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Contributed Paper

Molecular Taxonomy and Characterization of Thermotolerant *Komagataeibacter* Species for Bacterial Nanocellulose Production at High Temperatures

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ABSTRACT

Nineteen isolates (MSKU 1-MSKU 19) were selected from 166 isolates of acetic acid bacteria (AAB) which in turn were isolated from 47 rotten fruit samples in Thailand. These isolates were identified as *Komagataeibacter* based on 16S rRNA gene and 16S-23S rRNA gene ITS sequences. All 19 isolates are thermotolerant bacterial nanocellulose (BNC) producers that can grow up to 37 °C. Among the 19 isolates, MSKU 12 was the most effective BNC producer at high temperatures. It exhibited the highest amount of BNC, 11.11 ± 1.50 µmol sugar per mg of protein (measured as sugar content), which was 7.8 fold higher than that of the amount produced by *K. oboediens* JCM 16937^T in YPGD-1A2E medium at 37 °C. In addition, MSKU 12 produced the highest amount of BNC of 7.6 ± 1.01 g/L dry weight in HS-1A2E medium at 30 °C. This was at least 1.5 fold higher than that of other *Komagataeibacter* strains. Interestingly, this isolate also produced BNC of 2.2 ± 0.03 g/L dry weight in HS-1A2E medium at 37 °C, which is impossible for the related type strain, *K. xylinus* BCC 49175^T. MSKU 12 possessed a stable BNC-producing activity due to the absence of the insertion sequence elements, the IS*1031* family, which were detected in the genome of its related type strain and the other type strains of *Komagataeibacter*. These findings indicate that *Komagataeibacter* sp. MSKU 12 is a potentially effective BNC producer at high temperatures.

Keywords: thermotolerant acetic acid bacteria, *Komagataeibacter*, 16S rRNA gene, bacterial nanocellulose

1. INTRODUCTION

AAB are Gram-negative or gram-variable obligate aerobic bacteria which play an important role in high value food and beverage products. They are widespread in various sugar- and alcoholic-rich habitats, such as fruits, flowers, fermented products, vinegar and alcoholic beverages, including soils and insect guts [1]. Currently, AAB are classified in term of eighteen genera within the family *Acetobacteraceae*. Among these genera, *Acetobacter* and *Komagataeibacter* (formerly *Gluconacetobacter*) have been used for industrial vinegar fermentation due to their strongly ethanol oxidizing ability, and their resistance to high concentrations of acetic acid and ethanol [2]. Moreover, the *Komagataeibacter* species are also well-known as BNC producers, which is an alternative source for plant-derived cellulose. BNC possesses exceptional physicochemical properties and mechanical features such as high purity, high crystallinity, high tensile strength, high water-holding capacity, high moldability, high hydrophilicity, no toxicity, good biocompatibility and good biodegradability [3]. These characteristics make it suitable for applications in a wide range of fields, including food, electronics, cosmetics and biomedical devices [4].

Although BNC produced using the *Komagataeibacter* species is commercially used for various applications, its fermentation process is mostly carried out at 28-30 °C [5]. Therefore, a cooling system and additional control are required to maintain the optimum temperature for their growth during the fermentation process especially under the era of global warming. To overcome this problem, high temperature fermentation technology employing the thermotolerant *Komagataeibacter* species, is expected to become one of the most economic next-generation fermentation technologies. This technology allows us to perform economic fermentation, which increase the fermentation rate, but reduces cooling costs, contamination risks and operational costs.

The enzymes required for BNC biosynthesis in terms of the *Komagataeibacter* species are encoded by the bacterial cellulose synthase (*bcs*) gene operon [6]. However, there have been several reports that the transposition of indigenous insertion sequence (IS) elements, IS*1031* family, into the *bcs* operon were associated with a loss of the ability to produce BNC in spontaneous mutations of *K. xylinus* and *K. hansenii* [7-9].

In this study, we aim to isolate, identify and characterize thermotolerant BNC-producing *Komagataeibacter* from rotten fruits in Thailand, especially in terms of their growth and BNC production at high temperatures. In addition, we also aim to investigate the distribution of insertion sequence elements (IS*1031*) in the newly isolated BNC producers, to select the most promising strain for stable BNC production.

2. MATERIALS AND METHODS

2.1 Bacterial Strains, Culture Media and Culture Condition

Seven type strains, *K. hansenii* NBRC 14820^T, *K. maltaceti* NBRC 14815^T, *K. intermedius* JCM 16936^T, *K. oboediens* JCM 16937^T, *K. swingsii* JCM 17123^T, *K. nataicola* BCC 36443^T and *K. xylinus* BCC 49175^T were obtained from the NBRC (NITE Biological Resource Center, Chiba, Japan) culture collection, the JCM (Japan Collection of

Microorganisms, Ibaraki, Japan) culture collection and the BCC (BIOTEC Culture Collection, Pathum Thani, Thailand) culture collection, respectively. The type strains were grown in different media according to the instructions of the culture collections. The new isolates were generally cultivated in rich YPGD medium (1% yeast extract, 1% polypeptone, 2% glycerol and 0.5% D-glucose; % w/v) that was modified from YPGD medium [10]. The growth characteristics of these isolates were determined in YPGD medium containing 1% (v/v) acetic acid and 2% (v/v) ethanol (YPGD-1A2E medium), both of which were aseptically added after sterilization. BNC production was evaluated in YPGD-1A2E medium and Hestrin-Schramm medium [11] containing 1% (v/v) acetic acid and 2% (v/v) ethanol (HS-1A2E medium). A pre-culture of all the isolates and the type strains was grown in 5 ml of rich YPGD medium and the recommended media, respectively, containing cellulase (0.5 mg/ml, final concentration). The bacterial cultures were incubated at 30 °C under shaking conditions (200 rpm) until the turbidity reached 150 Klett units as measured by a Klett-Summerson photoelectric colorimeter.

2.2 Isolation and Identification of Thermotolerant BNC-producing AAB

Forty seven samples of rotten fruits were collected from northern and central regions of Thailand. The thermotolerant AAB were isolated by the enrichment condition method as previously described [12]. BNC-producing AAB that could produce a white pellicle floating on the surface of the rich YPGD liquid medium under static incubation at 30 °C for 7 days were selected from the isolates. Identification of BNC-producing isolates based on sequence similarities of 16S rRNA gene and 16S-23S rRNA gene ITS region was investigated [13]. The phylogenetic tree based on the 16S-23S rRNA gene ITS sequences was constructed by the maximum likelihood method, using MEGA6 software involving the bootstrap analysis of 1,000 replications [14].

2.3 Determination of Growth Characteristics at Various Temperatures

The bacterial growth characteristics were observed from dot-spot analysis as previously described by Matsutani *et al.* [15]. The pre-culture was diluted to 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} using a sterilized 0.85% (w/v) NaCl solution, and the diluted cell suspension (5 µl) were spotted onto YPGD-1A2E agar plates. These plates were incubated in an incubator (EYELA multi-thermo incubator MTI-201, Japan) at 30, 37 and 39 °C for 3 days.

2.4 Quantification of BNC Production at Various Temperatures

2.4.1 Determination of BNC sugar content

The pre-culture of each strain was centrifuged at 9,000 rpm for 10 min to separate cells and medium. The cells were washed with sterile distilled water to remove cellulase and were then suspended by the same original volume of sterile distilled water. The cell suspension (1%, v/v) was then inoculated in YPGD-1A2E medium and statically incubated at 30 and 37 °C for 7 days. The BNC pellicle was harvested and prepared as previously described by Matsutani *et al.* [16] with a slight modification. The pellicle was digested with cellulase (2 mg/ml, final concentration) at 30 °C for 6 h, and was used for further analysis. The sugar content was measured by the phenol-sulfuric acid method [17] using glucose

as a standard. The protein content was measured using the modified Lowry method. The sugar content was expressed as the micromole of polysaccharide (as the glucose amounts) per mg of proteins.

2.4.2 Determination of BNC dry weight

The cellulase-free culture of each strain (1%, v/v) was cultivated in HS-1A2E medium and statically incubated at 30, 35 and 37 °C for 7 days. The BNC pellicle was harvested and treated with 0.5 M NaOH at 90 °C for 1 h to remove the trapped cells. The pellicle was carefully washed in distilled water until a neutral pH was obtained, and then dried in an oven at 105 °C to obtain a constant weight. The BNC yield was expressed as grams of BNC dry weight per liter of a cultured medium [18].

2.5 Investigation of Insertion Sequence Elements (IS*1031*) in the *Komagataeibacter* Species

The distribution of the insertion sequence elements, IS*1031*, in the genomic DNA of BNC-producing strains and type strains of the genus *Komagataeibacter*, was determined by PCR. The specific primers were IS1031F (5′-GCG AAG AGA TGG AGT GAA TGG T-3′) for a forward primer and IS1031R (5′-ACT GAC AAT GCC TTG CGG TT-3′) for a reverse primer. These primers were designed within the conserved region of the IS*1031* elements (IS*1031*A, IS*1031*C and IS*1031*D) from whole genome shotgun sequence of *K. hansenii* ATCC 23769^T available in the GenBank database (accession no. M80805, M98777 and M98778) [8]. The amplified fragment (approximately 930 bp in length) obtained from a positive strain (*K. hansenii* NBRC 14820^T) was sequenced to confirm the PCR product. Then, the IS elements of all the isolated strains and of the type strains were amplified by the following conditions: initial step at 95 °C for 5 min; 30 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 40 s; and a final step at 72 °C for 10 min.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Thermotolerant BNC-producing AAB

In this study, 166 isolates of AAB were obtained from 47 fruit samples. All of them were gram-negative short rod and showed clear zone around colonies on an agar plate containing $CaCO_3$ and ethanol, indicating that these isolates can oxidize ethanol to acetic acid [12]. Among those isolates, 19 were selected as BNC-producing strains, based on their ability to produce a NaOH-resistant and cellulase-sensitive white pellicle [19]. These 19 isolates of the BNC producers were obtained from 12 kinds of fruit as shown in Table 1. All 19 isolates designated as MSKU 1-MSKU 19 are phenotypically characteristics of *Komagataeibacter* based on acetic acid production from ethanol and BNC production [20]. Furthermore, the 16S rRNA gene sequence analysis of the 19 isolates (accession numbers: MG971325-MG971343) indicated that these isolates were identified as species of the genus *Komagataeibacter*. The pairwise comparison of the isolates and their closely related type species showed a very high similarity, ranged from 99.71-100% (Table 1). Thus, the pairwise similarities based on 16S-23S rRNA gene ITS sequence were further used for species identification among *Komagataeibacter* species (Table 1). Phylogenetic tree based on the 16S-23S rRNA gene ITS sequences of 565 bases constructed using the maximum likelihood method confirmed that all 19 isolates were clustered within the genus *Komagataeibacter* (Figure 1). Consequently, the 19 isolates were determined as closely related to 7 type species, *K. intermedius* DSM 11804^T (MSKU 2, MSKU 8, MSKU 9 and

MSKU 15), *K. oboediens* LMG 18849^T (MSKU 3, MSKU 5, MSKU 11, MSKU 14, MSKU 16 and MSKU 17), *K. swingsii* LMG 22125^T (MSKU 7), *K. nataicola* LMG 1536^T (MSKU 1), *K. xylinus* LMG 1515^T (MSKU 6, MSKU 10 and MSKU 12), *K. hansenii* LMG 1527^T (MSKU 4 and MSKU 19) and *K. maltaceti* LMG 1529^T (MSKU 13 and MSKU 18). The subsequent reconstruction of the phylogenetic relationship based on 16S-23S rRNA gene ITS sequence allows a better species identification of these isolates, especially when the similarities of 16S rRNA gene are highly similar or even identical [21]. The most favorable fruits for isolation of the BNC-producing AAB in this study were bananas, rose apples and sapodillas, since at least 2 species of BNC-producing *Komagataeibacter* could be isolated from these fruits. However, their respective type strains were isolated from the tea fungus beverage (Kombucha), mountain-ash berry, nata de coco and various kinds of vinegar [22]. This could be attributed to the isolation approach adopted in this study that showed a reasonable selection, not only for thermotolerant AAB, but also for thermotolerant BNC-producing *Komagataeibacter* from sugar-rich fruits.

Table 1. Identification of thermotolerant BNC-producing strains of MSKU 1- MSKU 19 assigned to genus *Komagataeibacter*.

Isolates	Isolation source	Pairwise similarity (%) to the closely related type strains	
		16S rRNA gene	16S-23S rRNA gene ITS
MSKU 1	Banana	*K. nataicola* (99.93)	*K. nataicola* (100)
		K. swingsii (99.93)	*K. intermedius* (92.3)
		K. europaeus (99.93)	*K. oboediens* (93.5)
MSKU 2	Banana	*K. nataicola* (99.85)	*K. intermedius* (96.0)
		K. swingsii (99.85)	*K. oboediens* (94.5)
		K. europaeus (99.85)	*K. saccharivorans* (93.5)
MSKU 3	Banana	*K. intermedius* (100)	*K. oboediens* (99.7)
		K. oboediens (99.85)	*K. intermedius* (97.0)
MSKU 4	Banana	*K. hansenii* (99.85)	*K. hansenii* (99.0)
MSKU 5	Coconut	*K. intermedius* (100)	*K. oboediens* (99.7)
		K. oboediens (99.85)	*K. intermedius* (95.8)
MSKU 6	Grape	*K. xylinus* (99.71)	*K. nataicola* (95.8)
		K. intermedius (99.71)	*K. xylinus* (95.1)
		K. oboediens (99.71)	*K. swingsii* (93.4)
		K. sucrofermentans (99.71)	*K. oboediens* (93.1)
MSKU 7	Jack fruit	*K. swingsii* (99.78)	*K. swingsii* (93.8)
		K. europaeus (99.78)	*K. oboediens* (92.6)
MSKU 8	Lychee	*K. nataicola* (99.93)	*K. intermedius* (96.2)
		K. swingsii (99.93)	*K. oboediens* (94.7)
		K. europaeus (99.93)	*K. saccharivorans* (93.6)

Table 1. Continued.

Isolates	Isolation source	Pairwise similarity (%) to the closely related type strains	
		16S rRNA gene	16S-23S rRNA gene ITS
MSKU 9	Persimmon	*K. nataicola* (99.86)	*K. intermedius* (96.2)
		K. swingsii (99.86)	*K. oboediens* (94.7)
		K. europaeus (99.86)	*K. saccharivorans* (93.6)
MSKU 10	Pomegranate	*K. xylinus* (99.71)	*K. nataicola* (95.8)
		K. intermedius (99.71)	*K. xylinus* (94.8)
		K. oboediens (99.71)	*K. swingsii* (93.4)
		K. sucrofermentans (99.71)	*K. oboediens* (93.1)
MSKU 11	Rambai	*K. intermedius* (99.93)	*K. oboediens* (99.8)
		K. oboediens (99.78)	*K. intermedius* (95.9)
MSKU 12	Rose apple	*K. xylinus* (99.71)	*K. nataicola* (94.8)
		K. intermedius (99.71)	*K. xylinus* (93.9)
		K. oboediens (99.71)	*K. oboediens* (93.4)
		K. sucrofermentans (99.71)	*K. swingsii* (93.2)
MSKU 13	Rose apple	*K. maltaceti* (100)	*Ga. entanii* (97.7)
		Ga. entanii (99.85)	*K. maltaceti* (97.1)
MSKU 14	Santol	*K. intermedius* (99.93)	*K. oboediens* (99.8)
		K. oboediens (99.78)	*K. intermedius* (95.9)
MSKU 15	Sapodilla	*K. nataicola* (99.85)	*K. intermedius* (96.0)
		K. swingsii (99.85)	*K. oboediens* (94.5)
		K. europaeus (99.85)	*K. saccharivorans* (93.6)
MSKU 16	Sapodilla	*K. intermedius* (99.85)	*K. oboediens* (99.5)
		K. oboediens (99.85)	*K. intermedius* (95.9)
MSKU 17	Sapodilla	*K. intermedius* (99.93)	*K. oboediens* (99.8)
		K. oboediens (99.78)	*K. intermedius* (95.9)
MSKU 18	Star apple	*K. maltaceti* (100)	*Ga. entanii* (97.7)
		Ga. entanii (99.85)	*K. maltaceti* (97.1)
MSKU 19	Rose apple	*K. hansenii* (99.78)	*K. hansenii* (99.3)

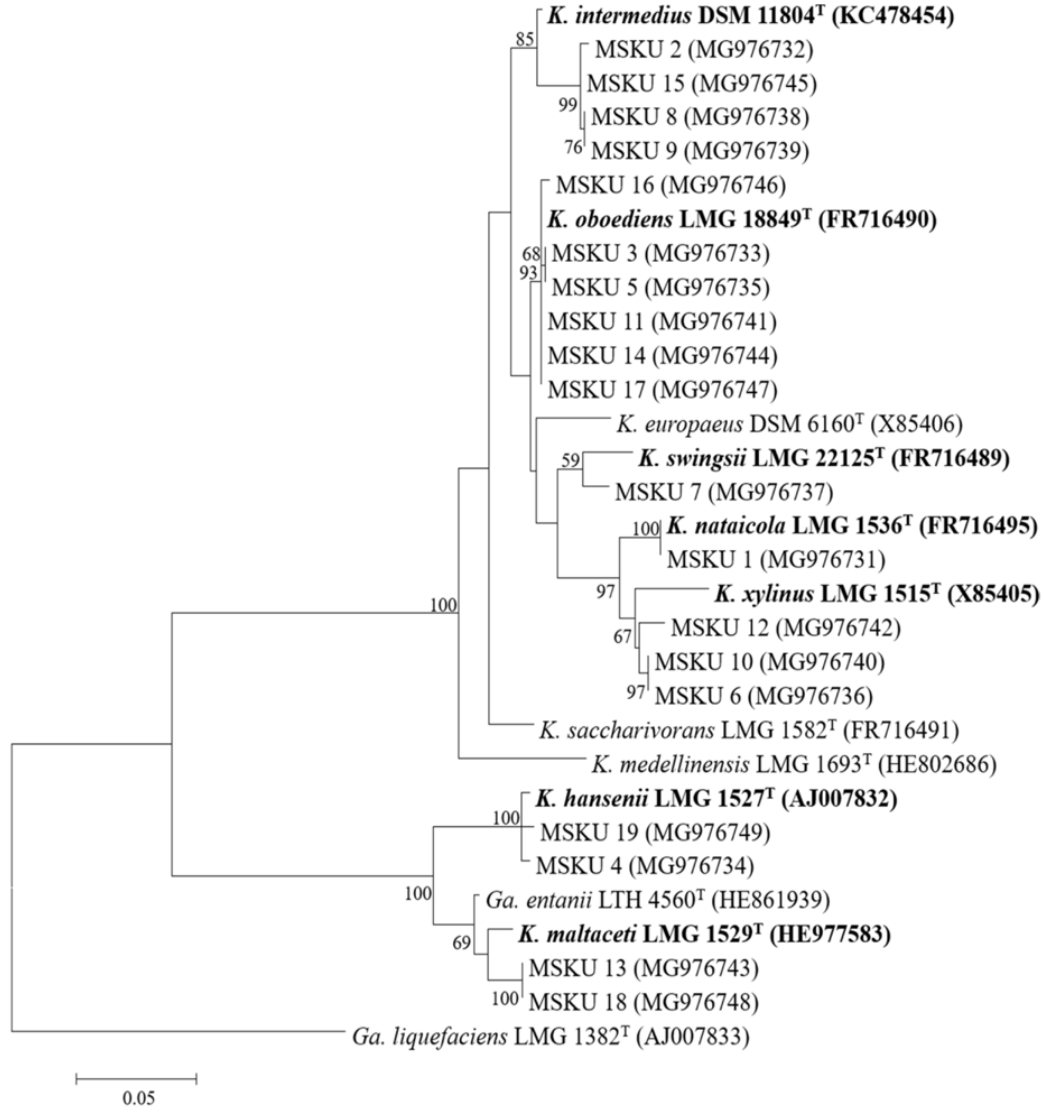

Figure 1. Phylogenetic relationships of *Komagataeibacter* sp. MSKU 1- MSKU 19 and related type strains. The phylogenetic tree based on the 16S-23S rRNA gene ITS sequences of 565 bases was constructed using the maximum likelihood method. The numerals at the nodes indicate bootstrap values (%) derived from 1,000 replications. *Gluconacetobacter liquefaciens* LMG 1382^T was used as outgroup.

3.2 Growth Characteristics of Thermotolerant BNC-producing AAB at Various Temperatures

The growth characteristics of the MSKU isolates along with closely related type strains at various temperatures (30, 37 and 39 °C) on YPGD-1A2E agar for 3 days, are shown in Table 2. Generally, all 19 isolates and the tested type strains, except for *K. nataicola* BCC 36443^T, could grow well on YPGD-1A2E agar at 30 °C. It is noted that *K. nataicola* BCC 36443^T could not grow on the medium containing 1% acetic acid as previously reported [19]. Nonetheless, even when tested on YPGD agar without acetic acid, *K. nataicola* BCC 36443^T could only grow at 30 °C (data not shown). Moreover, the growth characteristics of the *Komagataeibacter* species had not been previously reported at temperatures over 30 °C. In this study, 16 newly isolated *Komagataeibacter* species and 2 type strains (*K. intermedius* JCM 16936^T and *K. maltaceti* 14815^T) exhibited good growth at 37 °C. In addition, 9 of 16 isolates and only one type strain, *K. maltaceti* NBRC 14815^T, could also grow up to 39 °C, although some showing poor growth. Interestingly, MSKU 3 and MSKU 5 could grow well even at 39 °C, at which temperature the related type strain *K. oboediens* JCM 16937^T could not grow. Based on the results obtained from this experiment, it was clearly confirmed that thermotolerant BNC-producing *Komagataeibacter* have been successfully isolated from tropical fruits in Thailand.

Table 2. Growth characteristics and BNC production of thermotolerant BNC-producing strains, MSKU 1- MSKU 19, and related type strains *Komagataeibacter* species.

Isolates	Grow (°C)			BNC (µmol/mg)
	30	37	39	at 30 °C
K. hansenii NBRC 14820^T	good	poor	no	0.51
K. intermedius JCM 16936^T	good	good	no	7.73
K. maltaceti NBRC 14815^T	good	good	poor	0.48
K. nataicola BCC 36443^T	no	no	no	3.16
K. oboediens JCM 16937^T	good	poor	no	3.45
K. swingsii JCM 17123^T	good	no	no	1.50
MSKU 1	good	poor	no	5.61
MSKU 2	good	good	no	8.63
MSKU 3	good	good	good	8.28
MSKU 4	good	good	no	2.79
MSKU 5	good	good	good	3.63
MSKU 6	good	poor	no	16.35
MSKU 7	good	good	no	8.52
MSKU 8	good	good	no	6.62
MSKU 9	good	good	poor	11.67
MSKU 10	good	good	no	16.38
MSKU 11	good	good	poor	6.60
MSKU 12	good	good	no	16.66
MSKU 13	good	good	poor	1.00
MSKU 14	good	good	poor	6.28

Table 2. Continued.

Isolates	Grow (°C)			BNC (µmol/mg)
	30	37	39	at 30 °C
MSKU 15	good	good	poor	10.03
MSKU 16	good	good	no	13.20
MSKU 17	good	good	poor	5.21
MSKU 18	good	good	poor	0.60
MSKU 19	good	poor	no	2.71

3.3 BNC Production of Thermotolerant BNC-producing AAB at Various Temperatures

Preliminary investigation of BNC production, measured as sugar content, showed that all strains of *Komagataeibacter* could produce BNC at 30 °C which is the optimum temperature for BNC production [5]. Generally, the newly isolated MSKU 1-MSKU 19 exhibited high amounts of BNC (0.60-16.66 µmol/mg) when compared to tested type strains (0.48-7.73 µmol/mg) (Table 2). The four most effective BNC-producing isolates, MSKU 6, MSKU 10, MSKU 12 and MSKU 16 along with *K. oboediens* JCM 16936^T were selected for further BNC production at 37 °C. The result showed that MSKU 12 produced much thicker BNC pellicle than MSKU 6, MSKU 10, MSKU 16 and *K oboediens* JCM 16936^T (Figure 2A). MSKU 12 produced the highest amount of BNC (11.11 ± 1.50 µmol sugar per mg of protein), which was 1.2, 1.5, 3.5 and 7.8 fold higher than that of MSKU 6, MSKU 10, MSKU 16 and *K oboediens* JCM 16936^T, respectively, in the same culture condition (Figure 2B).

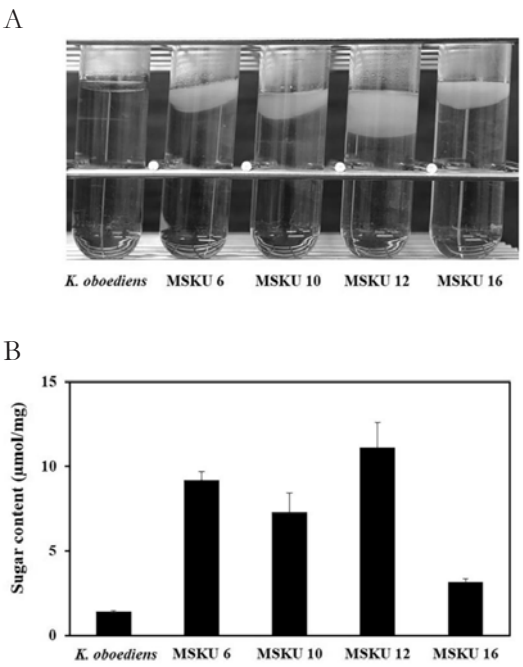

Figure 2. Pellicle formation (A) and sugar content (B) of BNC produced by *K. oboediens* JCM 16937^T, MSKU 6, MSKU 10, MSKU 12 and MSKU 16. All strains were grown in YPGD-1A2E medium and statically incubated at 37 °C for 7 days.

In order to compare the yield of BNC production with the previously reported strains [11], we further investigated the BNC production of MSKU 6, MSKU 10 and MSKU 12 in HS-1A2E medium along with *K. xylinus*, a prominent species for BNC production. The cultures were grown in HS-1A2E medium at various temperatures (30, 35 and 37 °C) for 7 days and the dry weights were determined. The pellicle formation by MSKU 6, MSKU 10, MSKU 12 and *K. xylinus* BCC 49175^T is shown in Figure 3A, and the obtained dry weight is shown in Figure 3B. The thickness of the pellicle which corresponded with the dry weight was obtained from the MSKU 6, MSKU 10 and MSKU 12 whereas the *K. xylinus* BCC 49175^T did not produce BNC. Although strains of *K. xylinus* were commonly studied for BNC production from sugars, non-BNC producing strains had been found [22]. Furthermore, the yields of the BNC (dry weight) synthesized by other *Komagataeibacter* strains after static cultivation in HS medium at 28-30 °C for 3-14 days were as follows: 4.40 ± 0.22 g/L for *K. rhaeticus* strain P 1463 [23], 3.10 g/L for *K. xylinus* ATCC 53524 [24], 2.80 g/L for *K. medellinensis* [25], 2.1 g/L for *K. swingsii* [26], 1.50 ± 0.33 g/L for *K. hansenii* UAC09 [27] and 0.217 ± 0.027 g/L for *K. xylinus* K3 [18]. Interestingly, MSKU 12 exhibited much higher BNC production than that of the other tested strains. It produced the highest yield with a total dry weight of 7.6 ± 1.01 g/L at 30 °C and 2.2 ± 0.03 g/L even at 37 °C. These results indicate that MSKU 12 is a potential producer for BNC, even at 37 °C. This study is the first report with regard to BNC production of a thermotolerant *Komagataeibacter* strain at 37 °C.

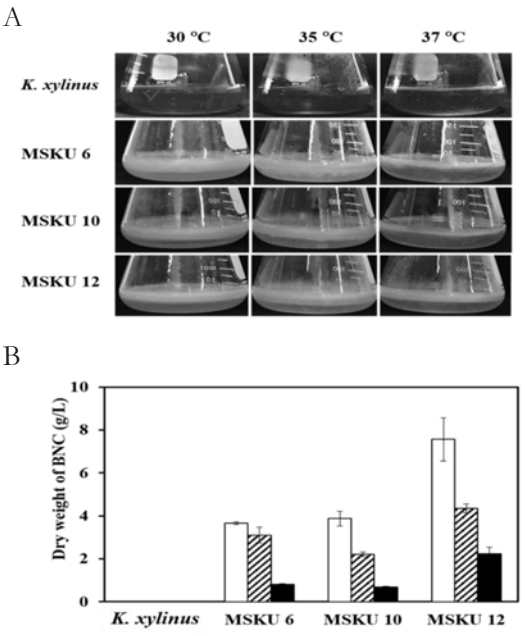

Figure 3. Pellicle formation (A) and dry weight of BNC (B) produced by *K. xylinus* BCC 49175^T, MSKU 6, MSKU 10 and MSKU 12 in HS-1A2E medium by static incubation at 30 °C (white bars), 35 °C (diagonal bars) and 37 °C (black bars) for 7 days.

3.4 Investigation of Insertion Sequence, IS*1031*, in the *Komagataeibacter* Species

The complete loss of BNC-producing ability in *K. hansenii* ATCC 23769 by the IS*1031*A element has been previously reported [7]. It is worth investigating the distribution of IS*1031* elements within the genome of the MSKU isolates and their type strains of the genus *Komagataeibacter*. All tested type strains, with the exception of *K. intermedius* JCM 16936^T and *K. swingsii* JCM 17123^T, harbor the IS*1031* elements in their genome (Figure 4). The IS elements was previously examined in a cellulose-negative mutant of *K. hansenii* ATCC 23769 [6-8]. This study revealed that the indigenous elements of

IS*1031* are commonly presented among the type strains of *Komagataeibacter*. Thus, the result indicates that genomic instability in the type strains may lead to a high frequency of spontaneous mutations, resulting in deficiency of the BNC synthesis. On the other hand, the IS elements were detected in the genome of 6 MSKU (MSKU 1, MSKU 9, MSKU 11, MSKU 14, MSKU 17 and MSKU 19) isolates (32%) so the newly isolated BNC producers lead to stable BNC production. Thus, these isolates, especially MSKU 12, is a promising strain for stable BNC production at high temperatures.

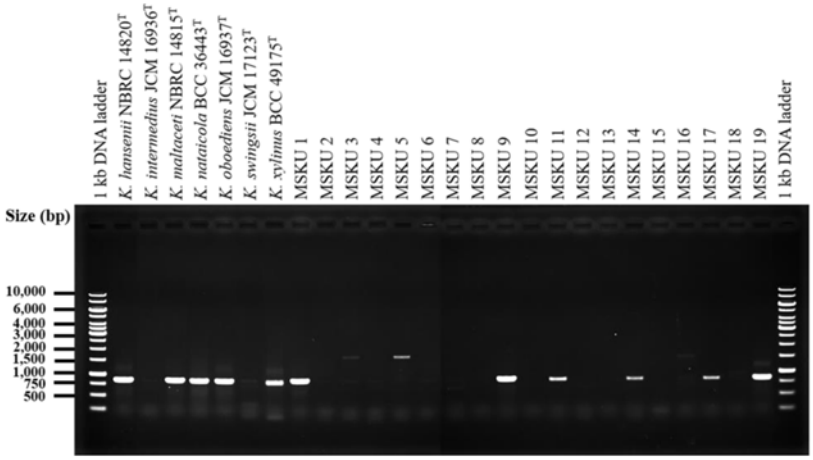

Figure 4. Agarose gel electrophoresis of PCR products of insertion sequence elements (IS*1031*) from type strains of *Komagataeibacter* and MSKU isolates.

4. CONCLUSION

From this study, we were able to isolate the most effective thermotolerant strain of the BNC-producing AAB, MSKU 12, from a rose apple, and it was closely related to *K. xylinus* based on molecular analysis. *Komagataeibacter* sp. MSKU 12 could grow and produce a high yield of BNC in a range of 30-37 °C. This isolate obviously exhibited the highest amount of the BNC (measured as sugar content and dry weight) at 30 °C, and even at 37 °C, a temperature under which the related type strain could not tolerate. In addition, MSKU 12 does not harbor the insertion sequence elements, IS*1031* family, whereas many type strains possess the IS*1031* element in their genomes. Thus, *Komagataeibacter* sp. MSKU 12 is clearly identified as a prominent strain for stable BNC production at high temperatures.

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