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A new cellulose-producing bacterium, *Rhodococcus* sp. MI 2: Screening and optimization of culture conditions

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ABSTRACT

A total of 59 bacterial strains were isolated from ripe fruits and vegetables and tested for their ability to produce cellulose. Only one identified as *Rhodococcus* sp. MI 2 based on its taxonomic characteristics and 16S rDNA sequence analysis. The glucose was the only product of digestion by cellulase confirmed by TLC and reversed phase HPLC. *Rhodococcus* sp. MI 2 produced significantly more cellulose with the SH medium containing glucose than with the coconut juice medium. *Rhodococcus* sp. MI 2 initially produced 3.91 ± 0.091, 2.20 ± 0.090 and 0.19 ± 0.051 g/L/6 days cellulose under static, agitated and stirred conditions, respectively, whereas *A. xylinum* 998 produced 1.17 ± 0.065, 1.34 ± 0.115 and 0.12 ± 0.046 g/L/6 days cellulose under the same conditions, respectively. The optimum culture conditions for cellulose production in SH medium were room temperature (25 °C), a 5% (v/v) inoculum, peptone 0.7%, yeast extract 0.9%, and sucrose 1.5%, at a pH of 3.5. The cellulose yield was increased by adding 0.5% $CaCO_3$ to the SH medium with sucrose but added agar had no effect. The cellulose yield under optimum and static conditions was increased about twice, from 3.7 to 7.4 g/L/14 days.

1. Introduction

Cellulose is one of the most common carbohydrate polymers found in the world especially tropical areas with excellent plant growth. Although it is a major structural polymer of all plants, various bacteria are also able to produce it. Cellulose from plants is normally mixed with lignin and hemicellulose, but cellulose from bacteria or bacterial cellulose (BC) consists of only glucose that is why BC has certain better characteristics for most uses than cellulose from plants, for example, it has an ultra fine network structure, is highly biodegradable (Yamanaka et al., 1989), and has unique mechanical strength (Castro et al., 2011; Yamanaka et al., 1989), water-holding capacity (Saibuatong & Phisalaphong, 2010), degree of polymerization (Dahman, Jayasuriya, & Kalis, 2010), and high crystallinity (Klemm, Schumann, Udhardt, & Marsch, 2001).

There have been many reports of cellulose being produced by Gram negative bacteria: *Gluconacetobacter xylinus* or *Acetobacter xylinum*, *Agrobacterium*, *Achromobacter*, *Aerobacter*, *Azotobacter*, *Pseudomonas*, and *Rhizobium*, but only one genus of Gram positive bacteria namely *Sarcina* (Jonas & Farah, 1998). *Gluconacetobacter* has been used for producing commercial amounts of cellulose and has become the most popular strain so far for uses not only as a food component but also for drug-delivery systems (Domb & Kost, 1997), artificial skin (Fontana et al., 1990), blood vessels for microsurgery (Klemm et al., 2001), flexible display screens (Nakagaito, Nogi, & Yano, 2010), composite reinforced, electronic paper (Jonas & Farah, 1998), and so on. However, it is difficult to obtain a high productivity using this bacterium in a large-scale fermentation system due to its low yield under agitated conditions.

The aim of this study was to screen for bacteria capable of producing bacterial cellulose, with high yields under static, agitated and stirred conditions, and to optimize conditions for bacterial cellulose production that could serve for commercialization and industrial applications.

2. Materials and methods

2.1. Materials

Schramm and Hestrin (SH) medium contains per liter 20 g glucose, 5 g peptone, 5 g yeast extract, 2.7 g anhydrous disodium phosphate, and 1.5 g citric acid monohydrate. 1.5% agar is added for the solid SH medium. A synthetic medium used contained per liter 15 g glucose, 2 g ammonium sulfate, 3 g potassium dihydrogen phosphate, 3 g disodium hydrogen phosphate, 0.8 g magnesium sulfate, 0.005 g ferric sulfate, 0.003 g borate, 0.0005 g nicotinamide, and 6 mL of 95% ethanol. A seed culture medium tested contained per liter 25 g D-mannitol, 5 g yeast extract, 3 g tryptone. A coconut medium contained 1000 mL of coconut juice from ripe fruit, 50 g sucrose, 5 g ammonium sulfate, and 10 mL of acetic acid. The pH of

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all media mentioned above was adjusted to 5.0 with 1 N acetic acid. Cellulase (6 units/mg solid) from *Trichoderma reesei* ATCC 26921 was from Sigma–Aldrich. Thin layer chromatography (TLC) silica gel 60 F_{254} was from MERCK. All chemicals used were of analytical grades.

2.2. A reference strain

A reference strain used in this study that was known to produce good yields of cellulose was *Acetobacter xylinum* 998 obtained from the Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkla.

2.3. Isolation of bacteria from ripe fruits and vegetables

Ripe fruits and vegetables used for the isolation of bacteria producing BC were papaya, cantaloupe, mango, banana, palm fruit, lychee, pineapple, dragon fruit, *Manilkara zapota* or sapodilla, lamai (in Thai), apple, guava, coconut, corn, Chinese lettuce, morning glory, and tomato. Three samples from each fruit type were collected for isolation. Bacterial colonies were isolated by a plate streaking method on SH plate agar. They were incubated at room temperature for 3–5 days. Each single colony was restreaked and selected from the same medium for further studies.

2.4. Screening of bacteria producing cellulose

The SH broth was used for cultivation of each isolated strain to screen for cellulose production. After subculturing each strain into SH broth, the cultures were incubated at room temperature for 72 h. A loopfull of the strain was then resubcultured under static conditions for 3–14 days. A cellulose positive strain was one that produced a pellicle of cellulose on the liquid medium that was not easily destroyed by a high speed vortex mixer or by centrifugation. The presence of cellulose was further confirmed by detecting glucose by reversed phase HPLC on a cellulase digestion of the pellicle.

2.5. Cultivation, harvest and determination of cellulose production

The SH liquid medium was used for cultivating the strains. A 250-mL flask containing 100 mL of SH medium was inoculated with 2%, v/v of starter. A bacterial isolate was inoculated into the SH medium under static conditions. After finishing the cultivation, the cellulose was picked from the surface and boiled in 0.1 N NaOH for 10 min. It was immersed in distilled water for at least 2 h or overnight, and then rinsed extensively with distilled water before drying at 60 °C for 48 h or until no change of weight. The dried weight of cellulose was determined.

2.6. Identification of bacteria

An isolate producing bacterial cellulose was identified by taxonomic characteristics accordingly to Bergey's Manual of Systematic Bacteriology (Kandler & Weiss, 1986): Gram staining, cell shape, endospore forming, catalase and oxidase production, indole test, MR-VP test, various carbohydrate fermentation tests, various sole carbon source tests, effects of crystal violet, sodium chloride and phenol, and growth at various temperatures.

2.7. 16S rDNA sequence analysis

Genomic DNA of a potentially useful strain was extracted by the standard method (Sambrook, Fritsch, & Maniatis, 1989) and 16S rDNA was amplified using GeneAmp PCR System 9600. The F-primer was 5′-GGGTGAGTACACGTGGGTGA-3′ and the R-primer was 5′-GCCCAGAGACCCGCCTTCGC-3′. The PCR product size was 1507 bp. A 603 bp region of the amplified DNA was partially sequenced using the ABI 377 DNA sequencer. The BLAST program from the NCBI database (Altschul et al., 1997) was used to identify the most closely related species to the strain.

2.8. Determination of cellulose by detecting glucose as the major product of digestion by cellulase using thin layer chromatography and reversed phase high performance liquid chromatography

The dried piece of cellulose obtained as described in Section 2.4 was cut into small 1 mm diameter pieces. The small pieces of cellulose were digested with 5 units of cellulase at pH 5 for 3 h at 37 °C. The separations of the digested products of cellulose by thin-layer chromatography (TLC) on silica gel (5 cm × 10 cm) was performed. The TLC plate was developed at room temperature using a mobile phase of a saturated isopropanol: 1-butanol: distilled water solvent (12:5:4). Detection was made by spraying the dried plate with 5% sulfuric acid in ethanol then heating at 150 °C for 1–2 min (modified from Godin, Poirier, Blomquist, & Tremblay, 1999). The products appeared as brown spots on a white background. Cellulose without digestion, cellubiose and glucose were used as controls. To carry out the HPLC separation the sorbent layer with the same R_f value as glucose was removed into a test tube. The sorbent layer was extracted with the same solvent used for the TLC. Reversed phase high performance liquid chromatography was then performed to analyze for glucose as a product of the digested cellulose. A standard of glucose was added to the sample to check the retention time and refractive index. Separation of the digested cellulose for detecting glucose was investigated by HPLC using a Zorbax NH2 column 4.6 mm × 250 mm, 5 μm performed at 25 °C. The mobile phase was acetonitrile: water (75: 25) with a flow rate of 0.5 mL/min.

2.9. Comparison of bacterial cellulose production in static, agitated and stirred culture

The potentially useful isolate was incubated at room temperature (25 °C) statically, or in a rotary shaker with a speed of 180 rpm, and also with a magnetic stirrer for the static, agitated, and stirred culture, respectively, for 6 day-incubation period in the SH medium. The cellulose produced under agitated and stirred conditions were harvested by centrifugation at 10,000 rpm for 10 min, and that produced under static condition following the procedure described in Section 2.5 to determine the yields. The experiments were done in triplicate.

2.10. Devising the optimum culture conditions for bacterial cellulose production

The effects of various kinds of media, incubation times, and use of various sugars substrates were investigated. They were as follows, media: SH medium, synthetic medium, seed culture medium and coconut juice medium; incubation time: 5, 6, 7–14 days; inoculum-size: 2, 5, 6, 8, 10, 12, 14, 16, 18, 20 and 22%; sugars: glucose, fructose, sucrose, lactose, sorbitol and mannitol. Various incubation times were used in place of the basal incubation time of 14 days. The experiments were done in triplicate.

2.11. Devising the optimum values for some of the ingredients and the pH in the SH medium that might be suitable for commercial production

The amounts of the major ingredients, yeast extract, peptone and sucrose, in the SH medium were optimized. They were as follows: peptone, 0.1, 0.3, 0.5, 0.7 and 0.9%; yeast extract, 0.1, 0.3,

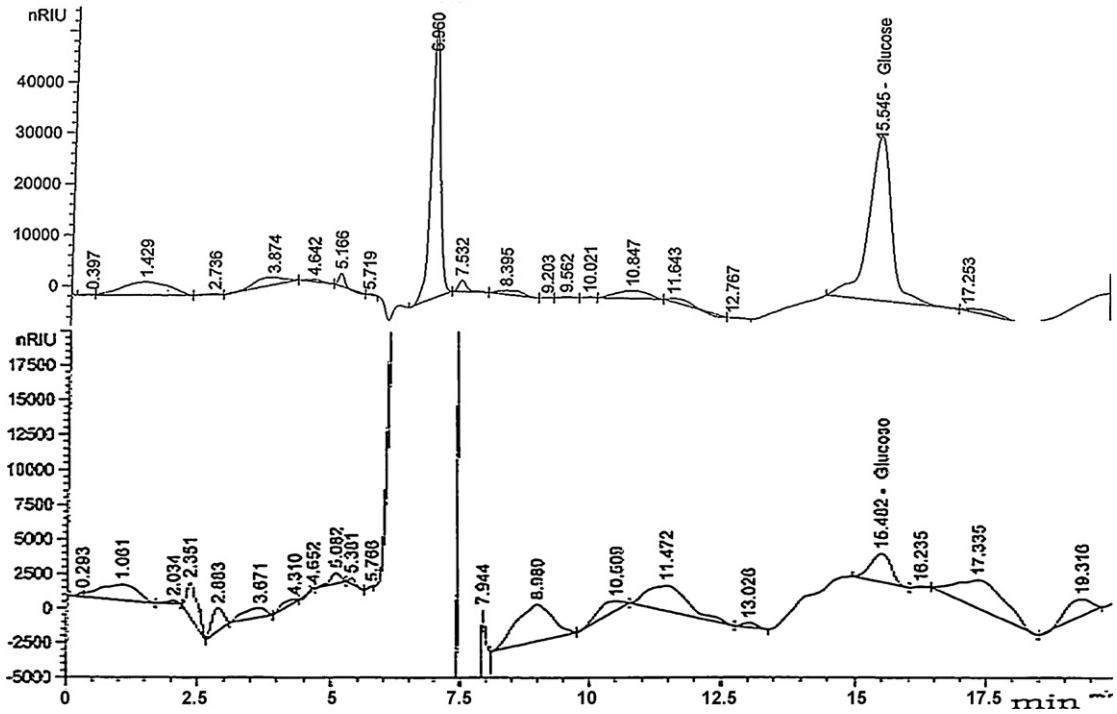

Fig. 1. Chromatogram of reversed phase HPLC of products of *Rhodococcus* sp. MI 2's cellulose after digestion by cellulase: (a) standard glucose and (b) digested products of *Rhodococcus* sp. MI 2.

0.5, 0.7 and 0.9%; sucrose, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0%; pH, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0. The experiments were done in triplicate.

2.12. Effects of $CaCO_3$ and agar

To determine if the bacterial cellulose production was affected by $CaCO_3$ and by the presence of agar, each of them was added to the standard SH medium. They were as follows: $CaCO_3$, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0%; agar, 0, 0.4, 0.5, 0.6, 0.7 and 0.8%. The experiments were done in triplicate.

2.13. Statistical analysis

All statistical analyses were performed with SPSS 11.5 (SPSS Inc., Chicago, IL, USA) for the results obtained from three replications. Means and standard deviations for each treatment were calculated. Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were used for comparing differences of the mean values at the 0.05 confidence level.

3. Results

3.1. Isolation of bacteria from ripe fruits and vegetables and determination of an isolated strain producing bacterial cellulose

A total of 59 strains were isolated from fruits and vegetables. There was only one strain named MI 2, screened from *Manilkara zapota* (Sapodilla), that produced significant amounts of cellulose. The mat of cellulose in the phase boundary between the gas and liquid on the surface of the culture broth was not destroyed by a high speed of a vortex mixer or by centrifugation. This isolate was then kept in glycerol solution at −70 °C as the stock culture, for further studies.

3.2. Identification of bacteria by taxonomic characteristics and 16S rDNA sequence analysis

The strain MI 2 formed short rods. It was nonmotile and did not produce endospores or conidia. It was indole and Voges-Proskauer-negative. Carbohydrate fermentation tests showed that it produced acid from only glucose and sucrose but grew with glucose, fructose, lactose, mannitol, sorbitol, ethanol and glycerol as sole carbon sources: no growth was observed on L-tyrosine (0.1%, w/v), nor in the presence of crystal violet (0.001%, w/v), sodium chloride (5.0 and 7.0%, w/v), and phenol (0.1%, w/v) (Table 1). It was catalase and methyl red positive, and grew in the presence of crystal violet (0.0001% w/v) (Table 1). It did not grow at 10, 40 and 45 °C. Unlike the reference strain, *Rhodococcus* sp. MI 2 was not able to ferment sorbitol and grow in the presence of sodium chloride (5% w/v), but did grow in the presence of crystal violet (0.0001% w/v). Regarding the 16S rDNA sequence analysis, the results revealed that the MI 2 had 100% homology with a *Rhodococcus* sp. Thus, the MI 2 was identified as *Rhodococcus* sp. MI 2 based on its taxonomic characteristics and 16S rDNA sequence analysis.

3.3. Cellulose confirmation by reversed phase HPLC

Identification of glucose was carried out by comparing its retention time and the amounts by refractive index. The refractive index of glucose was used for the quantification of the glucose content compared with the standard. Regarding the glucose determination by HPLC analysis with a refractive index detector, it was performed in 15.5 min with acceptable resolution (Fig. 1).

3.4. Comparison of cellulose production from Rhodococcus sp. MI 2 and A. xylinum 998 in static, agitated and stirred culture in SH medium

Cellulose production of *Rhodococcus* sp. MI 2 in static, agitated and stirred culture in SH medium was 3.91 ± 0.091, 2.20 ± 0.090

Table 1
Comparison of the characteristics of MI 2 isolate and a reference strain.

Characteristics	MI 2	*Rhodococcus* sp.
Gram staining	+	+
Cell shape	Rod	Rod
Oxidase test	–	nd
Catalase test	+	+
Indole test	–	nd
Methyl red test	+	nd
Voges–Proskauer	–	nd
Carbohydrate fermentation test		
Glucose	+	+
Sucrose	+	+
Fructose	–	–
Lactose	–	–
Mannitol	–	–
Sorbitol	–	+
Growth on sole carbon source (% w/v)		
Glucose (1.0)	+	+
Sucrose (1.0)	+	+
Sorbitol (1.0)	+	+
Mannitol (1.0)	+	+
L-tyrosine (0.1)	–	–
Ethanol (1.0)	+	+
Glycerol (1.0)	+	+
Growth in presence of (% w/v)		
Crystal violet (0.001)	–	d
Crystal violet (0.0001)	+	–
Sodium chloride (5.0)	–	+
Sodium chloride (7.0)	–	–
Phenol (0.1)	–	d
Growth at		
10 °C	–	+
40 °C	–	+
45 °C	–	–

Note: +, positive test; –, negative test; d, 11–89% of the strains are positive; nd, not detected.

and 0.19 ± 0.051 g/L all significantly higher than those by *A. xylinum* 998 after a 6 day-incubation period ($p < 0.05$) (Fig. 2). *Rhodococcus* sp. MI 2 and *A. xylinum* 998 produced a similar form of cellulose as both had a three dimensional interconnected reticular pellicle under static condition, but *Rhodococcus* sp. MI 2 produced cellulose mostly in small granules that were like tapioca-pearls with a few irregular shapes under agitated conditions (Fig. 3a and b), and feather like shapes under stirred conditions (Fig. 3c and d). In contrast the cellulose from *A. xylinum* 998 aggregated in a large size with an irregular shape under agitation (Fig. 3e and f) and stirred conditions (Fig. 3g and h).

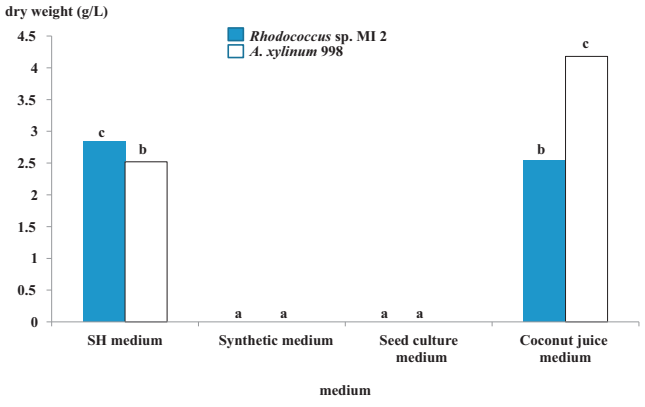

Fig. 2. Cellulose production by *Rhodococcus* sp. MI 2 and *A. xylinum* 998 under static, agitated and stirred conditions.

3.5. Devising optimum culture conditions for bacterial cellulose production

Rhodococcus sp. MI 2 produced the highest cellulose yield of 3.91 ± 0.091 g/L/14 days in the basal SH medium with glucose which was significantly higher than the 2.54 ± 0.067 g/L/14 days in coconut juice medium while *A. xylinum* 998 in SH gave the highest yield, 4.18 ± 0.100 g/L/14 days in coconut juice medium which was significantly higher than the 2.52 ± 0.125 g/L/14 days in SH medium ($p < 0.05$) (Fig. 4). The synthetic medium and seed culture medium gave no BC production from either strain (Fig. 4). Comparison of cellulose yield after 5–14 day-incubation period indicated that there was significantly different ($p < 0.05$) (Fig. 5). The results showed that after 14 day-incubation period in SH medium, *Rhodococcus* sp. MI 2 had the highest cellulose formation, 3.94 ± 0.096 g/L (Fig. 5).

3.6. Devising the compositions of major ingredients and pH of SH medium with sucrose

SH medium containing fructose instead of glucose gave significantly higher cellulose than that containing sorbitol, mannitol, sucrose and glucose, respectively ($p < 0.05$) (Fig. 6a). The optimum culture conditions in SH medium with sucrose at room temperature (25 °C) were found to be as follows: inoculum size 5% (v/v) (Fig. 6b), peptone 0.7% (Fig. 6c), yeast extract 0.9% (Fig. 6d), sucrose 1.5% (Fig. 7a), and pH 3.5 (Fig. 7b). A significant difference in each experiment ($p < 0.05$) was observed.

3.7. Effects of $CaCO_3$ and agar

A $CaCO_3$ concentration of 0.5% was found to significantly increase the cellulose production ($p < 0.05$) (Fig. 7c), but agar added up to 0.8% in the SH medium had no effect on the cellulose production (Fig. 7d).

4. Discussion

The bacterial strain capable of producing cellulose in this study was obtained from *Manilkara zapota* (sapodilla) a Thai fruit. This is the first report of a *Rhodococcus* sp. producing cellulose, and the second report of a Gram positive bacterium 13 years after Jonas and Farah's made their first report (Jonas & Farah, 1998). Seto, Kojima, Tonouchi, Tsuchida, & Yoshinaga (1997) screened 346 bacterial strains from corn steep liquor-sucrose medium for cellulose production and found 94 potential strains (Seto et al., 1997). There were also 3 highly productive strains isolated from melon, cherry and grape, respectively. In addition, other bacterial strains capable of cellulose production have been detected from other sources, fruits, fermented foods, beverages and vinegar (Aydin & Aksoyb, 2010).

After digestion of the cellulose produced by *Rhodococcus* sp. MI 2 with cellulase, glucose was the product detected by TLC and confirmed by reversed phase HPLC. There was a report that *Rhodococcus* sp. RHA1 produced an exocellular polysaccharide (EPS), but it was not cellulose. Analysis showed that it was a high-molecular-mass polymer of a repeating tetrasaccharide unit composed of D-glucuronic acid, D-glucose, D-galactose, L-fucose and an O-acetyl substituent in EPS (1:1:1:1:1) (Perry, MacLean, Patrauchan, & Vinogradov, 2007). The physical properties of the cellulose produced in this study were examined including the wet membrane and dry membrane thickness, the water holding capacity (data not shown). In addition, the wet form of the cellulose of this strain was resistant to cracks, tearing and gas pressure up to 15 psi. Regarding the visual appearance, physical, mechanical and

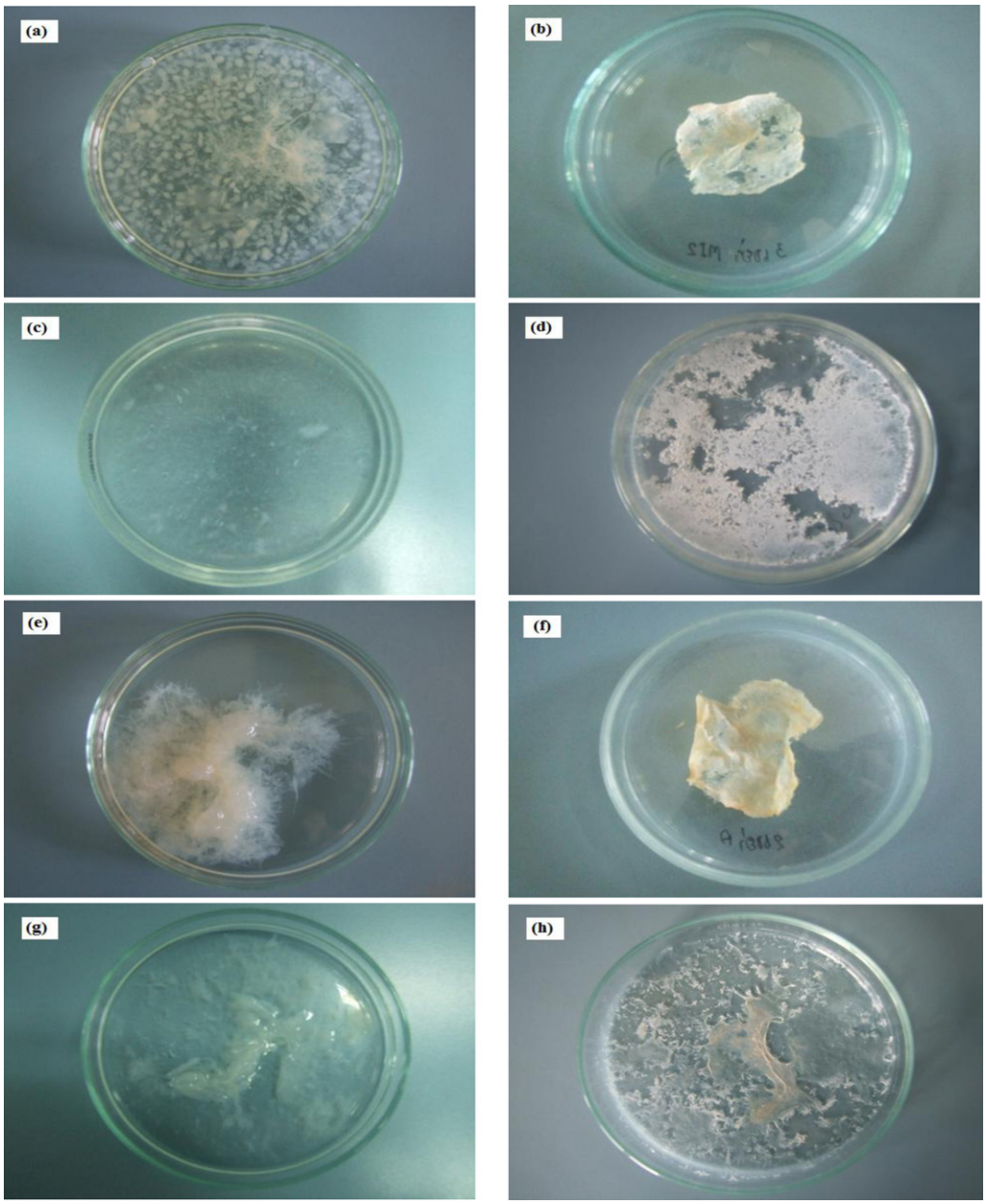

Fig. 3. Visual aspect of cellulose produced by *Rhodococcus* sp. MI 2 and *A. xylinum* 998 in agitated and stirred cultures: (a) wet form and (b) dried form of agitated culture; (c) wet form and (d) dried form of stirred culture of *Rhodococcus* sp. MI 2; (e) wet form and (f) dried form of agitated culture; (g) wet form and (h) dried form of stirred culture of *A. xylinum* 998.

chemical properties, the cellulose produced by *Rhodococcus* sp. MI 2 was extracellular.

Rhodococcus sp. MI 2 grew and produced cellulose in SH and coconut juice medium. A rich medium containing yeast extract and peptone (SH medium) has been found to be good for bacterial cellulose production (Fontana et al., 1997). Coconut juice contains organic compounds and minerals that supported the growth and cellulose production well for both strains, especially *A. xylinum* 998. The synthetic medium and seed culture medium were not suitable for the growth of *Rhodococcus* sp. MI 2, so cellulose was not produced. In contrast, *Acetobacter* sp. V6 could produce more cellulose in synthetic medium than in SH medium (Son et al., 2003).

Rhodococcus sp. MI 2 and *Acetobacter xylinum* 998 produced a similar form of cellulose as a three dimensional interconnected reticular pellicle under static conditions. Under agitated condition the cellulose of *Rhodococcus* sp. MI 2 was formed mostly in small granules or like tapioca-pearls and a few irregular shapes whereas that of *A. xylinum* 998 was aggregated in a bigger size with an irregular shape with a lower yield. Moreover, under stirred condition, the cellulose of *Rhodococcus* sp. MI 2 was feather like in shape and present in slightly higher amounts than by *A. xylinum* 998 that was similar in shape to that under agitated conditions. The different forms of cellulose produced under static, agitated and stirred conditions, could be correlated to the disrupting effect of strong aeration on the formation of hydrogen bonds between cellulose chains thus affecting the supramolecular organization of the microfibrils (Bootten, Harris, Melton, & Newman, 2008).

The cultivation time of *Rhodococcus* sp. MI 2 for the highest BC production was after 14 day-incubation period. Although after 6 day-cultivation the maximum of the dry weight of BC was obtained, the thickness and the wet weight of BC were less than those after 7, 8 and 9 days of cultivation (data not shown). This is maybe due to

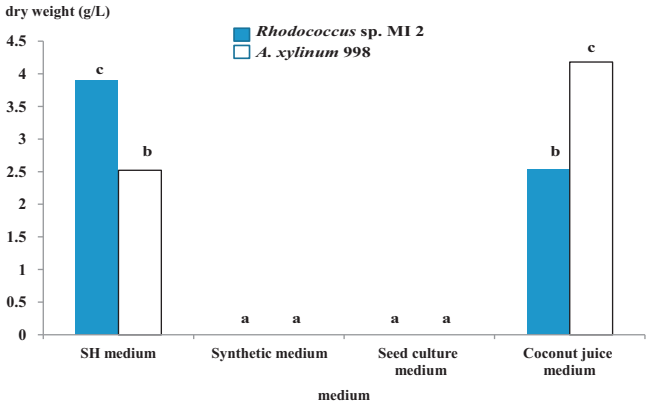

Fig. 4. BC yield from various kinds of medium of *Rhodococcus* sp. MI 2 and *A. xylinum* 998 under static conditions.

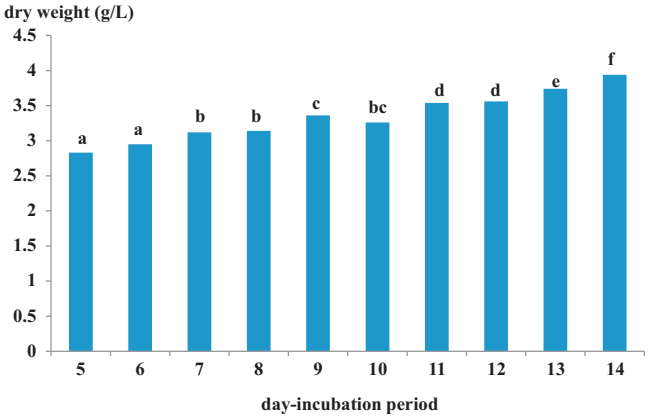

Fig. 5. Effect of incubation period on cellulose production of *Rhodococcus* sp. MI 2 under static conditions.

the water holding capacity of BC being higher after a longer time. Sheykhnazari, Tabarsa, Ashori, Shakeri, & Golalipour (2011) also reported that the cellulose production increased with time, but no increase was observed after 14 days of incubation (Sheykhnazari et al., 2011).

Rhodococcus sp. MI 2 gave the maximum cellulose yield in SH medium containing fructose in contrast with *Gluconacetobacter sacchari* which produced higher cellulose amounts (2.7 g/L in 96 h) in SH based medium containing glucose than that containing

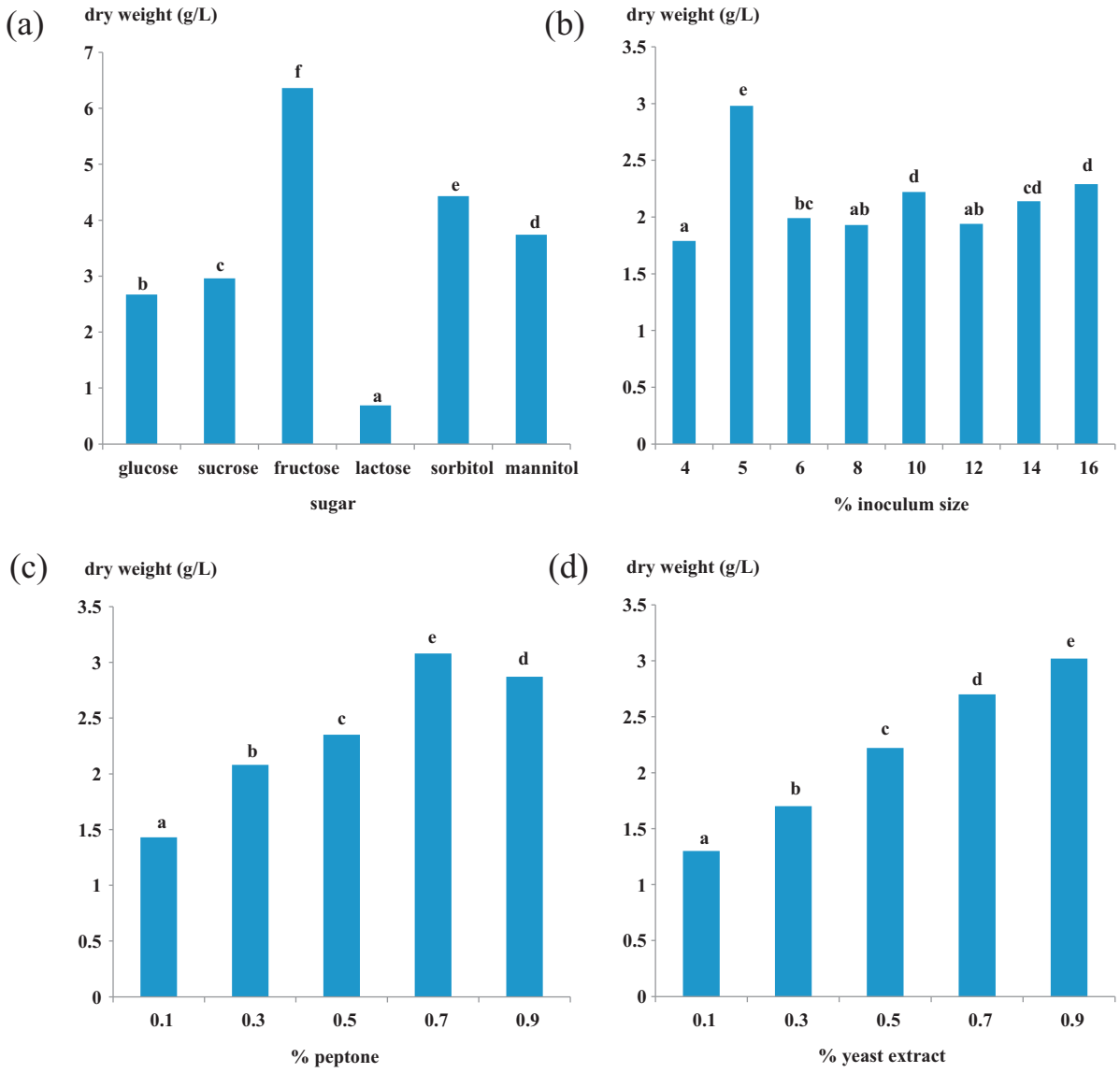

Fig. 6. Effect of various kinds of sugars (a), inoculums size (b), amount of peptone (c) and yeast extract (d) on BC production.

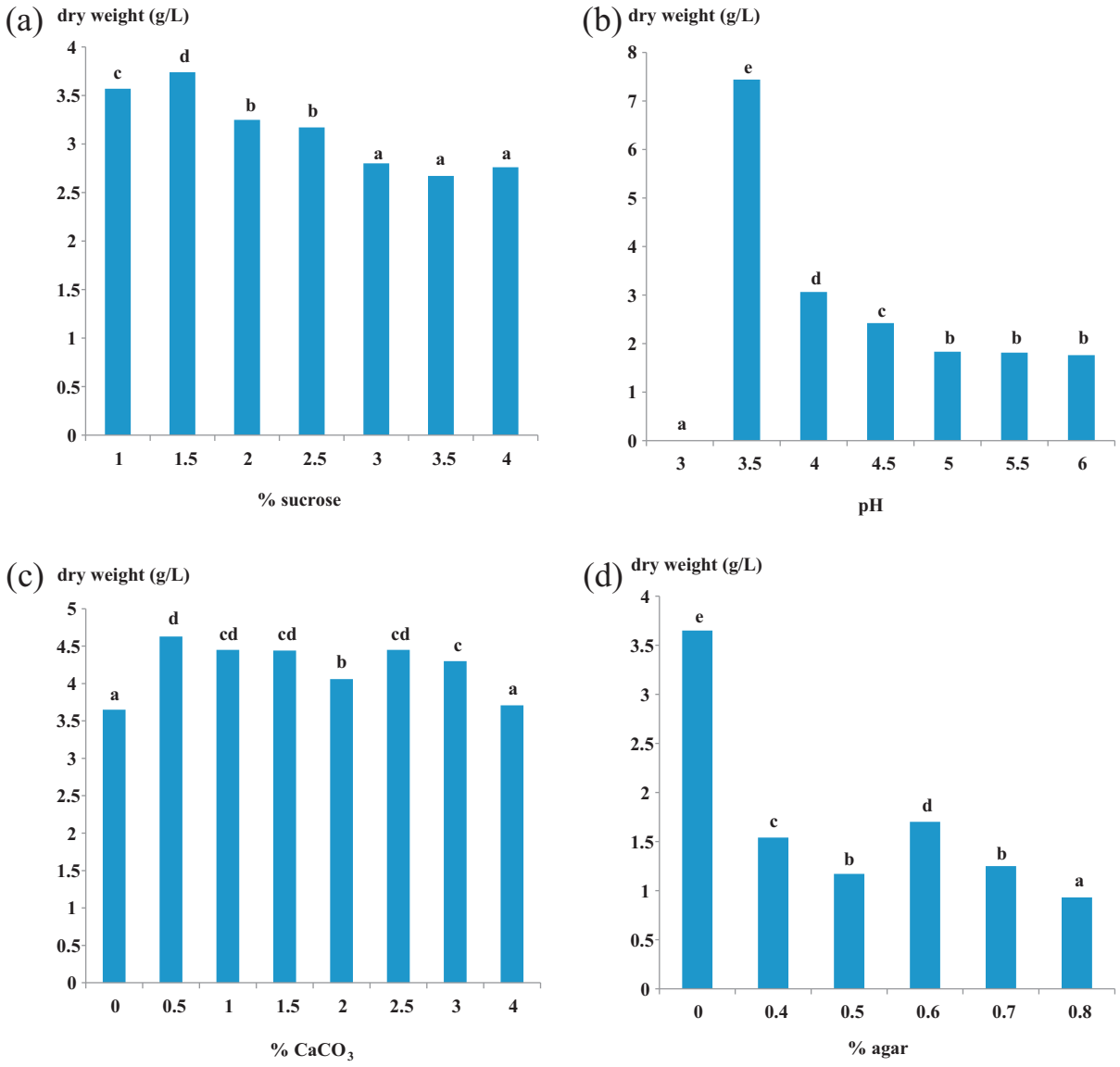

Fig. 7. Effect of amount of sucrose (a), pH (b), amount of $CaCO_3$ (c) and agar (d) on BC production.

sucrose, or fructose (Trovatti, Serafim, Freire, Silvestre, & Pascoal Neto, 2011). Vandamme, de Baets, Vanbaelen, Joris, & de Wulf (1998) reported that sucrose or glucose is the best carbon source for growth and cellulose production of bacteria because it needs to be used for conversion to a precursor, uridine diphosphoglucose, before cellulose formation (Vandamme et al., 1998), and Pourramezan, Roayaei, & Qezelbash (2009) reported that the highest cellulose yields of *Acetobacter* sp. 4B-2 were obtained in SH medium containing sucrose, followed by that containing glucose, xylose and lactose, respectively (Pourramezan et al., 2009). In Thailand, fructose is 10 times more expensive than sucrose, but the results demonstrated that fructose enhanced the yield of cellulose only 2.5 times over that from sucrose. Thus in this study we continued to use sucrose as carbon source for the optimization of cellulose production by *Rhodococcus* sp. MI 2.

The optimum culture conditions in SH medium at room temperature (25 °C) were as follows: 5% (v/v) inoculum, peptone 0.7%, yeast extract 0.9%, and sucrose 1.5%, at a pH of 3.5. Regarding the percentage of inoculum size, the result revealed a significant difference between the 4 and 5% inoculum: a 5% inoculum gave 3 times more cellulose than the 4%. With any inoculum of more than 5%, a lower amount of cellulose was produced. The initial pH of the SH medium for the strain should not be lower than 3.5 because below that there was no growth or cellulose-produced. Pourramezan et al. (2009) also reported that the optimum concentration of sucrose, initial pH and temperature were 1.5%, pH of 7, and at 30 °C, respectively (Pourramezan et al., 2009).

The cellulose yield was significantly increased by adding $CaCO_3$ and at the concentration of 0.5% gave the maximum yield of 4.63 ± 0.087 g/L/14 days compared with 3.65 ± 0.062 g/L/14 days without $CaCO_3$. This result was in agreement with a previous report on the effect of calcium ions on increasing BC production (Hong & Qiu, 2008). Addition of agar in the medium had no effect on the cellulose yield unlike another report on the influence of agar on the cellulose production of *A. xylinum* BPR 2001 in a jar fermenter with 2 turbines because it increased in viscosity of the culture, prevented aggregation and enhanced the dispersion of cellulose pellets (Bae, Sugano, & Shoda, 2004). We speculated that this study was done under static condition, so the viscosity of the culture did not affect the cell growth and cellulose production of *Rhodococcus* sp. MI 2.

The cellulose yield under optimum and static conditions was increased by about twice, from 3.7 to 7.4 g/L/14 days. This led to the

differences in the cellulose productivity of *Rhodococcus* sp. MI 2 and *A. xylinum* 998 from the beginning until optimal conditions were determined. Moreover, the resulting different forms of cellulose produced under agitated and stirred conditions could be considered to provide a number of potential advantages. More information is required on the cellulose produced by *Rhodococcus* sp. MI 2 such as explanations for the differences in structures and properties of cellulose produced under static, agitated and stirred conditions. These will require scanning electron microscope analysis of microfibrils, their tensile properties, and the metabolic pathways involved in cellulose production.

5. Conclusions

Rhodococcus sp. MI 2 is a Gram positive bacterium capable of producing cellulose under static, agitated and stirred conditions with higher yields than from *A. xylinum* 998. The appearance of cellulose from *Rhodococcus* sp. MI 2, especially under agitated and stirred conditions was totally different from that of the cellulose from *A. xylinum* 998. This could engender considerable commercial and scientific interest because the small size of the cellulose would be easy to modify or change its structure. Moreover, *Rhodococcus* sp. MI 2 gave higher yields under all conditions tested, so it could play an important role industrially and could be applied to wider areas of cellulose applications as well as for use as an alternative to cellulose obtained from *Acetobacter* (*Gluconacetobacter*) or plants.

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