed by GOV23, GOV25, GOV20, GOV21, GOV17, GOV12, GOV10, GOV18, GOV16, GOV2, GOV11, GOV19, GOV3, GOV24, GOV1, GOV22, GOV8, GOV4, GOV14, GOV6, GOV7, GOV5 and GOV13 with the cellulose yields of 0.48, 0.47, 0.47, 0.47, 0.46, 0.46, 0.46, 0.46, 0.45, 0.45, 0.45, 0.45, 0.45, 0.44, 0.44, 0.44, 0.44, 0.44, 0.44, 0.44, 0.43, 0.43, 0.43 and 0.43 g/L, respectively. Isolate GOV9 showed significantly ($p < 0.05$) lowest cellulose yield of 0.40 g/L.

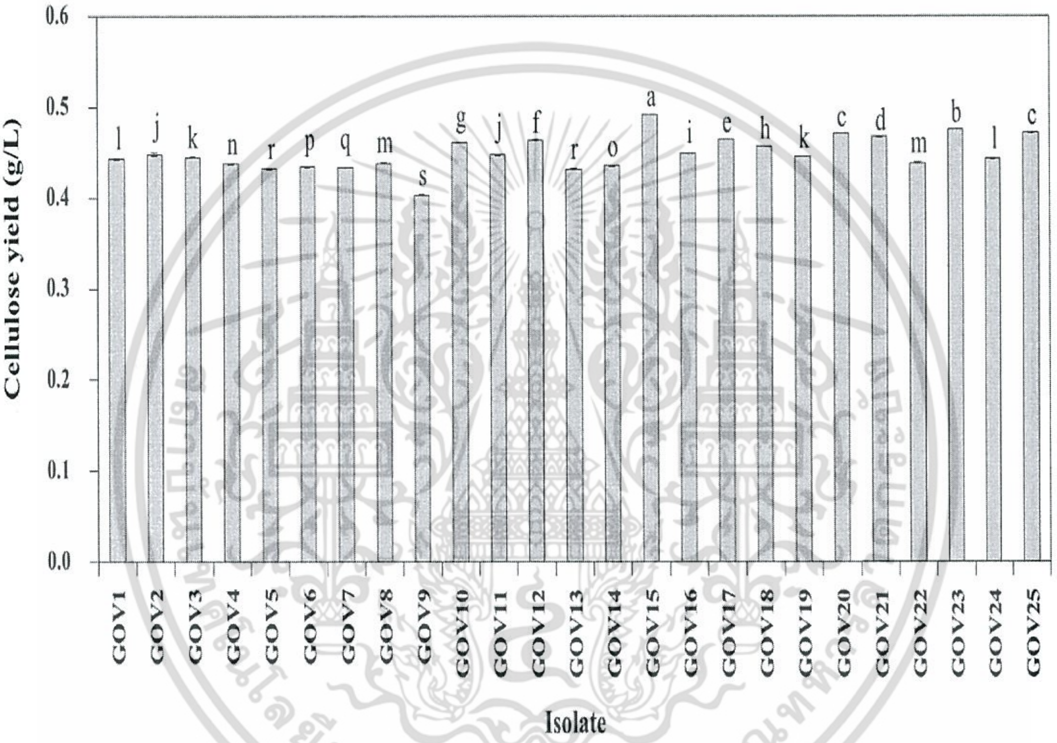


Figure 4.3 Cellulose production by 25 strains isolated from governor's plum. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.4 Grape

Cellulose production by all 11 strains isolated from grape is displayed in Figure 4.4. Isolate GRA8 showed significantly ($p < 0.05$) highest cellulose yield of 0.68 g/L followed by GRA6, GRA3, GRA10, GRA7, GRA1, GRA9, GRA4, GRA5 and GRA11 with the cellulose yields of 0.66, 0.65, 0.62, 0.62, 0.60, 0.60, 0.60, 0.58 and 0.58 g/L, respectively. Isolate GRA2 showed significantly ($p < 0.05$) lowest cellulose yield of 0.54 g/L.

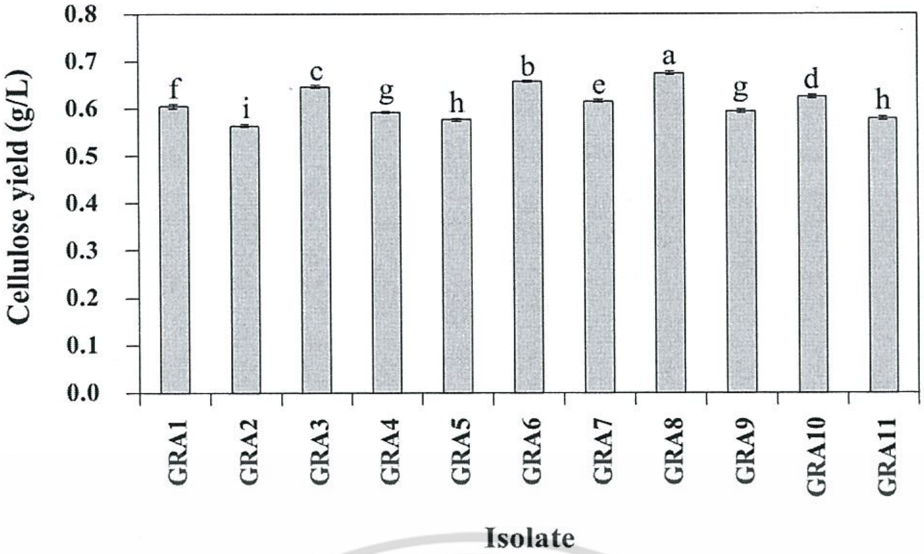


Figure 4.4 Cellulose production by 11 strains isolated from grape. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.5 Java plum

Cellulose production by all three strains isolated from java plum is displayed in Figure 4.5. Isolate JAV1 showed significantly ($p < 0.05$) highest cellulose yield of 0.70 g/L followed by JAV2 with the cellulose yields of 0.65 g/L. Isolate JAV3 showed significantly ($p < 0.05$) lowest cellulose yield of 0.62 g/L.

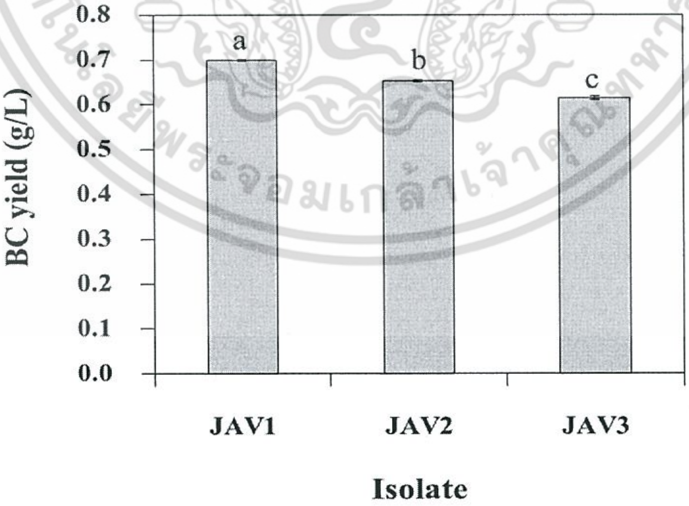


Figure 4.5 Cellulose production by three strains isolated from java plum. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.6 Lady's finger banana

Isolate LAD1 produced 0.43 g/L of cellulose yield.

4.2.7 Lychee

Cellulose production by all 15 strains isolated from lychee is displayed in Figure 4.6. Isolate LYC8 showed significantly ($p < 0.05$) highest cellulose yield of 0.63 g/L followed by LYC1, LYC12, LYC2, LYC14, LYC9, LYC11, LYC13, LYC3, LYC6, LYC4, LYC15, LYC5 and LYC10 with the cellulose yields of 0.62, 0.61, 0.61, 0.61, 0.59, 0.59, 0.59, 0.57, 0.57, 0.56, 0.55, 0.54, and 0.53 g/L, respectively. Isolate LYC7 showed significantly ($p < 0.05$) lowest cellulose yield of 0.51 g/L.

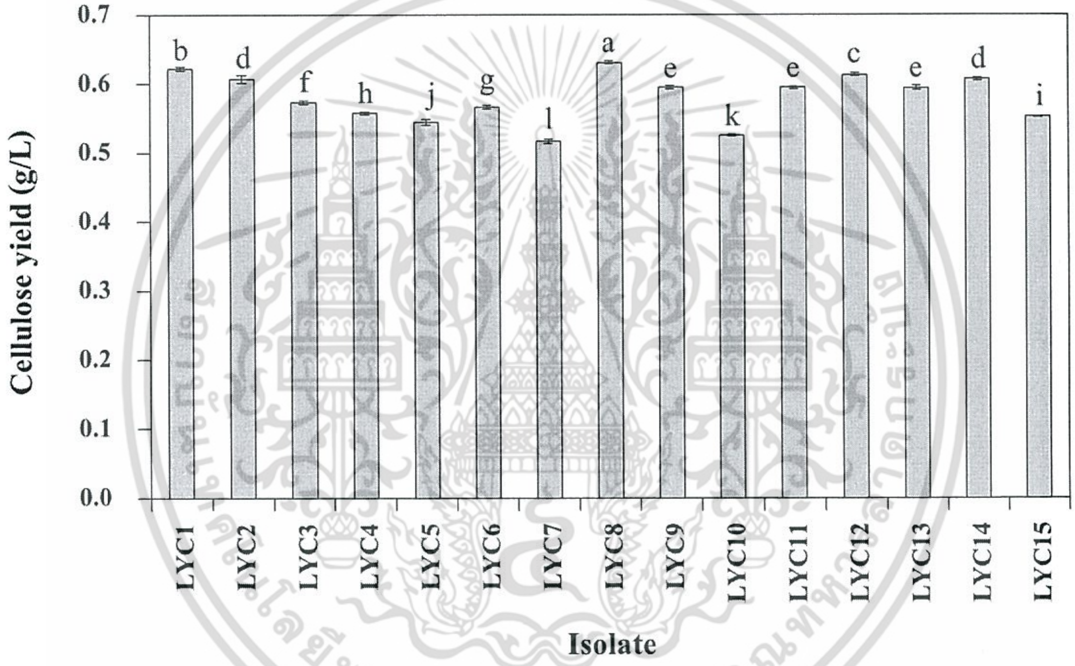


Figure 4.6 Cellulose production by 15 strains isolated from lychee. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.8 Mamao

Cellulose production by all 4 strains isolated from mamao is displayed in Figure 4.7. Isolate MAM4 showed significantly ($p < 0.05$) highest cellulose yield of 0.65 g/L followed by MAM1 and MAM3 with the same cellulose yields of 0.63 g/L. Isolate MAM2 showed significantly ($p < 0.05$) lowest cellulose yield of 0.61 g/L.

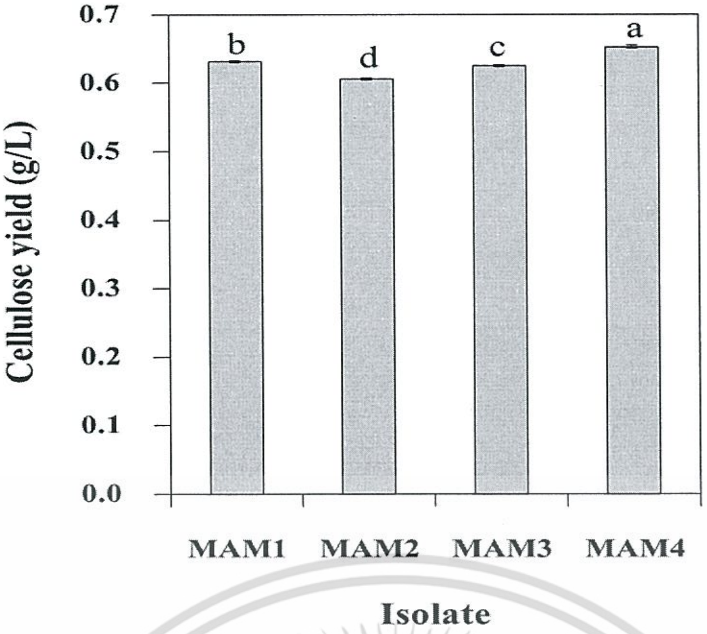


Figure 4.7 Cellulose production by 4 strains isolated from mamao. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.9 Mangosteen

Cellulose production by all 23 strains isolated from mangosteen is displayed in Figure 4.8. Isolate MAG15 showed significantly ($p < 0.05$) highest cellulose yield of 0.59 g/L followed by MAG23, MAG17, MAG10, MAG13, MAG21, MAG2, MAG19, MAG7, MAG18, MAG5, MAG11, MAG1, MAG20, MAG14, MAG22, MAG8, MAG16, MAG12, MAG3, MAG9 and MAG4 with the cellulose yields of 0.58, 0.56, 0.55, 0.54, 0.53, 0.53, 0.53, 0.51, 0.50, 0.50, 0.50, 0.50, 0.50, 0.49, 0.49, 0.48, 0.48, 0.48,0.48, 0.48, 0.47 and 0.47 g/L, respectively. Isolate MAG6 showed significantly ($p < 0.05$) lowest cellulose yield of 0.45 g/L.

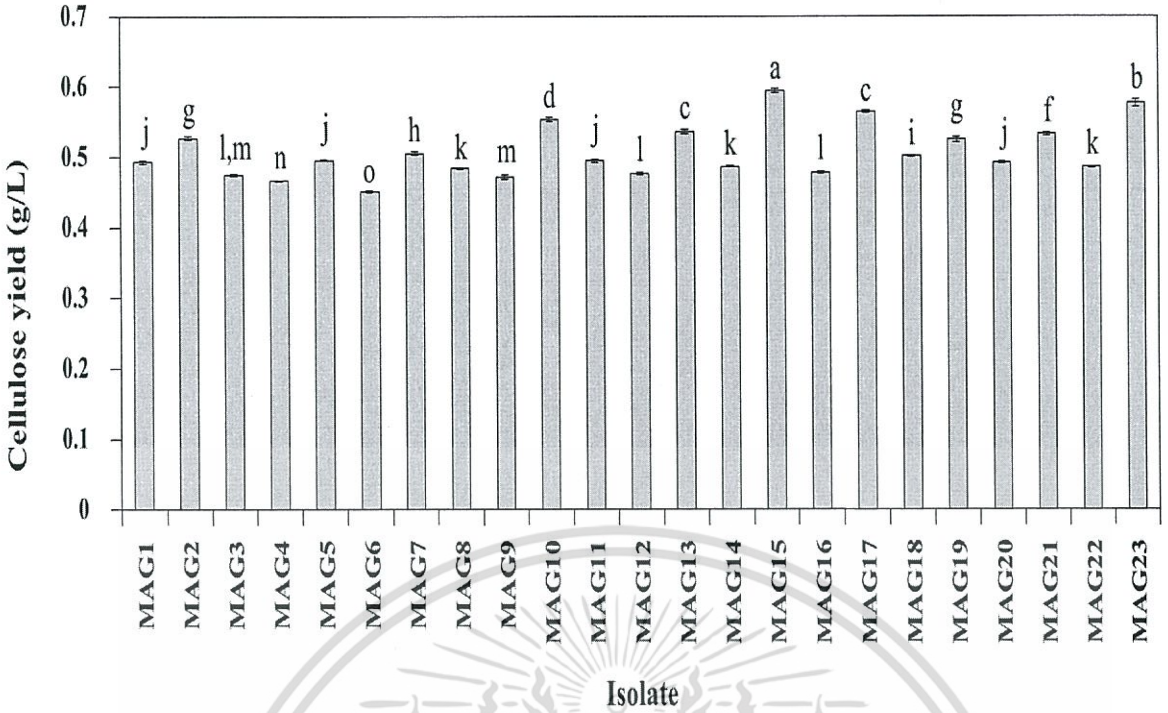


Figure 4.8 Cellulose production by 23 strains isolated from mangosteen. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.10 Papaya

Isolate PAP1 produced 0.79 g/L of cellulose while isolate PAP2 produced 0.78 g/L of cellulose.

4.2.11 Rambutan

Cellulose production by all 20 strains isolated from rambutan is displayed in Figure 4.9. Isolate RAM4 showed significantly ($p < 0.05$) highest cellulose yield of 0.61 g/L followed by RAM20, RAM19, RAM11, RAM15, RAM3, RAM7, RAM17, RAM9, RAM14, RAM10, RAM16, RAM12, RAM5, RAM8, RAM18, RAM13, RAM2 and RAM6 with the cellulose yields of 0.60, 0.60, 0.57, 0.57, 0.57, 0.54, 0.53, 0.53, 0.53, 0.52, 0.52, 0.49, 0.49, 0.49, 0.49, 0.48, 0.48 and 0.46 g/L, respectively. Isolate RAM1 showed significantly ($p < 0.05$) lowest cellulose yield of 0.44 g/L.

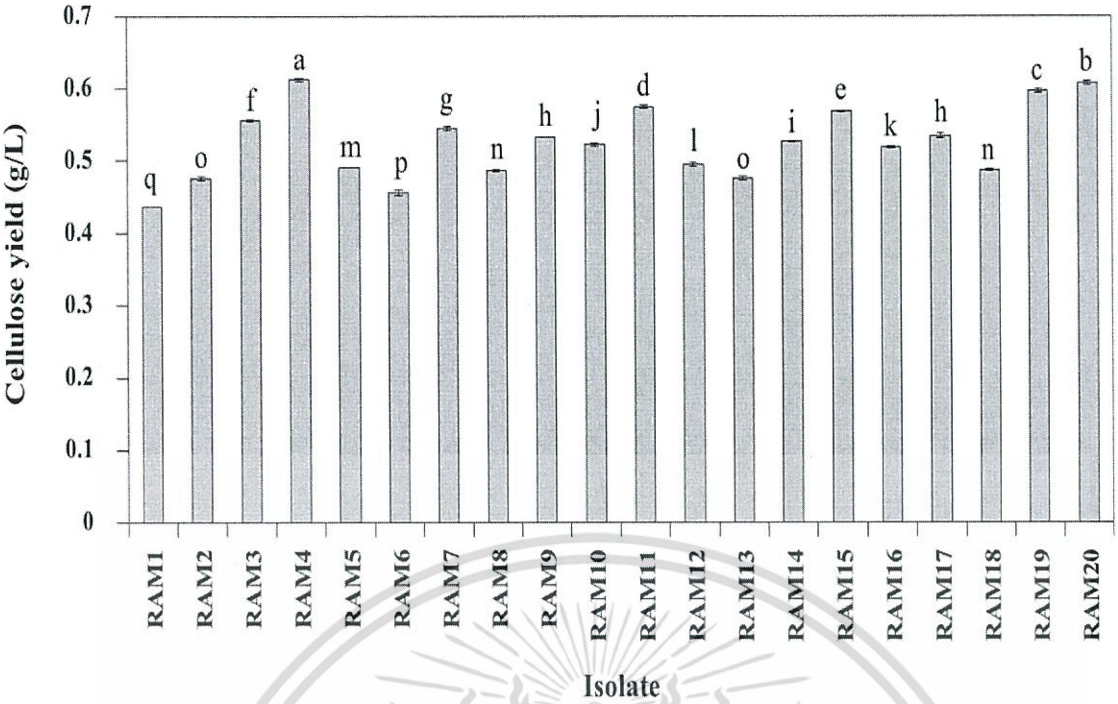


Figure 4.9 Cellulose production by 20 strains isolated from rambutan. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.12 Sapodilla

Cellulose production by all 23 strains isolated from sapodilla is displayed in Figure 4.10. Isolate SPO4 showed significantly ($p < 0.05$) highest cellulose yield of 0.54 g/L followed by SPO21, SPO6, SPO13, SPO20, SPO19, SPO7, SPO12, SPO22, SPO1, SPO18, SPO11, SPO9, SPO2, SPO5, SPO10, SPO17, SPO3, SPO23, SPO14, SPO8 and SPO16 with the cellulose yields of 0.53, 0.52, 0.51, 0.50, 0.50, 0.49, 0.49, 0.48, 0.48, 0.48, 0.47, 0.47, 0.47, 0.47, 0.46, 0.46, 0.45, 0.44, 0.42, 0.42, and 0.41 g/L, respectively. Isolate SPO15 showed significantly ($p < 0.05$) lowest cellulose yield of 0.38 g/L.

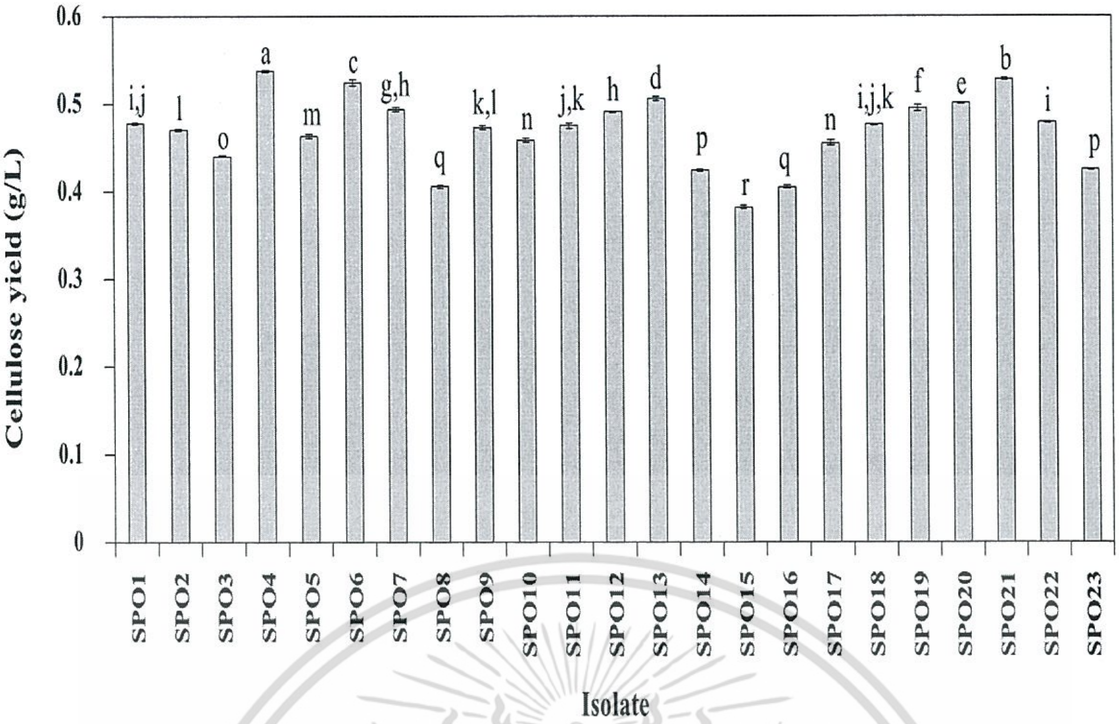


Figure 4.10 Cellulose production by 23 strains isolated from sapodilla. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.13 Star fruit

Cellulose production by all 21 strains isolated from star fruit is displayed in Figure 4.11. Isolate STA5 showed significantly ($p < 0.05$) highest cellulose yield of 0.53 g/L followed by STA4, STA9, STA19, STA17, STA14, STA21, STA11, STA3, STA20, STA13, STA8, STA12, STA18, STA7, STA2, STA15, STA16, STA10 and STA1with the cellulose yields of 0.52, 0.52, 0.52, 0.52, 0.52, 0.52, 0.52, 0.52, 0.51, 0.51, 0.51, 0.51, 0.51, 0.51, 0.51, 0.51, 0.51, 0.51, and 0.51 g/L, respectively. Isolate STA6 showed significantly ($p < 0.05$) lowest cellulose yield of 0.50 g/L.

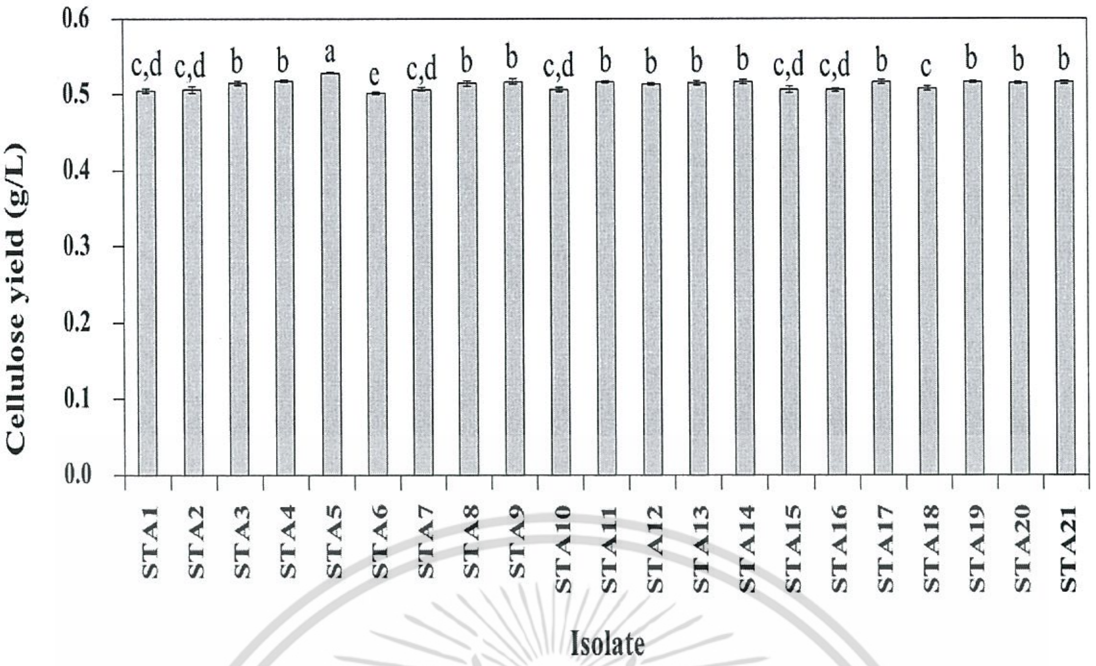


Figure 4.11 Cellulose production by 21 strains isolated from star fruit. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.14 Sugar apple

Cellulose production by all 20 strains isolated from sugar apple is displayed in Figure 4.12. Isolate SUG8 showed significantly ($p < 0.05$) highest cellulose yield of 0.46 g/L followed by SUG17, SUG15, SUG19, SUG11, SUG1, SUG4, SUG18, SUG10, SUG9, SUG16, SUG13, SUG7, SUG6, SUG3, SUG14, SUG2, SUG20 and SUG12 with the cellulose yields of 045, 0.45, 0.45, 0.44, 0.44, 0.44, 0.44, 0.44, 0.43, 0.43, 0.43, 0.42, 0.42, 0.42, 0.42, 0.42, 0.42, 0.41 and 0.41 g/L, respectively. Isolate SUG5 showed significantly ($p < 0.05$) lowest cellulose yield of 0.40 g/L.

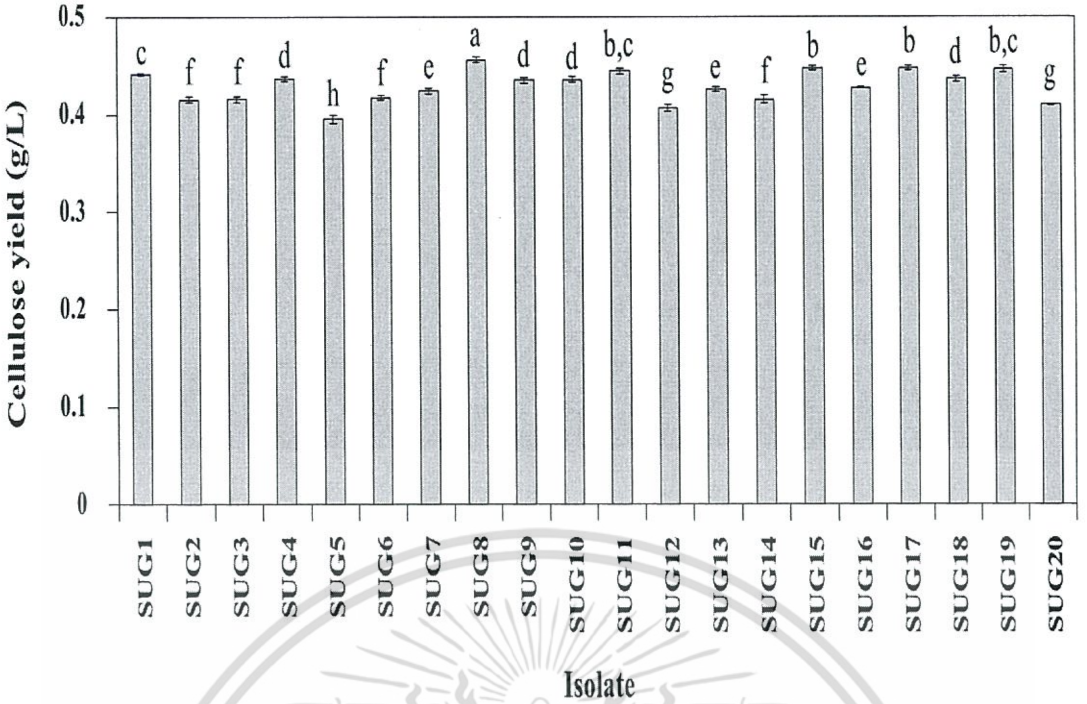


Figure 4.12 Cellulose production by 20 strains isolated from sugar apple. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.15 Water melon

Cellulose production by all 15 strains isolated from water melon is displayed in Figure 4.13. Isolate WAT11 showed significantly ($p < 0.05$) highest cellulose yield of 0.48 g/L followed by WAT7, WAT8, WAT2, WAT10, WAT3, WAT6, WAT12, WAT5, WAT13, WAT1, WAT4, WAT15 and WAT9 with the cellulose yields of 0.46, 0.46, 0.46, 0.46, 0.45, 0.45, 0.45, 0.45, 0.45, 0.45, 0.45, 0.43 and 0.42 g/L, respectively. Isolate WAT14 showed significantly ($p < 0.05$) lowest cellulose yield of 0.40 g/L.

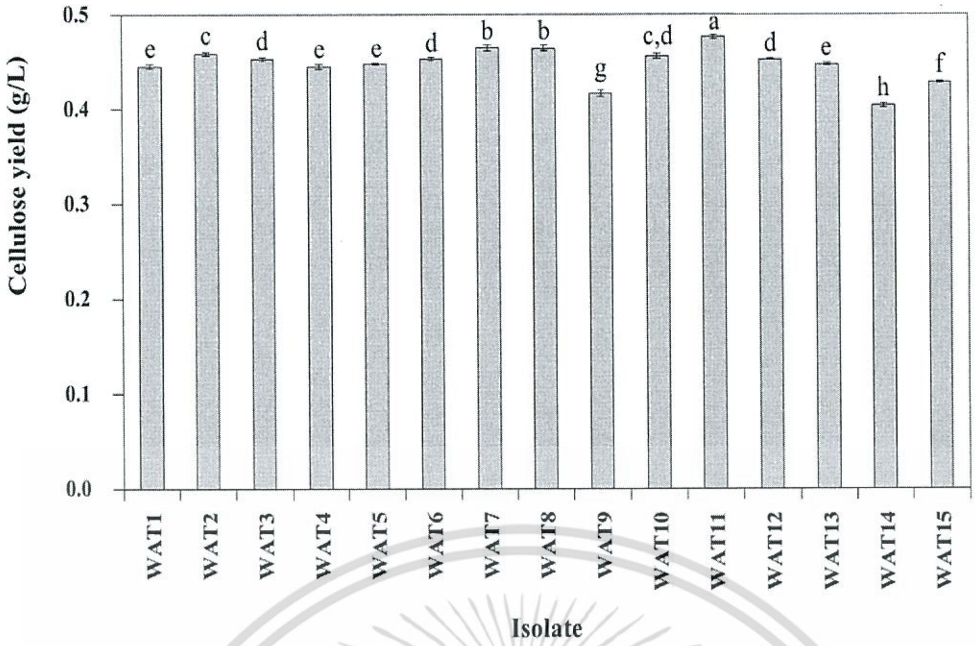


Figure 4.13 Cellulose production by 15 strains isolated from water melon. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.2.16 Wild lemon

Cellulose production by all three strains isolated from wild lemon is displayed in Figure 4.14. Isolate WIL2 showed significantly ($p < 0.05$) highest cellulose yield of 0.61 g/L followed by WIL1 with the cellulose yields of 0.57 g/L, respectively. Isolate WIL3 showed significantly ($p < 0.05$) lowest cellulose yield of 0.49 g/L.

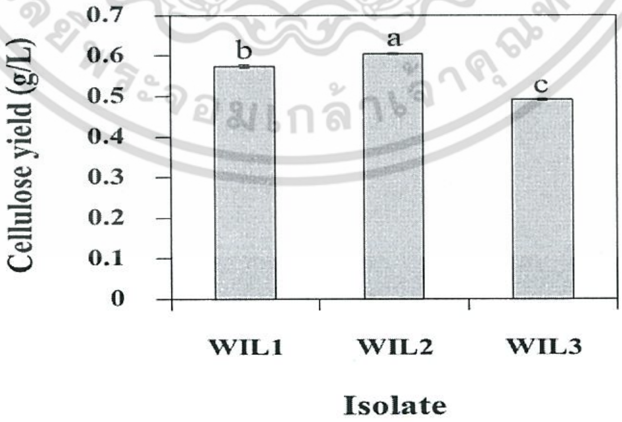


Figure 4.14 Cellulose production by three strains isolated from wild lemon. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

The highest and the lowest yield cellulose producing isolates were selected from each fruits for identification to species level. Since, they were expected that new species of cellulose producing bacteria will be found. As a result, 29 isolates including BEL1, BEL2, FET4, FET8, GOV9, GOV15, GRA2, GRA8, JAV1, JAV3, LAD1, LYC7, LYC8, MAG6, MAG15, MAM2, MAM4, PAP1, RAM1, RAM4, SPO4, SPO15, STA5, SUG5, SUG8, WAT11, WAT14, WIL2 and WIL3 were selected as representative cellulose producing strains from 204 cellulose producing isolates on the basis of cellulose yield. In the case of papaya, only one isolate was selected. Since, isolate PAP1 and isolate PAP2 produced cellulose in the same range and the colonies morphology of them were not differ. Similar reason, only one isolate (STA5) was also selected from star fruit and all of strains isolated from this fruit produced swollen cellulose pellicles.

4.3 Identification of representative cellulose producing isolates

All 29 representative cellulose producing isolates were identified up to species level based on morphological characteristics, biochemical tests and 16S rRNA gene sequence analysis.

4.3.1 Morphological characteristics

The colonies of the 29 representative cellulose producing isolates observed on HS agar plate after 48 hours grown were round with entire or undulate margin and convex or pulvinate or umbonate elevation in center with diameter approximately 3.9-18.3 μm. The surfaces of colonies were smooth or rough and their colors were cream. The colonies were mucous and covered with sticky substances. All the representative isolates were Gram-negative, ellipsoidal to rod-shaped in morphology approximately 0.6-3.4 μm long and 0.3-1.2 μm wide, and occurring singly or in pairs (Table 4.2). The colonies configurations and morphological results obtained were similar to reported of Dellaglio et al. (2005) and Yang et al. (2013b) who isolated *Gluconacetobacter* strains from fruits.

Table 4.2 Colony and morphological characteristics of selected strains.

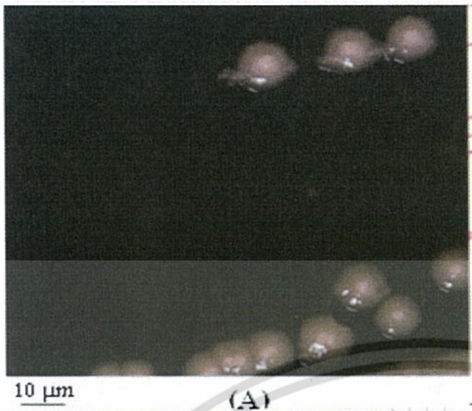
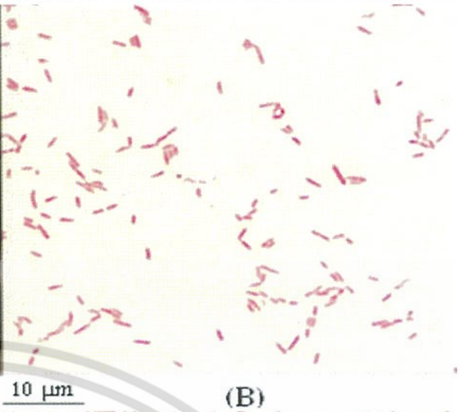
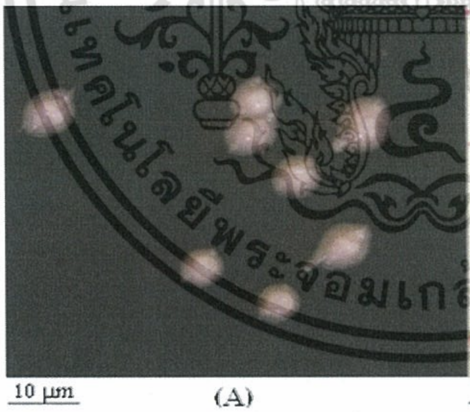
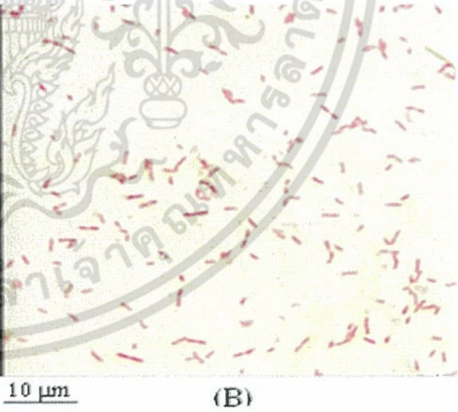
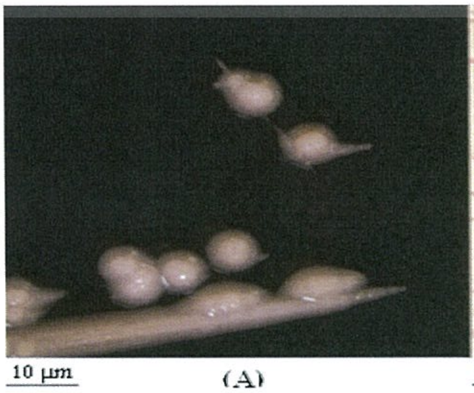
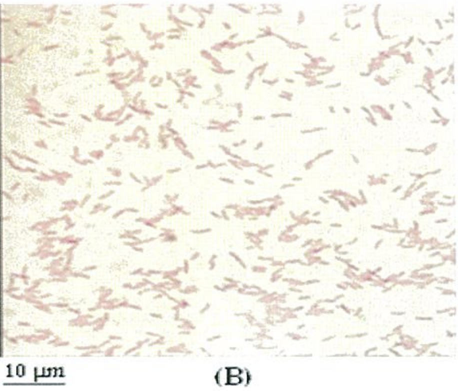
Isolate	Colony characteristic (100×)	Morphological characteristic (1000×)
BEL1		
BEL2		
FET4		
FET8		

Table 4.2 Colony and morphological characteristics of selected strains (continued).

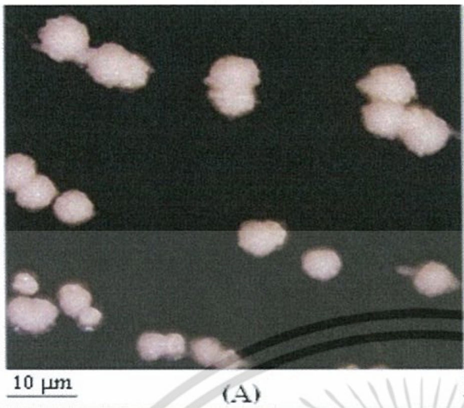
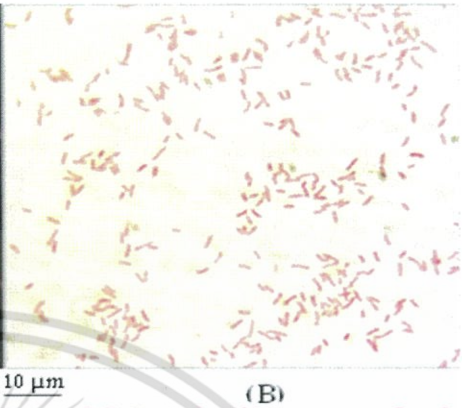
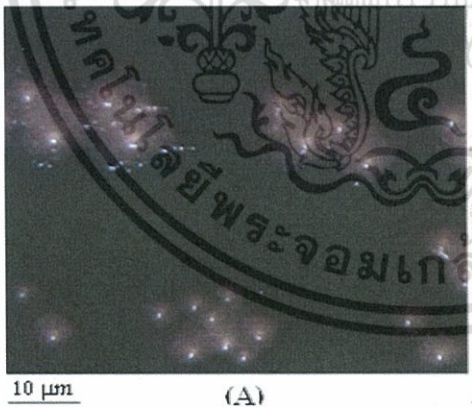
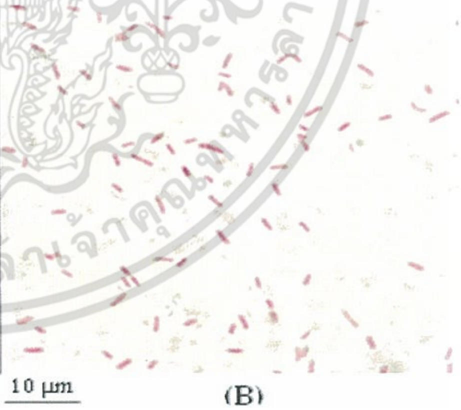
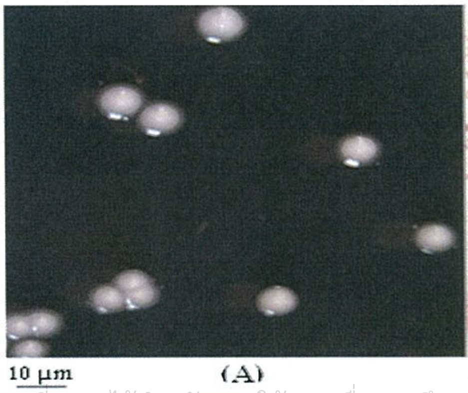
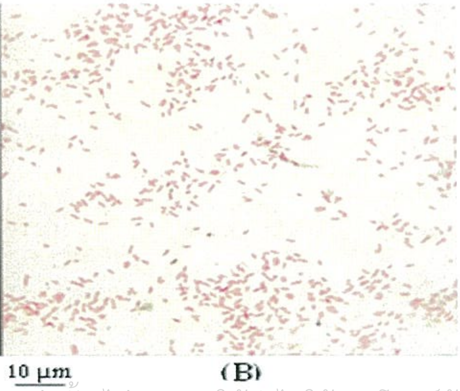
Isolate	Colony characteristic (100×)	Morphological characteristic (1000×)
GOV9	 10 μm (A)	 10 μm (B)
GOV15	 10 μm (A)	 10 μm (B)
GRA2	 10 μm (A)	 10 μm (B)
GRA8	 10 μm (A)	 10 μm (B)

Table 4.2 Colony and morphological characteristics of selected strains (continued).

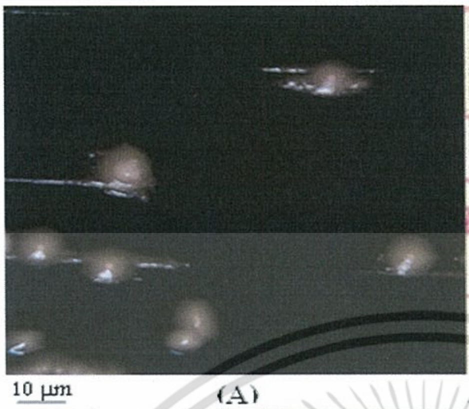
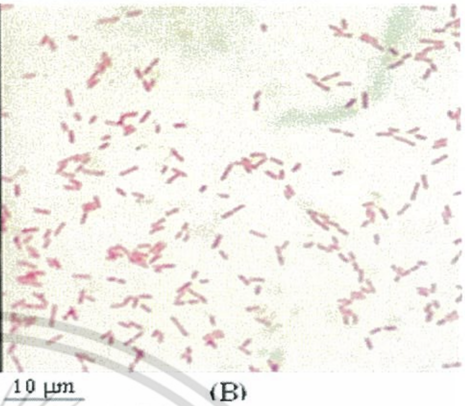
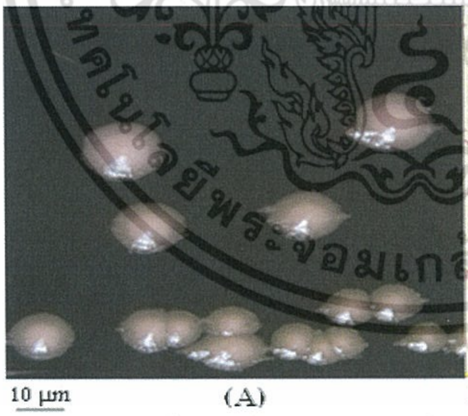
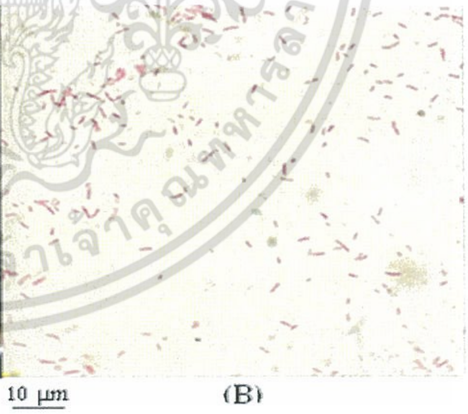
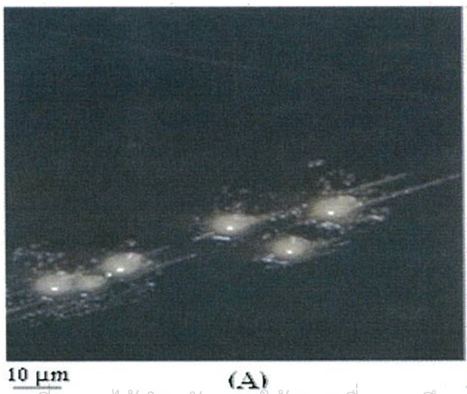
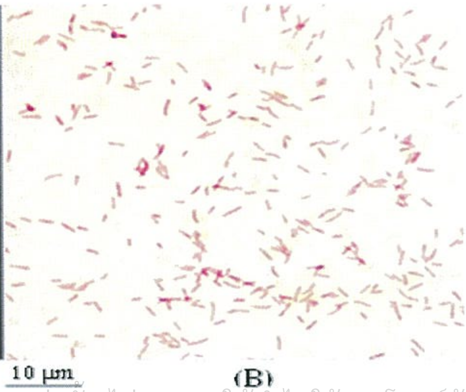
Isolate	Colony characteristic (100×)	Morphological characteristic (1000×)
JAV1		
JAV3		
LAD1		
LYC7		

Table 4.2 Colony and morphological characteristics of selected strains (continued).

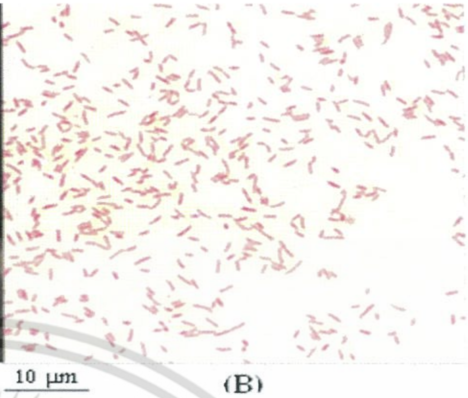
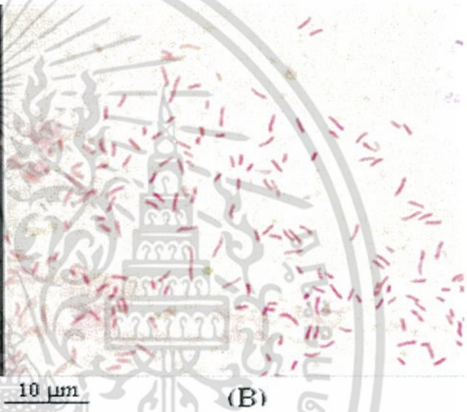
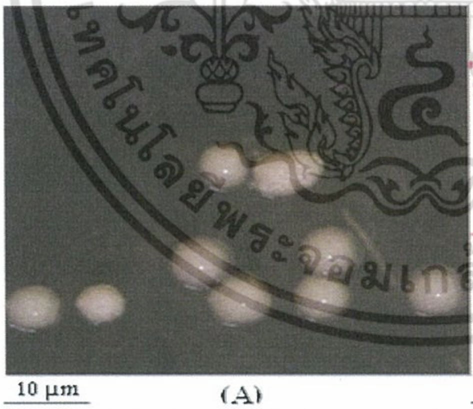
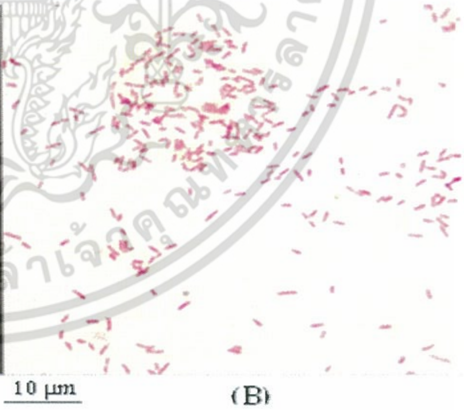
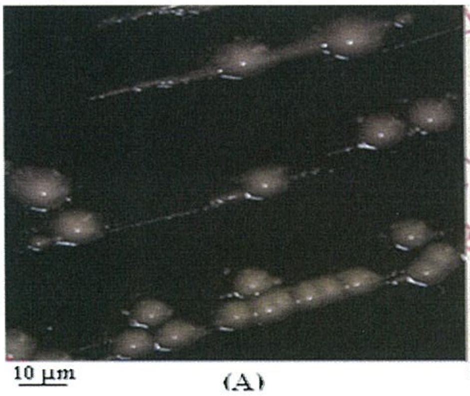
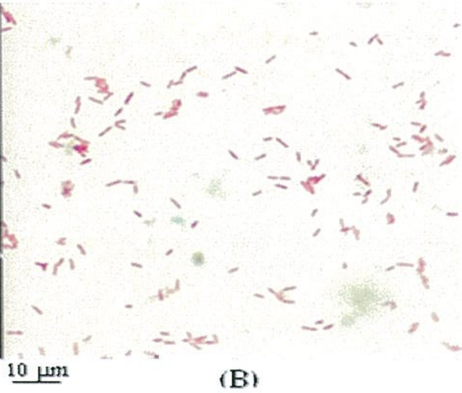
Isolate	Colony characteristic (100×)	Morphological characteristic (1000×)
LYC8		
MAM2		
MAM4		
MAG6		

Table 4.2 Colony and morphological characteristics of selected strains (continued).

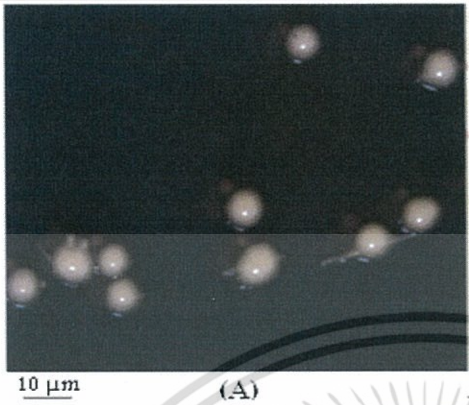
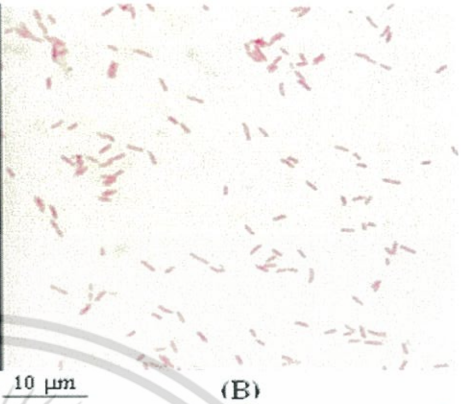
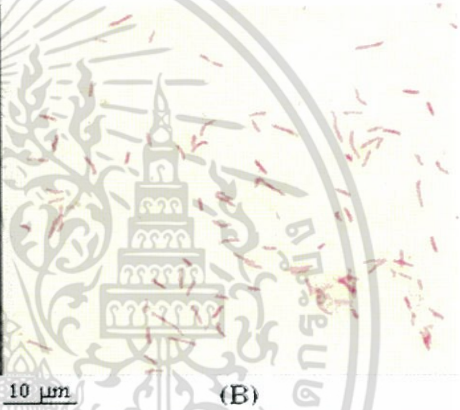
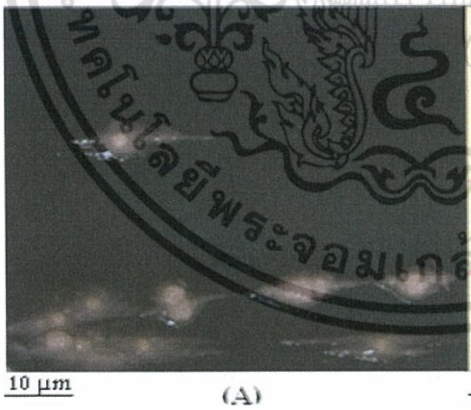
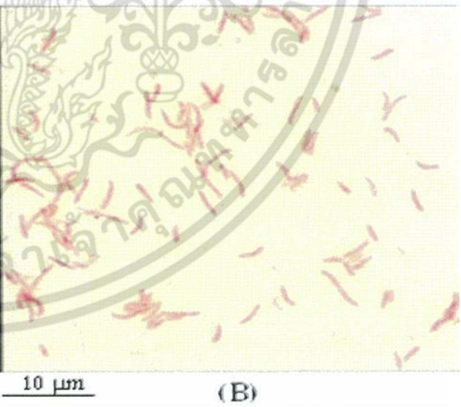
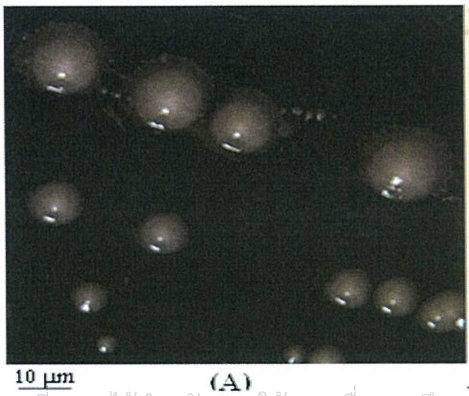
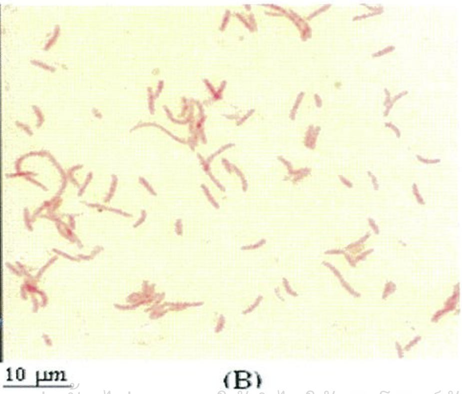
Isolate	Colony characteristic (100×)	Morphological characteristic (1000×)
MAG15		
PAP1		
RAM1		
RAM4		

Table 4.2 Colony and morphological characteristics of selected strains (continued).

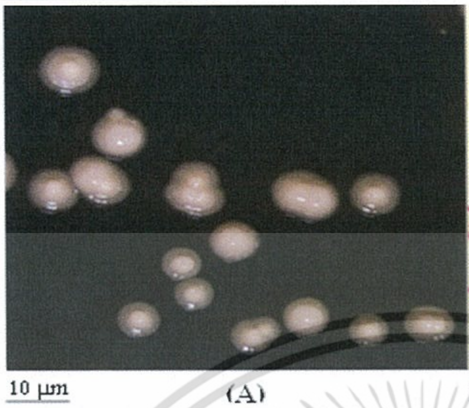
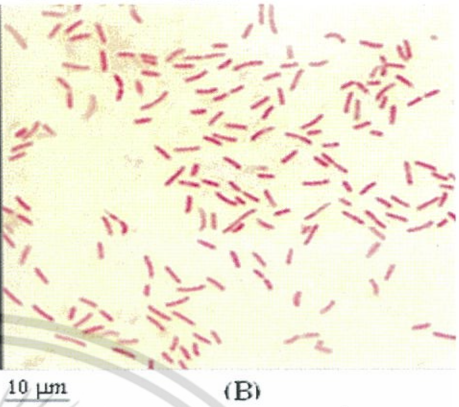
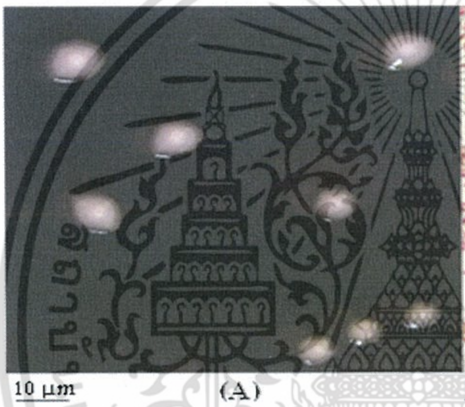
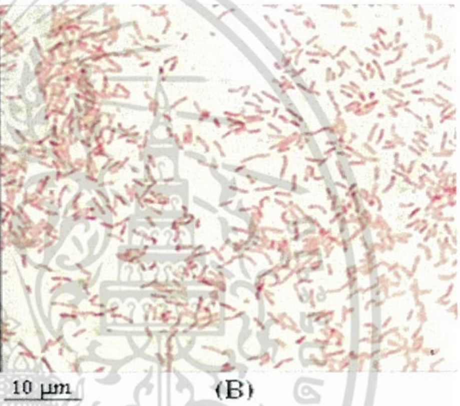
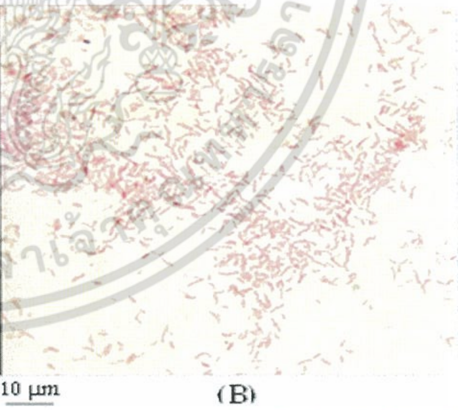
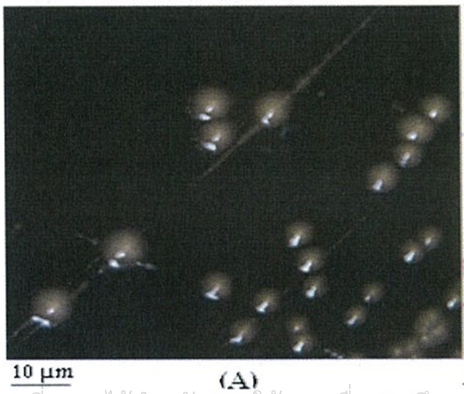
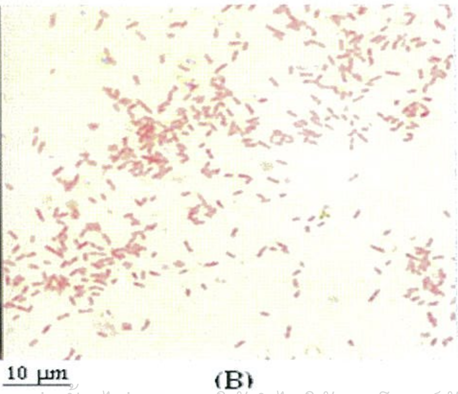
Isolate	Colony characteristic (100×)	Morphological characteristic (1000×)
SPO4		
SPO15		
STA5		
SUG5		

Table 4.2 Colony and morphological characteristics of selected strains (continued).

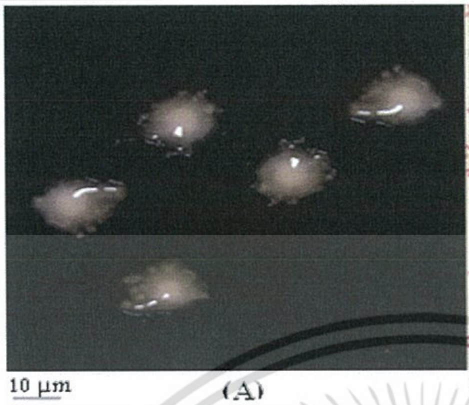
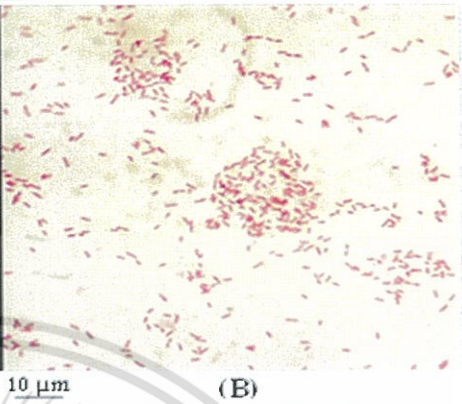
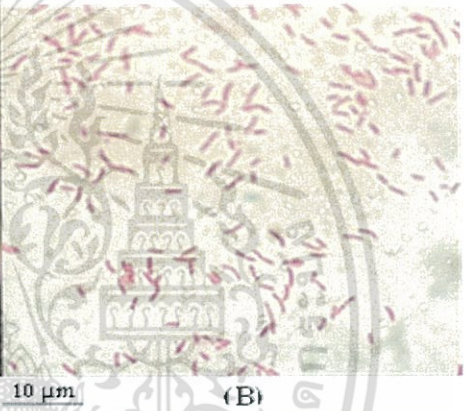
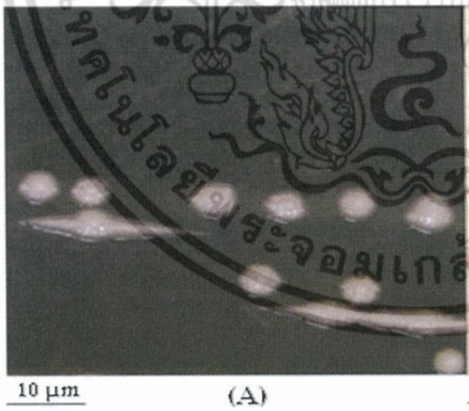
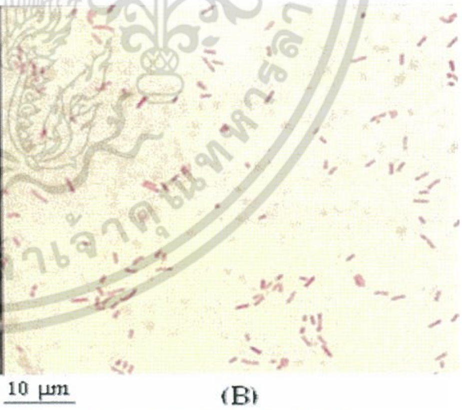
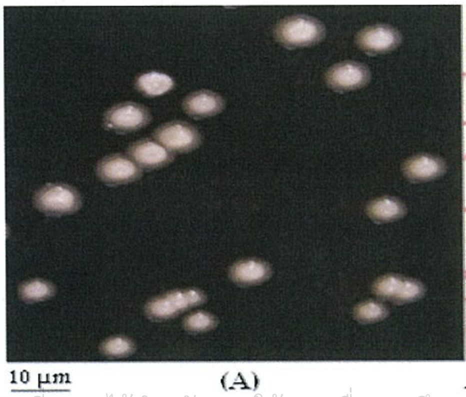
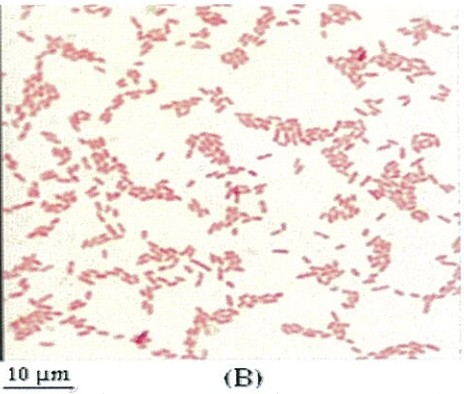
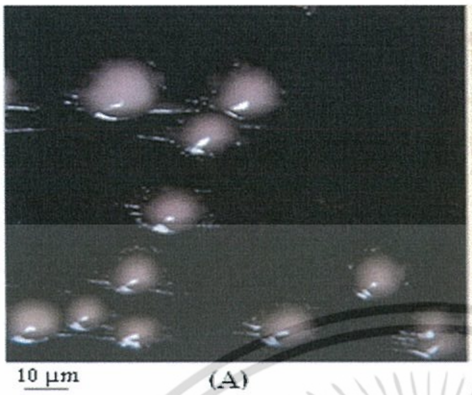
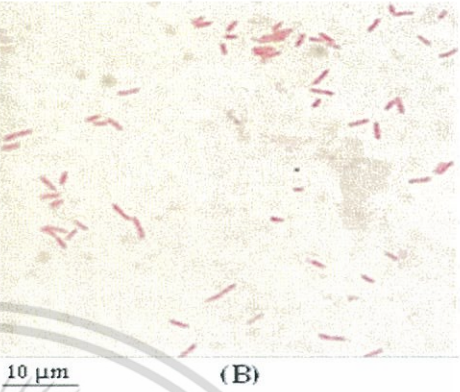
Isolate	Colony characteristic (100×)	Morphological characteristic (1000×)
SUG8	 (A)	 (B)
WAT11	 (A)	 (B)
WAT14	 (A)	 (B)
WIL2	 (A)	 (B)

Table 4.2 Colony and morphological characteristics of selected strains (continued).

Isolate	Colony characteristic (100×)	Morphological characteristic (1000×)
WIL3		

4.3.2 Biochemical tests

All the representative cellulose producing isolates showed catalase-positive reactions and growth at pH 3.0-7.0. They grew slowly at pH 3.0, 3.5 and 4.0, but the growth was better at pH 4.5-7.0. Testing for growth and acid production in different carbon sources indicated that almost representative isolates could grow in all carbon sources, but few isolates could not grow in D-arabinose, D-maltose, D-lactose, D-mannose, D-raffinose, D-sucrose, L-rhamnose, L-sorbose, ethanol and glycerol. All the representative isolates produced acid from D-glucose and D-sorbitol, but 9 out of 29 isolates also produced acid from D-arabinose, L-rhamnose and L-sorbose. For cellulose production, almost representative isolates could produce cellulose from D-fructose, D-glucose, D-mannitol and glycerol. Growing in different media indicated that all the isolates could not grow on sorbitol or methanol medium but grew well on glucose or sucrose medium. All representative isolates could grow on 30% (w/v) D-glucose and without 0.2% (v/v) acetic acid in standard HS medium. The results of biochemical tests of all 29 representative cellulose producing isolates are summarized in Table 4.3.

Table 4.3 Differential phenotypic characteristics of 29 representative cellulose producing isolates.

Subgroup	I	II					III		IV		V							VIa			VIb								
Characteristic	RAM1	GOV9	GOV15	MAM4	SPO4	WAT11	LYC7	FET8	SPO15	WAT14	BEL1	BEL2	FET4	LAD1	LYC8	STA5	WIL2	MAG6	SUG5	SUG8	GRA2	GRA8	JAV1	JAV3	MAG15	MAM2	PAP1	RAM4	WIL3
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at pH 3.0-7.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth on different media																													
Glucose medium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose medium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol medium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methanol medium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth on 30% D-glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth without acetic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth on different carbon sources																													
D-arabinose	+	+	+	-	+	+	+	+	+	-	+	+	-	-	-	+	-	+	-	-	+	+	-	+	+	+	+	+	+
D-arabitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+

Table 4.3 Differential phenotypic characteristics of 29 representative cellulose producing isolates (continued).

Subgroup	I	II					III		IV		V							VIa			VIb								
Characteristic	RAM1	GOV9	GOV15	MAM4	SPO4	WAT11	LYC7	FET8	SPO15	WAT14	BEL1	BEL2	FET4	LAD1	LYC8	STA5	WIL2	MAG6	SUG5	SUG8	GRA2	GRA8	JAV1	JAV3	MAG15	MAM2	PAP1	RAM4	WIL3
D-galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-lactose	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
D-maltose	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
D-mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-mannose	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
D-melibiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+
D-raffinose	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+
D-sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
D-sucrose	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
D-xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-arabinose	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
L-rhamnose	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	-
L-sorbose	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.3 Differential phenotypic characteristics of 29 representative cellulose producing isolates (continued).

Subgroup	I	II					III		IV		V							VIa			VIb								
Characteristic	RAM1	GOV9	GOV15	MAM4	SPO4	WAT11	LYC7	FET8	SPO15	WAT14	BEL1	BEL2	FET4	LAD1	LYC8	STA5	WIL2	MAG6	SUG5	SUG8	GRA2	GRA8	JAV1	JAV3	MAG15	MAM2	PAP1	RAM4	WIL3
Ethanol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-	+	+	+	+
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Meso-erythritol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid production from:																													
D-arabinose	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
D-arabitol	+	+	+	-	+	v	-	w	-	w	v	-	w	w	w	-	-	w	-	w	-	+	-	-	w	-	-	-	w
D-fructose	w	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-	+	-	+
D-galactose	w	+	+	w	+	-	-	-	+	+	+	+	+	+	+	+	+	v	-	w	+	+	-	+	+	w	-	+	w
D-glucose	+	+	+	+	+	+	+	w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-lactose	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+
D-maltose	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	+
D-mannitol	v	-	+	+	-	-	+	-	-	-	-	-	-	+	+	-	+	-	+	-	-	+	-	+	-	+	+	-	-
D-mannose	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+	w	w	+	+	+	+	+	w	w	+	-	-	+	-
D-melibiose	w	v	+	-	w	w	-	w	+	-	w	w	w	w	v	w	+	-	-	-	w	+	w	w	v	-	-	v	+
D-raffinose	+	+	+	-	+	+	+	+	+	+	-	-	+	-	+	-	-	+	+	+	+	+	-	-	+	-	-	+	+

Table 4.3 Differential phenotypic characteristics of 29 representative cellulose producing isolates (continued).

Subgroup	I	II					III		IV		V							VIa			VIb								
Characteristic	RAM1	GOV9	GOV15	MAM4	SPO4	WAT11	LYC7	FET8	SPO15	WAT14	BEL1	BEL2	FET4	LAD1	LYC8	STA5	WIL2	MAG6	SUG5	SUG8	GRA2	GRA8	JAV1	JAV3	MAG15	MAM2	PAP1	RAM4	WIL3
D-sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-sucrose	-	+	v	-	v	+	+	+	+	+	-	-	+	-	+	-	-	+	+	+	+	+	-	-	+	-	-	+	+
D-xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	w	+	+	+	+	+
L-arabinose	v	w	v	-	w	v	-	-	+	+	w	w	w	v	w	+	+	-	-	-	+	+	+	v	-	+	+	-	+
L-rhamnose	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	+	+	-	-	-	-	-	-	+	-	-	+	-	-
L-sorbose	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+
Ethanol	+	+	+	v	+	+	+	+	+	+	+	w	w	+	+	w	+	-	+	+	-	-	-	+	-	v	-	+	+
Glycerol	+	+	+	-	+	+	+	+	+	+	-	-	+	-	+	-	-	+	+	+	+	+	-	-	-	+	+	+	+
Meso-erythritol	w	-	v	-	+	-	+	+	+	+	-	+	+	w	+	w	w	+	-	+	+	+	-	w	+	-	w	w	+
Cellulose production from :																													
D-arabinose	-	+	+	-	+	+	+	+	+	-	+	+	-	-	-	-	-	+	-	-	+	+	-	+	+	+	+	+	+
D-arabitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
D-galactose	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
D-glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.3 Differential phenotypic characteristics of 29 representative cellulose producing isolates (continued).

Subgroup	I	II					III		IV		V							VIa			VIb								
Characteristic	RAM1	GOV9	GOV15	MAM4	SPO4	WAT11	LYC7	FET8	SPO15	WAT14	BEL1	BEL2	FET4	LAD1	LYC8	STA5	WIL2	MAG6	SUG5	SUG8	GRA2	GRA8	JAV1	JAV3	MAG15	MAM2	PAP1	RAM4	WIL3
D-lactose	+	+	+	-	+	+	+	+	+	-	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
D-maltose	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
D-mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-mannose	-	-	+	-	+	-	+	+	-	-	+	-	-	-	+	+	-	-	+	+	+	+	-	+	+	+	-	+	+
D-melibiose	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	-	+	+	+	+	+	+	-	+	+
D-raffinose	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+
D-sorbitol	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
D-sucrose	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
D-xylose	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
L-arabinose	+	+	+	-	+	+	+	+	+	-	+	+	-	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+
L-rhamnose	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	-
L-sorbose	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-
Ethanol	+	+	+	-	+	+	+	+	+	-	+	-	-	+	+	-	-	-	-	+	-	+	-	-	-	-	+	+	+
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Meso-erythritol	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+

+ = positive; - = negative; w = weakly positive; v= very weakly positive; I= *K. rhaeticus*; II= *K. oboediens*; III = *K. sucrofermentans*; IV = *K. swingsii*; *K. hansenii*; VIa and VIb = unidentified.

4.3.3 16S rRNA gene sequence analysis

For more accurate identification of all representative cellulose producing isolates, the nucleotide sequences of 1271 bp 16S rRNA gene were analyzed. By a phylogenetic tree construction analysis, all 29 representative cellulose producing isolates were belonged to the genus *Komagataeibacter* and were grouped into seven subgroups (Figure 4.15).

Subgroup I contained only one isolate, RAM1, and was identified as *K. rhaeticus*. This isolate showed 99.9% 16S rRNA gene sequence similarity to the type strain of *K. rhaeticus* (Table 4.4), and it was isolated from rambutan.

Subgroup II contained five isolates, GOV9, GOV15, MAM4, SPO4 and WAT11, and was not identified as *K. intermedius* but *K. obediens*. According to Lisdiyanti et al. (2006) reported that *K. intermedius* is a later subjective synonym of *K. oboediens*, although the isolates first constituted a cluster along with the type strain of *K. intermedius*. These isolates showed 99.6-99.7% 16S rRNA gene sequence similarities to the type strain of *K. intermedius* (Table 4.4), and they were isolated from governor's plum, mamao and water melon.

Subgroup III contained two isolates, LYC7 and FET8, and identified as *K. sucrofermentans*. These isolates showed 100% 16S rRNA gene sequence similarities to the type of *K. sucrofermentans* (Table 4.4), and they were isolated from lychee and fetid passionflower.

Subgroup IV contained two isolates, SPO15 and WAT14, which were located within the same cluster as *K. swingsii* and *K. europaeus* in the phylogenetic tree (Figure 4.15). On the basis of the growth test on 30% (w/v) D-glucose, and with or without 0.2% (v/v) acetic acid (Dutta and Gachhui, 2007), the isolates were identified as *K. swingsii*, but not as *K. europaeus*. Since, they showed the same results as the former but not the latter (Table 4.5). The two isolates had 99.8-99.9% 16S rRNA gene sequence similarities to the type strain of *K. swingsii* (Table 4.4), and they were isolated from sapodilla and water melon.

Subgroup V contained seven isolates, BEL1, BEL2, FET4, LAD1, STA5, WIL2 and LYC8, and was identified as *K. hansenii*. These isolates showed 99.6-99.8% 16S rRNA gene sequence similarities to the type strains of *K. hansenii* (Table 4.4), and they were isolated from beleric myrobaln, fetid passionflower, lady's finger banana, star fruit, wild lemon and lychee. Although isolate LYC8 was located in the cluster of *K. kombuchae* KG3^{T} (AY4688433), the isolate was identified as *G. hansenii*, as suggested by Cleenwerck et al. (2009) who reported that *K. kombuchae* is a later subjective synonym of *K. hansenii*.

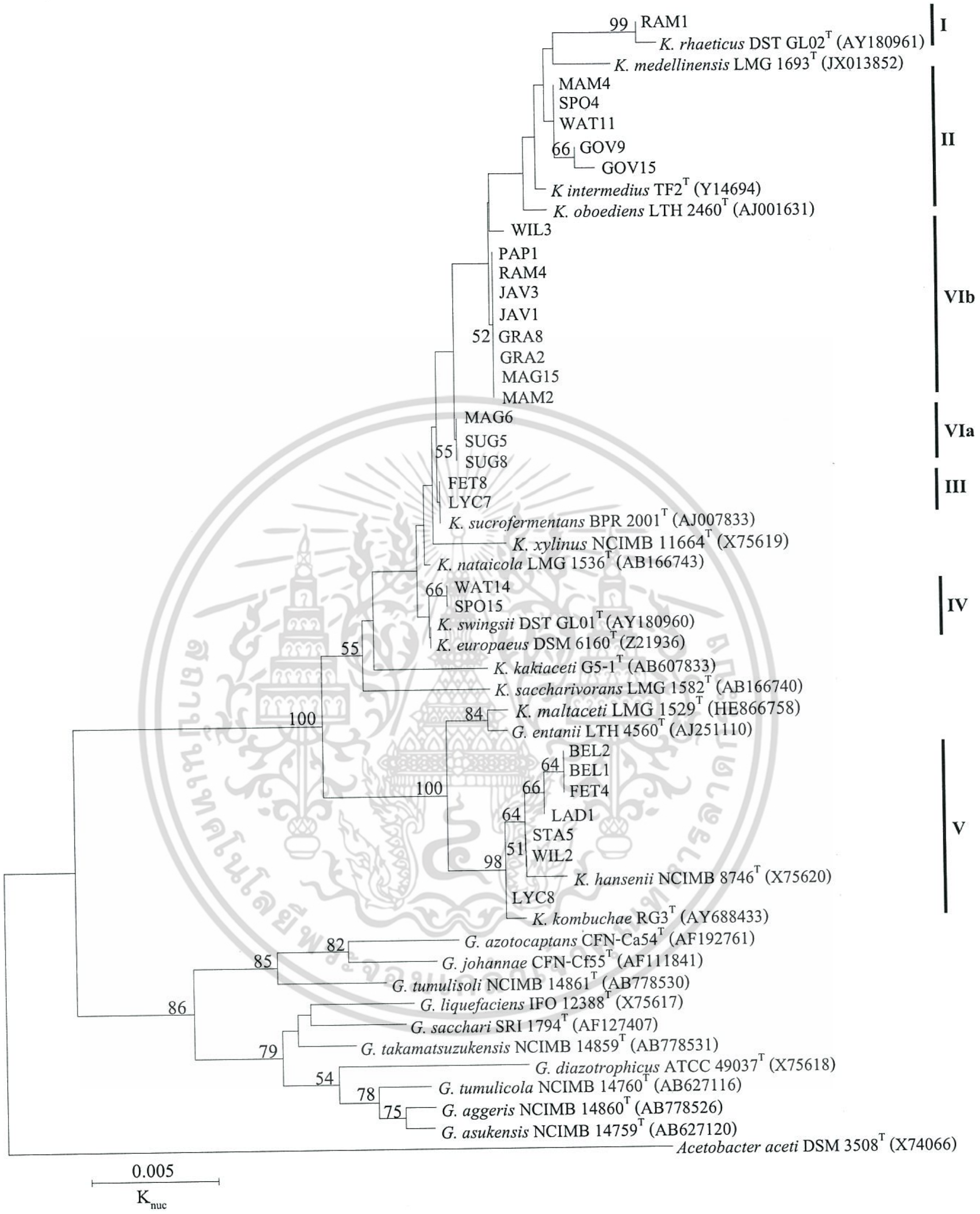


Figure 4.15 Phylogenetic relationships of cellulose producing bacteria. The numerals at the branching points indicated bootstrap values (%) derived from 1000 replications. Only values > 50% are indicated. *K. = Komagataeibacter*, *G. = Gluconacetobacter*.

Table 4.4 Similarity percentages of representative isolates and type strains.

Bacteria	RAM1	I	GOV9	II	LYC7	III	SPO15	IV	BEL1	V	MAG6	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
RAM1	100	99.9	99.4	99.4	99.1	99.1	99.2	99.2	98.6	98.6	99.2	99.3	99.2	99.4	99.1	98.8	98.7	99.4	99.2	98.9	98.9	98.7	97.0	95.6
I	99.9	100	99.3	99.3	99.0	99.0	99.1	99.2	98.5	98.5	99.1	99.2	99.2	99.3	99	98.7	98.6	99.3	99.1	98.8	98.9	98.6	96.9	95.6
GOV9	99.4	99.3	100	99.6	99.3	99.3	99.6	99.5	98.2	98.2	99.4	99.6	99.5	99.8	98.9	98.4	98.5	99.6	99.4	98.9	99.2	98.5	96.9	95.5
II	99.4	99.3	99.6	100	99.5	99.5	99.4	99.5	98.2	98.2	99.6	99.7	99.5	99.8	99.1	98.4	98.5	99.5	99.6	98.8	99.2	98.5	96.7	95.3
LYC7	99.1	99.0	99.3	99.5	100	100	99.7	99.8	98.5	98.5	99.9	99.7	99.8	99.5	99.2	98.7	98.8	99.2	99.9	99.1	99.6	98.8	97.2	95.3
III	99.1	99.0	99.3	99.5	100	100	99.7	99.8	98.5	98.5	99.9	99.7	99.8	99.5	99.2	98.7	98.8	99.2	99.9	99.1	99.6	98.8	97.2	95.3
SPO15	99.2	99.1	99.6	99.4	99.7	99.7	100	99.9	98.5	98.5	99.6	99.5	99.9	99.6	99.2	98.6	98.7	99.4	99.8	99.3	99.6	98.7	97.3	95.4
IV	99.2	99.2	99.5	99.5	99.8	99.8	99.9	100	98.5	98.5	99.7	99.6	100	99.5	99.2	98.7	98.8	99.3	99.9	99.2	99.6	98.8	97.2	95.3
BEL1	98.6	98.5	98.2	98.2	98.5	98.5	98.5	98.5	100	99.6	98.5	98.3	98.5	98.2	98.3	99.6	99.2	98.2	98.5	98.1	98.4	99.2	97.0	95.0
V	98.6	98.5	98.2	98.2	98.5	98.5	98.5	98.5	99.6	100	98.5	98.3	98.5	98.2	98.3	99.6	99.2	98.2	98.5	98.1	98.4	99.2	97.0	94.9
MAG6	99.2	99.1	99.4	99.6	99.9	99.9	99.6	99.7	98.5	98.5	100	99.8	99.7	99.6	99.2	98.6	98.7	99.2	99.8	99.0	99.6	98.7	97.1	95.2
GRA2	99.3	99.2	99.6	99.7	99.7	99.7	99.5	99.6	98.3	98.3	99.8	100	99.6	99.7	99.0	98.5	98.5	99.4	99.6	98.9	99.4	98.5	97.0	95.4
VI	99.2	99.2	99.5	99.5	99.8	99.8	99.9	100	98.5	98.5	99.7	99.6	100	99.5	99.2	98.7	98.8	99.3	99.9	99.2	99.6	98.8	97.2	95.3
VII	99.4	99.3	99.8	99.8	99.5	99.5	99.6	99.5	98.2	98.2	99.6	99.7	99.5	100	99.1	98.4	98.5	99.6	99.6	98.9	99.2	98.5	96.9	95.5
VIII	99.1	99.0	98.9	99.1	99.2	99.2	99.2	99.2	98.3	98.3	99.2	99.0	99.2	99.1	100	98.5	98.5	98.8	99.3	98.9	98.9	98.4	97.2	95.4
IX	98.8	98.7	98.4	98.4	98.7	98.7	98.6	98.7	99.6	99.6	98.6	98.5	98.7	98.4	98.5	100	99.4	98.4	98.6	98.3	98.5	99.4	97.0	95.2
X	98.7	98.6	98.5	98.5	98.8	98.8	98.7	98.8	99.2	99.2	98.7	98.5	98.8	98.5	98.5	99.4	100	98.3	98.7	98.4	98.6	99.8	96.9	94.9
XI	99.4	99.3	99.6	99.5	99.2	99.2	99.4	99.3	98.2	98.2	99.2	99.4	99.3	99.6	98.8	98.4	98.3	100	99.2	98.8	99.0	98.5	96.9	95.5
XII	99.2	99.1	99.4	99.6	99.9	99.9	99.8	99.9	98.5	98.5	99.8	99.6	99.9	99.6	99.3	98.6	98.7	99.2	100	99.2	99.6	98.7	97.1	95.2
XIII	98.9	98.8	98.9	98.8	99.1	99.1	99.3	99.2	98.1	98.1	99.0	98.9	99.2	98.9	98.9	98.3	98.4	98.8	99.2	100	98.9	98.4	97.3	95.6
XIX	98.9	98.9	99.2	99.2	99.6	99.6	99.6	99.6	98.4	98.4	99.6	99.4	99.6	99.2	99.2	98.5	98.6	99.0	99.6	98.9	100	98.6	97.1	95.2
XV	98.7	98.6	98.5	98.5	98.8	98.8	98.7	98.8	99.2	99.2	98.7	98.5	98.8	98.5	98.4	99.4	99.8	98.5	98.7	98.4	98.6	100	97.0	95.0
XVI	97.0	96.9	96.9	96.7	97.2	97.2	97.3	97.2	97.0	97.0	97.1	97.0	97.2	96.9	97.2	97.0	96.9	96.9	97.1	97.3	97.1	97.0	100	95.9
XVII	95.6	95.6	95.5	95.3	95.3	95.3	95.4	95.3	95.0	94.9	95.2	95.4	95.3	95.5	95.4	95.2	94.9	95.5	95.2	95.6	95.2	95.0	95.9	100

I = *K. rhaeticus* DST GL02^T, II = *K. oboediens* LTH 2460^T, III = *K. sucrofermentans* BPR 2001^T, IX = *K. swingsii* DST GL01^T, X = *K. hansenii* NCIMB 8746^T, XI = *K. europaeus* DSM 6160^T, XII = *K. intermedius* TF2^T, XIII = *K. kakiaceti* G5-1^T, IX = *K. kombuchae* RG3^T, X = *K. maltaceti* LMG 1529^T, XI = *K. medellinensis* LMG 1693^T, XII = *K. nataicola* LMG 1536^T, XIII = *K. saccharivorans* LMG 1582^T, XIV = *K. xylinus* NCIMB 11664^T, XV = *G. entanii* LTH 4560^T, XVI = *G. liquefaciens* IFO 12388^T, XVII = *A. aceti* DSM 3508^T, *K.* = *Komagataeibacter*, *G.* = *Gluconacetobacter* and *A.* = *Acetobacter*.

Table 4.5 Results of confirmative tests for identification of isolate SPO15 and WAT14.

Characteristic	SPO15	WAT14	*K. swingsii* LMG 22125$^{\mathrm{Ta}}$	*K. europeaus* LMG 18890$^{\mathrm{Tb}}$
Growth on 30% D-glucose	+	+	+	-
Growth without acetic acid	+	+	+	-
Growth on different carbon source				
Ethanol	+	+	+	-
D-mannitol	+	+	+	-
D-sorbitol	+	+	+	-
D-sucrose	+	+	+	-

The data of the reference strains are cited from, ^{a}Dellaglio et al. (2005) and ^{b}Sokollek et al. (1998).

Subgroup VI was divided into two subgroups, VIa and VIb. Subgroup VIa contained three isolates, MAG6, SUG5 and SUG8, and Subgroup VIb contained nine isolates, GRA2, GRA8, JAV1, JAV3, MAG15, MAM2, PAP1, RAM4 and WIL3, all of which were located in different phylogenetic positions from any other known species of the genus *Komagataeibacter* in the 16S rRNA gene sequence phylogenetic tree and assumed to constitute new species. They were isolated from grape, java plum, mangosteen, mamao, papaya, rambutan, sugar apple and wild lemon.

The genus *Komagataeibacter* was reclassified from genus *Gluconacetobacter* by Yamada et al. (2012). In the past, species of the genus *Gluconacetobacter* are divided into two groups, such as the *G. liquefaciens* group and the *G. xylinus* group (Yamada and Yukphan, 2008). The former group consisted of the non-nitrogen fixers such as *G. liquefaciens* and *G. sacchari* (Yamada et al., 1997; Franke et al., 1999), and the nitrogen fixers such as *G. diazotrophicus*, *G. azotocaptans* and *G. johannae* (Gillis et al., 1989; Fuentes-Ramírez et al., 2001). The latter group consisted of the non BC-producers such as *G. hansenii*, *G. europaeus,* etc. (Yamada et al., 1997), and the BC producers such as *G. xylinus*, *G. oboediens, G. intermedius, G. rhaeticus, G. nataicola*, etc. (Yamada et al., 1997; Yamada et al., 2000; Dellaglio et al., 2005; Lisdiyanti et al., 2006). Yamada and Yukphan (2008) suggested that the *G. liquefaciens* group and the *G. xylinus* group can be separated from each other at the generic level from these viewpoints.

(1) Morphologically, the former group is motile with peritrichous flagella, but the latter group is non-motile without any flagellation.

(2) Physiologically, the former group produces 2,5-diketo-D-gluconate γ-pyrone compounds from D- glucose and a water- soluble brown pigment, but the latter group does not. In acetate and lactate oxidation, the former group is more active than the latter group.

(3) Chemotaxonomically, the former group consists of lesser amount of Q-9 in addition to Q-10 as major quinones, but the latter group does not.

(4) Phylogenetically, (4.1) the calculated bootstrap value between the two groups in a 16S rRNA gene sequence phylogenetic tree constructed by the neighbor-joining method is 70%. This denoted that the two groups are phylogenetically not so tightly coupled. (4.2) The former group forms a large cluster along with the genera *Acidomonas*, *Asaia*, *Acetobacter*, *Gluconobacter*, *Kozakia*, *Neoasaia* and *Swaminathania* rather than the cluster of the latter group in a 16S rRNA gene sequence phylogenetic tree constructed by the maximum parsimony method. The calculated bootstrap value is 74%. (4.3) A similar clustering is found in a 16S rRNA gene sequence phylogenetic tree constructed by the maximum likelihood method. The calculated bootstrap value is 60%. Such a clustering never found in acetic acid bacteria. For example, in the genus *Gluconobacter*, the calculated bootstrap values from neighbor-joining method, maximum parsimony method and maximum likelihood method are 100%, 91% and 90%, respectively, in contrast to those of 70%, 74% and 60% in the genus *Gluconacetobacter*.

(5) Ecologically, the habitats are different between strains of the two groups. The strains of the former group are plant-associated and mostly isolated from flowers, sugarcane and coffee plants (Franke et al., 1999; Fuentes-Ramírez et al., 2001). In contrast, the strains of the latter group are not plant associated and mostly isolated from fermented foods such as tea fungus beverage, nata-de-coco, vinegar and wine (Dutta and Gachhui, 2007; Pourramezan et at., 2009; Rani et al., 2011b).

From these viewpoints, Yamada et al. (2012) separated the *G. xylinus* group from the *G. liquefaciens* group at the generic level and the species of the former group had been classified under a separate new genus. The name of the genus is *Komagataeibacter*. Yamada et al. (2012) reclassified *Komagataeibacter* from *Gluconacetobacter* (Yamada et al., 1998) with the following 12 species, *K. xylinus* (Brown, 1886), *K. hansenii* (Gosselé et al., 1983), *K. europaeus* (Sievers et al., 1992), *K. oboediens* (Sokollek et al., 1998), *K. intermedius* (Boesch et al., 1998), *K. swingsii* (Dellaglio et al., 2005), *K. rhaeticus* (Dellaglio et al., 2005), *K. saccharivorans* (Lisdiyanti et al., 2006), *K. nataicola* (Lisdiyanti et al., 2006), *K. kombuchae* (Dutta and Gachhui, 2007) and *K. sucrofermentans* (Toyosaki et al., 1995b). *Gluconacetobacter entanii* (Schüller et al., 2000) is not available in any culture collection including DSM. The species cannot be listed as a new combination, according to Rule 27 of the Bacteriological Code (Tindall et al., 2006). In addition, Yamada (2014) transferred of *G. kakiacetic* (Iino et al., 2012), *G. medellinensis* (Castro et al., 2013) and *G. maltaceti* (Slapšak et al., 2013) to the genus *Komagataeibacter* as *K. kakiaceti*, *K. medellinensis* and *K. maltaceti*, respectively.

In the present study, phylogenetic analysis based on 16S rRNA gene sequences showed that all the 29 representative cellulose producing isolates belonged to the genus *Komagataeibacter* and were divided into seven subgroups (Figure 4.1). Subgroup I was identified as *K. rhaeticus*. Subgroup II was identified as *K. oboediens*, type strain of which does not produce BC. Subgroup III was identified as *K. sucrofermentans*. Subgroup IV was identified as *K. swingsii* and Subgroup V was identified as *K. hansenii*, the type strain of which does not produce BC. However, it was remarkable in the present study that any strain assigned to *K. xylinus* were not isolated from tropical fruits collected in Thailand, suggesting that the distribution of the species might be rare in the tropical country. This phenomenon was in good accordance with the previous work of Lisdiyanti et al. (2003), who studied the diversity of acetic acid bacteria in South East Asia. In their work, isolation of acetic acid bacteria was conducted from 1994 to 1999 in Denpasar, Yogyakarta, and Bogor in Indonesia, Bangkok in Thailand, and Los Banõs in the Philippines. They found that, all of where no *K. xylinus* strains were isolated from tropical fruits and tropical flowers. For the remaining subgroups VIa and VIb were not identified. From the phylogenetic result obtained, it is obvious that these isolates constitute new species, which DNA-DNA hybridization or other molecular techniques could be used to identify them. Since this study was focused only on the ability in BC production of the isolated strains, therefore, these isolates were not identified to species level.

4.4 BC production by all representative cellulose producing bacteria

All 29 representative cellulose producing isolates were cultivated in a 250-mL flasks containing 90 mL of standard HS medium under static and shaking condition at 150 rpm for 7 days to ensure the cellulose production capacity and find the suitable cultivation method. Under static condition, BC was produced in a pellicle form over the surface of liquid medium and also became thicker with an increased cultivation time. The amount of BC yields in this condition ranged from 0.5 to 1.13 g/L (Figure 4.16). The lowest BC yield of 0.50 g/L was significantly ($p < 0.05$) found in isolate SPO15, and the highest BC yield of 1.13 g/L was significantly ($p < 0.05$) found in isolate PAP1. The pHs of culture media at the end of cultivation day were in a range of 3.2-4.3. On the other hand, under shaken condition BC was produced like pellets, granules or short fibrous spreading in the medium depending on bacterial strains. The amount of BC yields under shaking condition ranged from 0.32 to 0.65 g/L (Figure 4.16). The lowest BC yield of 0.32 g/L was found in isolate RAM4, and the highest BC yield of 0.65 g/L was found in isolate MAM2. The pH values of culture media at the end of cultivation were in a range of 3.0-4.5.

The lower BC yield under shaken condition could be explained by the shear stress and non cellulose producing mutant formation (Schramm and Hestrin, 1954). Ha et al. (2008) studied BC production by *G. hansenii* PJK under shaken condition. They found that, shaken culture converted cellulose producing (Cel^{+}) cells into non cellulose producing (Cel^{-}) mutants. The Cel^{-} cells became more enriched than the Cel^{+} cells since they grow faster, resulting in a lower BC production.

BC productions by all selected isolates under static and shaken conditions were compared and selected the most effective BC-producing isolate. The results showed that, under static condition isolate PAP1 was the most significantly effective cellulose producer whereas under shaken condition was isolate MAM2. However, BC production in all selected isolates under static condition gave the higher BC yield than under shaken condition, and this research was focused on the static condition because of the larger application potential for membranes of BC, produced in this way. Therefore, isolate PAP1 was chosen for further studies.

A lot of BC production studies have been reported Keshk and Sameshima (2006) reported that *A. xylinum* ATCC 10245 gave 1.15 g/L of BC when cultivated in HS medium under static condition for 7 days. The amounts of BC produced in the present study appeared to correspond to their results. Nguyen et al. (2008a) characterized cellulose production by *G. xylinus* strain isolated from kombucha and found that this bacterium produced BC yield of 0.28 g/L in HS medium when statically incubated at 30 °C for 7 days. Under the same condition, Park et al. (2003) reported that 0.35 g/L of BC was produced by *G. hansenii* strain isolated from rotten apple and, Jahan et al. (2012) also reported the maximum BC yield of 0.51 g/L was produced by *Gluconacetobacter* sp. F6 strain isolated from rotten apple. In the present study, isolate PAP1, which was isolate from rotten papaya, produced a large amount of BC with 1.15 g/L in standard HS medium.

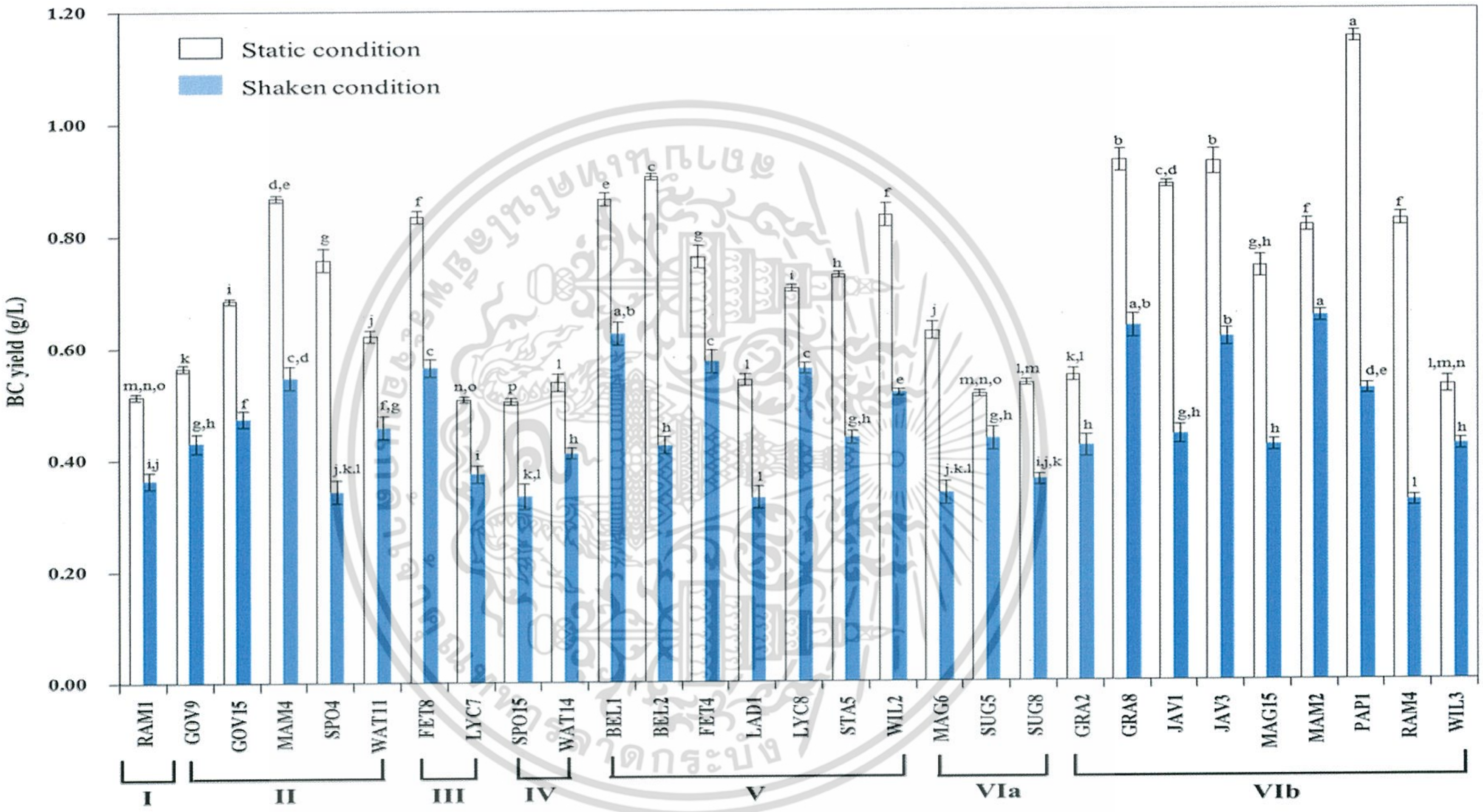


Figure 4.16 Cellulose production by all representatives BC-producing isolates. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

4.5 Characteristic of *Komagataeibacter* sp. PAP1

The characteristics of *Komagataeibacter* sp. PAP1 were summarized as the following section. Colonies of *Komagataeibacter* sp. PAP1 were circular with entire margin, convex elevation in center and with a diameter of 5.6-8.3 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short and long rod-shaped, approximately 1.6-1.7 μm long and 0.4-0.5 μm wide, and occurring singly or in short chains (Table 4.2). Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This bacterium could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-fructose, D-glucose, D-lactose, D-mannitol, D-sorbitol, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Cellulose was produced D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol (Table 4.3). This bacterium was isolated from papaya.

4.6 Growth and BC production by *Komagataeibacter* sp. PAP1 in standard HS medium

The most effective cellulose producing bacterium, *Komagataeibacter* sp. PAP1 was examined for viable cell counts, BC production and the pH change of culture medium during statically cultivation in standard HS medium at 30 °C for 14 days. As investigated in Figure 4.17, the viable cells and BC yields increased quite slowly for the first and second days and then rapidly increased from the third day. The viable cells and BC yields of *Komagataeibacter* sp. PAP1 increased exponentially after 2-day lag time and the stationary phase emerged after seven days of cultivation. The results obtained showed that BC production by *Komagataeibacter* sp. PAP1 was growth-associated production type and BC yield reached a maximum of 1.19 g/L after 14 days of cultivation. The pH of culture medium decreased from 6.0 to 3.35 to the cultivation time which could be explained by the nature of *Komagataeibacter*. The bacteria in this genus can convert carbohydrates to acetic acid and glucose to gluconic acid by producing and releasing fibrils of cellulose, which decreased pH in culture medium (Kongruang, 2008).

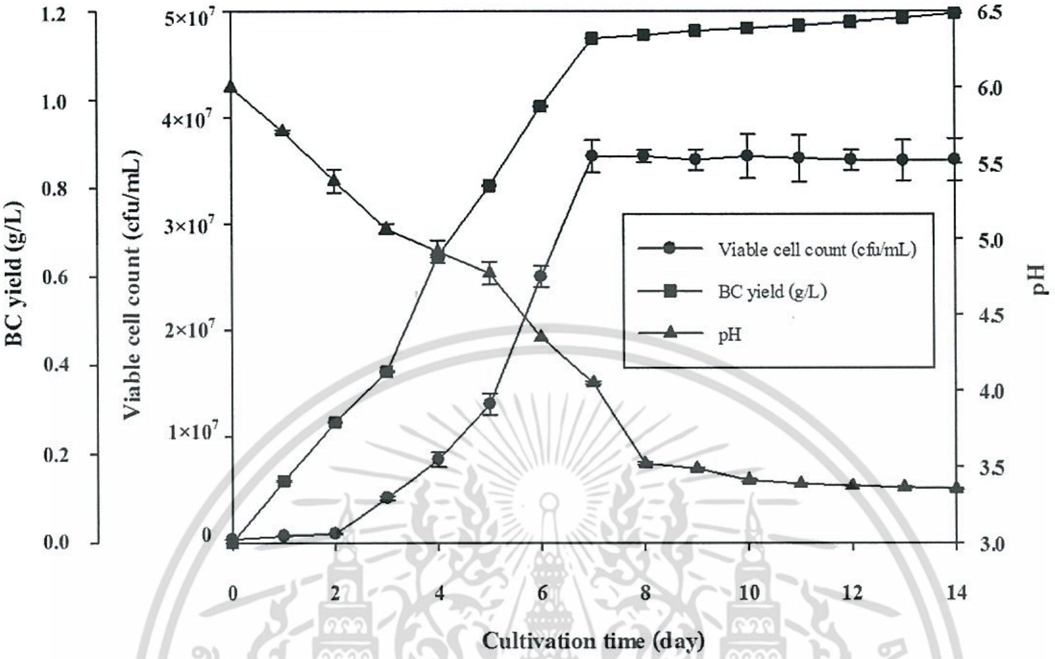


Figure 4.17 Time course of BC production by *Komagataeibacter* sp. PAP1 in the standard HS medium under static condition at 30 °C for 14 days. All the data were the means ± SD of triplicate analyses.

4.7 Evaluation of different carbon sources for BC production by *Komagataeibacter* sp. PAP1

Effect of various carbon sources on BC production by *Komagataeibacter* sp. PAP1 was studied by replacing the original carbon source (glucose) of standard HS medium by the same concentration (2.0% w/v or v/v) of galactose, fructose, maltose, lactose, sucrose, ethanol, glycerol, mannitol and sorbitol, respectively. The BC yields obtained after statically incubation at 30 °C for 7 days are shown in Figure 4.18. A high level of 3.50 g/L and 3.43 g/L BC productions were observed when mannitol and ethanol were used as a carbon source, respectively. There were no significant difference between BC production from mannitol and ethanol. In media containing glycerol, fructose, sucrose and sorbitol as a carbon source gave 2.89, 2.87, 2.64 and 2.09 g/L of BC yields, respectively. However, when lactose, maltose, glucose and galactose was used as a carbon source, low BC production of 1.99, 1.91, 1.17 and 1.11 g/L, respectively was observed. The pHs of all culture media were in the range of 3.6-5.9.

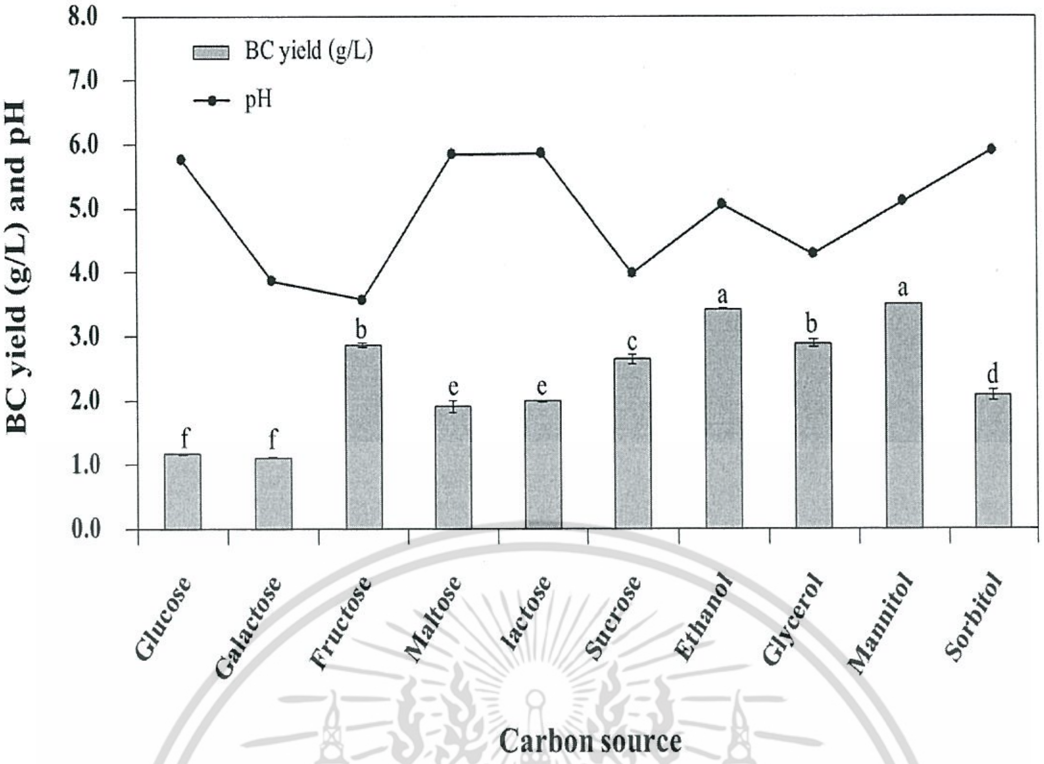


Figure 4.18 Effect of different carbon sources on BC production under static condition at 30 °C for 7 days by *Komagataeibacter* sp. PAP1. Bars show the standard deviations of the mean values. Different lowercase letters above bars for each isolates indicate a significant difference at $p < 0.05$.

It is well known that a Gram-negative acetic acid bacterium *K. xylinus* has long been used as a model organism for study of BC biosynthesis, since it can utilize a wide range of substrates such as 5- or 6-carbon monosaccharides (glucose, fructose and xylose), oligosaccharide, polysaccharide, sugar alcohols (mannitol and sorbitol), glycerol and ethanol, and industrial wastes (sugar cane molasses, coconut water, pineapple water, and hydrolyzed konjac powder) to generate high amounts of cellulose (Kongruang, 2008; Hong and Qiu, 2008; Keshk and Sameshima, 2006). *K. xylinus* has two main operative amphibolic pathways including the pentose phosphate pathway for oxidative of carbohydrates, and the Krebs cycle for the oxidation of organic acid and related compounds (Ross et al., 1991), which could be used for explain the mechanism of cellulose production in *Komagataeibacter* sp. PAP1.

In the present study, *Komagataeibacter* sp. PAP1 showed the capability of utilizing a wide variety of carbon sources for BC production. Efficient cellulose production by this isolate lies in its ability to synthesize glucose from various carbon sources, followed by glucose polymerization to cellulose. Mannitol and ethanol seemed to be the effective carbon sources for the production of BC by *Komagataeibacter* sp. PAP1. In *Komagataeibacter* spp. consisted of membrane-bound alcohol and aldehyde dehydrogenases which oxidized alcohol to acetic acid to generate electrons (De Faveri et al., 2003).

Mannitol is a sugar alcohol, has no double bonds and does not exist as a ring structure. It could provide electrons for bacterial metabolism and stimulated a higher yield of cellulose comparing with glucose (Brown, 1886). Mannitol was probably oxidized to fructose and then followed by second oxidation to 5-keto-D-fructose (Mowshowitz et al., 1974) (Figure 4.19). By precluding the formation of sugar acid (gluconic acid), the pH did not decrease drastically as with the glucose. As a result, bacterial cell growth and cellulose production were not inhibited (Hutchens et al., 2006).

Ethanol is a two carbon alcohol. It was transformed into acetate and entered the Krebs cycle, where oxaloacetate, water, carbon dioxide and ATP were produced. Then, oxaloacetate was transformed into phosphoenol pyruvate and entered the gluconeogenesis pathway to produce cellulose. Furthermore, it was believed that the presented of ethanol could improve BC synthesize by acting as an energy source for ATP generation instead of glucose. It could be explained by the production of ATP in the Krebs cycle. This process increased the level of intracellular ATP, which in turned decreased the activity of glucose-6-phosphate dehydrogenase and inhibited pentose phosphate pathway (Figure 4.19). Therefore, glucose-6-phosphate, a precursor of BC followed into BC synthesis pathway, resulted in higher BC production (Naritomi et al., 1998). Ethanol also showed the ability in preventing the bacterial cell forming a non-cellulose producing mutant (*Cel*$^{-}$) in shaken conditions (Park et al., 2003).

Glycerol is a three carbon sugar and could be utilized by *Komagataeibacter* sp. PAP1 (Figure 4.19). It was introduced into the two metabolic cycles at the triose phosphate level (Ross et al., 1991). Then triose phosphate was oxidized by pentose phosphate cycle and entered into the Krebs cycle to generate a precursor of cellulose production (Mikkelsen et al., 2009; Ruka et al., 2012).

Fructose is a hexose sugar and was metabolized to glucose-6-phosphate by fructose hexokinase and phosphoglucose isomerase, respectively (Figure 4.19). Then the generated glucose-6-phosphate entered into cellulose synthesis pathway via phosphoglucomutase or into pentose phosphate pathway via glucose-6-phosphate dehydrogenase (Naritomi et al., 1998).

Komagataeibacter sp. PAP1 could utilize fructose and produce a high BC yield but lower than mannitol.

Sucrose is a disaccharide made up of two hexose sugars (glucose and fructose). *Komagataeibacter* sp. PAP1 produced cellulose in a moderate level when using this sugar as a carbon source. Since sucrose could not be transported through the cell membrane. Sucrose required an additional step to hydrolyze in the periplasm to glucose and fructose for cellulose synthesis (Ruka et al., 2012).

Lactose is a disaccharide sugar derived from galactose and glucose. The low BC yield obtained in lactose could be explained by the lack of gene that encodes the β-galactosidase (*LacZ*) in *Komagataeibacter*. β-galactosidase is an enzyme that catalyzes the hydrolysis of lactose, which is the first step of lactose metabolic pathway in microorganisms (Battad-Bernardo et al., 2004). The formation of BC from lactose was probably due to other components of HS medium such as peptone or yeast extract (Carreira et al., 2011).

In the case of sorbitol, galactose and maltose, sorbitol is a sugar alcohol and an isomer of mannitol, galactose is a hexose sugar and maltose is a disaccharide sugar from formed two unit of glucose. These sugars gave low BC yields in *Komagataeibacter* sp. PAP1. Metabolism of these carbons to cellulose in isolate PAP1 did not occur very efficiently because of the inefficient uptake from the medium. It appeared that this bacterium was unable to transport sorbitol, galactose and maltose efficiently across the cell membrane. The small amounts of these carbons which were taken up only sufficient to maintain cell growth and essential cellular functions (Mikkelsen et al., 2009).

Glucose is a hexose sugar and has a structure similar to fructose. This sugar is a main carbon source in cellulose synthesis as an energy source and cellulose precursor (Figure 4.19) (Klemm et al., 2001). It was interesting that glucose which has been reported as producing the highest cellulose yield in HS medium by many works (Carreira et al., 2011; Trovatti et al., 2011; Jahan et al., 2012) but low cellulose yield produced by *Komagataeibacter* sp. PAP1. It could hypothesize that glucose was used for cell growth more than cellulose synthesis.

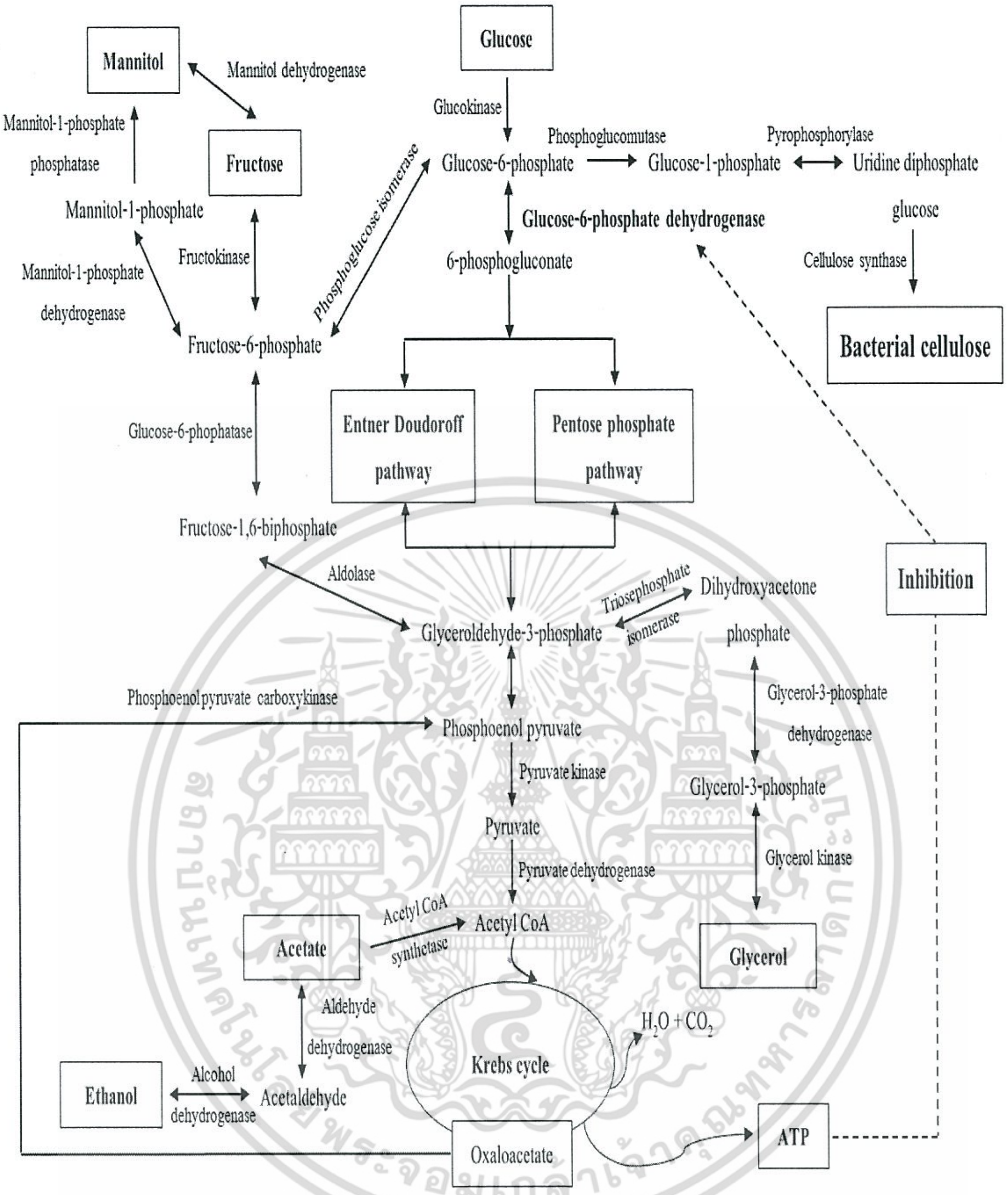


Figure 4.19 Predicted biochemical pathways of BC production from various carbon sources.

The results obtained were consistent with the previous works describing the use of several monosaccharides, disaccharides and various alcohols as carbon sources for BC synthesis by many species of *Komagataeibacter* (Keshk and Sameshima, 2005; Nguyen et al., 2008a). Nguyen et al. (2008a) studied cellulose production by *G. xylinus* isolated from Kombucha tea. They found that this bacterium gave the maximum BC yield of 0.82 g/L in HS medium containing mannitol as sole carbon source. However, the capability of a certain carbon sources for BC production also seemed to depend on the bacterial strains. For example *G. xylinus* ATCC 10245 and *Gluconacetobacter* sp. RKY5 isolated from persimmon vinegar showed the highest BC yields of 1.33 g/L and 2.45 g/L, respectively in glycerol (Keshk and Sameshima, 2005; Kim et al., 2006). *G. sacchari* isolated from Kombucha and *Gluconacetobacter* sp. F6 strain isolated from rotten apple gave the highest amount BC of 2.70 g/L and 1.34 g/L, respectively in glucose (Trovatti et al., 2011; Jahan et al., 2012). Furthermore, the low level of BC production obtained from lactose was consistent with the work of Nguyen et al. (2008a). They found that *G. xylinus* produced low BC yield of 0.07 g/L in HS medium containing lactose as sole carbon source. The results obtained indicated that factors affecting BC production are bacterial strain and carbon source.

4.8 Optimization culture condition for BC production

However, BC yield produced by *Komagataeibacter* sp. PAP1 was still low with a high production cost. Therefore, the optimization culture condition and utilization of agricultural waste as substrate were studied in order to increase BC productivity and decrease production cost. Soybean whey is a liquid by-product resulting from the protein coagulation of soybean milk during tofu production. Soybean whey is approximately 79-80% of the total soybean milk (Fung et al., 2008). Production of one ton of tofu generates ten tons of soybean whey as by-product (Lu et al., 2004). Soybean whey has a high biological (BOD) of 13730 ppm (Smith et al., 1962) and a high chemical oxygen demand (COD) of 25000 mg/L (Mitra et al., 2010). Due to the increasing demand of tofu in Thailand every year, a large amount of soybean whey is generated as waste. If soybean whey is discarded without any treatment, it will cause pollution. However, soybean whey is a rich source of nutrients (Smith et al., 1962), which could be used as substrate for microbial fermentation (Mitra et al., 2010). Therefore, soybean whey was considered as an alternative substrate for BC production by *Komagataeibacter* sp. PAP1 to reduce the BC production cost and environmental pollution. Soybean whey components were examined using standardized AOAC 2005 method and it composed of 98.87% moisture, 0.34% protein, 0.02% fat, 0.51% ash, 0.26% total carbohydrate and 0.02% lactose with a pH of 4.5 (Figure 4.20).

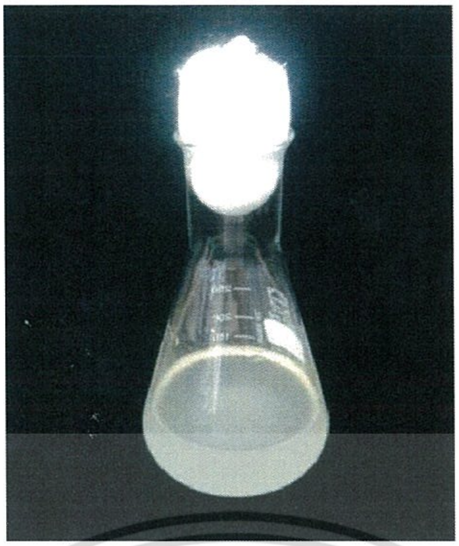


Figure 4.20 The characteristic of soybean whey.

4.8.1 Optimization of fermentation factors for BC production by *Komagataeibacter* sp. PAP1

In general, microbial cell growth and BC productivity depend on medium components (carbon source, nitrogen source and supplementary substances), environmental conditions (pH and temperature) and cultivation methods (static and shaken). Changes of these factors could stimulate or decrease cell growth and BC synthesis directly or indirectly. Microbial cells will grow rapidly and produce more cellulose when grow in medium supplied with appropriate carbon source, nitrogen source at the optimal pH and temperature (Chawla et al., 2009). Therefore, the three main fermentation factors, including initial pH of culture medium (X1), concentration of carbon source (X2) and incubation temperature (X3), were chosen for the optimization of cellulose production by *Komagataeibacter* sp. PAP1 in soybean whey. Nitrogen source was not selected for the optimization because soybean whey is a rich source of protein. From the results obtained, isolate PAP1 gave the maximum BC yield when mannitol and ethanol were used as a carbon source. However, on cost consideration one gram of mannitol per 3.23 baht (Univar, Australia) while one milliliter of ethanol per 0.05 baht (The Excise department) as a result, ethanol was selected as a carbon source for optimization.

A central composite design (CCD) of response surface methodology (RSM) was used to identify the optimal condition for the culture medium instead of the conventional method, one-variable-at-a-time (OVAT) method. Since, it can minimize error in determining the effect of parameters, explain the relationship when interaction between different components takes place and less-time consuming compared to the OVAT method (Liong and Shah, 2005). In CCD, each independent variable was analyzed at five levels including the lower limit ($-\alpha$), low (-1), central (0), high (+1) and upper limit ($+\alpha$). When, central is the position that expected to produce maximum yield, low and high are the positions that expected to produce lower yield than central, and lower limit and upper limit are the positions that expected to produce lowest yield or no production. Many studies were reported that various species of *Komagataeibacter* could grow and produce cellulose in pH ranged between 3.0-8.0, 1.0-5.0% concentration of carbon source and cultivation at 25-30 °C. However, most of researches have reported the maximum BC production at pH 6.0, 2.0% concentration of carbon source and cultivation at 30 °C (Hestrin and Schramm, 1954; Jonas and Farah, 1998; Jahan et al., 2012), and they were chosen as the central point in this study. BC production by isolate PAP1 were studied at pH ranged 2.64-9.36, 0-4.10% of carbon source and cultivation at 21.6-38.4 °C (Table 3.2).

The experimental design and the responses (BC yields) with the mean observed and predicted responses obtained from 20 differential experiments were shown in Table 4.6. The BC yields were determined using multiple regression analysis with $p \leq 0.05$ and used to construct second-order regression model capable of predicting the amounts of BC yield (Y) as a function of initial pH of culture medium, ethanol concentration, and incubation temperature. The derived regression equation for the optimization of the soybean whey-based medium is shown as equation 4.1.

$$Y = 3.95 + 0.08\,(X1) + 0.16\,(X2) - 0.48\,(X3) - 0.73\,(X1)^2 - 0.61\,(X2)^2 - 0.79(X3)^2 - 0.05(X1*X2) - 0.10\,(X1*X2) + 0.14\,(X1*X2) \qquad (4.1)$$

Where X1 is initial pH of culture medium, X2 is concentration of ethanol (%, v/v) and X3 is incubation temperature (°C).

Table 4.6 Experimental design and BC production by *Komagataeibacter* sp. PAP1 using central composite design.

Treatment	Code variable level			Response (BC yield, g/L)	
	X1	X2	X3	Observed	Predicted
1	-1	-1	-1	2.67 ± 0.02	2.05
2	-1	-1	+1	1.61 ± 0.04	0.99
3	-1	+1	-1	2.24 ± 0.02	2.20
4	-1	+1	+1	1.60 ± 0.03	1.71
5	+1	-1	-1	3.10 ± 0.02	2.51
6	+1	-1	+1	1.49 ± 0.03	1.06
7	+1	+1	-1	2.29 ± 0.04	2.44
8	+1	+1	+1	1.41 ± 0.02	1.56
9	-1.682	0	0	1.29 ± 0.02	1.76
10	+1.682	0	0	1.81 ± 0.02	2.01
11	0	-1.682	0	0.83 ± 0.02	2.33
12	0	+1.682	0	2.95 ± 0.05	2.50
13	0	0	-1.682	2.09 ± 0.06	2.53
14	0	0	+1.682	0.67 ± 0.02	0.90
15	0	0	0	3.60 ± 0.06	3.95
16	0	0	0	3.81 ± 0.06	3.95
17	0	0	0	4.00 ± 0.04	3.95
18	0	0	0	4.01 ± 0.04	3.95
19	0	0	0	4.01 ± 0.02	3.95
20	0	0	0	4.35 ± 0.03	3.95

The results of the second order response surface model in the form of analysis of variance (ANOVA) were shown in Table 4.7. The quality of fit of quadratic polynomial equation was expressed by R^2 value, probability (p) value and lack-of-fit test. The R^2 value indicates the percentage of the variability of the optimization parameter that is explained by the model when p value < 0.05. The lack-of-fit test is used to determine whether the constructed model is adequate to explain the observed data. When the estimated p value for the lack-of-fit is less than 0.05, there is statistically significant lack-of-fit at the 95% confidence level. That means that the model does not adequately represent the data (Rigas et al., 2000). As shown in Table 4.7, the R^2 value was found by calculation to be 0.835 with the *p* value was found to be significant ($p < 0.05$), indicating that 83.50 % of the variations in BC yield could be explained by the model. No significant lack-of-fit ($p > 0.05$) was observed. The results showed that, the generated second-order regression model was adequate in predicting the BC yield.

Table 4.7 Regression analysis (ANOVA) of BC yields.

Source of variation	df	SS	MS	*F*-value	*p*-value
Regression					
Model	9	67.070	7.453	34.154	0.000
Residual	50	10.910	0.218		
Total	59	77.980			
Lack of fit	1	0.360	0.360	1.643	0.207
Pure error	45	0.980	0.220		
Total error	50	1.340			
Coefficient of determination (R^2)			0.835		

Df = degree of freedom, SS = sum of square, MS = mean square.

The *p* value is used to examine the significance of each coefficient and also indicate the interaction strength between each independent variable (Rigas et al., 2000). As indicated in Table 4.8, statistical analysis showed significant effect of ethanol concentration and incubation temperature on BC production by *Komagataeibacter* sp. PAP1. From the linear coefficients of second-order in equation 4.1, initial pH of culture medium and ethanol concentration showed a positive effect on BC production, while incubation temperature showed a negative effect.

Table 4.8 Co-efficient of the regression equation.

Code	Co-efficient	*t*- value	*p*-value
Constant	3.946	35.880	0.000
X1	0.077	1.055	0.296
X2	0.163	2.234	0.030
X3	-0.484	-6.628	0.000
X1X2	-0.054	-0.569	0.572
X1X3	-0.099	-1.043	0.302
X2X3	0.141	1.484	0.144
X1X1	-0.730	-10.264	0.000
X2X2	-0.610	-8.571	0.000
X3X3	-0.791	-11.123	0.000

X1 = initial pH of culture medium, X2 = concentration of ethanol, X3 = incubation temperature.

4.8.2 Effects of three fermentation factors on BC production by *Komagataeibacter* sp. PAP1

The second-order regression model (equation 4.1) was used to generate response surface plots which are shown in Figure 4.21, 4.22 and 4.23. The response surface plots showed the complex interaction between the two variables by keeping other one variable at the central level (Table 3.2).

4.8.2.1 Effects of initial pH of culture medium and ethanol concentration on BC yield

Figure 4.21 shows the response surface curve of BC production as a function of initial pH of culture medium and ethanol concentration by keeping the incubation temperature at 30 °C (central level). At the lowest level of pH, BC production increased with increase in ethanol concentration. This may be due to the inhibition of glucose-6-phosphate dehydrogenase by the increased intracellular ATP (Figure 4.19). However, when the level of pH was more than 6.0, BC production decreased. Similar, at the lowest level of ethanol concentration, BC production increased with increase in level of pH up to 6.0. When the concentration of ethanol was more than 2.0%, BC production decreased because of increased in the residual acetate (Figure 4.19). Naritomi et al. (1998), reported that addition more than 1.5% ethanol led to an increase in residual acetate resulting in a decreased in BC formation. A high concentration of acetate inhibited cell growth, resulting in low BC production. As shown in Figure 4.21, the optimal pH and ethanol concentration were ranging from 4.75 to 7.50 and 0.9 to 2.4%, respectively. A maximum BC production of 3.30 g/L was obtained at pH 6.21 and 1.61% of ethanol (v/v). The results obtained in this study were consistent with the previous reports indicated the optimal pH range for BC production was 4.0-7.0 (Jonas and Farah, 1998; Jahan et al., 2012). Jonas and Farah (1998) reported that for BC production in large scale commercial, a pH 4.0 and 4.5 gave better BC yield. Jahan et al. (2012) determined the optimal condition for BC production by *Gluconacetobacter* sp. F6 and found that the optimal pH of this strain was 6.0 with the maximum BC yield of 0.92 g/L. In contrast, Castro et al. (2012) studied BC production by *G. medellinensis* isolated from fermentation of Colombian homemade vinegar. The maximum BC production of 4.50 g/L was obtained at pH 3.5, which most of other bacterial strains could not function so well. This supports that the optimal pH depends on the bacterial strains and varies from strain to strain.

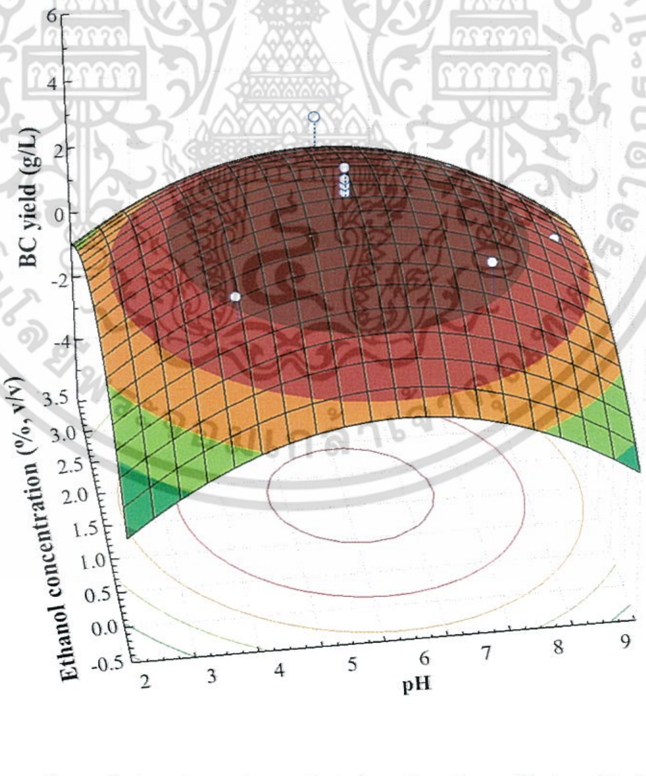


Figure 4.21 Response surface plot and contour plot showing the effects of initial pH of culture medium and ethanol concentration on BC production.

4.8.2.2 Effects of initial pH of culture medium and incubation temperature on BC yield

Figure 4.22 shows the response surface curve of BC production as a function of initial pH of culture medium and incubation temperature by keeping the concentration of ethanol at 2.0% (v/v) (central level). At the lowest (2.64) and the highest (9.36) levels of initial pH of culture medium, the amounts of BC production increased with an increased level of incubation temperature up to 30 °C (central level) and then decreased. The optimal pH and incubation temperature were ranging from 4.5 to 4.8 and 24.0 to 32.5 °C, respectively. The BC yield of 3.53 g/L was the maximum at initial pH of culture medium of 6.21 and incubation temperature of 28.4 °C. Many researches have been reported the optimal growth temperature for BC production was 25 to 30 °C (Cannon and Anderson, 1991; Jonas and Farah, 1998; Jahan et al., 2012). However, in mostly studied, the maximum BC production was obtained between 28 to 30 °C (Hestrin and Schramm, 1954; Keshk, 2006; Pourramezan et al., 2009).

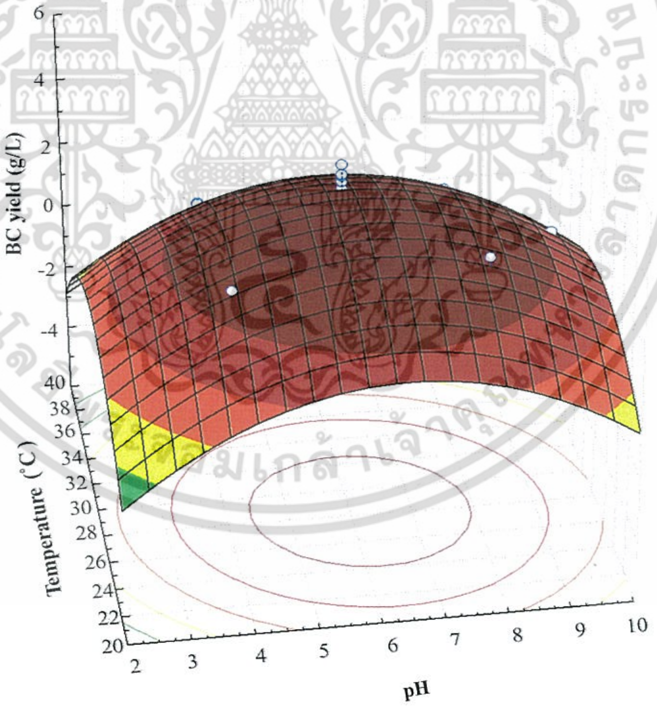


Figure 4.22 Response surface plot and contour plot showing the effects of initial pH of culture medium and incubation temperature on BC production.

4.8.2.3 Effects of ethanol concentration and incubation temperature on BC yield

Figure 4.23 shows the effect of ethanol concentration and incubation temperature on BC yield at an initial pH of culture medium at 6.0 (central level). The BC production increased with increase concentration of ethanol and temperature up to 2.0% (v/v) and 30 °C, respectively, and then decreased. The optimal ethanol concentration and incubation temperature were ranging from 0.6 to 2.5 % and 25 to 32 °C. The maximum BC production of 3.42 g/L was obtained at 1.61% ethanol (v/v) and incubation temperature of 28.4 °C. Based on Equation 4.1, the optimal initial pH of the culture medium, ethanol concentration and incubation temperature were 6.21, 1.61% and 28.4 °C, respectively, with the maximum BC yield of 4.03 g/L.

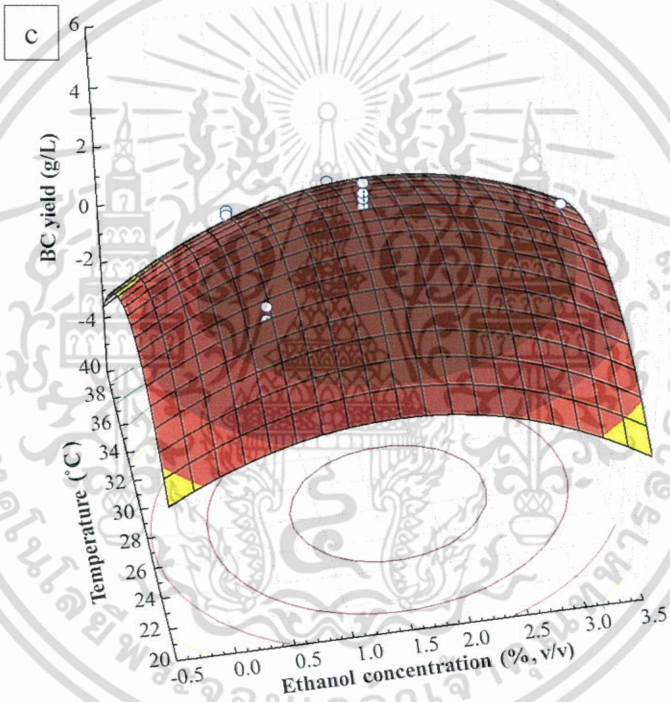


Figure 4.23 Response surface plot and contour plot showing the effects of ethanol concentration and incubation temperature on BC production.

4.8.3 Growth and BC production in optimized soybean whey-based medium

To verify the predicted response, *Komagataeibacter* sp. PAP1 was cultivated in optimized soybean whey-based medium under static condition for 14 days. As displayed in Figure 4.24, cells of *Komagataeibacter* sp. PAP1 increased rather slowly during the first two days and then increased exponentially from the third to seventh day. The stationary phase was observed after seven days of cultivation, and the viable cell count reached the maximum value of 8.2×10^7

cfu/mL. BC production started from the first day of cultivation and increased rapidly from the third day. The maximum BC production of 4.10 g/L was obtained on the seventh day of cultivation after which it stabilized. The sugar residual was decreased from 7.15 g/L at the beginning to less than 3.44 g/L after 14 days of cultivation similar to the pH of the culture medium decreased from 6.21 at the beginning to less than 3.50 after 14 days of cultivation. Under these conditions, the BC yield was relatively close to the predicted response (Table 4.5). Therefore, they were used as optimal conditions in this study.

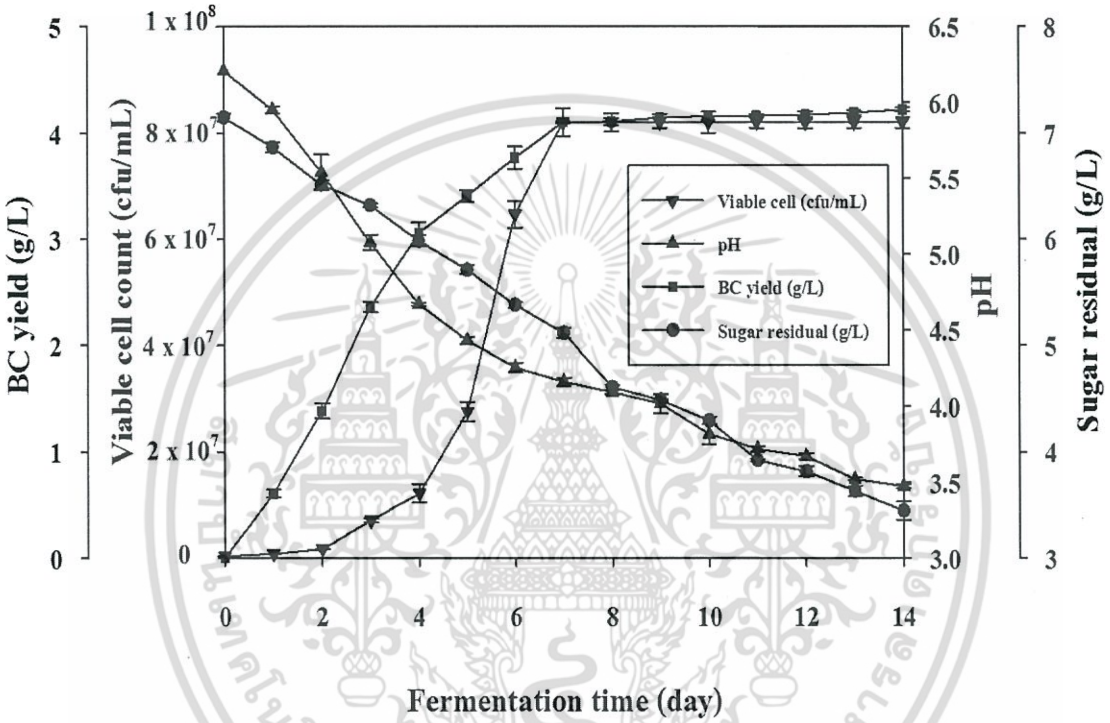


Figure 4.24 Time course of BC production by *Komagataeibacter* sp. PAP1 in optimized soybean whey-based medium under static condition for 14 days. All the data were the means ± SD of triplicate analyses.

4.9 Comparison of BC production in the optimized soybean whey-based medium and standard HS medium

Table 4.9 shows that optimized soybean whey-based medium gave better BC yield than the standard HS medium. The BC production in the optimized soybean whey-based medium was 4.14 ± 0.02 g/L and 3.6 times higher than that (1.15 ± 0.03 g/L) of standard HS medium. The difference is highly significant ($p < 0.01$). The variation in BC yields between the two media could be explained by the presence of some saccharides and protein in soybean whey that promotes cell growth and BC production by *Komagataeibacter* sp. PAP1. Similar results were obtained in the work of Lu et al. (2004), who studied BC production by *A. xylinum* C5 using soybean whey. They found that *A. xylinum* C5 could grow in soybean whey without adding any carbon and nitrogen source, indicating soybean whey had some oligosaccharides and nutrients which could be used by *A. xylinum* C5. When BC production by this bacterium was compared between the media prepared with soybean whey and distilled water. The results showed that, soybean whey medium gave better BC yield and more cell growth than those using the normal medium. They concluded that, soybean whey had some accelerative factors which could increase both cell growth and cellulose formation.

Table 4.9 BC yields produced in optimized soybean whey-based medium and standard HS medium.

Medium	BC yield (g/L)
Optimized soybean whey-based medium	4.14 ± 0.02*
Standard HS medium	1.15 ± 0.03

Results obtained are the mean of triplicates ± SD. Data were analysis using student's *t*-test.
*Highly significant ($p < 0.01$).

Many agricultural by-products and wastes have been successfully used as substrates for BC production by various species of *Komagataeibacter* because of their low cost. Bae and Shoda (2005) studied the use of molasses which is a by-production from sugar industry as carbon source for BC production by *G. xylinus* BPR 2001. When molasses was treated with sulfuric acid and heat, the BC production increased to 76% more than those using untreated molasses, and also increased two times of specific growth rate. The production cost of BC using untreated molasses was estimated at 0.26 Yen/g of BC which was lower than using sucrose (10.8 Yen/g) and fructose (26.0 Yen/g), respectively. The results indicated that utilization of molasses as carbon source is advantageous for economical BC production. However, molasses has some undesirable components for example coloring substances, heavy metals and unknown compounds may inhibit microbial cell growth. Moreover, crude molasses need to be diluted and treated with acid and heat. Ha et al. (2008) produced BC by *G. hansenii* PJK using the waste from beer fermentation broth (WBFB) under static condition. The WBFB contained 24.44 g/L total carbohydrate, 0.67

g/L total protein content and more than 4.0% ethanol. After 5 days of fermentation, WBFB gave BC yield of 4.52 g/L and much higher than 0.45 g/L produced from a buffered Schramm and Hestrin (BSH) chemically defined medium. Kongruang (2008) reported the used of coconut water and pineapple water as low cost substrate for BC production by three *Acetobacter* strains including *A. xylinum* TISTR 998, 975 and 893. All three strains gave a high level of BC in both substrate but coconut water seems to be a better substrate than pineapple water. The BC productivity on both substrates was very high with the low production cost. Castro et al. (2011) characterized BC production by *G. swingsii* sp. using pineapple peel juice which is Colombian agroindustrial waste, as culture medium. BC production from pineapple peel juice (2.80 g/L) was better than that produced from standard HS medium (2.10 g/L). The nutrients in pineapple peel juice were sufficient for cell growth and BC synthesis by *G. swingsii* sp. The ribbon-shaped microfibrils with a width of 20-70 nm were produced in pineapple peel juice medium. Yang et al. (2013b) examined the possibility to use citrus waste as culture medium for BC production by *G. intermedius* Cls26. Citrus waste was prepared by peeling and squeezing of mandarin fruits. The remained dregs waste was mixed with water at the ratio of 1:8 (w/w), 150 U/mL pectinase and 50 U/mL cellulase, and hydrolyzed at 45 °C for 2 hours. The samples were then filtered and resulting filtrate was collected as citrus waste solution. BC yield in citrus waste modified HS was 5.70 g/L which was higher than 2.10 g/L from standard HS medium. The results indicated that citrus waste could stimulate BC synthesis by *G. intermedius* Cls26 and could be used for the production of BC in commercial scale. On the other hand, Carreira et al. (2011) used residues from agro-forest industries as carbon source and nutrient sources for BC production by *G. sacchari*. After 4 days of fermentation, the low BC yields of 0.10, 0.08, 0.10 and 0 g/L were obtained from grape skin aqueous extract, cheese whey, crude glycerol and sulfite pulping liquor, respectively. Such the low BC yields were probably due to the presence of inhibitors derived from the industrial processes which obstructing microbial cell growth and BC production. In sulfite pulping liquor is a complex mixture derived from wood pulping, including lignosulfonates (sulfonated phenolic compounds), hydroxyl acids and hemicelluloses degradation that could be hindered cell growth and formation of BC.

In this study, the high productivity of BC in the optimized soybean whey-based medium compared to standard HS medium, may significantly lower its production cost. The BC production cost using optimized soybean whey-based medium was estimated 0.02 baht/g dry weight of BC which was 675 times lower than using standard HS medium (13.50 baht/g dry weight of BC) (Appendix G).

4.10 Characterization of BC paper

4.10.1 Surface morphology of BC paper by scanning electron microscopy analysis

As displayed in Figure 4.25a to 4.25d, the scanning electron micrographs of BC paper produced from the derived cellulose in optimized soybean whey-based medium (SBW paper) and standard HS medium (HS paper), had three-dimensional porous network structure consisting of randomly arranged ribbon-shaped nanofibrils. The BC microfibrils in both two kinds of BC papers were extremely thin with transverse diameters ranging 26 to 63 nm. They were thinner than wood pulp fiber and cotton fiber (Moon et al., 2011). The resulting BC structure was consistent with those previously reported (Park et al., 2003; Castro et al., 2011; Yang et al., 2013b). Castro et al. (2011) and Cavka et al. (2013) studied the structure of BC pellicles and found that BC consisted of ribbon-shaped nanofibrils that were 15 to 100 nm wide and self-assembled to form a three dimensional mesh like structure. The released nanofibrils from bacterial cells aggregated and became thick, and compact based on theirs production rate. Figure 4.25a and 4.25b indicated that nanofibrils in SBW paper were wider and thicker than those in HS paper (Figure 4.25c and 4.25d). The BC reticulated structure was close-knitted in SBW paper while in HS paper was fine and loose-knitted structure. This was likely due to the higher production rate of BC in optimized soybean whey-based medium than in standard HS medium. Tang et al. (2010) reported that fermentation conditions and carbon sources were important factors affecting BC thickness and porosity. When extended cultivation time, increased inoculation and using glucose as a carbon source, more nanofibrils were released by bacterial cells, resulting in a more compact structure and lowest porosity.

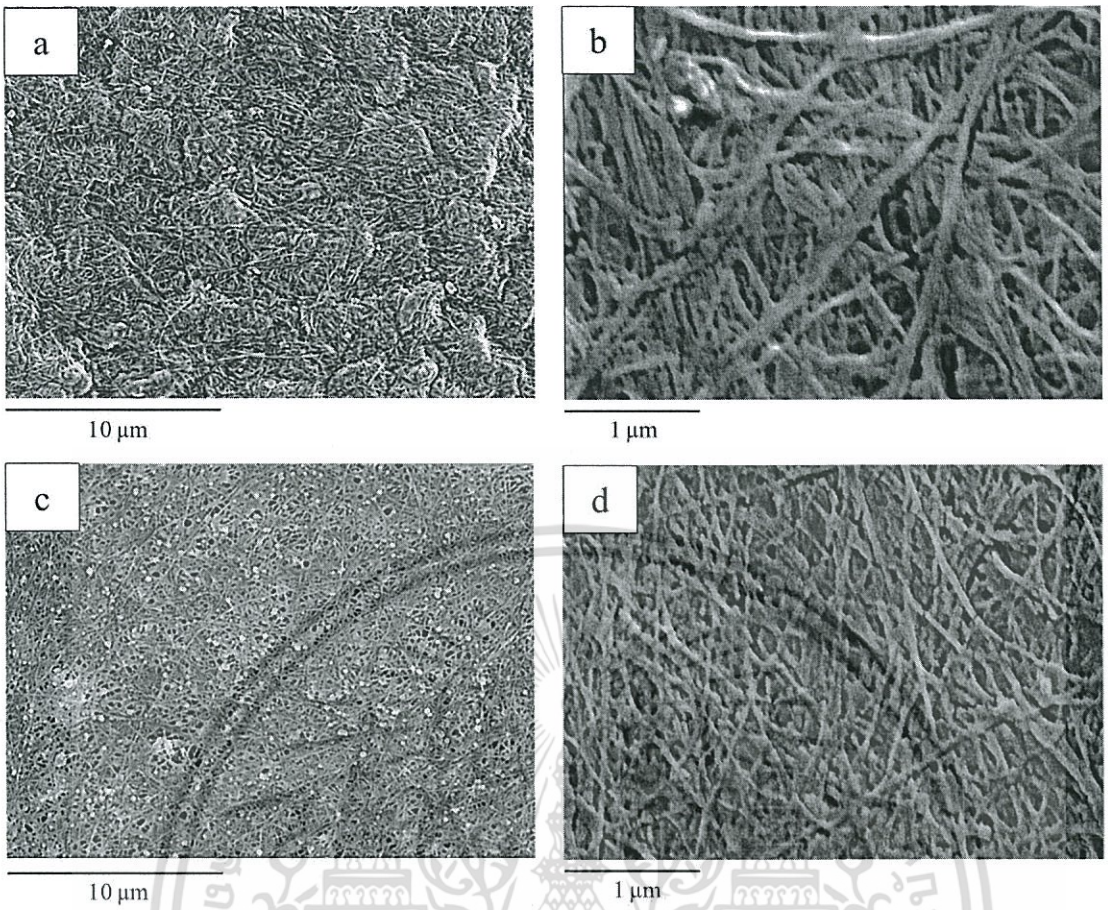


Figure 4.25 Scanning electron micrograph of BC papers produced from optimized soybean whey-based medium with 5,000× (a) and 30,000× (b), and the standard HS medium with 5,000× (c) and with 30,000× (d).

4.10.2 Mechanical properties

The mechanical properties of SBW paper and HS paper are shown in Table 4.10. The tensile strength and Young's modulus of HS paper were 23.58 MPa and 265.51 MPa, respectively. While tensile strength and Young's modulus of SBW paper were 49.16 MPa and 2498.69 MPa, respectively. The increase of 108.48% in tensile strength and 841.09% in Young's modulus of SBW paper in comparison to that of HS paper could be explained by scanning electron microscopy analysis. The scanning electron micrographs (Figure 4.25a to 4.25d) show that the fibers of the SBW paper were thicker, wider and more compact when compared to HS paper. This resulted in the BC produced in optimized soybean whey-based medium having greater mechanical properties. The wider ribbon-shaped nanofibrils gave the higher tensile strength and Young's modulus because of the increasing in effective cross-sectional area that produced an increase in cross-sectional momentum, and increasing in the number of 1,4 covalent bonds

(Keshk, 2006). The tensile strength of BC papers produced in this study was quite similar and in some cases even higher than those previously reports. Rani et al. (2011b) characterized properties of BC produced in grape medium by *Gluconacetobacter* sp. The tensile strength of BC paper from grape medium was 41.16 MPa whereas from HS medium was 16.56 MPa. In comparison with synthetic polymer indicated that BC papers from grape medium to be better than low density polyethylene, which had values of 16.08 to 17.17 MPa. Ul-Isam et al. (2013) studied the mechanical properties of BC produced in medium containing 0, 1.0, 2.0 and 4.0% of glucuronic acid, respectively. The tensile strength of BC0, BC1, BC2 and BC4 were 16.73, 25.05, 20.76 and 19.77 MPa, respectively.

It was observed that the elongation percentage (%E) of SBW paper was 3.28% which was less than 11.17% of HS paper (Table 4.10). The decreased in 70.60% elongation of SBW paper could be explained by the structure of BC pellicle. The thick and compact fibrils enhanced the toughness of BC and decreased its elasticity (Ul-Isam et al., 2013).

4.10.3 Barrier properties of SBW paper and HS paper

Barrier properties including water vapor transmission rate (WVTR) and oxygen gas transmission rate (O$_2$GTR) are summarized in Table 4.10. The WVTR and O$_2$GTR of SBW paper were 1986 g/m^2/day and 23.1 cm^3/m^2/day, respectively. While, the WVTR and O$_2$GTR of HS paper were 2,549 g/m^2/day and 173 cm^3/m^2/day, respectively. The SBW paper showed a decreased of 22.0% in WVTR and 86.60 % in O$_2$GTR, respectively. The results indicated that SBW paper was an efficient barrier against water vapor and oxygen than HS paper. Similar to the present study, Rani et al. (2011b) reported that BC paper produced from grape medium had a better barrier to oxygen (415.27 cm^3/m^2/day) than that from HS medium (1962.67 cm^3/m^2/day). They also found that BC papers had higher WVTR and lower O$_2$GTR (2448.28 g/m^2/day and 415.27 g/m^2/day) comparing with a synthetic polymer (18-20 g/m^2/day and 8000-1400 cc/m^2/day).

The transmission rate of a vapor or a gas in polymer, is a complex phenomenon and depend on degree of crystallinity (Chytiri et al., 2006). A higher degree of cystallinity results in lower permeability since the crystalline regions are relatively impermeable compared with the amorphous region. The lower WVTR and O$_2$GTR of SBW paper indicated that BC produced in optimized soybean whey-based medium had more crystalline regions when compared with that produced in standard HS medium.

4.10.4 Water absorption capacity

The water absorption capacity of SBW paper and HS paper are listed in Table 4.10. The SBW paper and HS paper showed a great water absorption capacity of 232.29% and 257.83%, respectively. The variation of water absorption capacity in SBW paper and HS paper could be explained by theirs porous structure. As revealed by scanning electron micrograph in Figure 4.25a to 4.25d, fibers of SBW paper were thicker, more compact and smaller size of pores. The water absorption capacity of both kinds of BC paper was over two times of theirs weight. Since, BC had a three-dimensional porous network structure which could keep a large amount of water (Czaja et al., 2006; Tang et al., 2010).

Table 4.10 Properties of BC paper produced from the optimized soybean whey-based medium and standard HS medium.

Property	SBW paper	HS paper
Tensile strength (MPa)	49.16 ± 0.12	23.58 ± 0.38
Young's modulus (MPa)	2498.69 ± 2.13	265.51 ± 2.47
Elongation at break (%)	3.28 ± 0.07	11.17 ± 0.09
WVTR ($g/m^2/day$)	1986	2549
O_2GTR ($cm^3/m^2/day$)	23.1	173
WAC (%)	232.29 ± 0.14	257.83 ± 0.19

WVTR = water vapor transmission rate, O_2GTR = oxygen gas transmission rate, WAC = water absorption capacity.

4.10.5 Color analysis

The color of SBW paper and HS paper were opaque and white, respectively (Figure 4.26) as evidenced from the L* values had shifted slightly towards the lighter side (Table 4.11), indicated that no bleaching was required for either of them. The results obtained were consistent with a previous report on the use of coconut juice and pineapple juice for BC production by *A. xylinum* strains (Kongruang, 2008). The BC papers produced from both agricultural wastes showed a white color and opaque yellow color, respectively, no need for an additional step of de-colorization.

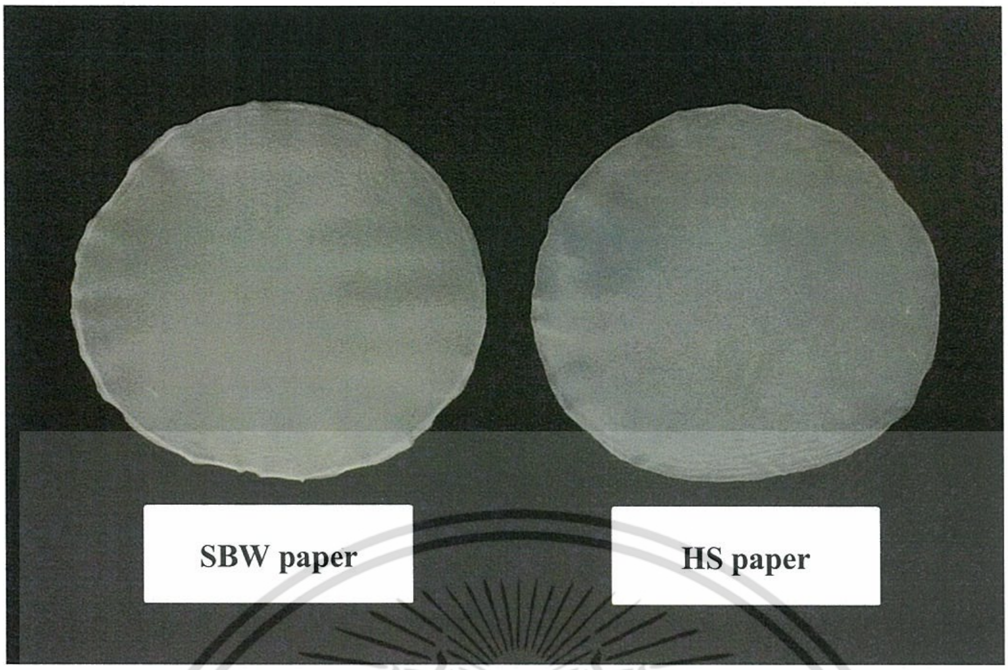


Figure 4.26 The characterization of BC paper produced from optimized soybean whey-based medium and standard HS medium.

Table 4.11 Color comparison of BC paper produced from optimized soybean whey-based medium and standard HS medium.

Sample	CIE Lab value ± standard error		
	L*	a*	b*
SBW paper	57.72 ± 0.34	-2.06 ± 0.17	-5.49 ± 0.06
HS paper	58.56 ± 0.10	-1.19 ± 0.03	-5.31 ± 0.21

CHAPTER 5

CONCLUSIONS

Forty-eight rotten tropical fruits were collected from different parts of Thailand (nine provinces) and used for isolation of cellulose producing bacteria. Two hundred and four cellulose producing strains were isolated from 16 rotten tropical fruits and 29 representative isolates were selected from each fruit. Based on morphological, physiological and biochemical characterizations and 16S rRNA gene sequence analysis, all 29 representative isolates were bacteria belonging to genus *Komagataeibacter* and grouped into 7 subgroups. Subgroup I was identified as *K. rhaeticus* which contained one isolate, RAM1. Subgroup II was identified as *K. oboediens* which contained five isolates, GOV9, GOV15, MAM4, SPO4 and WAT11. Subgroup III was identified as *K. sucrofermentans* which contained two isolates, LYC7 and FET8. Subgroup IV was identified as *K. swingsii* which contained two isolates, SPO15 and WAT14. Subgroup V was identified as *K. hansenii* which contained seven isolates, BEL1, BEL2, FET4, LAD1, STA5, WIL2 and LYC8. The remaining isolates could not be identified to species level and grouped into Subgroup VIa and VIb. Subgroup VIa contained three isolates, MAG6, SUG5 and SUG8. Subgroup VIb contained nine isolates, GRA2, GRA8, JAV1, JAV8, MAG15, MAM2, PAP1, RAM4 and WIL3. It can be concluded that fruits in Thailand are a rich source of cellulose producing bacteria and *Komagataeibacter* is the most cellulose producer which commonly found in natural source more than other cellulose producing bacteria.

Isolate PAP1 was the most effective cellulose producing bacteria which was isolated from papaya and identified as *Komagataeibacter* sp. PAP1. The static condition seems to be better than shaken condition. *Komagataeibacter* sp. PAP1 gave a significantly maximum BC yield of 1.15 g/L in standard HS medium and ethanol was the most suitable carbon source with 3.43 g/L of BC yield.

The optimal conditions for BC production by *Komagataeibacter* sp. PAP1 in soybean whey-based medium were pH 6.21, 1.61% ethanol concentration and temperature of 30 °C. Under the optimal conditions, *Komagataeibacter* sp. PAP1 produced 4.04 g/L which was 3.5 times when compared with standard HS medium. The BC production cost using optimized soybean whey-based medium was 290 times lower than using standard HS medium. Therefore, soybean whey can be used as low cost substrate for large scale BC production to reduce the production cost and environmental pollution.

Bacterial cellulose paper from cellulose pellicle produced in optimized soybean whey-based medium (SBW paper) has a three-dimensional porous network structure with the wider and compact ribbon-shaped microfibrils than those BC paper from cellulose pellicle produced in standard HS medium (HS paper). SBW paper showed a greater tensile strength (49.16 MPa) and Young's modulus (2498.69 MPa) than those HS paper. SBW paper was more resistant to water vapor and oxygen gas with the lower WVTR (1986 g/m^2/day) and O2GTR (23.1 cm^3/m^2/day) when compared to HS paper. Moreover, SBW paper indicated the high water absorption capacity of 232.29% without any additional step of de-colorization. It can be concluded that *Komagataeibacter* sp. PAP1 can be used as an effective cellulose producing bacteria to produce a high level of BC for development into eco-friendly food packaging for oxygen sensitive products, wound dressing or other applications using soybean whey as an alternative low cost substrate.

BIBLIOGRAPHY

American Organization of Analytical Chemist. 2005. **Official methods of analysis of AOAC international.** 18th ed. Maryland : AOAC International.

Aqileware Pty Ltd. 2014. **PCR method.** [Online]. Available : enfo.agt.bme.hu.

Asai, T., Iizuka, H. and Komagata, K. 1964. "The flagellation and taxonomy of genera *Gluconobacter* and *Acetobacter* with reference to the existence of intermediate strains." **The Journal of General and Applied Microbiology**. 10(2) : 95-126.

ASTM International. 2010. **Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor.** [Online]. Available : http://enterprise2.astm.org/DOWNLOAD/D3985.1272742-1.pdf.

ASTM International. 2010. **Standard test method for water absorption of plastics.** [Online]. Available : http://enterprise2.astm.org/DOWNLOAD/D570.1272742-1.pdf.

ASTM International. 2013. **Standard test method for water vapor transmission rate of sheet materials using dynamic relative humidity measurement.** [Online]. Available : http://-enterprise2.astm.org/DOWNLOAD/E398.1272742-1.pdf.

Bae, S.O. and Shoda, M. 2005. "Production of bacterial cellulose by *Acetobacter xylinum* BPR2001 using molasses medium in a jar fermentor." **Applied Microbiology and Biotechnology**. 67(1) : 45-51.

Barrington, S. and Kim, J.W. 2008. "Response surface optimization of medium components for citric acid production by *Aspergillus niger* NRRL 567 grown in peat moss." **Bioresource Technology.** 99(2) : 368-377.

Battad-Bernardo, E., McCrindle, S.L., Couperwhite, I. and Neilan, B.A. 2004. "Insertion of an *E. coli* lacZ gene in *Acetobacter xylinus* for the production of cellulose in whey." **FEMS Microbiology Letters.** 231(2) : 253-260.

Baum, M.M., Kainović, A., O'Keeffe, T., Pandita, R., McDonald, K., Wu, S. and Webster, P. 2009. "Characterization of structures in biofilms formed by a *Pseudomonas fluorescens* isolated from soil." **BMC Microbiology.** 9: 103-116.

Boesch, C., Trček, J., Sievers, M. and Teuber, M. 1998. "*Acetobacter intermedius*, sp. nov." **Systematic and Applied Microbiology.** 21(2) : 220-229.

Box, G.E.P. and Hunter, J.S. 1957. "Multi-factor experimental designs for exploring response surfaces." **The Annals of Mathematical and Statisticals.** 28(1) : 195-241.

Brosius, J., Dull, T.J., Sleeter, D.D. and Noller, H.F. 1981. "Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*." **Journal of Molecular Biology.** 148(2) : 107-127.

Brown, A. 1886. "On an acetic ferment which forms cellulose." **Journal of the Chemical Society Transactions.** 49 : 432-439.

Brown, R.M., Jr., Willison, J.H. and Richardson, C.L. 1976. "Cellulose biosynthesis in *Acetobacter xylimum*: visualization of the site of synthesis and direct measurement of the in vivo process." **Proceedings of the National Academy of Sciences of the United States of America.** 73(12) : 4565-4569.

Cannon, R.E. and Anderson, S.M. 1991. "Biogenesis of bacterial cellulose." **Critical Reviews in Microbiology.** 17(6) : 435-447.

Cappuccino, J.A. and Sherman, N. 2000. **Microbiology: a laboratory manual.** New York : The Banjamin/Cummings Publishing Company.

Carr, J.G. 1968. "Methods for identifying acetic acid bacteria." In: Gibbs, B.M. and Shapton, D.A. (Eds), Identification methods for microbiologists **The Society for Applied Bacteriology Technical series no.2, part B**. London : Acadamic Press.

Carreira, P., Mendes, J.A.S., Trovatti, E., Serafim, L.S., Freire, C.S.R., Silvestre, A.J.D. and Neto, C.P. 2011. "Utilization of residues from agro-forest industries in the production of high value bacterial cellulose." **Bioresource Technology.** 102(15) : 7354-7360.

Castro, C., Cleenwerck, I., Trček, J., Zuluaga, R., De Vos, P., Caro, G., Aguirre, R., Putaux, J.L. and Gañán, P. 2013. "*Gluconacetobacter medellinensis* sp. nov., cellulose-and non-cellulose-producing acetic acid bacteria isolated from vinegar." **International Journal of Systematic and Evolutionary Microbiology.** 63(3) : 1119-1125.

Castro, C., Zuluaga, R., Álvarez, C., Putaux, J.L., Caro, G., Rojas, O.J., Mondragon, I. and Gañán, P. 2012. "Bacterial cellulose produced by a new acid-resistant strain of *Gluconacetobacter* genus." **Carbohydrate Polymers.** 89(4): 1033-1037.

Castro, C., Zuluaga, R., Putaux, J.L., Caro, G., Mondragon, I. and Gañán, P. 2011. "Structural characterization of bacterial cellulose produced by *Gluconacetobacter swingsii* sp. from Colombian agroindustrial wastes." **Carbohydrate Polymers.** 84(1) : 96-102.

Cavka, A., Guo, X., Tang, S.J., Winestrand, S., Jönsson, L.J. and Hong, F. 2013. "Production of bacterial cellulose and enzyme from waste fiber sludge." **Biotechnology for Biofuels.** 6 : 25-35.

Chawla, P.R., Bajai, I.B., Survase, S.A. and Singhal, R.S. 2009. "Microbial cellulose: fermentative production and applications." **Food Technology and Biotechnology.** 47(2) : 107-124.

Chen, P., Cho, S.Y. and Jin, H.J. 2010. "Modification and applications of bacterial celluloses in polymer science." **Macromolecular Research.** 18(4) : 309-320.

Chytiri, S., Goulas, A.E., Riganakos, K.A. and Kontominas, M.G. 2006. "Thermal, mechanical and permeation properties of gamma-irradiated multilayer food packing films containing a buried layer of recycled low density polyethylene." **Radiation Physics and Chemistry.** 75(3) : 461-423.

Cleenwerck, I. and De Vos, P. 2008. " Polyphasic taxonomy of acetic acid bacteria: an overview of the currently applied methodology." **International Journal of Food Microbiology.** 125(1) : 2-14.

Cleenwerck, I., De Wachter, M., González, Á., De Vuyst, L. and De Vos, P. 2009. "Differentiation of species of the family *Acetobacteraceae* by AFLP DNA fingerprinting: *Gluconacetobacter kombuchae* is a later heterotypic synonym of *Gluconacetobacter hansenii*." **International Journal of Systematic and Evolutionary Microbiology.** 59(7) : 1771-1786.

Cleenwerck, I., Vandemeulebroecke, K., Janssens, D. and Swings, J. 2002. "Re-examination of the genus *Acetobacter*, with descriptions of *Acetobacter cerevisiae* sp. nov. and *Acetobacter malorum* sp. nov." **International Journal of Systematic and Evolutionary Microbiology.** 52(5) : 1551-1558.

Coronel, L.M. and Joson, L.M. 1986. "Isolation, screening and characterization of cellulose utilizing bacteria." **Philippine Journal of Science.** 2: 223-232.

Crawford, R.L. 1981. **Lignin biodegradation and transformation.** New York : John Wiley and Sons.

Czaja, W., Krystynowicz, A., Bielecki, S. and Brown, R.M., Jr. 2006. "Microbial cellulose-the natural power to heal wounds." **Biomaterials.** 27(2) : 145-151.

De Faveri, D., Torre, P., Molinari, F., Perego, P. and Converti, A. 2003. "Carbon material balances and bioenergetics of 2,3-butanediol bio-oxidation by *Acetobacter hansenii.*" **Enzyme and Microbial Technology.** 33(5) : 708-719.

Deinema, M.H. and Zevenhuizen, L.P. 1971. "Formation of cellulose fibrils by Gram-negative bacteria and their role in bacterial flocculation." **Archives of Microbiology.** 78(1) : 42-51.

Dellaglio, F., Cleenwerck, I., Felis, G.E., Engelbeen, K., Janssens, D. and Marzotto, M. 2005. "Description of *Gluconacetobacter swingsii* sp. nov. and *Gluconacetobacter rhaeticus* sp. nov., isolated from Italian apple fruit." **International Journal of Systematic and Evolutionary Microbiology.** 55(6) : 2365-2370.

De Ley, J., Gillis, M. and Swings, J. 1984. "In: Krieg, N.R. and Holt, J.G. (Eds), Family VI. *Acetobacteraceae*, 1th Ed. **Bergey's Manual of Systematic Bacteriology, vol 1.1.** Williams and Wikins Co, Baltimore, Maryland, p. 276-278.

Dreywood, R. 1946. "Qualitative test for carbohydrate material." **Industrial and Engineering Chemistry Analytical Edition.** 18(8) : 499.

Dutta, D. and Gachhui, R. 2007. "Nitrogen-fixing and cellulose-producing *Gluconacetobacter kombuchae* sp. nov., isolated from kombucha tea." **International Journal of Systematic and Evolutionary Microbiology.** 57(2) : 353-357.

Embuscado, M.E., Marks, J.S. and BeMiller, J.N. 1994. "Bacterial cellulose. II. Optimization of cellulose production by *Acetobacter xylinum* through response surface methodology." **Food Hydrocolloids.** 8(5) : 419-430.

Entani, E., Ohmori, S., Masai, H. and Suzuki, K.I. 1985. "*Acetobacter polyoxogenes* sp. nov., a new species of an acetic acid bacterium useful for producing vinegar with high acidity." **The Journal of General and Applied Microbiology.** 31(5) : 475-490.

Falanghe, H., Smith, A.K., and Rackis, J.J. 1964. "Production of fungal mycelial protein in submerged culture of soybean whey." **Applied Microbiology.** 12(4) : 330-334.

Felsenstein, J. 1985. "Confidence limits on phylogenies: an approach using the bootstrap." **Evolution.** 39(4) : 783-791.

Franke, I.H., Fegan, M., Hayward, C., Leonard, G., Stackebrandt, E. and Sly, L.I. 1999. "Description of *Gluconacetobacter sacchari* sp. nov., a new species of acetic acid bacterium isolated from the leaf sheath of sugar cane and from the pink sugar-cane mealy bug." **International Journal of Systematic and Bacteriology.** 49(4) : 1681-1693.

Frateur, J. 1950. "Essai sur la systématique des acetobacters." **Cellule.** 53: 285-392.

Fuentes-Ramírez, L.E., Bustillos-Cristales, R., Tapia-Hernández, A., Jiménez-Salgado, T., Wang, E.T., Martínez-Romero, E. and Caballero-Mellado, J. 2001. "Novel nitrogen-fixing acetic acid bacteria, *Gluconacetobacter johannae* sp. nov. and *Gluconacetobacter azotocaptans* sp. nov., associated with coffee plants." **International Journal of Systematic and Evolutionary Microbiology.** 51(4) : 1305-1314.

Fung, W.Y., Woo, Y.P. and Liong, M.T. 2008. "Optimization of growth of *Lactobacillus acidophilus* FTCC 0291 and evaluation of growth characteristics in soy whey medium : a response surface methodology approach." **Journal of Agricultural Food Chemistry.** 56(17) : 7910-7918.

Gawande, B.N. and Patkar, A.Y. 1999. "Application of factorial designs for optimization of cyclodextrin glycosyltransferase production from *Klebsiella pneumoniae* AS-22." **Biotechnology and Bioengineering.** 64(2) : 168-173.

George, J., Ramana, K.V., Sabapathy, S.N., Jagannath, J.H. and Bawa, A.S. 2005. "Characterization of chemically treated bacterial (*Acetobacter xylimum*) biopolymer: some thermo-mechanical properties." **International Journal of biological Macromolecules.** 37(4) : 189-194.

Geyer, U., Heinze, T., Stein, A., Klemm, D., Marsch, S., Schumann, D. and Schmauder, H.P. 1994. "Formation, derivatization and applications of bacterial cellulose." **International Journal of Biological Macromolecules.** 16(6) : 343-347.

Gilbert, S.F. 2013. Techniques of molecular biology. [Online]. Available : qe.devbio.com.

Gillis, M., Kersters, K., Hoste, B., Janssens, D., Kroppenstedt, R.M., Stephan, M.P., Teixeira, K.R.S., Döbereiner, J. and De Ley, J. 1989. "*Acetobacter diazotrophicus* sp. nov., a nitrogen-fixing acetic acid bacterium associated with sugarcane." **International Journal of Systematic Bacteriology.** 39(3) :361-364.

Gosselé. F., Swings, J., Kersters, K., Pauwels, P. and De Ley, J. 1983. "Numerical analysis of phenotypic features and protein gel electrophoregrams of a wide variety of *Acetobacter* strains. Proposal for the improvement of the taxonomy of the genus *Acetobacter* Beijerinck 1898, 215." **Systematic and Applied Microbiology.** 4(3) : 338-368.

Gromet-Elhanan, Z. and Hestrin, S. 1963. "Synthesis of cellulose by *Acetobacter xylinum* VI. growth on citric acid-cycle intermediates." **Journal of Bacteriology.** 85(2) : 284-292.

Gualdi, L., Tagliabue, L., Bertagnoli, S., Ieranò, T., De Castro, C. and Landini, P. 2008. "Cellulose modulates biofilm formation by counteracting curli-mediated colonization of solid surfaces in *Escherichia coli*." **Microbiology.** 154(7) : 2017-2024.

Ha, J.H., Shah, N., Ul-Isam, M., Khan, T. and Park, J.K. 2011. "Bacterial cellulose production from a single sugar α-linked glucuronic acid-based oligosaccharide." **Process Biochemistry.** 46(9) : 1717-1723.

Ha, J.H., Shehzad, O., Khan, S., Lee, S.Y., Park, J.W., Khan, T. and Park, J.K. 2008. "Production of bacterial cellulose by a static cultivation using the waste from beer culture broth." **Korean Journal of Chemical Engineering.** 25(4) : 812-815.

Hestrin, S. and Schramm, M. 1954. "Synthesis of cellulose by *Acetobacter xylimum*. 2. preparation of freeze-dried cells capable of polymerizing glucose to cellulose." **Biochemical Journal.** 58(2) : 345-352.

Hong, F. and Qiu, K. 2008. "An alternative carbon source from konjac powder for enhancing production of bacterial cellulose in static cultures by a model strain *Acetobacter aceti* subsp. *xylinus* ATCC 23770." **Carbohydrate Polymers.** 72(3) : 545-549.

Hungund, B.S. and Gupta, S.G. 2010. "Production of bacterial cellulose from *Enterobacter amnigenus* GH-1 isolated from rotten apple." **World Journal of Microbiology and Biotechnology.** 26(10) : 1823-1828.

Hutchens, S.A., Benson, R.S., Evans, B.R., O'Neill, H.M. and Rawn, C.J. 2006. "Biomimetic synthesis of calcium-deficient hydroxyapatite in a natural hydrogel." **Biomaterials.** 27(26) : 4661-4670.

Hwang, J.W., Yang, Y.K., Hwang, J.K., Pyun, Y.R. and Kim, Y.S. 1999. "Effect of pH and dissolved oxygen on cellulose production by *Acetobacter xylimum* BRC5 in agitated culture." **Journal of Bioscience and Bioengineering.** 88(2) : 183-188.

Iguchi, M., Mitsuhashi, S., Ichimura, K., Nishi, Y., Uryu, M., Yamanaka, S. and Watanabe, K. 1988. "Bacterial cellulose-containing molding material having high dynamic strength." US patent 4742164.

Iguchi, M., Yamanaka, S. and Budhiono, A. 2000. "Bacterial cellulose-a masterpiece of nature's arts." **Journal of Materials Science.** 35(2) : 261-270.

Iino, T., Suzuki, R., Tanaka, N., Kosako, Y., Ohkuma, M., Komagata, K. and Uchimura, T. 2012. "*Gluconacetobacter kakiaceti* sp. nov., an acetic acid bacterium isolated from a traditional Japanese fruit vinegar." **International Journal of Systematic and Evolutionary Microbiology.** 62(7) : 1465-1469.

Ishihara, M., Matsunaga, M., Hayashi, N. and Tišler, V. 2002. "Utilization of D-xylose as carbon source for production of bacterial cellulose." **Enzyme and Microbial Technology.** 31(7) : 986-991.

Jagannath, A., Kalaiselvan, A., Manjunatha, S.S., Raju, P.S. and Bawa, A.S. 2008. "The effect of pH, sucrose and ammonium sulphate concentrations on the production of bacterial cellulose (*Nata-de-coco*) by *Acetobacter xylimum*." **World Journal of Microbiology and Biotechnology.** 24(11) : 2593-2599.

Jahan, F., Kumar, V. and Rawat, G. 2012. "Production of microbial cellulose by a bacterium isolated from fruit. **Applied Biochemistry and Biotechnology.** 167(5) : 1157-1171.

Jia, S., Ou, H., Chen, G., Choi, D., Cho, K. and Okabe, M. 2004. "Cellulose production from *Gluconobacter oxydans* TQ-B2." **Biotechnology and Bioprocess Engineering.** 9(3) : 166-170.

Johnson, D.C. and Neogi, A.N. 1989. "Sheeted products formed from reticulated microbial cellulose." US patent 4863565.

Jonas, R. and Farah, L.F. 1998. "Production and application of microbial cellulose." **Polymer Degradation and Stability.** 59(1-3) : 101-106.

Jung, H.I., Lee, O.M., Jeong, J.H., Jeong, Y.D., Park, K.H., Kim, H.S., An, W.G. and Son, H.J. 2010. "Production and characterization of cellulose by *Acetobacter* sp. V6 using a cost-effective molasses-corn steep liquor medium." **Applied Biochemistry and Biotechnology.** 162(2) : 486-497.

Jung, J.Y., Park, J.K. and Chang, H.N. 2005. "Bacterial cellulose production by *Gluconacetobacter hansenii* in an agitated culture without living non-cellulose producing cells." **Enzyme and Microbial Technology.** 37(3) : 347-354.

Karahan, A.G., Akoğlu, A., Çakir, Í., Kart, A., Çakakçi, M.L., Uygun, A. and Göktepe, F. 2011. "Some properties of bacterial cellulose produced by new native strain *Gluconacetobacter* sp. A06O2 obtained from Turkish vinegar." **Journal of Applied Polymer Science.** 121(3) : 1823-1831.

Kaur, V., Bera, M.B., Panesar, P.S. and Chopra, H.K. 2013. "Production and characterization of exopolysaccharide produced by *Alcaligenes faecalis* B14 isolated from indigenous soil." **International Journal of Biotechnology and Bioengineering Research.** 4(4) : 365-374.

Keshk, S. 2006. "Physical properties of bacterial cellulose sheets produced in presence of lignosulfonate." **Enzyme and Microbial Technology.** 40(1) : 9-12.

Keshk, S.M.A.S. and Sameshima, K. 2005. "Evaluation of different carbon sources for bacterial cellulose production." **African Journal of Biotechnology.** 4(6) : 478-482.

Keshk, S. and Sameshima, K. 2006. "The utilization of sugar cane molasses with/without the presence of lignosulphonate for the production of bacterial cellulose." **Applied Microbiology and Biotechnology.** 72(2) : 291-296.

Kim, S.Y., Kim, J.N., Wee, Y.J., Park, D.H. and Ryu, H.W. 2006. "Production of bacterial cellulose by *Gluconacetobacter* sp. RKY5 isolated from persimmon vinegar." **Applied Biochemistry and Biotechnology.** 131(1-3) : 705-715.

Kimura, M. 1980. "A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences." **Journal of Molecule Evolution.** 16(2) : 111-120.

Klemm, D., Schumann, D., Udhardt, U. and Marsch, S. 2001. "Bacterial synthesized cellulose-artificial blood vessels for microsurgery." **Progress in Polymer Science.** 26(9) : 1561-1603.

Komagata, K. and Suzuki, K. 1987. "Lipid and cell-wall analysis in bacterial systematics." **Methods Microbiology.** 19 : 161-207.

Kongruang, S. 2008. "Bacterial cellulose production by *Acetobacter xylimum* strains from agricultural waste products." **Applied Biochemistry and Biotechnology.** 148(1-3) : 245-256.

Krieg, N.R. and Holt, J.G. 1984. **Bergey's manual of systematic bacteriology.** London : Williams and Wilkins.

Krystynowicz, A., Czaja, W., Wiktorowska-Jezierska, A., Gonçalves-Miśkiewicz, M., Turkiewicz, M. and Bielecki, S. 2002. "Factors affecting the yield and properties of bacterial cellulose." **Journal of Industrial Microbiology and Biotechnology.** 29(4) : 189-195.

Kudlicka, K. 1989. "Terminal complexes in cellulose synthesis." **Postçpy Biologii Komórki** 16 : 197-212.

Lapuz, M.M., Gallardo, E.G. and Palo, M.A. 1967. "The nata organism cultural requirements, characteristics and identity." **Philippine Journal of Science.** 96(2) : 91-109.

Liong, M.T. and Shah, N.P. 2005. "Optimization of cholesterol removal by probiotics in the presence of prebiotics by using a response surface method." **Applied and Environmental Microbiology.** 71(4) : 1745-1753.

Lisdiyanti, P., Katsura, K., Potacharoen, W., Navarro, R.R., Yamada, Y., Uchimura, T. and Komagata, K. 2003. "Diversity of acetic acid bacteria in Indonesia, Thailand, and the Philippines." **Microbiology and Culture Collections.** 19(2) : 91-99.

Lisdiyanti, P., Navarro, R.R., Uchimura, T. and Komagata, K. 2006. "Reclassification of *Gluconacetobacter hansenii* strains and proposals of *Gluconacetobacter saccharivorans* sp. nov. and *Gluconacetobacter nataicola* sp. nov." **International Journal of Systematic and Evolutionary Microbiology.** 56(9) : 2101-2111.

Lu, X., Qian, Y. and Yan, T. 2004. "Bacterial cellulose production by soybean whey based medium." **Kmitl Science Journal.** 4(1) : 100-107.

Malisorn, C. and Suntornsuk, W. 2008. "Optimization of β-carotene production by *Rhodotorula glutinis* DM28 in fermented radish brine." **Bioresource Technology.** 99(7) : 2281-2287.

Marmur, J. and Doty, P. 1962. "Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature." **Journal of Molecular Biology.** 5(1) : 109-118.

Masaoka, S., Ohe, T. and Sakota, N. 1993. "Production of cellulose from glucose by *Acetobacter xylimum.*" **Journal of Fermentation and Bioengineering.** 75(1) : 18-22.

Matsuoka, M., Tsuchida, T., Matsushita, K., Adachi, O. and Yoshinaka, F. 1996. "A synthetic medium for bacterial cellulose production by *Acetobacter xylimum* subsp. *sucrofermentans.*" **Bioscience Biotechnology and Biochemistry.** 60(4) : 575-579.

Matthysse, A.G., Marry, M., Krall, L., Kaye, M., Ramey, B.E., Fuqua, C. and White, A.R. 2005. "The effect of cellulose overproduction on binding and biofilm formation roots by *Agrobacterium tumefaciens.*" **The American Phytopathological Society.** 18(9) : 1002-1010.

Mesbah, M., Premachandran, U. and Whitman, W.B. 1989. "Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography." **International Journal of Systematic Bacteriology.** 39(2) : 159-167.

Mikkelsen, D., Flanagan, B.M., Dykes, G.A., and Gidley, M.J. 2009. "Influence of different carbon sources on bacterial cellulose production by *Gluconacetobacter xylinus* strain ATCC 53524." **Journal of Applied Microbiology.** 107(2) : 576-583.

Mitra, D., Pometto III, A.L., Khanal, S.K., Karki, B., Brehm-Stecher, B.F. and Leeuwen Van, J.H. 2010. "Value-added production of nisin from soy whey." **Applied Biochemistry and Biotechnology.** 162(162) : 1819-1833.

Monajjemi, M., Aminin, A.L.N., Ilkhani, A.R. and Mollaamin, F. 2012. "Nano study of antioxidant activities of fermented soy whey prepared with lactic acid bacteria and kefir." **African Journal of Microbiology Research.** 6(2) : 426-430.

Moon, R.J., Martini, A., Nairn, J., Simonsen, J. and Youngblood, J. 2011. "Cellulose nanomaterials review: structure, properties and nanocomposites." **Chemical Society Reviews.** 40(7) :3941-3994.

Mowshowitz, S., Avigad, G. and Englard, S. 1974. "5-keto-D-fructose: formation and utilization in the course of D-fructose assimilation by *Gluconobacter cerinus*." **Journal of Bacteriology**. 118(3) : 1051-1058.

Napoli, C., Dazzo, F. and Hubbell, D. 1975. "Production of cellulose microfibrils by *Rhizobium*." **Applied Microbiology.** 30(1) : 123-131.

Naritomi, T., Kouda, T., Yano, H. and Yoshinaga, F. 1998. "Effect of ethanol on bacterial cellulose production from fructose in continuous culture." **Journal of Fermentation and Bioengineering.** 85(6) : 598-603.

National Innovation Agency. 2014. **Moist Wound Dressing of Bacteria Cellulose.** [Online]. Available : www.nia.or.th.

Navarro, R.R., Uchimura, T. and Komagata, K. 1999. "Taxonomic heterogeneity of strains comprising *Gluconacetobacter hansenii*." **The Journal of General and Applied Microbiology.** 45(6) : 295-300.

Ng, C.C. and Shyu, Y.T. 2004. "Development and production of cholesterol-lowering *Monascus*-nata complex." **World Journal of Microbiology and Biotechnology.** 20(20) : 875-879.

Nguyen, V.T., Flanagan, B., Gidley, M.J. and Dykes, G.A. 2008a. "Characterization of cellulose production by a *Gluconacetobacter xylinus* strain from kombucha." **Current Microbiology.** 57(7) : 449-453.

Nguyen, V.T., Gidley, M.J. and Dykes, G.A. 2008b. "Potential of a nisin-containing bacterial cellulose film to inhibit *Listeria monocytogenes* on processed meats." **Food Microbiology.** 25(3) : 471-478.

Pal, A. and Khanum, F. 2010. "Production and extraction optimization of xylanase from *Aspergillus niger* DFR-5 through solid-state-fermentation." **Bioresource Technology.** 101(19) : 7563-7569.

Park, J.K., Jung, J.Y. and Park, Y.K. 2003. "Cellulose production by *Gluconacetobacter hansenii* in a medium containing ethanol." **Biotechnology Letters.** 25(24) : 2055-2059.

Parthiban, K., Manikandan, S. and Genesapandian, S. 2011. "Production of cellulose I microfibrils from *Rhizobium* sp. and its wound healing activity on mice." **Asian Journal of Applied Sciences.** 4(3) : 247-254.

Photisarach, S. 2011. "Optimization of bacterial cellulose production from *Acetobacter xylinum* Agr60 cultured in tofu whey." Master Thesis Chiang Mai University.

Pourramezan, G.Z., Roayaei, A.M. and Qezelbash, Q.R. 2009. "Optimization of culture conditions for bacteria cellulose production by *Acetobacter* sp. 4B-2." **Biotechnology.** 8(1) : 150-154.

Priest, F. and Austin, B. 1993. **Modern bacterial taxonomy.** 2nd ed. London : Chapman & Hall 2-6 Boundary Row.

Ramana, K.V., Tomar, A. and Singh, L. 2000. "Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter xylimum.*" **World Journal of Microbiology and Biotechnology.** 16(3) : 245-248.

Rani, M.U., Rastogi, N.K., Appaiah, K.A.A. 2011a. "Statistical optimization of medium composition for bacterial cellulose production by *Gluconacetobacter hansenii* UAC09 using coffee cherry husk extract-an agro-industry waste." **Journal of Microbiology and Biotechnology.** 21(7) : 739-745.

Rani, M.U., Udayasankar, K. and Appaiah, K.A.A. 2011b. "Properties of bacterial cellulose produced in grape medium by native isolate *Gluconacetobacter* sp." **Journal of Applied Polymer Science.** 120(5) : 2835-2841.

Re, S.D., Quéré, B.L., Ghigo, J.M. and Beloin, C. 2007. "Tight modulation of *Escherichia coli* bacterial biofilm formation through controlled expression of adhesion factors." **Applied and Environmental Microbiology.** 73(10) : 3391-3403.

Rigas, F., Dritsa, V., Marchant, R., Papadopoulou, K., Avramides, E.J. and Hatzianestis, I. 2005. "Biodegradation of lindane by *Pleurotus ostreatus* via central composite design." **Environmental International.** 31(2) : 191-196.

Rigas, F., Panteleos, P. and Laoudis, C. 2000. "Central composite design in a refinery's wastewater treatment by air flotation." **Global NEST: the International Journal.** 2(3) : 245-253.

Rivas, B., Moldes, A.B., Domínguez, J.M. and Parajó, J.C. 2004. "Development of culture media containing spent yeast cells of *Debaryomyces hansenii* and corn steep liquor for lactic acid production with *Lactobacillus rhamnosus*." **International Journal of Food Microbiology.** 97(1) : 93-98.

Römling, U. 2002. "Molecular biology of cellulose production in bacteria." **Research in Microbiology.** 153(4) : 205-212.

Ross, P., Mayer, R. and Benziman, M. 1991. "Cellulose biosynthesis and function in bacteria." **Microbiological Reviews.** 55(1) : 35-58.

Ruka, D.R., Simon, G.P. and Dean, K.M. 2012. "Altering the growth conditions of *Gluconacetobacter xylinus* to maximize the yield of bacterial cellulose." **Carbohydrate Polymers.** 89(2) : 613-622.

Saitou, N. and Nei, M. 1987. "The neighbor-joining method: a new method for reconstructing phylogenetic trees." **Molecular Biology and Evolution.** 4(4) : 406-425.

Schramm, M. and Hestrin, S. 1954. "Factors affecting production of cellulose at the air/liquid interface of a culture of *Acetobacter xylinum*." **Journal of General Microbiology.** 11(1) : 123-129.

Schüller, G., Hertel, C. and Hammes, W.P. 2000. "*Gluconacetobacter entanii* sp. nov., isolated from submerged high-acid industrial vinegar fermentations." **International Journal of Systematic and Evolutionary Microbiology.** 50(6) : 2013-2020.

Seto, A., Kojima, Y., Tonouchi, N., Tsuchida, T. and Yoshinaga, F. 1997. "Screening of bacterial cellulose-producing *Acetobacter* strains suitable for sucrose as a carbon source." **Bioscience Biotechnology and Biochemistry.** 61(4) : 735-736.

Shah, J. and Brown, R.M., Jr. 2005. "Towards electronic paper displays made from microbial cellulose." **Applied Microbiology and Biotechnology.** 66(4) : 352-355.

Shoda, M. and Sugano, Y. 2005. "Recent advances in bacterial cellulose production." **Biotechnology and Bioprocess Engineering.** 10(1) : 1-8.

Singh, A. and Banerjee, R. 2013. "Peptide enriched function food adjunct from soy whey : a statistical optimization study." **Food Science and Biotechnology.** 22(5) : 65-71.

Sievers, M., Sellmer, S. and Teuber, M. 1992. "*Acetobacter europaeus* sp. nov., a main component of industrial vinegar fermenters in central Europe." **Systematic and Applied Microbiology.** 15(3) : 386-392.

Slapšak, N., Cleenwerck, I., De Vos, P. and Trček. J. 2013. "*Gluconacetobacter maltaceti* sp. nov., a novel vinegar producing acetic acid bacterium." **Systematic and Applied Microbiology.** 36(1) : 17-21.

Smith, A.K., Nash, A.M., Eldridge, A.C. and Wolf, W.J. 1962. "Recovery of soybean whey protein with edible gums and detergents." **Agricultural and Food Chemistry.** 10(4) : 302-304.

Sokollek, S.J., Hertel, C. and Hammes, W.P. 1998. "Description of *Acetobacter oboediens* sp. nov. and *Acetobacter pomorum* sp. nov., two new species isolated from industrial vinegar fermentations." **International Journal of Systematic Bacteriology.** 48(3) : 935-940.

Son, H.J., Heo, M.S., Kim, Y.G. and Lee, S.J. 2001. "Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated *Acetobacter* sp. A9 in shaking cultures." **Biotechnology and Applied Biochemistry.** 33(1) : 1-5.

Son, C.J., Chung, S.Y., Lee, J.E., Kim, S.J. 2002. "Isolation and cultivation characteristics of *Acetobacter xylinum* KJ-1 producing bacterial cellulose in shaking cultures." **Journal of Microbiology and Biotechnology.** 12(5) : 722-728.

Son, H.J., Kim, H.G., Kim, K.K., Kim, H.S., Kim, Y.G. and Lee, S.J. 2003. "Increased production of bacterial cellulose by *Acetobacter* sp. V6 in synthetic media under shaking culture conditions." **Bioresource Technology.** 86(3) : 215-219.

Stanbury, P and Whitaker, F. 1984. **Principle of fermentation technology.** Oxford : Pergamon Press.

Surma-Ślusarska, B., Presler, S. and Danielewicz, D. 2008. "Characteristics of bacterial cellulose obtained from *Acetobacter xylimum* for application in papermaking." **Fibres and Textile in Eastern Europe.** 16(69) : 108-111.

Suwannapinunt, N., Burakorn, J. and Thaenthanee, S. 2007. "Effect of culture conditions on bacterial cellulose (BC) production from *Acetobacter xylimum* TISTR 976 and physical properties of BC parchment paper." **Suranaree Journal of Science and Technology.** 14(4) : 357-365.

Svensson, A., Nicklasson, E., Harrah, T., Panilaitis, B., Kaplan, D.L., Brittberg, M. and Gatenholm, P. 2005. "Bacterial cellulose as a potential scaffold for tissue engineering of cartilage." **Biomaterials.** 26(4) : 419-431.

Swings, J. 1992. "The genera *Acetobacter* and *Gluconobacter* . In: Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H. (Eds). **The Prokaryotes.** New York : Springer-Verlag.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. "MEGA6: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0." **Molecular Biology Evoluation.** 24(8) : 1596-1599.

Tang, W., Jia, S., Jia, Y. and Yang, H. 2010. "The influence of fermentation conditions and post-treatment method on porosity of bacterial cellulose membrane." **World Journal of Microbiology and Biotechnology.** 26(1) : 125-131.

Tantratian, S., Tammarate, P., Krusong, W., Bhattarakosol, P. and Phunsri, A. 2005. "Effcet of dissolved oxygen on cellulose production by *Acetobacter* sp." **The Journal of Scientific Research Chulalongkorn University.** 30(2) : 179-186.

Teeri, T.T. 1997. "Crystalline cellulose degradation: new insight into the function of cellobiohydrolases." **Trends in Biotechnology.** 15(12) : 160-167.

Thai Industrial Standard Inatitute. 1997. **Standard test methods for paper and paper board**. Industrial Standards Institute, Ministry of Industry, Bangkok.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. 1994. "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice." **Nucleic Acids Research.** 22(2) : 4673-4680.

Tindall, B.J., Kämpfer, P., Euzéby, J.P. and Oren, A. 2006. "Valid publication of names of prokaryotes according to the rules of nomenclature: past history and current practice."

International Journal of Systematic and Evolutionary Microbiology. 56(11) : 2715-2720.

Tonouchi, N., Tsuchida, T., Yoshinaga, F., Beppu, T. and Horinouchi, S. 1996. "Characterization of the biosynthetic pathway of cellulose from glucose and fructose in *Acetobacter xylimum*." **Bioscience Biotechnology and Biochemistry.** 60(8) : 1377-1379.

Toyosaki, H., Naritomi, T., Seto, A., Matsuoka, M., Tsuchida, T. and Yoshinaga, F. 1995a. "Screening of bacterial cellulose-producing *Acetobacter* strains suitable for agitated culture." **Bioscience Biotechnology and Biochemistry.** 59(8) : 1498-1502.

Toyosaki, H., Kojima, Y., Tsuchida, T., Hoshino, K., Yamada, Y. and Yoshinaga, F. 1995b. "The characterization of an acetic acid bacterium useful for producing bacterial cellulose in agitation cultures: the proposal of *Acetobacter xylinum* subsp. *sucrofermentans* subsp. nov." **The Journal of General and Applied Microbiology.** 41(4) : 307-314.

Trovatti, E., Serafim, L.S., Freire, C.S.R., Silvestre, A.J.D. and Neto, C.P. 2011. "*Gluconacetobacter sacchari* : an efficient bacterial cellulose cell-factory." **Carbohydrate Polymers.** 86(3) : 1417-1420.

Tsuchida, T. and Yoshinaga, F. 1997. "Production of bacterial cellulose by agitation culture systems." **Pure Applied Chemistry.** 69(11) : 2453-2458.

Ude, S., Arnold, D.L., Moon, C.D., Wilson, T.T. and Spiers, A. 2006. "Biofilm formation and cellulose expression among diverse environmental *Pseudomonas* isolates." **Environmental Microbiology.** 8(11) : 1997-2011.

Ul-Islam, M., Ha, J.H., Khan, T. and Park, J.K. 2013. "Effect of glucuronic and oligomers on the production, structure and properties of bacterial cellulose." **Carbohydrate Polymers.** 92(1) : 360-366.

Updegraff, D.M. 1969. "Semimicro determination of cellulose in biological materials." **Analytical Biochemistry.** 32(3) : 420-424.

Valla, S. and Kjosbakken, J. 1982. "Cellulose-negative mutants of *Acetobacter xylimum*." **Journal of General Microbiology.** 128(1) : 1401-1408.

Watanabe, K., Tabuchi, M., Morinaga, Y. and Yoshinaga, F. 1998. "Structural feature and properties of bacterial cellulose produced in agitated culture." **Cellulose.** 5(3) : 187-200.

Watanabe, K. and Yamanaka, S. 1995. "Effects of oxygen tension in the gaseous phase on production and physical properties of bacterial cellulose formed under static culture conditions." **Bioscience Biotechnology and Biochemistry.** 59(1) : 65-68.

White, D.G. and Brown, R.M. 1989. "Prospects for the commercialization of the biosynthesis of microbial cellulose In: Schuerech C^{ed} **Cellulose and wood- chemistry and technology.** New York : Wiley.

Williams, W.S. and Cannon, R.E. 1989. "Alternative environmental roles for cellulose produced by *Acetobacter xylimum*." **Applied and Environmental Microbiology.** 55(10) : 2448-2452.

Yamada, Y. 2000. "Transfer of *Acetobacter oboediens* Sokollek *et al.*, 1998 and *Acetobacter intermedius* Boesch *et al.*, 1998 to the genus *Gluconacetobacter* as *Gluconacetobacter oboediens* comb. nov. and *Gluconacetobacter intermedius* conb. nov." **International Journal of Systematic and Evolutionary Microbiology.** 50(6) : 2225-2227.

Yamada, Y. 2014. Transfer of *Gluconacetobacter kakiaceti, Gluconacetobacter medellinensis* and *Gluconacetobacter maltaceti* to the genus *Komagataeibacter* as *Komagataeibacter kakiaceti* comb. nov., *Komagataeibacter medellinensis* comb. nov. and *Komagataeibacter maltaceti* comb. nov." **International Journal of Systematic and Evolutionary Microbiology.** Doi: 10.1099/ijs.0.054494-0.

Yamada, Y., Aida, K. and Uemura, T. 1969. "Enzymatic studies on the oxidation of sugar and sugar alcohol. V. Ubiquinone of acetic acid bacteria and its relation to classification of*Gluconobacter* and *Acetobacter*, especially of the so-called intermediate strains." **The Journal of General and Applied Microbiology.** 15(2) : 186-196.

Yamada, Y., Hoshino, K. and Ishikawa, T. 1997. "The phylogeny of acetic acid bacteria based on the partial sequences of 16S ribosomal RNA: the elevation of the subgenus *Gluconacetobacter* to the generic level." **Bioscience Biotechnology and Biochemistry**. 61 (8) : 1244-1251.

Yamada, Y., Hoshino, K. and Ishikawa, T. 1998. "Validation list no 64. Validation of publication of new names and new combinations previously effective published outside the IJJB. **International Journal of Systematic Bacteriology.** 48(1) : 327-328.

Yamada, Y. and Yukphan, P. 2008. "Genera and species in acetic acid bacteria." **International Journal of Food Microbiology.** 125(1) : 15-24.

Yamada, Y., Yukphan, P., Vu, H.T.L., Muramatsu, Y., Ochaikul, D., Tanasupawat, S. and Nakagawa, Y. 2012. "Description of *Komagataeibacter* gen. nov., with proposals of new combinations (*Acetobacteraceae*)." The **Journal of General and Applied Microbiology.** 58(5) : 397-404.

Yamanaka, S., Ishihara, M. Sugiyama, J. 2000. "Structural modification of bacterial cellulose." **Cellulose.** 7(3) : 213-225.

Yamanaka, S., Watanabe, K. and Kitamura, N. 1989. "The structure and mechanical properties of sheets prepared from bacterial cellulose." **Journal of Materials Science.** 24(9) : 3141-3145.

Yang, X.Y., Huang, C., Guo, H.J., Xiong, L., Li, Y.Y., Zhang, H.R. and Chen, X.D. 2013b. "Bioconversion of elephant grass (*Pennisetum purpureum*) acid hydrolysate to bacterial cellulose by *Gluconacetobacter xylinus*." **Journal of Applied Microbiology.** 115(4) : 995-1002.

Yang, Y., Jia, J., Xing, J., Chen, J. and Lu, S. 2013a. "Isolation and characteristics analysis of a novel high bacterial cellulose producing strain *Gluconacetobacter intermedius* Cls26." **Carbohydrate Polymers.** 92(2) : 2012-2017.

Yoshinaga, F., Tonouchi, N. and Watanabe, K. 1997. "Research progress in production of bacterial cellulose by aeration and agitation culture and its application as a new industrial material." **Bioscience Biotechnology and Biochemistry.** 61(2) : 219-224.

Yoshino, T., Asakura, T. and Toda, K. 1996. "Cellulose production by *Acetobacter pasteurianus* on silicone membrane." **Journal of Fermentation and Bioengineering.** 81(1) : 32-36.

Yukphan, P., Malimas, T., Takahashi, M., Potacharoen, W., Busabun, T., Tanasupawat, S., Nakagawa, Y., Tanticharoen, M. and Yamada, Y. 2004. "Re-identification of *Gluconobacter* strains based on the restriction analysis of 16S-23S rDNA internal transcribed spacer regions." **The Journal of General and Applied Microbiology.** 50(4) : 189-195.

Zaar, K. 1979. "Visualization of pores (export sites) correlated with cellulose production in the envelope of the Gram-negative bacterium *Acetobacter xylimum*." **Journal of Cell Biology.** 80(3) : 773-777.

Zeng, X., Small, D.P. and Wan, W. 2011. "Statistical optimization of culture conditions for bacterial cellulose production by *Acetobacter xylinum* BPR2001 from maple syrup." **Carbohydrate Polymers.** 85(1) : 506-513.

APPENDIX A

Isolation source

1. Bangkok province

1.1 Barbados cherry (*Malpighia glabra*)

1.2 Cantaloupe (*Cucumis melo*)

1.3 Fetid passionflower (*Passiflora foetida*)

1.4 Grape (*Vitis vinifera*)

1.5 Guava (*Psidium guajava*)

1.6 Java apple (*Syzygium samarangense*)

1.7 Lady's finger banana (*Musa acuminate*)

1.8 Long kong (*Lansium domesticum*)

1.9 Lychee (*Litchi chinensis*)

1.10 Mango (*Mangifera indica*)

1.11 Mangosteen (*Garcinia mangostana*)

1.12 Marian plum (*Bouea macrophylla*)

1.13 Musk melon (*Cucumis melo*)

1.14 Papaya (*Carica papaya*)

1.15 Pineapple (*Ananas comosus*)

1.16 Pisang awak banana (*Musa sapientum*)

1.17 Plum (*Prunus domestica*)

1.18 Rambutan (*Nephelium lappaceum*)

1.19 Salak (*Salacca zalacca*)

1.20 Sapodilla (*Manikara achras*)

1.21 Sugar apple (*Annona squamosal*)

1.22 Sugar plam (*Borassus flabellifer*)

1.23 Tangerine (*Citrus reticulata*)

1.24 Water melon (*Citrullus lanatus*)

2. Changmai province

2.1 Strawberry (*Fragaria ananassa*)

3. Chantaburi province

3.1 Burmese grape (*Baccaurea ramiflora*)

3.2 Longon (*Dimocarpus longan*)

4. Chumporn province

4.1 Bilimbi (*Averrhoa bilimbi*)

4.2 Passion fruit (*Passiflora edulis*)

4.3 Star fruit (*Averrhoa carambola*)

5. Kampangpat

5.1 Beleric myrobalan (*Terminalia bellerica*)

5.2 Garcina (*Garcinia schomburgkiana*)

6. Nakhonphanom province

6.1 Dragon fruit (*Hylocercus undatus*)

6.2 Governor's plum (*Flacourtia indica*)

6.3 Jack fruit (*Artocarpus heterophyllus*)

6.4 Java plum (*Syzygium cumini*)

6.5 Mahuat (*Lepisanthes rubiginosa*)

6.6 Monkey apple (*Ziziphus mauritiana*)

6.7 Star gooseberry (*Phyllanthus acidus*)

6.8 Tamarrind (*Tamarindus indica*)

7. Nakhon Ratchasima province

7.1 Look yee (*Dialium indum*)

7.2 Makokkean (*Canarium subulatum*)

7.3 Mulberry (*Morus alba*)

7.4 Wild lamon (Unkown species)

8. Rayoung province

8.1 Durian (*Durio zibethinus*)

8.2 Santol (*Sandoricum koetjape*)

9. Sakholnakhon province

9.1 Kholaen (*Nephelium hypoleucum*)

9.2 Mamao (*Antidesma thwaiteaianum*)

APPENDIX B

Medium

1. Medium for isolation of cellulose producing microorganism

1.1 Standard Hestrin & Schramm (HS) medium

Glucose	2.0%
Yeast extract	0.5%
Peptone	0.5%
Disodium hydrogenphosphate	0.27%
Citric acid	0.12%

pH 6.0

All components are dissolved in distilled water and mixed them together. The mixed solution is sterilized at 121°C for 15 minutes.

1.2 Glucose ethanol yeast extract (GEY) medium

Glucose	2.0%
Ethanol	5.0%
Yeast extract	1.0%
$CaCO_3$	0.3%

All components are dissolved and mixed them together in distilled water. After sterilization at 121°C for 15 minutes, filtered ethanol is added under aseptic condition into the medium after cooling up to 50 °C.

2. Medium for test of growth on pH 3.0-7.0

Glucose	2.0%
Ethanol	0.5%
Yeast extract	0.5%
Peptone	0.3%

All components are dissolved in distilled water and adjusted pH to 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0, respectively by hydrochloric acid or sodium hydroxide. All media are sterilized at 121°C for 15 minutes.

3. Medium for test of growth and acid production on different carbon sources

Basal medium A

Yeast extract	0.5%
Bromocresol purple (dissolved in acetone)	0.2%

pH 6.8

All ingredients are mixed together in distilled water and adjusted pH to 6.8 by sodium hydroxide. After sterilization at 121°C for 15 minutes, 1.9 mL of basal medium A is added with 0.1 mL of 20% filtrated (0.2 micron filter) carbon source under aseptic condition.

4. Medium for test of growth on different media

4.1 Glucose medium

Glucose	1.5%
Ethanol	0.5%
Peptone	0.3%
Yeast extract	0.3%
Acetic acid	0.35%

pH 3.5

All the ingredients, except ethanol are mixed in distilled water. After sterilization at 121°C for 15 minutes, filtered (0.2 micron filter) ethanol is added under aseptic conditions into the medium after cooling up to 30 °C.

4.2 Methanol medium

Methanol	0.8%
Peptone	0.3%
Yeast extract	0.3%

pH 4.0

All the ingredients, except methanol are mixed in distilled water. After sterilization at 121°C for 15 minutes, filtered (0.2 micron filter) methanol is added under aseptic conditions into the medium after cooling up to 30 °C.

4.3 Sorbitol medium

Sorbitol	2.0%
Peptone	0.3%

Yeast extract	0.3%

pH 3.5

All components are dissolved in distilled water and sterilization at 121°C for 15 minutes.

4.4 Sucrose medium

Sucrose	2.0%
Peptone	0.3%
Yeast extract	0.3%
Acetic acid	0.35%

pH 3.5

All components are dissolved in distilled water and sterilization at 121°C for 15 minutes.

5 Medium for the test of growth with or without 0.2% (v/v) acetic acid

Glucose	2.0%
Yeast extract	0.5%
Peptone	0.5%
Disodium hydrogenphosphate	0.27%
Citric acid	0.12%

pH 6.0

All components are dissolved in distilled water and mixed them together. The 0.2% of acetic acid is added in to standard HS medium and then sterilized at 121°C for 15 minutes.

6. Medium for the test of growth on 30% (w/v) D-glucose

Peptone	1.0%
Yeast extract	1.0%
Acetic acid	1.0%
Agar	1.5%
D-glucose	30.0%

All ingredients, except glucose are dissolved and mixed together in distilled water. The mixed solution is sterilized at 121°C for 15 minutes. D-glucose is dissolved in distilled water and sterilized 121°C for 15 minutes. The sterilized D-glucose is added to the sterilized mixed solution under aseptic condition.

7. Starter medium for optimization of culture condition

Sucrose	5.0%
Ammonium sulphate	0.1%
Acetic acid	1.0%
Soybean whey	
pH 4.5	

All components are dissolved in soybean whey and mixed them together. The mixed solution is sterilized at 110°C for 20 minutes.

APPENDIX C

Analysis method

1. Proximate analysis

1.1 Moisture content

The crucible is dried in hot air oven at 105 °C for 2 hours. The dried crucible is cooled in desiccators and weighted (W1). Later, two grams of sample is put into dried crucible and weighted (W2). Next the crucible with sample is dried in hot air oven at 105 °C for 3 hours, cooled in desiccators and then weighted (W3). Finally, moisture content is calculated using equation (1.1).

$$\% \text{ moisture content} = \frac{(W3-W1)}{(W2-W1)} x100 \tag{1.1}$$

1.2 Crude protein

Nitrogen is examined using micro-kjeldahl method. Firstly, 1.0 gram of dried sample is transferred into digestion tube containing 3.5 g of K_2SO_4 and 0.4 g of $CuSO_4.5H_2O$, and the sample is digested by adding 15 mL of sulfuric acid. Digestion of sample to convert nitrogen into ammonium sulfate is performed at 420 °C for 60 minutes in kjeldahl digesters. Subsequently, digested sample is added into kjeldahl flask which contains 50 mL of distilled water, 50 mL of 40% sodium hydroxide and 6-8 piece of pumic stones, and the kjeldahl flask is placed in kjeldahl distillation. The nitrogen is released from digested sample and it is collected in H_3BO_3 solution, and titrated with 0.1 N sulfuric acid using methyl red as indicator. Finally, total nitrogen and crude protein are calculated according to equation 1.2 and 1.3 respectively.

$$\% \text{ Total nitrogen} = \frac{1.4007 \text{ x } (Vs-Vb) \text{x } M}{W} \tag{1.2}$$

Where Vs and Vb = mL of sulfuric acid titrants used for sample and blank, respectively

M = molarity of sulfuric acid

W = weight of sample (g)

$$\% \text{ Crude protein} = \%\text{nitrogen x } 6.25 \tag{1.3}$$

1.3 Crude fat

Two grams of sample (W1) is added into thimble and covered with cotton wool. Next, the thimble with sample is dried at 100 °C for 3 hours in hot air oven and cooled in desiccators. The beaker is dried at 100 °C for 1 hours and the dried beaker is weighted (W2). Later, sample is transferred into dried beaker and extracted using 50 ml of petroleum ether for 4 hours in soxhlet extractor. The solvent is removed, and the residue is dried at 100 °C for 30 minutes and weighted (W3). Crude fat is calculated using equation 1.4

$$\%\ \text{Crude fat} = \frac{(W3-W2)}{W1} \text{x} 100 \tag{1.4}$$

1.4 Crude fiber

The crucible is dried in hot air oven at 100 °C for 1 hour, cooled in desiccators and dried crucible is weighted (W1). The 1.02 grams of fat extracted sample (W2) is put into dried crucible. The sample is extracted with 1.25% of sulfuric acid by heating for 30 minutes. The acid extracted sample is washed with hot water until bubbles is not observed. Later, acid extracted sample is extracted with 1.25% of sodium hydroxide by heating for 30 minutes again. The basic extracted sample in crucible is washed with acetone three times, and the crucible is dried in hot air oven at 100 °C for 5 hours, cooled to room temperature and weighted (W3). Then the crucible is burned at 550 °C for 2 hours in furnace, cooled down, and weighted (W4). The crude fiber is calculated following as the described equation.

$$\%\ \text{Crude fiber} = \frac{(W4-W3)}{W2} \text{x} 100 \tag{1.5}$$

1.5 Ash

Porcelain dish is dried in hot air oven at 100 °C for 1 hours, cooled in desiccators and dried porcelain dish is weighted (W1). Next, the dried porcelain dish with two grams of sample (W2) is burned at 550 °C for 3 hours, cooled in desiccators and weighted (W3). Ash is calculated as the below equation.

$$\%\ \text{Ash} = \frac{(W3-W2)}{W1} \text{x} 100 \tag{1.6}$$

1.6 Carbohydrate

Carbohydrate content in sample is calculated using equation 1.7

% Total carbohydrate = 100-(%moisture + %crude protein + % fat + % crude fiber + % ash (1.7)

2. Anthrone method

The 0.2 grams of anthrone reagent is dissolved in 5 mL of ethanol and then adjusted volume up to 100 mL using 75% (v/v) of sulfuric acid. The reagent is allowed to stand for 30-40 minutes with occasional shaking until it become clear. Subsequently, 1.0 mL of sample is transferred into test tubes contained 5 mL of anthrone solution and mixed together. The test tubes are chilled in ice water and fitted with lids. Next, test tubes are boiled for 10 minutes and cooled in ice water. Finally, the sample is measured absorption spectra at 620 nm. Glucose is used as a standard.

APPENDIX D

Statistical analysis

1. Selection of representative isolated strains

1.1 Beleric myrobalan

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
BEL1		3	.572433	.0023029	.0013296	.566713	.578154	.5702	.5748	
BEL2		3	.691567	.0022301	.0012875	.686027	.697107	.6899	.6941	
BEL3		3	.644967	.0037207	.0021481	.635724	.654209	.6409	.6482	
Total		9	.636322	.0520500	.0173500	.596313	.676331	.5702	.6941	
Model	Fixed Effects			.0028355	.0009452	.634009	.638635			
	Random Effects				.0346614	.487186	.785458			.0036016

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.022	2	.011	1344.863	.000
Within Groups	.000	6	.000		
Total	.022	8			

yield

Duncan[a]

BEL	N	Subset for alpha = 0.05		
		1	2	3
BEL1	3	.572433		
BEL3	3		.644967	
BEL2	3			.691567
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

1.2 Fetid passionflower

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
FET1		3	.531133	.0003055	.0001764	.530374	.531892	.5308	.5314	
FET2		3	.557233	.0005859	.0003383	.555778	.558689	.5568	.5579	
FET3		3	.490367	.0011150	.0006438	.487597	.493137	.4891	.4912	
FET4		3	.584233	.0031660	.0018279	.576369	.592098	.5809	.5872	
FET5		3	.455933	.0027025	.0015603	.449220	.462647	.4533	.4587	
FET6		3	.556200	.0002646	.0001528	.555543	.556857	.5560	.5565	
FET7		3	.539400	.0003606	.0002082	.538504	.540296	.5390	.5397	
FET8		3	.403100	.0030512	.0017616	.395520	.410680	.4006	.4065	
FET9		3	.544500	.0004000	.0002309	.543506	.545494	.5441	.5449	
FET10		3	.433600	.0029309	.0016921	.426319	.440881	.4313	.4369	
FET11		3	.505000	.0040000	.0023094	.495063	.514937	.5010	.5090	
FET12		3	.514100	.0022869	.0013204	.508419	.519781	.5124	.5167	
FET13		3	.503667	.0030551	.0017638	.496078	.511256	.5010	.5070	
FET14		3	.442333	.0023159	.0013371	.436580	.448086	.4404	.4449	
FET15		3	.473100	.0037000	.0021362	.463909	.482291	.4691	.4764	
Total		45	.502260	.0509894	.0076010	.486941	.517579	.4006	.5872	
Model	Fixed Effects			.0024067	.0003588	.501527	.502993			
	Random Effects				.0134650	.473380	.531140			.0027177

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.114	14	.008	1408.570	.000
Within Groups	.000	30	.000		
Total	.114	44			

yield

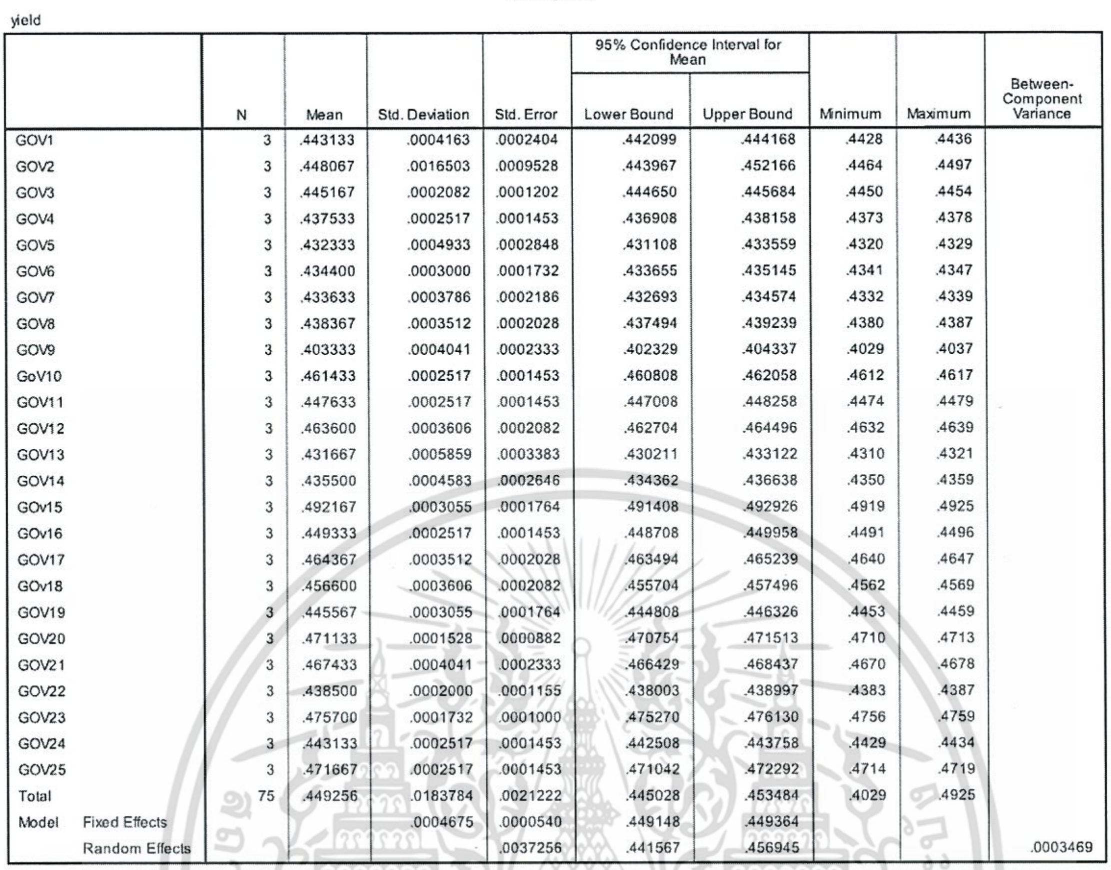
Duncan[a]

BEL		Subset for alpha = 0.05												
	N	1	2	3	4	5	6	7	8	9	10	11	12	13
FET8	3	.403100												
FET10	3		.433600											
FET14	3			.442333										
FET5	3				.455933									
FET15	3					.473100								
FET3	3						.490367							
FET13	3							.503667						
FET11	3							.505000						
FET12	3								.514100					
FET1	3									.531133				
FET7	3										.539400			
FET9	3											.544500		
FET6	3												.556200	
FET2	3												.557233	
FET4	3													.584233
Sig.		1.000	1.000	1.000	1.000	1.000	1.000	.503	1.000	1.000	1.000	1.000	.603	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

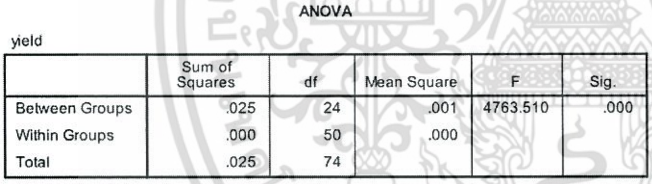
1.3 Governor's plum



Descriptives

yield

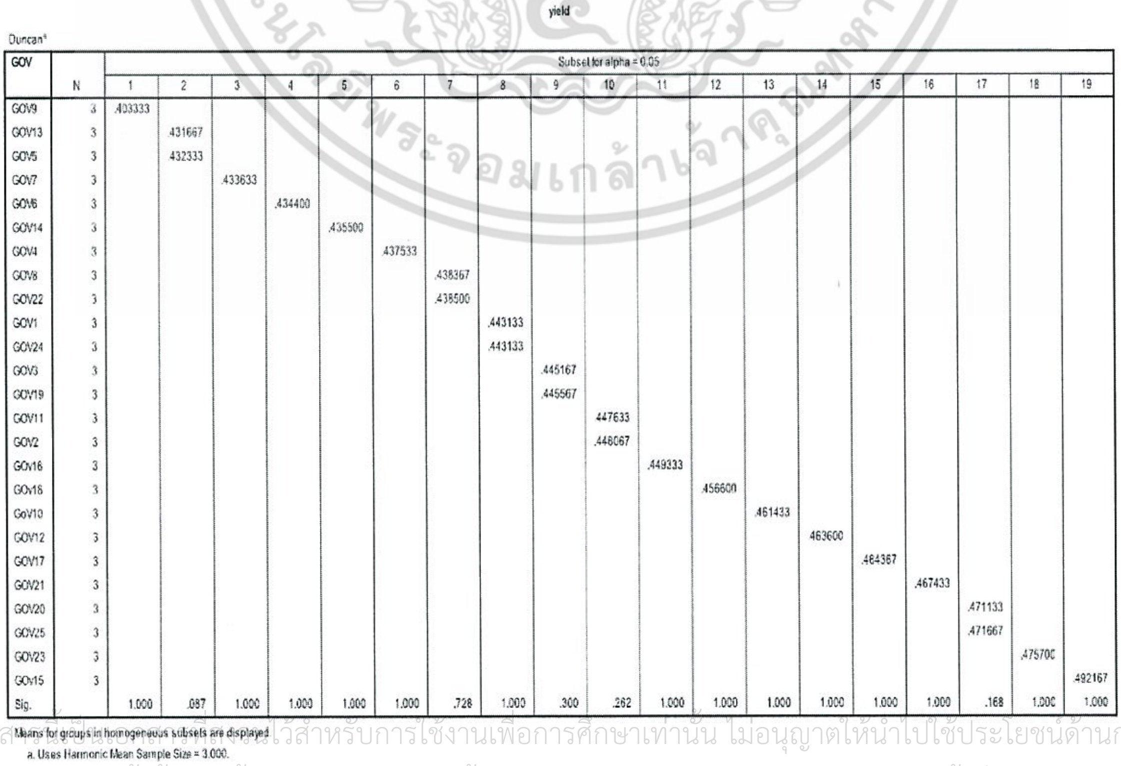
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
					Lower Bound	Upper Bound			
GOV1	3	.443133	.0004163	.0002404	.442099	.444168	.4428	.4436	
GOV2	3	.448067	.0016503	.0009528	.443967	.452166	.4464	.4497	
GOV3	3	.445167	.0002082	.0001202	.444650	.445684	.4450	.4454	
GOV4	3	.437533	.0002517	.0001453	.436908	.438158	.4373	.4378	
GOV5	3	.432333	.0004933	.0002848	.431108	.433559	.4320	.4329	
GOV6	3	.434400	.0003000	.0001732	.433655	.435145	.4341	.4347	
GOV7	3	.433633	.0003786	.0002186	.432693	.434574	.4332	.4339	
GOV8	3	.438367	.0003512	.0002028	.437494	.439239	.4380	.4387	
GOV9	3	.403333	.0004041	.0002333	.402329	.404337	.4029	.4037	
GoV10	3	.461433	.0002517	.0001453	.460808	.462058	.4612	.4617	
GOV11	3	.447633	.0002517	.0001453	.447008	.448258	.4474	.4479	
GOV12	3	.463600	.0003606	.0002082	.462704	.464496	.4632	.4639	
GOV13	3	.431667	.0005859	.0003383	.430211	.433122	.4310	.4321	
GOV14	3	.435500	.0004583	.0002646	.434362	.436638	.4350	.4359	
GOv15	3	.492167	.0003055	.0001764	.491408	.492926	.4919	.4925	
GOv16	3	.449333	.0002517	.0001453	.448708	.449958	.4491	.4496	
GOV17	3	.464367	.0003512	.0002028	.463494	.465239	.4640	.4647	
GOv18	3	.456600	.0003606	.0002082	.455704	.457496	.4562	.4569	
GOV19	3	.445567	.0003055	.0001764	.444808	.446326	.4453	.4459	
GOV20	3	.471133	.0001528	.0000882	.470754	.471513	.4710	.4713	
GOV21	3	.467433	.0004041	.0002333	.466429	.468437	.4670	.4678	
GOV22	3	.438500	.0002000	.0001155	.438003	.438997	.4383	.4387	
GOV23	3	.475700	.0001732	.0001000	.475270	.476130	.4756	.4759	
GOV24	3	.443133	.0002517	.0001453	.442508	.443758	.4429	.4434	
GOV25	3	.471667	.0002517	.0001453	.471042	.472292	.4714	.4719	
Total	75	.449256	.0183784	.0021222	.445028	.453484	.4029	.4925	
Model Fixed Effects			.0004675	.0000540	.449148	.449364			
Random Effects				.0037256	.441567	.456945			.0003469



ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.025	24	.001	4763.510	.000
Within Groups	.000	50	.000		
Total	.025	74			



yield

Duncan[a]

GOV	N	Subset for alpha = 0.05																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
GOV9	3	.403333																		
GOV13	3		.431667																	
GOV5	3		.432333																	
GOV7	3			.433633																
GOV6	3				.434400															
GOV14	3					.435500														
GOV4	3						.437533													
GOV8	3							.438367												
GOV22	3							.438500												
GOV1	3								.443133											
GOV24	3								.443133											
GOV3	3									.445167										
GOV19	3									.445567										
GOV11	3										.447633									
GOV2	3										.448067									
GOv16	3											.449333								
GOv18	3												.456600							
GoV10	3													.461433						
GOV12	3														.463600					
GOV17	3															.464367				
GOV21	3																.467433			
GOV20	3																	.471133		
GOV25	3																	.471667		
GOV23	3																		.475700	
GOv15	3																			.492167
Sig.		1.000	.087	1.000	1.000	1.000	1.000	.728	1.000	.300	.262	1.000	1.000	1.000	1.000	1.000	1.000	.168	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

1.4 Grape

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
GRA1		3	.604933	.0042477	.0024524	.594381	.615485	.6011	.6095	
GRA2		3	.564467	.0024583	.0014193	.558360	.570573	.5629	.5673	
GRA3		3	.646267	.0028184	.0016272	.639265	.653268	.6431	.6485	
GRA4		3	.592600	.0022605	.0013051	.586985	.598215	.5901	.5945	
GRA5		3	.576167	.0026083	.0015059	.569687	.582646	.5732	.5781	
GRA6		3	.657000	.0019672	.0011358	.652113	.661887	.6549	.6588	
GRA7		3	.615767	.0027683	.0015983	.608890	.622643	.6132	.6187	
GRA8		3	.675400	.0039661	.0022898	.665548	.685252	.6721	.6798	
GRA9		3	.594600	.0041581	.0024007	.584271	.604929	.5901	.5983	
GRA10		3	.624233	.0034847	.0020119	.615577	.632890	.6205	.6274	
GRA11		3	.575200	.0036346	.0020984	.566171	.584229	.5721	.5792	
Total		33	.611512	.0350214	.0060964	.599094	.623930	.5629	.6798	
Model	Fixed Effects			.0032171	.0005600	.610351	.612674			
	Random Effects				.0108740	.587283	.635741			.0012972

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.039	10	.004	377.007	.000
Within Groups	.000	22	.000		
Total	.039	32			

yield

Duncan[a]

GRA	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
GRA2	3	.564467								
GRA11	3		.575200							
GRA5	3		.576167							
GRA4	3			.592600						
GRA9	3			.594600						
GRA1	3				.604933					
GRA7	3					.615767				
GRA10	3						.624233			
GRA3	3							.646267		
GRA6	3								.657000	
GRA8	3									.675400
Sig.		1.000	.716	.455	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

1.5 Java plum

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
JAV1		3	.699200	.0015524	.0008963	.695344	.703056	.6976	.7007	
JAV2		3	.653100	.0024000	.0013856	.647138	.659062	.6507	.6555	
JAV3		3	.615100	.0040951	.0023643	.604927	.625273	.6123	.6198	
Total		9	.655800	.0365580	.0121860	.627699	.683901	.6123	.7007	
Model	Fixed Effects			.0028833	.0009611	.653448	.658152			
	Random Effects				.0243151	.551181	.760419			.0017709

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.011	2	.005	640.057	.000
Within Groups	.000	6	.000		
Total	.011	8			

yield

Duncan[a]

JAV	N	Subset for alpha = 0.05		
		1	2	3
JAV3	3	.615100		
JAV2	3		.653100	
JAV1	3			.699200
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

1.6 Lychee

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
LYC1		3	.621867	.0024685	.0014252	.615735	.627999	.6198	.6246	
LYC2		3	.606833	.0053126	.0030672	.593636	.620030	.6007	.6100	
LYC3		3	.573200	.0028160	.0016258	.566205	.580195	.5708	.5763	
LYC4		3	.557433	.0020648	.0011921	.552304	.562563	.5560	.5598	
LYC5		3	.544433	.0036638	.0021153	.535332	.553535	.5406	.5479	
LYC6		3	.565700	.0028513	.0016462	.558617	.572783	.5628	.5685	
LYC7		3	.517067	.0033546	.0019368	.508733	.525400	.5132	.5192	
LYC8		3	.630867	.0016503	.0009528	.626767	.634966	.6292	.6325	
LYC9		3	.594800	.0024515	.0014154	.588710	.600890	.5924	.5973	
LYC10		3	.525367	.0017616	.0010171	.520991	.529743	.5234	.5268	
LYC11		3	.594367	.0024007	.0013860	.588403	.600330	.5926	.5971	
LYC12		3	.613000	.0024556	.0014177	.606900	.619100	.6109	.6157	
LYC13		3	.593833	.0037112	.0021427	.584614	.603053	.5903	.5977	
LYC14		3	.606533	.0024583	.0014193	.600427	.612640	.6037	.6081	
LYC15		3	.552000	.0012767	.0007371	.548828	.555172	.5509	.5534	
Total		45	.579820	.0343514	.0051208	.569500	.590140	.5132	.6325	
Model	Fixed Effects			.0028797	.0004293	.578943	.580697			
	Random Effects				.0090564	.560396	.599244			.0012275

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.052	14	.004	445.062	.000
Within Groups	.000	30	.000		
Total	.052	44			

yield

Duncan^a

LYC	N	Subset for alpha = 0.05											
		1	2	3	4	5	6	7	8	9	10	11	12
LYC7	3	.517067											
LYC10	3		.525367										
LYC5	3			.544433									
LYC15	3				.552000								
LYC4	3					.557433							
LYC6	3						.565700						
LYC3	3							.573200					
LYC13	3								.593833				
LYC11	3								.594367				
LYC9	3								.594800				
LYC14	3									.606533			
LYC2	3									.606833			
LYC12	3										.613000		
LYC1	3											.621867	
LYC8	3												.630867
Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	.702	.899	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

1.7 Mamao

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
MAM1		3	.632000	.0011533	.0006658	.629135	.634865	.6308	.6331	
MAM2		3	.606000	.0018520	.0010693	.601399	.610601	.6039	.6074	
MAM3		3	.625100	.0017436	.0010066	.620769	.629431	.6239	.6271	
MAM4		3	.652767	.0017214	.0009939	.648490	.657043	.6514	.6547	
Total		12	.628967	.0175181	.0050570	.617836	.640097	.6039	.6547	
Model	Fixed Effects			.0016404	.0004735	.627875	.630059			
	Random Effects				.0096525	.598248	.659685			.0003718

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.003	3	.001	415.507	.000
Within Groups	.000	8	.000		
Total	.003	11			

yield

Duncan[a]

MAM	N	Subset for alpha = 0.05			
		1	2	3	4
MAM2	3	.606000			
MAM3	3		.625100		
MAM1	3			.632000	
MAM4	3				.652767
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

1.8 Mangosteen

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
MAG1		3	.493300	.0023580	.0013614	.487442	.499158	.4913	.4959	
MAG2		3	.527133	.0024007	.0013860	.521170	.533097	.5245	.5292	
MAG3		3	.475267	.0017214	.0009939	.470990	.479543	.4733	.4765	
MAG4		3	.466667	.0007572	.0004372	.464786	.468548	.4658	.4672	
MAG5		3	.496167	.0011372	.0006566	.493342	.498992	.4949	.4971	
MAG6		3	.451900	.0017578	.0010149	.447533	.456267	.4506	.4539	
MAG7		3	.505533	.0029023	.0016756	.498324	.512743	.5022	.5075	
MAG8		3	.483733	.0009292	.0005364	.481425	.486041	.4827	.4845	
MAG9		3	.472467	.0006110	.0003528	.470949	.473984	.4718	.4730	
MAG10		3	.553900	.0031048	.0017926	.546187	.561613	.5509	.5571	
MAG11		3	.494833	.0023459	.0013544	.489006	.500661	.4922	.4967	
MAG12		3	.476500	.0023000	.0013279	.470786	.482214	.4742	.4788	
MAG13		3	.536700	.0029816	.0017214	.529293	.544107	.5335	.5394	
MAG14		3	.486367	.0007234	.0004177	.484570	.488164	.4859	.4872	
MAG15		3	.593900	.0031607	.0018248	.586048	.601752	.5909	.5972	
MAG16		3	.478467	.0012342	.0007126	.475401	.481533	.4771	.4795	
MAG17		3	.564833	.0013650	.0007881	.561442	.568224	.5639	.5664	
MAG18		3	.501400	.0008888	.0005132	.499192	.503608	.5007	.5024	
MAG19		3	.525133	.0039829	.0022995	.515239	.535027	.5217	.5295	
MAG20		3	.492067	.0014503	.0008373	.488464	.495669	.4906	.4935	
MAG21		3	.532900	.0022716	.0013115	.527257	.538543	.5303	.5345	
MAG22		3	.485433	.0004509	.0002603	.484313	.486553	.4850	.4859	
MAG23		3	.576333	.0050817	.0029339	.563710	.588957	.5705	.5798	
Total		69	.507432	.0369911	.0044532	.498546	.516318	.4506	.5972	
Model	Fixed Effects			.0023038	.0002773	.506874	.507990			
	Random Effects				.0078189	.491216	.523647			.0014043

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.093	22	.004	794.801	.000
Within Groups	.000	46	.000		
Total	.093	68			

yield

Duncan[a]

MAG	N	Subset for alpha = 0.05														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MAG6	3	.451900														
MAG4	3		.466667													
MAG9	3			.472467												
MAG3	3			.475267	.475267											
MAG12	3				.476500											
MAG16	3				.478467											
MAG8	3					.483733										
MAG22	3					.485433										
MAG14	3					.486367										
MAG20	3						.492067									
MAG1	3						.493300									
MAG11	3						.494833									
MAG5	3						.496167									
MAG18	3							.501400								
MAG7	3								.505533							
MAG19	3									.525133						
MAG2	3									.527133						
MAG21	3										.532900					
MAG13	3											.536700				
MAG10	3												.553900			
MAG17	3													.564833		
MAG23	3														.576333	
MAG15	3															.593900
Sig.		1.000	1.000	.143	.114	.193	.051	1.000	1.000	.293	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

1.9 Rambutan

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
RAM1		3	.437167	.0003786	.0002186	.436226	.438107	.4369	.4376	
RAM2		3	.475567	.0026502	.0015301	.468983	.482150	.4729	.4782	
RAM3		3	.556300	.0011358	.0006557	.553479	.559121	.5555	.5576	
RAM4		3	.612333	.0023159	.0013371	.606580	.618086	.6104	.6149	
RAM5		3	.490633	.0003512	.0002028	.489761	.491506	.4903	.4910	
RAM6		3	.456167	.0037541	.0021674	.446841	.465492	.4521	.4595	
RAM7		3	.544433	.0029670	.0017130	.537063	.551804	.5422	.5478	
RAM8		3	.486900	.0012124	.0007000	.483888	.489912	.4856	.4880	
RAM9		3	.532433	.0004041	.0002333	.531429	.533437	.5320	.5328	
RAM10		3	.522533	.0020841	.0012032	.517356	.527710	.5207	.5248	
RAM11		3	.574200	.0023580	.0013614	.568342	.580058	.5716	.5762	
RAM12		3	.494667	.0026312	.0015191	.488130	.501203	.4930	.4977	
RAM13		3	.475733	.0031660	.0018279	.467869	.483598	.4724	.4787	
RAM14		3	.526267	.0003512	.0002028	.525394	.527139	.5259	.5266	
RAM15		3	.567700	.0011136	.0006429	.564934	.570466	.5667	.5689	
RAM16		3	.518597	.0016784	.0009690	.514427	.522766	.5167	.5199	
RAM17		3	.534133	.0036460	.0021050	.525076	.543191	.5302	.5374	
RAM18		3	.486533	.0017616	.0010171	.482157	.490909	.4851	.4885	
RAM19		3	.595633	.0025482	.0014712	.589303	.601963	.5927	.5973	
RAM20		3	.607100	.0030790	.0017776	.599451	.614749	.6037	.6097	
Total		60	.524752	.0491804	.0063492	.512047	.537456	.4369	.6149	
Model	Fixed Effects			.0022569	.0002914	.524163	.525340			
	Random Effects				.0111803	.501351	.548152			.0024983

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.143	19	.008	1472.413	.000
Within Groups	.000	40	.000		
Total	.143	59			

yield

Duncan[a]

RAM		Subset for alpha = 0.05																
	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
RAM1	3	.437167																
RAM6	3		.456167															
RAM2	3			.475567														
RAM13	3			.475733														
RAM18	3				.486533													
RAM8	3				.486900													
RAM5	3					.490633												
RAM12	3						.494667											
RAM16	3							.518597										
RAM10	3								.522533									
RAM14	3									.526267								
RAM9	3										.532433							
RAM17	3										.534133							
RAM7	3											.544433						
RAM3	3												.556300					
RAM15	3													.567700				
RAM11	3														.574200			
RAM19	3															.595633		
RAM20	3																.607100	
RAM4	3																	.612333
Sig.		1.000	1.000	.928	.843	1.000	1.000	1.000	1.000	1.000	.362	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

1.10 Sapodilla

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
SPO1		3	.477267	.0012097	.0006984	.474262	.480272	.4759	.4782	
SPO2		3	.470000	.0011136	.0006429	.467234	.472766	.4690	.4712	
SPO3		3	.440533	.0009504	.0005487	.438172	.442894	.4396	.4415	
SPO4		3	.537567	.0013317	.0007688	.534259	.540875	.5361	.5387	
SPO5		3	.463067	.0019140	.0011050	.458312	.467821	.4613	.4651	
SPO6		3	.523833	.0033843	.0019539	.515426	.532240	.5201	.5267	
SPO7		3	.493633	.0028308	.0016344	.486601	.500665	.4919	.4969	
SPO8		3	.405700	.0017436	.0010066	.401369	.410031	.4037	.4069	
SPO9		3	.472967	.0024379	.0014075	.466911	.479023	.4702	.4748	
SPO10		3	.457767	.0025325	.0014621	.451476	.464058	.4549	.4597	
SPO11		3	.474900	.0033451	.0019313	.466590	.483210	.4711	.4774	
SPO12		3	.490633	.0004509	.0002603	.489513	.491753	.4902	.4911	
SPO13		3	.505633	.0023029	.0013296	.499913	.511354	.5031	.5076	
SPO14		3	.423900	.0015000	.0008660	.420174	.427626	.4224	.4254	
SPO15		3	.381967	.0018903	.0010914	.377271	.386662	.3805	.3841	
SPO16		3	.405067	.0021127	.0012197	.399819	.410315	.4037	.4075	
SPO17		3	.454833	.0029263	.0016895	.447564	.462103	.4529	.4582	
SPO18		3	.476200	.0004359	.0002517	.475117	.477283	.4759	.4767	
SPO19		3	.495233	.0036350	.0020987	.486203	.504263	.4923	.4993	
SPO20		3	.500467	.0005508	.0003180	.499099	.501835	.5001	.5011	
SPO21		3	.527700	.0009165	.0005292	.525423	.529977	.5269	.5287	
SPO22		3	.478700	.0009000	.0005196	.476464	.480936	.4778	.4796	
SPO23		3	.424767	.0006110	.0003528	.423249	.426284	.4241	.4253	
Total		69	.468797	.0400787	.0048249	.459169	.478425	.3805	.5387	
Model	Fixed Effects			.0020343	.0002449	.468304	.469290			
	Random Effects				.0084753	.451220	.486374			.0016507

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.109	22	.005	1197.628	.000
Within Groups	.000	46	.000		
Total	.109	68			

yield

Duncan[a]

SPO	N	Subset for alpha = 0.05																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
SPO15	3	.381967																
SPO16	3		.405067															
SPO8	3		.405700															
SPO14	3			.423900														
SPO23	3			.424767														
SPO3	3				.440533													
SPO17	3					.454833												
SPO10	3					.457767												
SPO5	3						.463067											
SPO2	3							.470000										
SPO9	3							.472967	.472967									
SPO11	3								.474900	.474900								
SPO18	3								.476200	.476200	.476200							
SPO1	3									.477267	.477267							
SPO22	3										.478700							
SPO12	3											.490633						
SPO7	3											.493633	.493633					
SPO19	3												.495233					
SPO20	3													.500467				
SPO13	3														.505633			
SPO6	3															.523833		
SPO21	3																.527700	
SPO4	3																	.537567
Sig.		1.000	.705	.604	1.000	.084	1.000	.081	.071	.186	.162	.077	.340	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

1.11 Star fruit

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
STA1		3	.505067	.0032517	.0018774	.496989	.513144	.5014	.5076	
STA2		3	.506133	.0037820	.0021835	.496738	.515528	.5021	.5096	
STA3		3	.515167	.0028290	.0016333	.508139	.522194	.5126	.5182	
STA4		3	.518233	.0013429	.0007753	.514897	.521569	.5167	.5192	
STA5		3	.528700	.0011000	.0006351	.525967	.531433	.5276	.5298	
STA6		3	.502133	.0013577	.0007839	.498761	.505506	.5007	.5034	
STA7		3	.506367	.0020599	.0011893	.501250	.511484	.5042	.5083	
STA8		3	.514567	.0035076	.0020251	.505853	.523280	.5112	.5182	
STA9		3	.517000	.0040286	.0023259	.506992	.527008	.5124	.5199	
STA10		3	.505600	.0028000	.0016166	.498644	.512556	.5024	.5076	
STA11		3	.515967	.0014295	.0008253	.512416	.519518	.5144	.5172	
STA12		3	.513667	.0016166	.0009333	.509651	.517682	.5122	.5154	
STA13		3	.514800	.0025515	.0014731	.508462	.521138	.5129	.5177	
STA14		3	.516167	.0027502	.0015878	.509335	.522998	.5134	.5189	
STA15		3	.506100	.0038588	.0022279	.496514	.515686	.5021	.5098	
STA16		3	.505867	.0024132	.0013932	.499872	.511861	.5032	.5079	
STA17		3	.516300	.0025120	.0014503	.510060	.522540	.5134	.5178	
STA18		3	.507433	.0025482	.0014712	.501103	.513763	.5045	.5091	
STA19		3	.516367	.0019604	.0011319	.511497	.521237	.5143	.5182	
STA20		3	.514833	.0016010	.0009244	.510856	.518811	.5132	.5164	
STA21		3	.515967	.0024786	.0014310	.509810	.522124	.5133	.5182	
Total		63	.512497	.0066008	.0008316	.510834	.514159	.5007	.5298	
Model	Fixed Effects			.0026109	.0003289	.511833	.513161			
	Random Effects				.0013845	.509609	.515385			.0000380

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	20	.000	17.715	.000
Within Groups	.000	42	.000		
Total	.003	62			

yield

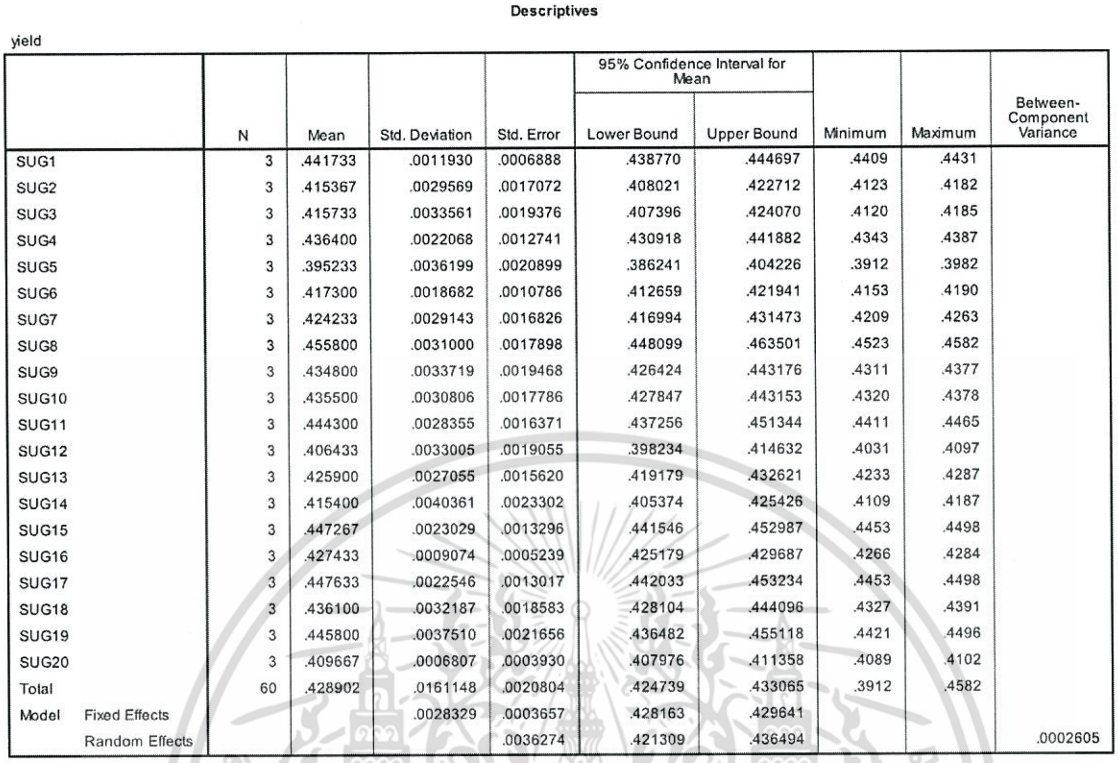
Duncan[a]

STA	N	Subset for alpha = 0.05			
		1	2	3	4
STA6	3	.502133			
STA1	3	.505067	.505067		
STA10	3	.505600	.505600		
STA16	3	.505867	.505867		
STA15	3	.506100	.506100		
STA2	3	.506133	.506133		
STA7	3	.506367	.506367		
STA18	3		.507433		
STA12	3			.513667	
STA8	3			.514567	
STA13	3			.514800	
STA20	3			.514833	
STA3	3			.515167	
STA11	3			.515967	
STA21	3			.515967	
STA14	3			.516167	
STA17	3			.516300	
STA19	3			.516367	
STA9	3			.517000	
STA4	3			.518233	
STA5	3				.528700
Sig.		.092	.346	.079	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

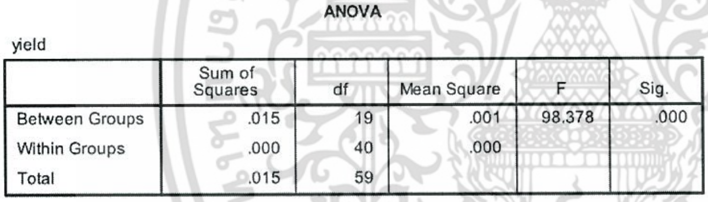
1.12 Sugar apple



Descriptives

yield

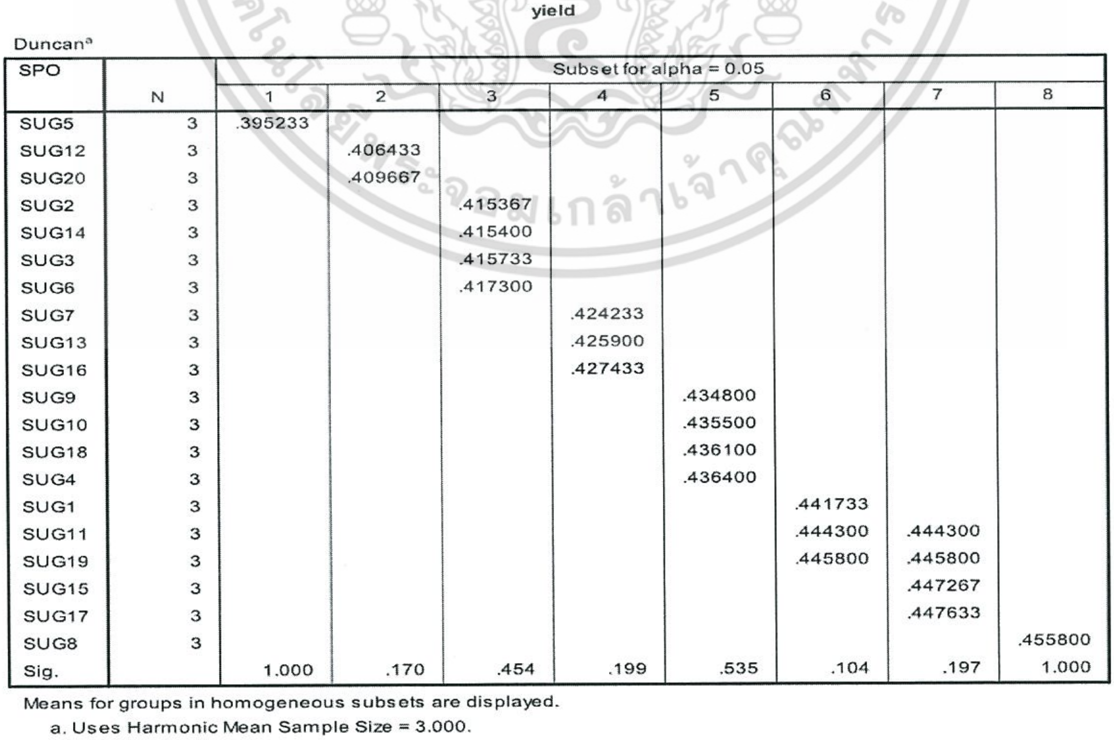
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum	Between-Component Variance
SUG1	3	.441733	.0011930	.0006888	.438770	.444697	.4409	.4431	
SUG2	3	.415367	.0029569	.0017072	.408021	.422712	.4123	.4182	
SUG3	3	.415733	.0033561	.0019376	.407396	.424070	.4120	.4185	
SUG4	3	.436400	.0022068	.0012741	.430918	.441882	.4343	.4387	
SUG5	3	.395233	.0036199	.0020899	.386241	.404226	.3912	.3982	
SUG6	3	.417300	.0018682	.0010786	.412659	.421941	.4153	.4190	
SUG7	3	.424233	.0029143	.0016826	.416994	.431473	.4209	.4263	
SUG8	3	.455800	.0031000	.0017898	.448099	.463501	.4523	.4582	
SUG9	3	.434800	.0033719	.0019468	.426424	.443176	.4311	.4377	
SUG10	3	.435500	.0030806	.0017786	.427847	.443153	.4320	.4378	
SUG11	3	.444300	.0028355	.0016371	.437256	.451344	.4411	.4465	
SUG12	3	.406433	.0033005	.0019055	.398234	.414632	.4031	.4097	
SUG13	3	.425900	.0027055	.0015620	.419179	.432621	.4233	.4287	
SUG14	3	.415400	.0040361	.0023302	.405374	.425426	.4109	.4187	
SUG15	3	.447267	.0023029	.0013296	.441546	.452987	.4453	.4498	
SUG16	3	.427433	.0009074	.0005239	.425179	.429687	.4266	.4284	
SUG17	3	.447633	.0022546	.0013017	.442033	.453234	.4453	.4498	
SUG18	3	.436100	.0032187	.0018583	.428104	.444096	.4327	.4391	
SUG19	3	.445800	.0037510	.0021656	.436482	.455118	.4421	.4496	
SUG20	3	.409667	.0006807	.0003930	.407976	.411358	.4089	.4102	
Total	60	.428902	.0161148	.0020804	.424739	.433065	.3912	.4582	
Model Fixed Effects			.0028329	.0003657	.428163	.429641			
Random Effects				.0036274	.421309	.436494			.0002605



ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.015	19	.001	98.378	.000
Within Groups	.000	40	.000		
Total	.015	59			



yield

Duncan^a

SPO	N	Subset for alpha = 0.05 1	2	3	4	5	6	7	8
SUG5	3	.395233							
SUG12	3		.406433						
SUG20	3		.409667						
SUG2	3			.415367					
SUG14	3			.415400					
SUG3	3			.415733					
SUG6	3			.417300					
SUG7	3				.424233				
SUG13	3				.425900				
SUG16	3				.427433				
SUG9	3					.434800			
SUG10	3					.435500			
SUG18	3					.436100			
SUG4	3					.436400			
SUG1	3						.441733		
SUG11	3						.444300	.444300	
SUG19	3						.445800	.445800	
SUG15	3							.447267	
SUG17	3							.447633	
SUG8	3								.455800
Sig.		1.000	.170	.454	.199	.535	.104	.197	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

1.13 Water melon

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
WAT1		3	.445567	.0023502	.0013569	.439729	.451405	.4432	.4479	
WAT2		3	.458533	.0014572	.0008413	.454914	.462153	.4569	.4597	
WAT3		3	.453100	.0018248	.0010536	.448567	.457633	.4519	.4552	
WAT4		3	.445300	.0025865	.0014933	.438875	.451725	.4425	.4476	
WAT5		3	.447667	.0010970	.0006333	.444942	.450392	.4468	.4489	
WAT6		3	.452900	.0017436	.0010066	.448569	.457231	.4509	.4541	
WAT7		3	.464733	.0030501	.0017610	.457156	.472310	.4617	.4678	
WAT8		3	.464167	.0032347	.0018676	.456131	.472202	.4616	.4678	
WAT9		3	.416867	.0036254	.0020931	.407861	.425873	.4127	.4193	
WAT10		3	.455667	.0025929	.0014970	.449225	.462108	.4527	.4575	
WAT11		3	.476200	.0024759	.0014295	.470050	.482350	.4734	.4781	
WAT12		3	.452700	.0007550	.0004359	.450825	.454575	.4519	.4534	
WAT13		3	.447300	.0016823	.0009713	.443121	.451479	.4460	.4492	
WAT14		3	.403600	.0022869	.0013204	.397919	.409281	.4019	.4062	
WAT15		3	.428267	.0006506	.0003756	.426650	.429883	.4276	.4289	
Total		45	.447504	.0184078	.0027441	.441974	.453035	.4019	.4781	
Model	Fixed Effects			.0022617	.0003371	.446816	.448193			
	Random Effects				.0048396	.437124	.457884			.0003496

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.015	14	.001	206.054	.000
Within Groups	.000	30	.000		
Total	.015	44			

yield

Duncan[a]

WAT	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
WAT14	3	.403600							
WAT9	3		.416867						
WAT15	3			.428267					
WAT4	3				.445300				
WAT1	3				.445567				
WAT13	3				.447300				
WAT5	3				.447667				
WAT12	3					.452700			
WAT6	3					.452900			
WAT3	3					.453100			
WAT10	3					.455667	.455667		
WAT2	3						.458533		
WAT8	3							.464167	
WAT7	3							.464733	
WAT11	3								.476200
Sig.		1.000	1.000	1.000	.252	.152	.131	.761	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

1.14 Wild lemon

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
WIL1		3	.574300	.0036014	.0020793	.565354	.583246	.5711	.5782	
WIL2		3	.605533	.0016503	.0009528	.601434	.609633	.6039	.6072	
WIL3		3	.492367	.0020526	.0011851	.487268	.497466	.4901	.4941	
Total		9	.557400	.0506645	.0168882	.518456	.596344	.4901	.6072	
Model	Fixed Effects			.0025760	.0008587	.555299	.559501			
	Random Effects				.0337435	.412213	.702587			.0034137

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.020	2	.010	1544.353	.000
Within Groups	.000	6	.000		
Total	.021	8			

yield

Duncan[a]

WIL	N	Subset for alpha = 0.05		
		1	2	3
WIL3	3	.492367		
WIL1	3		.574300	
WIL2	3			.605533
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

2. BC production by all representative strains

2.1 static condition

Descriptives

Yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
RAM1		3	.5133	.00577	.00333	.4990	.5277	.51	.52	
GOV9		3	.5633	.00577	.00333	.5490	.5777	.56	.57	
GOV15		3	.6833	.00577	.00333	.6690	.6977	.68	.69	
MAM4		3	.8667	.00577	.00333	.8523	.8810	.86	.87	
SPO4		3	.7567	.02082	.01202	.7050	.8084	.74	.78	
WAT11		3	.6200	.01000	.00577	.5952	.6448	.61	.63	
FET8		3	.8333	.01155	.00667	.8046	.8620	.82	.84	
LYC7		3	.5067	.00577	.00333	.4923	.5210	.50	.51	
SPO15		3	.5033	.00577	.00333	.4890	.5177	.50	.51	
WAT14		3	.5367	.01528	.00882	.4987	.5746	.52	.55	
BEL1		3	.8633	.01155	.00667	.8346	.8920	.85	.87	
BEL2		3	.9033	.00577	.00333	.8890	.9177	.90	.91	
FET4		3	.7600	.02000	.01155	.7103	.8097	.74	.78	
LAD1		3	.5400	.01000	.00577	.5152	.5648	.53	.55	
LYC8		3	.7033	.00577	.00333	.6890	.7177	.70	.71	
STA5		3	.7267	.00577	.00333	.7123	.7410	.72	.73	
WIL2		3	.8333	.02082	.01202	.7816	.8850	.81	.85	
MAG6		3	.6267	.01528	.00882	.5887	.6646	.61	.64	
SUG5		3	.5133	.00577	.00333	.4990	.5277	.51	.52	
SUG8		3	.5333	.00577	.00333	.5190	.5477	.53	.54	
GRA2		3	.5467	.01155	.00667	.5180	.5754	.54	.56	
GRA8		3	.9300	.02000	.01155	.8803	.9797	.91	.95	
JAV1		3	.8867	.00577	.00333	.8723	.9010	.88	.89	
JAV3		3	.9267	.02309	.01333	.8693	.9840	.90	.94	
MAG15		3	.7400	.02000	.01155	.6903	.7897	.72	.76	
MAM2		3	.8133	.01155	.00667	.7846	.8420	.80	.82	
PAP1		3	1.1500	.01000	.00577	1.1252	1.1748	1.14	1.16	
RAM4		3	.8233	.01155	.00667	.7946	.8520	.81	.83	
WIL3		3	.5267	.01528	.00882	.4887	.5646	.51	.54	
Total		87	.7148	.16882	.01810	.6788	.7508	.50	1.16	
Model	Fixed Effects			.01269	.00136	.7121	.7175			
	Random Effects				.03166	.6500	.7797			.02901

ANOVA

Yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.442	28	.087	541.894	.000
Within Groups	.009	58	.000		
Total	2.451	86			

Yield

Duncan[a]

Static	N	Subset for alpha = 0.05														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
SPO15	3	.5033														
LYC7	3	.5067	.5067													
RAM1	3	.5133	.5133	.5133												
SUG5	3	.5133	.5133	.5133												
WIL3	3		.5267	.5267	.5267											
SUG8	3			.5333	.5333											
WAT14	3				.5367											
LAD1	3				.5400											
GRA2	3				.5467	.5467										
GOV9	3					.5633										
WAT11	3						.6200									
MAG6	3						.6267									
GOV15	3							.6833								
LYC8	3							.7033								
STA5	3								.7267							
MAG15	3								.7400	.7400						
SPO4	3									.7567						
FET4	3									.7600						
MAM2	3										.8133					
RAM4	3										.8233					
FET8	3										.8333					
WIL2	3										.8333					
BEL1	3											.8633				
MAM4	3											.8667	.8667			
JAV1	3												.8867	.8867		
BEL2	3													.9033		
JAV3	3														.9267	
GRA8	3														.9300	
PAP1	3															1.1500
Sig.		.387	.082	.082	.090	.113	.522	.058	.203	.072	.082	.749	.058	.113	.749	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

2.2 Shaken condition

Descriptives

Yield

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
					Lower Bound	Upper Bound			
RAM1	3	.3633	.01528	.00882	.3254	.4013	.35	.38	
GOV9	3	.4300	.01732	.01000	.3870	.4730	.42	.45	
GOV15	3	.4733	.01528	.00882	.4354	.5113	.46	.49	
MAM4	3	.5467	.02082	.01202	.4950	.5984	.53	.57	
SPO4	3	.3433	.02082	.01202	.2916	.3950	.32	.36	
WAT11	3	.4567	.02082	.01202	.4050	.5084	.44	.48	
FET8	3	.5633	.01528	.00882	.5254	.6013	.55	.58	
LYC7	3	.3733	.01528	.00882	.3354	.4113	.36	.39	
SPO15	3	.3333	.02309	.01333	.2760	.3907	.32	.36	
WAT14	3	.4100	.01000	.00577	.3852	.4348	.40	.42	
BEL1	3	.6233	.02082	.01202	.5716	.6750	.60	.64	
BEL2	3	.4233	.01528	.00882	.3854	.4613	.41	.44	
FET4	3	.5733	.02082	.01202	.5216	.6250	.55	.59	
LAD1	3	.3300	.02000	.01155	.2803	.3797	.31	.35	
LYC8	3	.5600	.01000	.00577	.5352	.5848	.55	.57	
STA5	3	.4367	.01155	.00667	.4080	.4654	.43	.45	
WIL2	3	.5167	.00577	.00333	.5023	.5310	.51	.52	
MAG6	3	.3367	.02082	.01202	.2850	.3884	.32	.36	
SUG5	3	.4333	.02082	.01202	.3816	.4850	.41	.45	
SUG8	3	.3600	.01000	.00577	.3352	.3848	.35	.37	
GRA2	3	.4200	.02000	.01155	.3703	.4697	.40	.44	
GRA8	3	.6333	.02082	.01202	.5816	.6850	.61	.65	
JAV1	3	.4400	.01732	.01000	.3970	.4830	.43	.46	
JAV3	3	.6133	.01528	.00882	.5754	.6513	.60	.63	
MAG15	3	.4200	.01000	.00577	.3952	.4448	.41	.43	
MAM2	3	.6500	.01000	.00577	.6252	.6748	.64	.66	
PAP1	3	.5200	.01000	.00577	.4952	.5448	.51	.53	
RAM4	3	.3200	.01000	.00577	.2952	.3448	.31	.33	
WIL3	3	.4200	.01000	.00577	.3952	.4448	.41	.43	
Total	87	.4594	.10040	.01076	.4380	.4808	.31	.66	
Model Fixed Effects			.01637	.00175	.4559	.4629			
Random Effects				.01869	.4211	.4977			.01005

ANOVA

Yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.851	28	.030	113.529	.000
Within Groups	.016	58	.000		
Total	.867	86			

Yield

Duncan[a]

Static	N	Subset for alpha = 0.05											
		1	2	3	4	5	6	7	8	9	10	11	12
RAM4	3	.3200											
LAD1	3	.3300											
SPO15	3	.3333	.3333										
MAG6	3	.3367	.3367	.3367									
SPO4	3	.3433	.3433	.3433									
SUG8	3		.3600	.3600	.3600								
RAM1	3			.3633	.3633								
LYC7	3				.3733								
WAT14	3					.4100							
GRA2	3					.4200							
MAG15	3					.4200							
WIL3	3					.4200							
BEL2	3					.4233							
GOV9	3					.4300	.4300						
SUG5	3					.4333	.4333						
STA5	3					.4367	.4367						
JAV1	3					.4400	.4400						
WAT11	3						.4567	.4567					
GOV15	3							.4733					
WIL2	3								.5167				
PAP1	3								.5200	.5200			
MAM4	3									.5467	.5467		
LYC8	3										.5600		
FET8	3										.5633		
FET4	3										.5733		
JAV3	3											.6133	
BEL1	3											.6233	.6233
GRA8	3											.6333	.6333
MAM2	3												.6500
Sig.		.125	.072	.072	.353	.060	.079	.217	.804	.051	.072	.163	.063

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

3. Test carbon sources

Descriptives

yield

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
glucose		3	1.1720	.00900	.00520	1.1496	1.1944	1.16	1.18	
galactose		3	1.1140	.01411	.00814	1.0790	1.1490	1.10	1.13	
fructose		3	2.8720	.03118	.01800	2.7946	2.9494	2.85	2.91	
maltose		3	1.9073	.09335	.05390	1.6754	2.1392	1.85	2.02	
lactose		3	1.9940	.00781	.00451	1.9746	2.0134	1.99	2.00	
sucrose		3	2.6433	.07234	.04177	2.4636	2.8230	2.56	2.69	
ethanol		3	3.4283	.00569	.00328	3.4142	3.4425	3.42	3.43	
glycerol		3	2.8873	.06048	.03492	2.7371	3.0376	2.84	2.95	
mannitol		3	3.5060	.00100	.00058	3.5035	3.5085	3.51	3.51	
sorbitol		3	2.0850	.08664	.05002	1.8698	2.3002	1.99	2.15	
Total		30	2.3609	.81819	.14938	2.0554	2.6664	1.10	3.51	
Model	Fixed Effects			.05144	.00939	2.3413	2.3805			
	Random Effects				.26778	1.7552	2.9667			.71617

ANOVA

yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.360	9	2.151	813.039	.000
Within Groups	.053	20	.003		
Total	19.413	29			

yield

Duncan^a

Sugar	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
galactose	3	1.1140					
glucose	3	1.1720					
maltose	3		1.9073				
lactose	3		1.9940				
sorbitol	3			2.0850			
sucrose	3				2.6433		
fructose	3					2.8720	
glycerol	3					2.8873	
ethanol	3						3.4283
mannitol	3						3.5060
Sig.		.183	.052	1.000	1.000	.719	.079

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

4. BC production in optimized soybean whey-based medium and standard HS medium

Group Statistics

Medium			Statistic	Bootstrap[a]			
				Bias	Std. Error	95% Confidence Interval	
						Lower	Upper
yield	Optimized soybean whey-based medium	N	3				
		Mean	4.1433	-.0003[b]	.0079[b]	4.1300[b]	4.1600[b]
		Std. Deviation	.01528	-.00444[c]	.00618[c]	.00000[c]	.02121[c]
		Std. Error Mean	.00882				
	standard HS medium	N	3				
		Mean	1.1467	.0005[d]	.0127[d]	1.1200[d]	1.1700[d]
		Std. Deviation	.02517	-.00738[e]	.00973[e]	.00000[e]	.03536[e]
		Std. Error Mean	.01453				

a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples

b. Based on 983 samples

c. Based on 884 samples

d. Based on 987 samples

e. Based on 910 samples

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
yield	Equal variances assumed	.643	.468	176.308	4	.000	2.99667	.01700	2.94948	3.04386
	Equal variances not assumed			176.308	3.298	.000	2.99667	.01700	2.94523	3.04810

a. No statistics are computed for one or more split files

APPENDIX E

Characteristic of representative strains

1. Subgroup I : *K. rhaeticus*

1.1 Isolate RAM1

Colonies of isolate RAM1 were round with entire margin, convex elevation in center and with a diameter of 14.2-18.3 µm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, long rod-shaped, straight or slightly curved, approximately 1.8-3.4 µm long and 0.3-0.5 µm wide, and occurring individually or in pairs. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-xylose, L-arabinose, ethanol, glycerol and meso-erithritol. Cellulose was produced from D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-mannitol, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate showed 99.9% 16S rRNA gene sequence similarity to the type strain of *K. rheaticus*, and was isolated from rambutan.

2. Subgroup II: *K. obediens*

2.1 Isolate GOV9

Colonies of isolate GOV9 were circular with undulate margin, pulvinate elevation in center and with a diameter of 4.2-5.8 µm on standard HS agar. The surfaces of colonies were rough and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short rod-shaped, approximately 1.5-2.6 µm long and 0.6-0.7 µm wide, and occurring singly or in pairs. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-galactose, D-glucose, D-lactose, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, ethanol and glycerol. Cellulose was produced from D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate showed 99.6% 16S rRNA gene sequence similarity to the type strain of *K. obediens*, and was isolated from governor's plum.

2.2 Isolate GOV15

Colonies of isolate GOV15 were circular with entire margin, pulvinate elevation in center and with a diameter of 6.4-6.7 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 0.6-1.6 μm long and 0.5-0.6 μm wide, and occurring singly or in pairs. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, ethanol, glycerol and meso-erithritol. Cellulose was produced from D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate showed 99.6% 16S rRNA gene sequence similarity to the type strain of *K. obediens*, and was isolated from governor's plum.

2.3 Isolate MAM4

Colonies of isolate MAM4 were circular with entire margin, pulvinate elevation in center and with a diameter of 5.2-5.6 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 1.8-2.0 μm long and 0.5-0.7 μm wide, and occurring singly, in pairs or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabitol, D-fructose, D-galactose, D-glucose, D-maltose, D-mannitol, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-galactose, D-glucose, D-mannitol, D-sorbitol, D-xylose and ethanol. Cellulose was produced from D-arabitol, D-fructose, D-glucose, D-maltose, D-mannitol, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-rhamnose, L-sorbose, glycerol and meso-erythritol. The isolate showed 99.7% 16S rRNA gene sequence similarity to the type strain of *K. obediens*, and was isolated from mamao.

2.4 Isolate SPO4

Colonies of isolate SPO4 were circular with entire margin, umbonate elevation in center and with a diameter of 6.2-8.2 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short and long rod-shaped, approximately 2.0-3.0 μm long and 0.7-0.9 μm wide, and occurring singly, in pairs or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-galactose, D-glucose, D-lactose, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, ethanol, glycerol and meso-erithritol. Cellulose was produced from D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate showed 99.7% 16S rRNA gene sequence similarity to the type strain of *K. obediens*, and was isolated from sapodilla.

2.5 Isolate WAT11

Colonies of isolate WAT11 were circular with entire margin, umbonate elevation in center and with a diameter of 6.4-7.1 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 1.9-2.7 μm long and 0.5-0.8 μm wide, and occurring individually or in pairs. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-glucose, D-lactose, D-maltose, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, ethanol and glycerol. Cellulose was produced from D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate showed 99.7% 16S rRNA gene sequence similarity to the type strain of *K. obediens*, and was isolated from water melon.

3. Subgroup III: *K. sucrofermentans*

3.1 Isolate LYC7

Colonies of isolate LYC7 were circular with entire margin, convex elevation in center and with a diameter of 7.4-8.4 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short rod-shaped, approximately 1.5-2.1 μm long and 0.5-0.6 μm wide, and occurring singly or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol.

Acid was produced from D-fructose, D-glucose, D-lactose, D-mannitol, D-mannose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, ethanol, glycerol and meso-erithritol. Cellulose was produced from D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate showed 100% 16S rRNA gene sequence similarity to the type strain of *K. sucrofermentans*, and isolated from lychee.

3.2 Isolate FET8

Colonies of isolate FET8 were circular with entire margin, umbonate elevation in center and with a diameter of 6.7-8.4 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short rod-shaped, approximately 1.1-2.2 μm long and 0.4-0.6 μm wide, and occurring singly or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-fructose, D-glucose, D-lactose, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, ethanol, glycerol and meso-erithritol. Cellulose was produced from D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate showed 100% 16S rRNA gene sequence similarity to the type strain of *K. sucrofermentans*, and isolated from fetid passionflower.

4. Subgroup IV : *K. swingsii*

4.1 Isolate SPO15

Colonies of isolate SPO15 were circular with entire margin, pulvinate elevation in center and with a diameter of 5.4-7.1 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 1.2-2.9 μm long and 0.5-0.6

μm wide, and occurring singly or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-fructose, D-galactose, D-glucose, D-lactose, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, ethanol, glycerol and meso-erithritol. Cellulose was produced from D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate showed 99.9% 16S rRNA gene sequence similarity to the type strain of *K. swingsii*, and isolated from sapodilla.

4.2 Isolate WAT14

Colonies of isolate WAT14 were circular with undulate margin, umbonate elevation in center and with a diameter of 3.9-4.6 mm on standard HS agar. The surfaces of colonies were rough and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 0.8-1.4 μm long and 0.4-0.6 μm wide, and occurring singly or in pairs. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-mannose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, ethanol, glycerol and meso-erithritol. Cellulose was produced from D-arabitol, D-fructose, D-glucose, D-lactose, D-maltose, D-mannitol, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, glycerol and meso-erythritol. The isolate showed 99.8% 16S rRNA gene sequence similarity to the type strain of *K. swingsii*, and isolated from water melon.

5. Subgroup V : *K. hansenii*

5.1 Isolate BEL1

Colonies of isolate BEL1 were circular with entire margin, umbonate elevation in center and with a diameter of 9.6-12.7 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short rod-shaped, approximately 1.5-2.14 μm long and 0.6-0.9 μm wide, and occurring singly, in pairs or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-mannitol, D-mannose, D-melibiose, D-sorbitol, D-xylose, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-galactose, D-glucose, D-lactose, D- melibiose, D-sorbitol, D-xylose, L-arabinose, L-rhamnose and ethanol. Cellulose was produced from D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-mannitol, D-mannose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate showed 99.6% 16S rRNA gene sequence similarity to the type strain of *K. hansenii* and isolated from beleric myrobalan.

5.2 Isolate BEL2

Colonies of isolate BEL2 were circular with entire margin, umbonate elevation in center and with a diameter of 4.0-8.0 μm on standard HS agar. The surfaces of colonies were rough and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 1.2-1.5 μm long and 0.6-0.7 μm wide, and occurring singly. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-maltose, D-mannitol, D-melibiose, D-raffinose, and D-sucrose. Acid was produced from D-arabinose, D-galactose, D-glucose, D-melibiose, D- sorbitol, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol and meso-erithritol. Cellulose was produced from D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-maltose, D-mannitol, D-melibiose, D-raffinose, D-sorbitol, D-xylose, L-arabinose, L-rhamnose, L-sorbose, glycerol and meso-erythritol. The isolate showed 99.6% 16S rRNA gene sequence similarity to the type strain of *K. hansenii* and isolated from beleric myrobalan.

5.3 Isolate FET4

Colonies of isolate FET4 were circular with entire margin, umbonate elevation in center and with a diameter of 5.7-6.5 μm on standard HS agar. The surfaces of colonies were rough and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 1.1-1.7 μm long and 0.4-0.5 μm wide, and occurring singly or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-galactose, D-glucose, D-lactose, D-maltose, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, ethanol, glycerol and meso-erithritol. Cellulose was produced from D-arabitol, D-fructose, D-galactose, D-glucose, D-mannitol and glycerol. The isolate showed 99.6% 16S rRNA gene sequence similarity to the type strain of *K. hansenii* and isolated from fetid passionflower.

5.4 Isolate LAD1

Colonies of isolate LAD1 were circular with undulate margin, convex elevation in center and with a diameter of 11.9-16.0 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 1.0-1.7 μm long and 0.3-0.5 μm wide, and occurring singly or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-mannitol, D-mannose, D-melibiose, D-sorbitol, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-galactose, D-glucose, D-lactose, D-mannitol, D-mannose, D-melibiose, D-sorbitol, D-xylose, L-arabinose, L-rhamnose, ethanol and meso-erithritol. Cellulose was produced from D-arabitol, D-fructose, D-galactose, D-glucose, D-mannitol, D-sorbitol, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate showed 99.7% 16S rRNA gene sequence similarity to the type strain of *K. hansenii* and isolated from lady's finger banana.

5.5 Isolate STA5

Colonies of isolate STA5 were circular with undulate margin, umbonate elevation in center and with a diameter of 9.6-13.4 μm on standard HS agar. The surfaces of colonies were rough and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short and long rod-shaped, approximately 1.2-2.6 μm long and 0.4-0.8 μm wide, and occurring singly. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-mannitol, D-mannose, D-melibiose, D-xylose, L-arabinose, ethanol, glycerol and meso-erythritol. Acid was produced from D-galactose, D-glucose, D-lactose, D-mannose, D-melibiose, D-sorbitol, D-xylose, L-arabinose, L-rhamnose, ethanol and meso-erithritol. Cellulose was produced from D-arabitol, D-fructose, D-glucose, D-lactose, D-mannitol, D-mannose, D-melibiose, glycerol and meso-erythritol. The isolate showed 99.8% 16S rRNA gene sequence similarity to the type strain of *K. hansenii* and isolated from star fruit.

5.6 Isolate WIL2

Colonies of isolate WIL2 were circular with entire margin, pulvinate elevation in center and with a diameter of 5.1-6.5 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short rod-shaped, approximately 1.6-2.1 μm long and 0.6-1.2 μm wide, and occurring singly, in pairs or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabitol, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, ethanol, glycerol and meso-erythritol. Acid was produced form D-galactose, D-glucose, D-lactose, D-mannitol, D-mannose, D-melibiose, D-sorbitol, D-xylose, L-arabinose, L-rhamnose, ethanol and meso-erithritol. Cellulose was produced from D-arabitol, D-fructose, D-glucose, D-mannitol, D-raffinose, D-sorbitol, D-sucrose, glycerol and meso-erythritol. The isolate showed 99.6% 16S rRNA gene sequence similarity to the type strain of *K. hansenii* and isolated from wild lemon.

5.7 Isolate LYC8

Colonies of isolate LYC8 were circular with undulate margin, umbonate elevation in center and with a diameter of 15.7-18 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 1.1-2.2 μm long and 0.6-0.7 μm wide, and occurring singly or in pairs. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, ethanol, glycerol and meso-erithritol. Cellulose was produced from D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate showed 99.7% 16S rRNA gene sequence similarity to the type strain of *K. hansenii* and isolated from lychee.

6. Subgroup VI : Unidentified

6.1 Subgroup VIa

6.1.1 Isolate MAG6

Colonies of isolate MAG6 were circular with entire margin, pulvinate elevation in center and with a diameter of 9.7-11.9 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 1.4-2.5 μm long and 0.5-0.8 μm wide, and occurring singly or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-galactose, D-glucose, D-lactose, D-mannose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, glycerol and meso-erithritol. Cellulose was produced from D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, glycerol and meso-erythritol. The isolate was isolated from mangosteen.

6.1.2. Isolate SUG5

Colonies of isolate SUG5 were circular with entire margin, convex elevation in center and with a diameter of 4.4-6.6 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 1.1-1.5 μm long and 0.3-0.5 μm wide, and occurring singly, in pairs or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-fructose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, ethanol and glycerol. Cellulose was produced D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, glycerol and meso-erythritol. The isolate was isolated from sugar apple.

6.1.3. Isolate SUG8

Colonies of isolate SUG8 were circular with undulate margin, pulvinate elevation in center and with a diameter of 12.6-14.2 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 1.1-1.3 μm long and 0.5-0.6 μm wide, and occurring singly, in pairs or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, ethanol, glycerol and meso-erithritol. Cellulose was produced D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate was isolated from sugar apple.

6.2 Subgroup VIb

6.2.1. Isolate GRA2

Colonies of isolate GRA2 were circular with entire margin, convex elevation in center and with a diameter of 5.1-7.1 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short rod-shaped, approximately 1.4-2.4 μm long and 0.5-0.7 μm wide, and occurring singly or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, glycerol and meso-erythritol. Acid was produced from D-galactose, D-glucose, D-lactose, D-maltose, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, glycerol and meso-erithritol. Cellulose was produced D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, glycerol and meso-erythritol. The isolate was isolated from grape.

6.2.2. Isolate GRA8

Colonies of isolate GRA8 were circular with entire margin, convex elevation in center and with a diameter of 6.3-7.0 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 0.9-1.6 μm long and 0.5-0.6 μm wide, and occurring singly or in pairs. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-sorbose, glycerol and meso-erithritol. Cellulose was produced D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate was isolated from grape.

6.2.3. Isolate JAV1

Colonies of isolate JAV1 were circular with entire margin, convex elevation in center and with a diameter of 7.9-11.1 µm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short rod-shaped, approximately 1.1-1.2 µm long and 0.5-0.7 µm wide, and occurring singly or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, glycerol and meso-erythritol. Acid was produced from D-glucose, D-mannose, D-melibiose, D-sorbitol and L-arabinose. Cellulose was produced D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, glycerol and meso-erythritol. The isolate was isolated from java plum.

6.2.4. Isolate JAV3

Colonies of isolate JAV3 were circular with entire margin, pulvinate elevation in center and with a diameter of 11.5-15.3 µm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short rod-shaped, approximately 1.5-2.4 µm long and 0.5-0.7 µm wide, and occurring singly, in pairs or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-galactose, D-glucose, D-lactose, D-mannitol, D-mannose, D-melibiose, D-sorbitol, D-xylose, L-arabinose, L-rhamnose, ethanol and meso-erithritol. Cellulose was produced D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, glycerol and meso-erythritol. The isolate was isolated from java plum.

6.2.5. Isolate MAG15

Colonies of isolate MAG15 were circular with entire margin, pulvinate elevation in center and with a diameter of 7.0-7.7 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, ellipsoidal to short rod-shaped, approximately 1.0-1.4 μm long and 0.4-0.5 μm wide, and occurring singly, in pairs or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose and meso-erithritol. Cellulose was produced D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, glycerol and meso-erythritol. The isolate was isolated from mangosteen.

6.2.6 Isolate MAM2

Colonies of isolate MAM2 were circular with entire margin, umbonate elevation in center and with a diameter of 4.1-4.9 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short and long rod-shaped, approximately 1.5-1.9 μm long and 0.6-0.9 μm wide, and occurring singly or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-galactose, D-glucose, D-mannitol, D-sorbitol, D-xylose, L-arabinose, ethanol and glycerol. Cellulose was produced D-arabinose, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, glycerol and meso-erythritol. The isolate was isolated from mamao.

6.2.7 Isolate PAP1 (Chapter 4).

6.2.8. Isolate RAM4

Colonies of isolate RAM4 were irregular with undulate margin, convex elevation in center and with a diameter of 12.4-14.4 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, long rod-shaped, approximately 2.8-5.2 μm long and 0.6-0.7 μm wide, and occurring singly, in pairs or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-galactose, D-glucose, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, ethanol, glycerol and meso-erithritol. Cellulose was produced D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-rhamnose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate was isolated from rambutan.

6.2.9. Isolate WIL3

Colonies of isolate WIL3 were circular with undulate margin, pulvinate elevation in center and with a diameter of 12.4-14.4 μm on standard HS agar. The surfaces of colonies were smooth and their colors were cream. The colonies were mucous and covered with sticky substances. Cells were Gram-negative, short rod-shaped, approximately 1.1-1.5 µm long and 0.4-0.7 µm wide, and occurring singly or in short chains. Catalase-positive. Growth was weakly at pH 3.0, 3.5 and 4.0, but more efficient at pH 4.5-7.0. This isolate could not grow on either sorbitol or methanol medium but grew well on glucose or sucrose medium. Growth was observed on 30% D-glucose and without acetic acid. Growth occurred on D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-sorbose, ethanol, glycerol and meso-erythritol. Acid was produced from D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-sorbose, ethanol, glycerol and meso-erithritol. Cellulose was produced D-arabinose, D-arabitol, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-sucrose, D-xylose, L-arabinose, L-sorbose, ethanol, glycerol and meso-erythritol. The isolate was isolated from wild lemon.

APPENDIX F

Sequence alignment of isolated strains

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                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                      5         15         25         35         45
RAM1                          TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
K. rhaeticus DST GL02^T       TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
GOV9                          TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
GOV15                         TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
MAM4                          TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
SPO4                          TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
WAT11                         TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
K. oboediens LTH 2460^T       CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
LYC7                          CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
FET8                          CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
K. sucrofermentans BPR 2001^T CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
SPO15                         TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
WAT14                         TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
K. swingsii  DST GL01^T       TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
BEL1                          TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
BEL2                          TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
FET4                          TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
LAD1                          TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
LYC8                          TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
STA5                          TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
WIL2                          TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
K. hansenii NCIMB 8746^T      TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
MAG6                          CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
SUG5                          CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
SUG8                          CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
GRA2                          CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
GRA8                          CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
JAV1                          CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
JAV3                          CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
MAG15                         CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
MAM2                          CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
PAP1                          CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
RAM4                          CGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
WIL3                          TGGGTGGGGG ATAACTTGGG AAACTGAAGC TAATACCGCA TGACACCTGA
Clustal consensus              ********* ********** ********** ********** **********

                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                     55         65         75         85         95
RAM1                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
K. rhaeticus DST GL02^T       GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
GOV9                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
GOV15                         GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
MAM4                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
SPO4                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
WAT11                         GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
K. oboediens LTH 2460^T       GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
LYC7                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
FET8                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
K. sucrofermentans BPR 2001^T GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
SPO15                         GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
WAT14                         GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
K. swingsii  DST GL01^T       GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
BEL1                          GGGTCAAAGG CGCGAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
BEL2                          GGGTCAAAGG CGCGAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
FET4                          GGGTCAAAGG CGCGAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
LAD1                          GGGTCAAAGG CGCGAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
LYC8                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
STA5                          GGGTCAAAGG CGCGAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
WIL2                          GGGTCAAAGG CGCGAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
K. hansenii NCIMB 8746^T      GGGTCAAAGG CGCGAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
MAG6                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
SUG5                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
SUG8                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
GRA2                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
GRA8                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
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JAV1                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
JAV3                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
MAG15                         GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
MAM2                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
PAP1                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
RAM4                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
WIL3                          GGGTCAAAGG CGCAAGTCGC CTGTGGAGGA ACCTGCGTTC GATTAGCTAG
Clustal consensus             ********** *** ****** ********** ********** **********

                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                    105        115        125        135        145
RAM1                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
K. rhaeticus DST GL02^T       TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
GOV9                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
GOV15                         TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
MAM4                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
SPO4                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
WAT11                         TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
K. oboediens LTH 2460^T       TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
LYC7                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
FET8                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
K. sucrofermentans BPR 2001^T TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
SPO15                         TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
WAT14                         TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
K. swingsii DST GL01^T        TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
BEL1                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
BEL2                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
FET4                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
LAD1                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
STA5                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
WIL2                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
K. hansenii NCIMB 8746^T      TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
MAG6                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
SUG5                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
SUG8                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
GRA2                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
GRA8                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
JAV1                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
JAV3                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
MAG15                         TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
MAM2                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
PAP1                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
RAM4                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
WIL3                          TTGGTGGGGT AAAGGCCTAC CAAGGCGATG ATCGATAGCT GGTCTGAGAG
Clustal consensus             ********** ********** ********** ********** **********

                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                    155        165        175        185        195
RAM1                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
K. rhaeticus DST GL02^T       GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
GOV9                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
GOV15                         GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
MAM4                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
SPO4                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
WAT11                         GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
K. oboediens LTH 2460^T       GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
LYC7                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
FET8                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
K. sucrofermentans BPR 2001^T GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
SPO15                         GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
WAT14                         GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
K. swingsii DST GL01^T        GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
BEL1                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
BEL2                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
FET4                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
LAD1                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
LYC8                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
STA5                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
WIL2                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
K. hansenii NCIMB 8746^T      GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
MAG6                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
SUG5                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
SUG8                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
GRA2                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
GRA8                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
JAV1                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
JAV3                          GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
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MAG15                             GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
MAM2                              GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
PAP1                              GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
RAM4                              GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
WIL3                              GATGATCAGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG
Clustal consensus                 ********** ********** ********** ********** **********

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                        205        215        225        235        245
RAM1                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
K. rhaeticus DST GL02^T           CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
GOV9                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
GOV15                             CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
MAM4                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
SPO4                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
WAT11                             CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
K. oboediens LTH 2460^T           CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
LYC7                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
FET8                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
K. sucrofermentans BPR 2001^T     CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
SPO15                             CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
WAT14                             CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
K. swingsii DST GL01^T            CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
BEL1                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
BEL2                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
FET4                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
LAD1                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
LYC8                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
STA5                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
WIL2                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
K. hansenii NCIMB 8746^T          CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
MAG6                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
SUG5                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
SUG8                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
GRA2                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
GRA8                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
JAV1                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
JAV3                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
MAG15                             CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
MAM2                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
PAP1                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
RAM4                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
WIL3                              CAGCAGTGGG GAATATTGGA CAATGGGCGC AAGCTGATCC AGCAATGCCG
Clustal consensus                 ********** ********** ********** ********** **********

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                        255        265        275        285        295
RAM1                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
K. rhaeticus DST GL02^T           CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
GOV9                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
GOV15                             CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
MAM4                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
SPO4                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
WAT11                             CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
K. oboediens LTH 2460^T           CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGATGAT
LYC7                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
FET8                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
K. sucrofermentans BPR 2001^T     CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
SPO15                             CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
WAT14                             CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
K. swingsii DST GL01^T            CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
BEL1                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
BEL2                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
FET4                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
LAD1                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
LYC8                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
STA5                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
WIL2                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
K. hansenii NCIMB 8746^T          CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
MAG6                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
SUG5                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
SUG8                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
GRA2                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
GRA8                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
JAV1                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
JAV3                              CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
MAG15                             CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
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MAM2                             CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
PAP1                             CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
RAM4                             CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
WIL3                             CGTGTGTGAA GAAGGTTTTC GGATTGTAAA GCACTTTCAG CGGGGACGAT
Clustal consensus                ********** ********** ********** ********** ****** ***

                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                       305        315        325        335        345
RAM1                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
K. rhaeticus DST GL02T           GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
GOV9                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
GOV15                            GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
MAM4                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
SPO4                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
WAT11                            GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
K. oboediens LTH 2460T           GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
LYC7                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
FET8                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
K. sucrofermentans BPR 2001T     GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
SPO15                            GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
WAT14                            GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
K. swingsii  DST GL01T           GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
BEL1                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
BEL2                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
FET4                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
LAD1                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
LYC8                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
STA5                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
WIL2                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
K. hansenii NCIMB 8746T          GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
MAG6                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
SUG5                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
SUG8                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
GRA2                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
GRA8                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
JAV1                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
JAV3                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
MAG15                            GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
MAM2                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
PAP1                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
RAM4                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
WIL3                             GATGACGGTA CCCGCAGAAG AAGCCCCGGC TAACTTCGTG CCAGCAGCCG
Clustal consensus                ********** ********** ********** ********** **********

                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                       355        365        375        385        395
RAM1                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
K. rhaeticus DST GL02T           CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
GOV9                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
GOV15                            CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
MAM4                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
SPO4                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
WAT11                            CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
K. oboediens LTH 2460T           CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
LYC7                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
FET8                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
K. sucrofermentans BPR 2001T     CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
SPO15                            CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
WAT14                            CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
K. swingsii  DST GL01T           CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
BEL1                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
BEL2                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
FET4                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
LAD1                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
LYC8                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
STA5                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
WIL2                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
K. hansenii NCIMB 8746T          CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
MAG6                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
SUG5                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
SUG8                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
GRA2                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
GRA8                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
JAV1                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
JAV3                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
MAG15                            CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
MAM2                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
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PAP1                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
RAM4                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
WIL3                             CGGTAATACG AAGGGGGCAA GCGTTGCTCG GAATGACTGG GCGTAAAGGG
Clustal consensus                ********** ********** ********** ********** **********

                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                       405        415        425        435        445
RAM1                             CGCGTAGGCG GTTGTTACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
K. rhaeticus DST GL02T           CGCGTAGGCG GTTGTTACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
GOV9                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
GOV15                            CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
MAM4                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
SPO4                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
WAT11                            CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
K. oboediens LTH 2460T           CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
LYC7                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
FET8                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
K. sucrofermentans BPR 2001T     CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
SPO15                            CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
WAT14                            CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
K. swingsii  DST GL01T           CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
BEL1                             CGCGTAGGCG GTTGTTACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
BEL2                             CGCGTAGGCG GTTGTTACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
FET4                             CGCGTAGGCG GTTGTTACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
LAD1                             CGCGTAGGCG GTTGTTACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
LYC8                             CGCGTAGGCG GTTGTTACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
STA5                             CGCGTAGGCG GTTGTTACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
WIL2                             CGCGTAGGCG GTTGTTACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
K. hansenii NCIMB 8746T          CGCGTAGGCG GTTGTTACAG TCAGATGTGA AATTCCCGGG CTTAACCTGG
MAG6                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
SUG5                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
SUG8                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
GRA2                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
GRA8                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
JAV1                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
JAV3                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
MAG15                            CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
MAM2                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
PAP1                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
RAM4                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
WIL3                             CGCGTAGGCG GTTGACACAG TCAGATGTGA AATTCCTGGG CTTAACCTGG
Clustal consensus                ********** ****  **** ********** ****** *** **********

                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                       455        465        475        485        495
RAM1                             GGGCTGCATT TGATACGTGA TGACTAGAGT GTGAGAGAGG GTTGTGGAAT
K. rhaeticus DST GL02T           GGGCTGCATT TGATACGTGA TGACTAGAGT GTGAGAGAGG GTTGTGGAAT
GOV9                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
GOV15                            GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGA GTTGTGGAAT
MAM4                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
SPO4                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
WAT11                            GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
K. oboediens LTH 2460T           GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
LYC7                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
FET8                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
K. sucrofermentans BPR 2001T     GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
SPO15                            GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
WAT14                            GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
K. swingsii  DST GL01T           GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
BEL1                             GGGCTGCATT TGATACGTGA CGACTAGAGT GTGAGAGAGA GTTGTGGAAT
BEL2                             GGGCTGCATT TGATACGTGA CGACTAGAGT GTGAGAGAGA GTTGTGGAAT
FET4                             GGGCTGCATT TGATACGTGA CGACTAGAGT GTGAGAGAGA GTTGTGGAAT
LAD1                             GGGCTGCATT TGATACGTGA CGACTAGAGT GTGAGAGAGA GTTGTGGAAT
LYC8                             GGGCTGCATT TGATACGTGA CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
STA5                             GGGCTGCATT TGATACGTGA CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
WIL2                             GGGCTGCATT TGATACGTGA CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
K. hansenii NCIMB 8746T          GGGCTGCATT TGATACGTGA CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
MAG6                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
SUG5                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
SUG8                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
GRA2                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
GRA8                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
JAV1                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
JAV3                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
MAG15                            GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
MAM2                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
PAP1                             GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
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RAM4                            GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
WIL3                            GGGCTGCATT TGATACGTGG CGACTAGAGT GTGAGAGAGG GTTGTGGAAT
Clustal consensus               ********** *********   ********* *********   *********

                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                      505        515        525        535        545
RAM1                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
K. rhaeticus DST GL02T          TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
GOV9                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
GOV15                           TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
MAM4                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
SPO4                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
WAT11                           TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
K. oboediens LTH 2460T          TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
LYC7                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
FET8                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
K. sucrofermentans BPR 2001T    TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
SPO15                           TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
WAT14                           TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
K. swingsii DST GL01T           TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
BEL1                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
BEL2                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
FET4                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
LAD1                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
LYC8                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
STA5                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
WIL2                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
K. hansenii NCIMB 8746T         TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
MAG6                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
SUG5                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
SUG8                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
GRA2                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
GRA8                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
JAV1                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
JAV3                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
MAG15                           TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
MAM2                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
PAP1                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
RAM4                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
WIL3                            TCCCAGTGTA GAGGTGAAAT TCGTAGATAT TGGGAAGAAC ACCGGTGGCG
Clustal consensus               ********** ********** ********** ********** **********

                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                      555        565        575        585        595
RAM1                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
K. rhaeticus DST GL02T          AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
GOV9                            AAGGCGGCAG CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
GOV15                           AAGGCGGCAG CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
MAM4                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
SPO4                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
WAT11                           AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
K. oboediens LTH 2460T          AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
LYC7                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
FET8                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
K. sucrofermentans BPR 2001T    AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
SPO15                           AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
WAT14                           AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
K. swingsii DST GL01T           AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
BEL1                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
BEL2                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
FET4                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
LAD1                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
LYC8                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
STA5                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
WIL2                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
K. hansenii NCIMB 8746T         AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGT
MAG6                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
SUG5                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
SUG8                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
GRA2                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
GRA8                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
JAV1                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
JAV3                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
MAG15                           AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
MAM2                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
PAP1                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
RAM4                            AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
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WIL3                              AAGGCGGCAA CCTGGCTCAT GACTGACGCT GAGGCGCGAA AGCGTGGGGA
Clustal consensus                 *********  ********** ********** ********** *********

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                        605        615        625        635        645
RAM1                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
K. rhaeticus DST GL02T            GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
GOV9                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
GOV15                             GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
MAM4                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
SPO4                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
WAT11                             GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
K. oboediens LTH 2460T            GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
LYC7                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
FET8                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
K. sucrofermentans BPR 2001T      GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
SPO15                             GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
WAT14                             GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
K. swingsii  DST GL01T            GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
BEL1                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
BEL2                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
FET4                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
LAD1                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
LYC8                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
STA5                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
WIL2                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
K. hansenii NCIMB 8746T           GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
MAG6                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
SUG5                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
SUG8                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
GRA2                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
GRA8                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
JAV1                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
JAV3                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
MAG15                             GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
MAM2                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
PAP1                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
RAM4                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
WIL3                              GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC GATGTGTGCT
Clustal consensus                 ********** ********** ********** ********** **********

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                        655        665        675        685        695
RAM1                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
K. rhaeticus DST GL02T            GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
GOV9                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
GOV15                             GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
MAM4                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
SPO4                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
WAT11                             GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
K. oboediens LTH 2460T            GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
LYC7                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
FET8                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
K. sucrofermentans BPR 2001T      GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
SPO15                             GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
WAT14                             GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
K. swingsii  DST GL01T            GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
BEL1                              GGATGTTGGA TGGCTTGGCC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
BEL2                              GGATGTTGGA TGGCTTGGCC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
FET4                              GGATGTTGGA TGGCTTGGCC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
LAD1                              GGATGTTGGA TGGCTTGGCC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
LYC8                              GGATGTTGGA TGGCTTGGCC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
STA5                              GGATGTTGGA TGGCTTGGCC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
WIL2                              GGATGTTGGA TGGCTTGGCC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
K. hansenii NCIMB 8746T           GGATGTTGGA TGGCTTGGCC ATTCGGTGTC GTAGTTAACG CGATAAGCAC
MAG6                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
SUG5                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
SUG8                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
GRA2                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
GRA8                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
JAV1                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
JAV3                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
MAG15                             GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
MAM2                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
PAP1                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
RAM4                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
WIL3                              GGATGTTGGG TGACTTTGTC ATTCAGTGTC GTAGTTAACG CGATAAGCAC
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Clustal consensus                 *********  ** *** * * **** ***** ********** **********

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         705        715        725        735        745
RAM1                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
K. rhaeticus DST GL02^T           ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
GOV9                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
GOV15                             ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
MAM4                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
SPO4                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
WAT11                             ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
K. oboediens LTH 2460^T           ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
LYC7                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
FET8                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
K. sucrofermentans BPR 2001^T     ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
SPO15                             ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
WAT14                             ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
K. swingsii  DST GL01^T           ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
BEL1                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
BEL2                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
FET4                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
LAD1                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
LYC8                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
STA5                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
WIL2                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
K. hansenii NCIMB 8746^T          ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
MAG6                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
SUG5                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
SUG8                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
GRA2                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
GRA8                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
JAV1                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
JAV3                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
MAG15                             ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
MAM2                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
PAP1                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
RAM4                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
WIL3                              ACCGCCTGGG GAGTACGGCG CAAGGTTGAA ACTCAAAGGA ATTGACGGGG
Clustal consensus                 ********** ********** ********** ********** **********

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         755        765        775        785        795
RAM1                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
K. rhaeticus DST GL02^T           GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
GOV9                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
GOV15                             GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
MAM4                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
SPO4                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
WAT11                             GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
K. oboediens LTH 2460^T           GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
LYC7                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
FET8                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
K. sucrofermentans BPR 2001^T     GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
SPO15                             GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
WAT14                             GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
K. swingsii  DST GL01^T           GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
BEL1                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
BEL2                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
FET4                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
LAD1                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
LYC8                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
STA5                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
WIL2                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
K. hansenii NCIMB 8746^T          GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
MAG6                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
SUG5                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
SUG8                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
GRA2                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
GRA8                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
JAV1                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
JAV3                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
MAG15                             GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
MAM2                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
PAP1                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
RAM4                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
WIL3                              GCCCGCACAA GCGGTGGAGC ATGTGGTTTA ATTCGAAGCA ACGCGCAGAA
Clustal consensus                 ********** ********** ********** ********** **********
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                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                   805        815        825        835        845
RAM1                          CCTTACCAGG GCTTGCATGC GGAGGCTGTG TCCAGAGATG GGCATTTCTC
K. rhaeticus DST GL02T        CCTTACCAGG GCTTGCATGC GGAGGCTGTG TCCAGAGATG GGCATTTCTC
GOV9                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
GOV15                         CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
MAM4                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
SPO4                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
WAT11                         CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
K. oboediens LTH 2460T        CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
LYC7                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
FET8                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
K. sucrofermentans BPR 2001T  CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
SPO15                         CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
WAT14                         CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
K. swingsii DST GL01T         CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
BEL1                          CCTTACCAGG ACTTGCATGC GGAGGCTGTG TCCAGAGATG GACATTTCTC
BEL2                          CCTTACCAGG ACTTGCATGC GGAGGCTGTG TCCAGAGATG GACATTTCTC
FET4                          CCTTACCAGG ACTTGCATGC GGAGGCTGTG TCCAGAGATG GACATTTCTC
LAD1                          CCTTACCAGG ACTTGCATGC GGAGGCTGTG TCCAGAGATG GGCATTTCTC
LYC8                          CCTTACCAGG ACTTGCATGC GGAGGCTGTG TCCAGAGATG GGCATTTCTC
STA5                          CCTTACCAGG ACTTGCATGC GGAGGCTGTG TCCAGAGATG GGCATTTCTC
WIL2                          CCTTACCAGG ACTTGCATGC GGAGGCTGTG TCCAGAGATG GGCATTTCTC
K. hansenii NCIMB 8746T       CCTTACCAGG ACTTGCATGC GGAGGCTGTG TCCAGAGATG GGCATTTCTC
MAG6                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
SUG5                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
SUG8                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
GRA2                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
GRA8                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
JAV1                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
JAV3                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
MAG15                         CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
MAM2                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
PAP1                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
RAM4                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
WIL3                          CCTTACCAGG GCTTGCATGC GGAGGCCGTG TCCAGAGATG GGCATTTCTC
Clustal consensus             **********  ********* ****** *** ********** * ********

                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                   855        865        875        885        895
RAM1                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
K. rhaeticus DST GL02T        GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
GOV9                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
GOV15                         GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
MAM4                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
SPO4                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
WAT11                         GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
K. oboediens LTH 2460T        GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
LYC7                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
FET8                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
K. sucrofermentans BPR 2001T  GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
SPO15                         GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
WAT14                         GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
K. swingsii DST GL01T         GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
BEL1                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
BEL2                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
FET4                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
LAD1                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
LYC8                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
STA5                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
WIL2                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
K. hansenii NCIMB 8746T       GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
MAG6                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
SUG5                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
SUG8                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
GRA2                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
GRA8                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
JAV1                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
JAV3                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
MAG15                         GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
MAM2                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
PAP1                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
RAM4                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
WIL3                          GCAAGAGACC TCCAGCACAG GTGCTGCATG GCTGTCGTCA GCTCGTGTCG
Clustal consensus             ********** ********** ********** ********** **********
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                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                     905        915        925        935        945
RAM1                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
K. rhaeticus DST GL02T          TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
GOV9                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
GOV15                           TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
MAM4                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
SPO4                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
WAT11                           TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
K. oboediens LTH 2460T          TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
LYC7                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
FET8                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
K. sucrofermentans BPR 2001T    TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
SPO15                           TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
WAT14                           TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
K. swingsii  DST GL01T          TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
BEL1                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
BEL2                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
FET4                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
LAD1                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
LYC8                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
STA5                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
WIL2                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
K. hansenii NCIMB 8746T         TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
MAG6                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
SUG5                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
SUG8                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
GRA2                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
GRA8                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
JAV1                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
JAV3                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
MAG15                           TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
MAM2                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
PAP1                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
RAM4                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
WIL3                            TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTCG CCTTTAGTTG
Clustal consensus               ********** ********** ********** ********** **********

                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                     955        965        975        985        995
RAM1                            CCATCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
K. rhaeticus DST GL02T          CCATCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
GOV9                            CCATCACGTT TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
GOV15                           CCATCACGTT TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
MAM4                            CCATCACGTT TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
SPO4                            CCATCACGTT TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
WAT11                           CCATCACGTT TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
K. oboediens LTH 2460T          CCATCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
LYC7                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
FET8                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
K. sucrofermentans BPR 2001T    CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
SPO15                           CCATCACGTT TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
WAT14                           CCATCACGTT TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
K. swingsii  DST GL01T          CCATCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
BEL1                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
BEL2                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
FET4                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
LAD1                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
LYC8                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
STA5                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
WIL2                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
K. hansenii NCIMB 8746T         CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
MAG6                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
SUG5                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
SUG8                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
GRA2                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
GRA8                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
JAV1                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
JAV3                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
MAG15                           CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
MAM2                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
PAP1                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
RAM4                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
WIL3                            CCAGCACGTC TGGGTGGGCA CTCTAAAGGA ACTGCCGGTG ACAAGCCGGA
Clustal consensus               *** *****  ********** ********** ********** **********
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                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1005       1015       1025       1035       1045
RAM1                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
K. rhaeticus DST GL02T            GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
GOV9                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
GOV15                             GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
MAM4                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
SPO4                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
WAT11                             GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
K. oboediens LTH 2460T            GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
LYC7                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
FET8                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
K. sucrofermentans BPR 2001T      GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
SPO15                             GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
WAT14                             GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
K. swingsii  DST GL01T            GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
BEL1                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
BEL2                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
FET4                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
LAD1                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
LYC8                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
STA5                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
WIL2                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
K. hansenii NCIMB 8746T           GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
MAG6                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
SUG5                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
SUG8                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
GRA2                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
GRA8                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
JAV1                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
JAV3                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
MAG15                             GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
MAM2                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
PAP1                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
RAM4                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
WIL3                              GGAAGGTGGG GATGACGTCA AGTCCTCATG GCCCTTATGT CCTGGGCTAC
Clustal consensus                 ********** ********** ********** ********** **********

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1055       1065       1075       1085       1095
RAM1                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
K. rhaeticus DST GL02T            ACACGTGCTA CAATGACGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
GOV9                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
GOV15                             ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
MAM4                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
SPO4                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
WAT11                             ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
K. oboediens LTH 2460T            ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
LYC7                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTAGC GATACCGAGC
FET8                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTAGC GATACCGAGC
K. sucrofermentans BPR 2001T      ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTAGC GATACCGAGC
SPO15                             ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTAGC GATACCGAGC
WAT14                             ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTAGC GATACCGAGC
K. swingsii  DST GL01T            ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTAGC GATACCGAGC
BEL1                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGCAGC GATGCCGAGC
BEL2                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGCAGC GATGCCGAGC
FET4                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGCAGC GATGCCGAGC
LAD1                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGCAGC GATGCCGAGC
LYC8                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGCAGC GATGCCGAGC
STA5                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGCAGC GATGCCGAGC
WIL2                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGCAGC GATGCCGAGC
K. hansenii NCIMB 8746T           ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGCAGC GATGCCGAGC
MAG6                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTAGT GATACCGAGC
SUG5                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTAGT GATACCGAGC
SUG8                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTAGT GATACCGAGC
GRA2                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
GRA8                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
JAV1                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
JAV3                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
MAG15                             ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
MAM2                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
PAP1                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
RAM4                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
WIL3                              ACACGTGCTA CAATGGCGGT GACAGTGGGA AGCAGGTGGT GACACCGAGC
Clustal consensus                 ********** ***** **** ********** ******  *  **  ******
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                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1105       1115       1125       1135       1145
RAM1                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
K. rhaeticus DST GL02T            CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
GOV9                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
GOV15                             CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
MAM4                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
SPO4                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
WAT11                             CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
K. oboediens LTH 2460T            CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
LYC7                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
FET8                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
K. sucrofermentans BPR 2001T      CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
SPO15                             CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
WAT14                             CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
K. swingsii  DST GL01T            CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
BEL1                              GGATCTCCAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
BEL2                              GGATCTCCAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
FET4                              GGATCTCCAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
LAD1                              GGATCTCCAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
LYC8                              GGATCTCCAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
STA5                              GGATCTCCAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
WIL2                              GGATCTCCAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
K. hansenii NCIMB 8746T           GGATCTCCAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
MAG6                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
SUG5                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
SUG8                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
GRA2                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
GRA8                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
JAV1                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
JAV3                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
MAG15                             CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
MAM2                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
PAP1                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
RAM4                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
WIL3                              CGATCTCAAA AAGCCGTCTC AGTTCGGATT GCACTCTGCA ACTCGAGTGC
Clustal consensus                  ****** ** ********** ********** ********** **********

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1155       1165       1175       1185       1195
RAM1                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
K. rhaeticus DST GL02T            ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
GOV9                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
GOV15                             ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
MAM4                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
SPO4                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
WAT11                             ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
K. oboediens LTH 2460T            ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
LYC7                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
FET8                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
K. sucrofermentans BPR 2001T      ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
SPO15                             ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
WAT14                             ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
K. swingsii  DST GL01T            ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
BEL1                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
BEL2                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
FET4                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
LAD1                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
LYC8                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
STA5                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
WIL2                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
K. hansenii NCIMB 8746T           ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
MAG6                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
SUG5                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
SUG8                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
GRA2                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
GRA8                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
JAV1                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
JAV3                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
MAG15                             ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
MAM2                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
PAP1                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
RAM4                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
WIL3                              ATGAAGGTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG CGGTGAATAC
Clustal consensus                 ********** ********** ********** ********** **********
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                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                   1205       1215       1225       1235       1245
RAM1                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
K. rhaeticus DST GL02T        GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
GOV9                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
GOV15                         GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
MAM4                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
SPO4                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
WAT11                         GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
K. oboediens LTH 2460T        GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
LYC7                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
FET8                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
K. sucrofermentans BPR 2001T  GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
SPO15                         GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
WAT14                         GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
K. swingsii DST GL01T         GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
BEL1                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
BEL2                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
FET4                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
LAD1                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
LYC8                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
STA5                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
WIL2                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
K. hansenii NCIMB 8746T       GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
MAG6                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
SUG5                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
SUG8                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
GRA2                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
GRA8                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
JAV1                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
JAV3                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
MAG15                         GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
MAM2                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
PAP1                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
RAM4                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
WIL3                          GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCATGGGA GTTGGTTTGA
Clustal consensus             ********** ********** ********** ********** **********

                              ....|....| ....|....| .
                                   1255       1265
RAM1                          CCTTAAGCCG GTGAGCGAAC C
K. rhaeticus DST GL02T        CCTTAAGCCG GTGAGCGAAC C
GOV9                          CCTTAAGCCG GTGAGCGAAC C
GOV15                         CCTTAAGCCG GTGAGCGAAC C
MAM4                          CCTTAAGCCG GTGAGCGAAC C
SPO4                          CCTTAAGCCG GTGAGCGAAC C
WAT11                         CCTTAAGCCG GTGAGCGAAC C
K. oboediens LTH 2460T        CCTTAAGCCG GTGAGCGAAC C
LYC7                          CCTTAAGCCG GTGAGCGAAC C
FET8                          CCTTAAGCCG GTGAGCGAAC C
K. sucrofermentans BPR 2001T  CCTTAAGCCG GTGAGCGAAC C
SPO15                         CCTTAAGCCG GTGAGCGAAC C
WAT14                         CCTTAAGCCG GTGAGCGAAC C
K. swingsii DST GL01T         CCTTAAGCCG GTGAGCGAAC C
BEL1                          CCTTAAGCCG GTGAGCGAAC C
BEL2                          CCTTAAGCCG GTGAGCGAAC C
FET4                          CCTTAAGCCG GTGAGCGAAC C
LAD1                          CCTTAAGCCG GTGAGCGAAC C
LYC8                          CCTTAAGCCG GTGAGCGAAC C
STA5                          CCTTAAGCCG GTGAGCGAAC C
WIL2                          CCTTAAGCCG GTGAGCGAAC C
K. hansenii NCIMB 8746T       CCTTAAGCCG GTGAGCGAAC C
MAG6                          CCTTAAGCCG GTGAGCGAAC C
SUG5                          CCTTAAGCCG GTGAGCGAAC C
SUG8                          CCTTAAGCCG GTGAGCGAAC C
GRA2                          CCTTAAGCCG GTGAGCGAAC C
GRA8                          CCTTAAGCCG GTGAGCGAAC C
JAV1                          CCTTAAGCCG GTGAGCGAAC C
JAV3                          CCTTAAGCCG GTGAGCGAAC C
MAG15                         CCTTAAGCCG GTGAGCGAAC C
MAM2                          CCTTAAGCCG GTGAGCGAAC C
PAP1                          CCTTAAGCCG GTGAGCGAAC C
RAM4                          CCTTAAGCCG GTGAGCGAAC C
WIL3                          CCTTAAGCCG GTGAGCGAAC C
Clustal consensus             ********** ********** *
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APPENDIX G

Calculation of BC production cost

1. Production cost of BC using standard HS medium

The BC yield of 1.15 g/L was produced in 90 mL of standard HS medium which contained 1.8 g glucose, 0.05 g yeast extract, 0.45 g peptone, 0.24 g disodium hydrogen phosphate and 0.11 g citric acid, respectively. The costs of each ingredient were estimated 4.14 baht (Sigma), 4.14 baht (Fluka), 4.84 baht (Sigma), 1.73 baht (BDH Prolabo) and 0.68 baht (Sigma), respectively and the cost of 90 mL standard HS medium was estimated 15.33 baht. The BC production cost was calculated as following:

BC production cost of 1.15 g has estimated 15.33 baht
BC production cost of 1.0 g has estimated 15.53/1.15 = 13.50 baht/g of BC

** Therefore, the BC production cost using standard HS medium was estimated 13.50 baht per gram dry weight of BC.

2. Production cost of BC using optimized soybean whey-based medium

The BC yield of 4.14 g/L was produced in 90 mL of optimized soybean whey-based medium which contained 1.45 mL of 95% ethanol and 88.55 mL of soybean whey, respectively. The costs of each ingredient were 0.07 baht (The Excise Department) and 0 baht, respectively and the cost of 90 mL optimized soybean whey-based medium was estimated 0.07 baht. The BC production cost was calculated as following:

BC production cost of 4.14 g has estimated 0.07 baht
BC production cost of 1.0 g has estimated 0.07/4.14 = 0.02 baht/g of BC

** Therefore, the BC production cost using standard HS medium was estimated 0.02 baht per gram dry weight of BC.

AUTHOR BIOGRAPHY

Name : Amornrat Suwanposri

Date of birth : 1 November 1983

Address : 31 M. 2 Anamai Road, Renunakhon, Nakonphanom, 48170

Email Address suwanposri@hotmail.com

Education :

2002-2005 Bachelor of Science in Biotechnology, B.Sc. Biotechnology, GPA 3.30
King Monkut's Institute of Technology Ladkrabang (KMITL), Bangkok, 10520, Thailand
Major-Biotechnology
Research : Paper production from bacterial cellulose with chitosan

Experience :

Assistant Teacher King Monkut's Institute of Technology Ladkrabang (KMITL)
Assigned work: Laboratory at Faculty of Science
Teaching in Principle of Biology Laboratory, Principle of Microbiology Laboratory, Microbial Genetic Laboratory, Principle of Zoology Laboratory and Principle of Botany Laboratory.

Publication:

Amornrat Suwanposri, Pattaraporn Yukphan, Yuzo Yamada and Duangjai Ochaikul, "Statistical optimization of culture conditions for biocellulose production by *Komagataeibacter* sp. PAP1 using soya bean whey." Maejo International of Science and Technology, 2014, 8(01): 1-14.

Amornrat Suwanposri, Pattaraporn Yukphan, Yuzo Yamada and Duangjai Ochaikul, "Identification and biocellulose production of *Gluconacetobacter* strains isolated from tropical fruits in Thailand." Maejo International of Science and Technology, 2013, 7(01): 70-82.

Amornrat Suwanposri, Pattaraporn Yukphan, Yuzo Yamada and Duangjai Ochaikul, "Optimization of culture condition for bio-cellulose production by *Gluconacetobacter nataicola* PAP5 isolated from rotten tropical fruits in Thailand." The 24th Annual Meeting of the Thai Society for Biotechnology"Renewable Energy and Global Care"2012.

Amornrat Suwanposri, Pattaraporn Yukphan, Yuzo Yamada and Duangjai Ochaikul, "Bio-cellulose paper production using agricultural waste by *Gluconacetobacter* sp. PAP1."International conference on sustainable environmental technologies (ICSET) 2012.

Amornrat Suwanposri, Pattaraporn Yukphan and Duangjai Ochaikul, "Isolation and identification of bacterial cellulose producers from local fruits in Thailand." The 8th International symposium on biocontrol and biotechnology 2010.