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Conference Paper

Isolation and Screening of Microbial Isolates from Kombucha Culture for Bacterial Cellulose Production in Sugarcane Molasses Medium

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Abstract

Kombucha tea is a traditional fermented beverage of Manchurian origins which is made of sugar and tea. The fermentation involves the application of a symbiotic consortium of bacteria and yeast (SCOBY) in which their metabolites provide health benefits for the consumer and subsequently allow the product to protect itself from contamination. Additionally, kombucha tea fermentation also produces a byproduct in the form of a pellicle composed of cellulose (Bacterial Cellulose, BC). Compared to plant cellulose, BC properties are more superior, which makes it industrially important. However, BC production at industrial scale has been faced with many challenges, including low yield and high fermentation medium cost. Many researchers have focused their studies on the use of alternative low cost media, such as molasses, which is a by-product of sugar refining process. To maximize the BC production in molasses medium, it is important to select the microbial strains that can grow and produce BC at high yield in molasses. This study aimed to isolate and characterize BC-producing bacteria and a dominant yeast from kombucha culture which had been previously adapted in molasses medium. The isolation of bacteria was performed using Nutrient Agar (NA) and Hestrin and Schramm (HS) supplemented with cycloheximide, while yeast was isolated using Potato Dextrose Agar (PDA) supplemented with chloramphenicol. The most dominant colonies were isolated and then subjected to microscopic observation for morphological analysis. The pure bacteria and yeast isolates were then identified by sequencing the 16S rRNA gene and D1/D2 region of the 26S rRNA, respectively. The bacteria isolates obtained were all from closely related genera: *Komagataeibacter sp. DS1MA.62A, Komagataeibacter xylinus, Komagataeibacter saccharivorans, Komagataeibacter xylinus* and *Gluconacetobacter saccharivorans.* The single isolated yeast was identified as *Brettanomyces bruxellensis.* This study helps to elucidate the BC-producing species which thrive in molasses medium for potential use in the BC production using molasses as alternative cheap carbon source. Also, the study revealed that the co-culture of *Komagataeibacter sp. DS1MA.62A* and *B. bruxellensis* could produce BC from molasses supplemented with caffeine and acetate buffer at an average yield of 27.7±1.83 g/L.

Keywords: Kombucha, SCOBY, bacterial cellulose, Acetobacter, Komagataeibacter, Brettanomyces, alternative medium, molasses, caffeine, acetate buffer.

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1. Introduction

Kombucha tea is a traditional fermented beverage originated from northeast China, Manchuria [1]. Nowadays, it has become very popular in Asia and Western world (North America and Europe) due to many health claims that are linked to its chemical composition resulted from microorganisms activity during fermentation process. The tea is traditionally made by fermenting sugar-rich black or green tea with indigenous microorganisms mostly known as SCOBY, a short for symbiotic consortium of bacteria and yeast [1, 2]. The main product coming from the fermentation is a slightly acidic or sour beverage known as kombucha tea. It is rich in organic acids such as acetic, gluconic, glucuronic, citric, L-lactic; and vitamins B1, B2, B6, B12, C, as well as 14 amino acids [1]. The fermentation not only produces the tea, but it also by-produces a transparent pellicle of cellulose called bacterial cellulose or BC.

BC has recently gained a huge interest due to better physical and chemical properties compared to plant cellulose such as higher purity, crystallinity, polymerization, tensile strength, and water holding capacity, although both have the same molecular formula $(C_6H_{10}O_5)_n$ [3--5]. Those superiority of BC leads to huge potential in industry, replacing cellulose derived from plant. Recent research has revealed the BC potential as biosorbent in waste management industry, thickening agent and stabilizer in the food industry, and wound healing agent in pharmacy and medical field [1, 3, 5].

Despite the enormous potential of BC, its industrial production is still hindered by high fermentation medium cost. Industrial scale fermentation generally utilizes pure sugars such as glucose, sucrose, mannitol, fructose, and arabitol as carbon source, which consume about 30% of the total production cost [6]. Recent studies have revealed the potential of carbon-rich agroindustrial waste such as molasses, wheat straw, rice bark, and many more as an alternative fermentation medium for producing BC [6--8]. The strategy of utilizing waste as alternative medium could not only lower the production cost, but also solve the environmental issues that might arise from unprocessed waste.

Fermentation process to produce kombucha tea beverage along with BC involves bacteria and yeast which exist as a symbiotic consortium (SCOBY). In the case where the fermentation uses complex sugar, the yeast helps the bacteria to digest the complex sugar sucrose into glucose and fructose. The sugar digestion by yeast will also produce ethanol through hydrolysis and glycolysis pathway [1, 9]. The glucose and ethanol derived from yeast metabolism are utilized as a food source by acetic acid bacteria where they oxidize them into gluconic acid and acetic acid [1, 9]. The metabolism process can be seen in **Figure 1**. Symbiotic relation between *S. cerevisiae* and *Acetobacter*

isolated from Haipao fermentation was reported by Liu et al. (1996). They mentioned that the yeast produced ethanol to drive the bacterial growth and thus proportionally promoting higher acetic acid production. The symbiotic relationship happened in a way that ethanol production by yeast can be further stimulated by acetic acid [10--12]. The presence of ethanol has also been confirmed by several previous studies to positively affect the BC yield [11, 12].
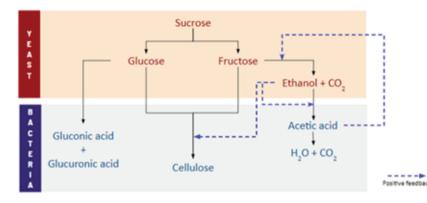

Figure 1: Microbial Interaction in Kombucha Tea Fermentation. (Villarreal-Soto et al., 2018, after modification)

Microbial composition of SCOBY may vary, depending on the inoculum source as well as fermentation condition; however, it is generally composed of acetic acid bacteria (AAB), lactic acid bacteria (LAB), and yeast [2, 13]. Several previous studies have successfully identified the most dominant bacteria and yeast species. The bacteria is generally coming from the genus *Gluconacetobacter*, *Agrobacterium*, *Aerobacter*, *Alcaligenes*, *Achromobacter*, *Azotobacter*, *Sarcina*, *Rhizobium*, *Salmonella*, and *Escherichia* [3--5]. The most well-known and well-studied cellulose-producing bacteria is *Komagataeibacter xylinus*, which was formerly known as *Acetobacter xylinum*. Other cellulose-producing bacteria that have also been identified are *Komagataeibacter kombuchae, Gluconacetobacter* sp A4, *Gluconacetobacter sacchari*, *Acetobacter aceti, Acetobacter nitrogenificans, Acetobacter pasteurianus, Acetobacter liquefaciens, Bacterium gluconicum,* and *Gluconobacter oxydans* [1, 14]. On the other hand, the yeast ecology in SCOBY is dominated by *Saccharomyces, Saccharomycodes, Zygosaccharomyces, Candida, Pichia, Schizosaccharomyces, Brettanomyces/Dekkera, Torulospora, Koleckera, Mycotorula*, and *Mycoderma* [1, 9, 15, 16]. All of them are described as osmotolerant, acid-tolerant, fermentative, and alcohol- as well as flavor-producing yeast.

In addition to suitable fermentation medium, the use of appropriate microbial strain also plays a key role in enhancing the BC production. To maximize the BC production in molasses, the microbial strains used should be able to grow and exhibit a high BC production capacity in molasses. Since molasses consists primarily of sucrose

(50%), the utilization of yeast could help break down the sucrose into fructose and glucose, which will subsequently be used by the bacteria to synthesize BC. In this study, kombucha cultured suspected to contain yeast and BC-producing bacteria was adapted in molasses. The predominant bacteria and yeast were then isolated and identified by sequencing the 16S rRNA gene and D1/D2 region of the 26S rRNA gene, respectively. BC production from molasses with co-culture consisting the dominant bacterial and yeast isolates was also addressed in this study.

2. Methods

2.1. Materials

Kombucha tea was kindly provided by PT Tujju Kombucha (Jakarta, Indonesia). Goalpara Perbawati black tea (Sukabumi, Indonesia) and Gulaku Murni table sugar from Sugar Group Companies (Lampung, Indonesia) were purchased from a local market in Indonesia and were used in the starter culture preparation step. Molasses that was used as the carbon source in the study was supplied by PT. Andalan Furnindo (Marunda, Indonesia). The characteristics of the molasses presented in **Table 1** was provided as well by the company. Pure caffeine with minimal purity of 99.5% used in the study was purchased from local online shop. Other chemicals used in this study were purchased from Sigma-Aldrich and Merck.

TABLE 1: Characteristics of Molasses Used in the Current Study.

Parameter	Unit	Result
Color	visual	Brown/Black
Brix	%	77.6
Sucrose	%	46.92
Inverted sugar	%	60.76
Purity	%	60.76
Specific gravity	%	1.39835
pH	-	5.12
Ash Conductivity	%	0.4

2.2. Kombucha culture adaptation in molasses

This step was performed in order to adapt the culture present in the kombucha tea to molasses medium. The adaptation process was done sequentially as described in **Figure 2**. The first adaptation was done in sugared tea medium that was prepared by boiling the black tea and table sugar at concentration of 100 g/L and 10 g/L, respectively, in type III water for 10 minutes. The tea mixture was then filtered through filter paper 125 mm $\varnothing$ (cat no 1004 125 Whatman) in a funnel into 100 ml Erlenmeyer flask and autoclaved at 121^OC for 20 minutes. After being autoclaved, the mixture was left to cool down and inoculated with kombucha tea; the size of the inoculum was 10% of the final volume. The setup was then left in a dark room at room temperature (28 ± 2 °C) for 7 days to be used in the second step of adaptation. The fermentation medium used in the second adaptation step was prepared by mixing molasses at 50 g/L and filtered sugared tea that was prepared by boiling tea at 10 g/L and table sugar at 50 g/L. The mixture was then autoclaved at 121-^OC for 20 minutes then left to cool down before being inoculated with culture from step 1. The set up was left in a dark room at room temperature for 5 days. The last adaptation part was done by inoculating culture from step 2 in sterilized molasses medium that was prepared by dissolving 100 g/L molasses in acetate buffer (200 mM, pH 4.75). The set up was left in a dark room for 6 days. The composition of fermentation in each step can be seen in **Table 2**.
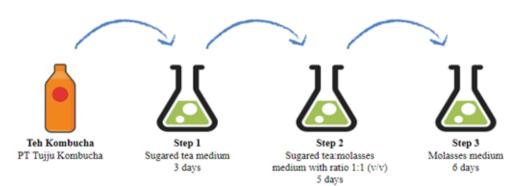

Figure 2: Schematic Procedure for Starter Culture Preparation. The composition of fermentation medium in each step can be seen in Table 2.

2.3. Isolation and Characterization of Microbes in Molasses-Adapted Kombucha Culture

TABLE 2: Composition of Medium Used for Adaptation.

	Step 1	Step 2	Step 3
Black tea (g/L)	10	10	-
Table sugar (g/L)	100	50	-
Molasses (g/L)	-	50	150
Total Volume (ml)	50	200	1000
Water (ml)	45	180	-
200mM acetate buffer pH 4.75 (ml)	-	-	900
Culture (ml)	5[a]	20[b]	100[c]

[a]Kombucha tea, [b]Fermentation broth from step 1, [c]Fermentation broth from step 2

2.3.1. Microbiological Culture Media Preparation

Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were obtained from Sigma-Aldrich. Both NA and PDA were prepared according to the manufacturer's instructions. Hestrin-Schramm (HS) agar was made by mixing 20 g/L of glucose, 5 g/L of peptone, 5 g/L of yeast extract, 2.7 g/L of Na_2HPO_4, and 1.15 g/L of citric acid. To prepare plates for enumeration of bacteria from kombucha fermentations, a 0.5 mg/mL solution of cycloheximide in ethanol was added to cooled, molten NA and HS. To prepare plates for enumeration of yeasts from kombucha fermentations, a 25 mg/mL solution of chloramphenicol in ethanol was added to cooled, molten PDA.

2.4. Isolation of Dominant Microbes (Bacteria and Yeast) in Kombucha Culture Adapted in Molasses

Fermentation broth from step 3 in adaptation part was cultured on 3 types of agar: PDA, NA, and HS. The sample (10 ml fermentation broth) was first serially diluted in sterile phosphate buffer saline (PBS). The bacteria was isolated on NA media that had been added with cycloheximide to inhibit the growth of yeast, which was then incubated at room temperature for 24-48 hours. On the other hand, the yeast was isolated on PDA which had been added with chloramphenicol to inhibit the growth of bacteria, followed by incubation at room temperature for 72 hours. Microbial samples were also grown on HS agar media at room temperature for 72 hours. The dominant isolates of each petri dish were selected based on colony morphology and the result of gram staining.

2.5. Characterization and Identification of Bacteria and Yeast Isolates

Bacterial isolates were identified through PCR method and gene sequencing of the 16S rRNA gene using universal primers (27F/1492R). Meanwhile yeast isolates were identified through PCR method and gene sequencing of the D1/D2 region of the 26S rRNA gene using primers NL-1 and NL-4. The sequence of the primers are listed in **Table 3**. PCR product was then run on electrophoresis in 1% agarose gel with Tris-borate-EDTA. Agarose gel was then soaked in ethidium bromide and visualized with the help of UV light. Then, the pure PCR results from bacterial 16S rRNA gene amplification and yeast 26S rRNA were sent for sequencing.

2.6. Evaluation of the Potential of Bacteria Isolates for Producing BC Pellicle

In this step, bacterial isolates were tested exclusively, as yeast are not cellulose-producing microbes. Bacteria isolates obtained from previous step were tested for their potential to produce BC by growing each isolate on a fermentation medium containing 150 g/L molasses and 500 mg/L pure caffeine dissolved in acetate buffer solution (200 mM, pH 4.75). This stage was carried out on 6-well plates and the fermentation was done at room temperature for 7 days. The isolate with the highest yield (significant BC yield expressed in g/L), was selected to produce BC in the next step.

2.7. BC Pellicle Production through Fermentation of Molasses with Co-culture of Isolated Microbes

According to the results obtained from previous steps, the most productive bacteria (*Komagataeibacter sp*. DS1MA.62A) was selected to be used for the following experiment. In this stage, BC was produced through fermentation with co-culture consisted of the bacteria and yeast isolate (*Brettanomyces bruxellensis*). The fermentation medium consisted of 500 mg/L caffeine and 150 g/L molasses prepared in acetate buffer (200 mM, pH 4.75). Fermentation volume was 3 mL, using 6-well plates. The medium was autoclaved first before being inoculated with co-culture of bacteria and yeast. The fermentation was carried out at room temperature for 14 days. After 14 days of fermentation, the BC pellicle was harvested and dried at 50^OC in an oven until the weight remained constant. The dry weight of BC pellicle was recorded and reported in this study as BC yield expressed in g/L, which was grams of dry weight BC produced

per 1 liter fermentation volume. The BC yield reported in this study was mean±standard deviation from triplicate experiments.

TABLE 3: Primers Used in the Study to Identify the Microbes.

	Primers	Sequence
Identification of Bacteria Isolates	27F	5'-AGAGTTTGATCCTGGCTCAG-3'
	1492R	5'-GGTTACCTTGTTACGACTT-3'
Identification of Yeast Isolates	NL1	5ʹ-GCATATCAATAAGCGGAGGAAAAG-3ʹ
	NL4	5ʹ-GGTCCGTGTTTCAAGACGG-3ʹ

3. Result

3.1. Screening of Bacterial Cellulose-producing Isolates

Bacteria which have been isolated and preserved from an adapted culture were chosen to be screened based on their visual colony difference. Prior to screening, 6 different colony types were subject to microscopy tests, showing that all bacteria were homogeneous in visual appearance, rod-shaped and pink when run through a gram staining procedure (**Figure 3**). This was an indication of acetic acid bacteria, common producer of BC.

After 14 days of static incubation, the single most productive bacteria, chosen through its exceptionally large thickness assessed through visual analysis, was selected for isolation. This isolate was the only bacterial inoculum used for the following experiments. This bacteria is stored in the i3L culture collection labeled ``13.7.KBC.HSA''.
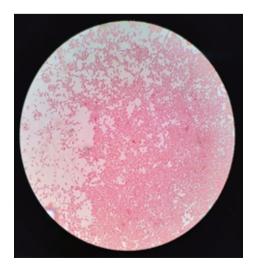

Figure 3: Visualization of gram stained bacteria under light microscope at 1000x magnification.

3.2. PCR analysis of Bacterial and Yeast Isolates

Colony PCR was successful without any pretreatment of bacterial DNA template, where 4 out of 6 samples shows single bands (**Figure 4**). The remaining samples, ``13.7.KBC.HSA'' and the yeast needed Wizard® Genomic DNA Purification Kit prior to PCR efforts before having its NA validated for purity.

3.3. Sequence Analysis

PCR product would then be subjected to DNA sequencing. The resulting sequencing data were processed with FinchTV software to select clean peaks for both forward and reverse primers. The forward primer was then aligned with the reverse-complement of the reverse primer to validate the integrity of both the forward and reverse identity with the help of CLC Viewer software, resulting in a ``Consensus'' sequence (**Appendix**). This consensus sequence was then screened using nucleotide BLAST to provide microbial identity. The identity chosen from the BLAST result list would be based on the accession link's journal status, as either published or submitted. Unpublished BLAST results would be overlooked. 5 bacteria and 1 yeast have been successfully sequenced and identified (**Table 4**). All 5 of the bacteria are from closely related genera. Single isolated yeast belongs to the *Brettanomyces* genus.
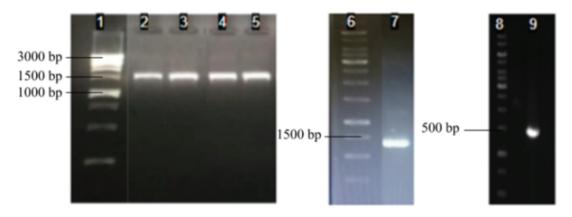

Figure 4: PCR gel electrophoresis showing 1. DNA Ladder 1 kb 2. ``13.7.KBC.HSB'' 3. ``13.7.KBC.HSC'' 4. ``13.7.KBC.HSD'' 5. ``13.7.KBC.HSE'' 6. DNA Ladder 1 kb 7. ``13.7.KBC.HSA'' 8. DNA Ladder 1 kb 9. ``13.7.KBC.HSF''.

3.4. Co-Culture Fermentation Yields

The resulting BC dry weight of the co-culture fermented for 14 days using molasses medium using 500 mg/L caffeine and 150 g/L molasses prepared in acetate buffer (200

TABLE 4: Isolated bacteria and Yeast Names, Primers, Identities and Their Accession.

No.	Sample Name	Forward Primer	Reverse Primer	Identity	Accession Number
1	13.7.KBC.HSA	27F	1492R	*Komagataeibacter sp. DS1MA.62A*	LN884048.1
2	13.7.KBC.HSB	27F	1492R	*Komagataeibacter xylinus*	MH511551.1
3	13.7.KBC.HSC	27F	1492R	*Komagataeibacter saccharivorans*	KY287776.1
4	13.7.KBC.HSD	27F	1492R	*Komagataeibacter xylinus*	MH511551.1
5	13.7.KBC.HSE	27F	1492R	*Gluconacetobacter saccharivorans*	JF718412.1
6	13.7.KBC.HSF	NL1	NL4	*Brettanomyces bruxellensis*	MH930867.1

mM, pH 4.75) was calculated into an average yield of 27.7±1.83 g/L of BC. This data was expressed as mean±standard deviation from triplicate experiments.

4. Discussion

PCR analysis of the microbes were intended to identify the bacteria responsible for the monoculture setups as well as to highlight the diversity of the kombucha consortium. A total of 6 samples, despite some not as efficient as the other with producing BC, were sequenced. Furthermore, as only a single phenotype of yeast colony has emerged in all of the isolation efforts, following the usage of this yeast in the experimental co-culture design, it could be of interest to investigate this particular yeast deeper. A single dominant yeast throughout every single time point in kombucha culture contradicts several studies which provide various species of yeast [9]. However, the prevalence of *Brettanomyces* in this experiment might be attributed to its affinity to the particular medium used in this experiment, as well as the lack of supportive medium for the uncultured yeast species.

Sequencing data has shown that the bacteria culture used in the co-culture experiments was identified as *Komagataeibacter sp. DS1MA.62A*. This BLAST result has been selected due to its journal status as submitted. *Komagataeibacter* is a genus of bacteria best known for its capability to produce cellulose. It is an acetic acid producing bacteria, gram-negative and aerobic. The particular species, *Komagataeibacter sp. DS1MA.62A,* has not been published in an article specifically for its BC producing capabilities. As for the yeast, it was identified as *Brettanomyces bruxellensis,* a yeast commonly used for

brewing alcoholic beverages. This particular yeast have been isolated from a kombucha fermentation and have shown to be part of a greater consortium [9], unlike in this study where it is the sole isolate of yeast grown on PDA. This might also be attributed to dissimilarity of isolation methods used in the mentioned study.

The average yield of BC produced with a co-culture of *Komagataeibacter sp. DS1MA.62A* and *Brettanomyces bruxellensis* was 27.7 g/L for 14 days. In comparison with previous study [17], a fermentation of 6 days using pineapple waste media with a pure *Acetobacter xylinum 0416* culture yields 28.3 g/L of BC. This sparse difference showed that the isolated culture had comparable potential with the known highest BC producing species.

5. Conclusion

This study isolated and screened the microbes in kombucha culture which were predominant after adaptation in molasses medium.. The result revealed 5 dominant bacteria and 1 dominant yeast. The bacterial isolates include *Komagataeibacter sp. DS1MA.62A, Komagataeibacter xylinus, Komagataeibacter saccharivorans, Komagataeibacter xylinus* and *Gluconacetobacter saccharivorans.* Meanwhile, the single isolated yeast was identified as *Brettanomyces bruxellensis*. Amongst the bacterial isolates, *Komagataeibacter sp. DS1MA.62A* was found to be the most potential BC producer, which has the ability to produce BC at significant amount in monoculture fermentation. In co-culture fermentation between the selected bacteria and the yeast, *Brettanomyces bruxellensis* yeast was present in the culture to help break down complex sugar and producing ethanol to enhance the fermentation process. The BC yield from co-culture fermentation reached 27.7±1.83 g/L. This finding could be further used to consider which species of bacteria and yeast used during bacterial cellulose production in industrial scale which utilized molasses agroindustrial waste.

Acknowledgment

Funding

Conflict of Interest

None declared.

APPENDICES

13.7.KBC.HSA, Komagataeibacter sp. DS1MA.62A

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CAGTCGCACGAACCTTTCGGGGTTAGTGGCGGACGGGTGAGTAACGCGTAGGGATCTATC
CACGGGTGGGGGATAACTTTGGGAAACTGAAGCTAATACCGCATGACACCTGAGGGTCA
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13.7.KBC.HSB, Komagataeibacter xylinus

GCAGTCGCACGAACCTTTCGGGGGTTAGTGGCGGACGGGTGAGTAACGCGTAGGGATCTGT
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TTGGTTTG

13.7.KBC.HSC, Komagataeibacter saccharivorans

CGCACGAACCTTTCGGGGTTAGTGGCGGACGGGTGAGTAACGCGTAGGGATCTGTCCATG
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13.7.KBC.HSD, Komagataeibacter xylinus

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CGGTAATACGAAGGGGGCAAGCGTTGCTCGGAATGACTGGGCGTAAAGGGCGCGTAGGC
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GAGACTAGAGTGTGAGAGAGGGTTGTGGAATTCCCAGTGTAGAGGTGAAATTCGTAGAT
ATTGGGAAGAACACCGGTGGCGAAGGCGGCAACCTGGCTCATTACTGACGCTGAGGCGC
GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTG
TGCTGGATGTTGGGTGACTTTGTCATTCAGTGTCGTAGTTAACGCGATAAGCACACCGCCT
GGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGGGCTTGACATGCGGA
GGCCGTGTCCAGAGATGGGCATTTCTCGCAAGAGACCTCCAGCACAGGTGCTGCATGGCT
GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCT
TTAGTTGCCATCACGTCTGGGTGGGCACTCTAGAGGAACTGCCGGTGACAAGCCGGAGGA
AGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGTCCTGGGCTACACACGTGCTACAA
TGGCGGTGACAGTGGGAAGCCAGGTAGCGATACCGAGCCGATCTCTAAAAGCCGTCTCA
GTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGGTGGAATCGCTAGTAATCGCGGATC
AGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAG
TTGGTTTG

13.7.KBC.HSE, Gluconacetobacter saccharivorans

CCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGTTTTCGGATTGTAAAGCACTTTCAGCG
GGGACGATGATGACGGTACCCGCAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGC
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GTTTTAACAGTCAGATGTGAAATTCCTGGGCTTAACCTGGGGGCTGCATTTGATACGTTG
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AAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTGT
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GGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGGGCTTGACATGCGGA
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GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCT
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AGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGTCCTGGGCTACACACGTGCTACAA
TGGCGGTGACAGTGGGAAGCCAGGTAGCGATACCGAGCCGATCTCTAAAAGCCGTCTCA
GTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGGTGGAATCGCTAGTAATCGCGGATC
AGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAG
TTGGTTTGACCTTAAGCCGGTGAGCGAACCG

13.7.KBC.HSF, Brettanomyces bruxellensis

GAAAAGAAACCAACAGGGATTGCCCCAGTAATGGCGAATGAAGCGGCAAGAGCCCAAAT
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TCGATTCTGTCGGAGGGTGTTATAGCGCGGACATCTTGTGGCTAGCCGGGACCGGGGACT
GCGGTGACTTGTCACCAAGGATGCTGGCAGAACGAGCAAATACCACCC

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