Journal of the Korean Applied Science and Technology
Vol. 39, No. 5. October, 2022. 632~643
ISSN 1225-9098 (Print)
ISSN 2288-1069 (Online)
http://dx.doi.org/10.12925/jkocs.2022.39.5.632

Characterization and Production of Low Molecular Weight of Biopolymer by *Weisella* sp. strain YSK01 Isolated from Traditional Fermented Foods

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(Received September 20, 2022; Revised October 24, 2022; Accepted October 24, 2022)

전통 발효식품으로부터 분리된 *Weisella* sp. strain YSK01에 의한 저분자 Biopolymer 발효생산 공정 및 생성물의 특성

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(2022년 9월 20일 접수: 2022년 10월 24일 수정: 2022년 10월 24일 채택)

Abstract : Although probiotics have been shown to improve health when consumed, recent studies have reported that they can cause unwanted side effects due to bacterial-human interactions. Therefore, the importance of prebiotics that can form beneficial microbiome in the gut has been emphasized. This study isolated and identified bacteria capable of producing biopoymer as a candidate prebiotic from traditional fermented foods. The isolated and identified strain was named WCYSK01 (*Wissella* sp. strain YSK01). The composition of the medium for culturing this strain was prepared by dissolving 3 g K_2HPO_4, 0.2 g $MgSO_4$, 0.05 g $CaCl_2$, 0.1 g NaCl in 1 L of distilled water. The LMBP(low molecular weight biopoymers) produced when fermentation was performed with sucrose and maltose as substrates were mainly consisted of DP3 (degree of polymer; isomaltotriose), DP4 (isomaltotetraose), DP5 (isomaltopentaose), and DP6 (isomaltoheptaose). The optimization of LMBP (low molecular weight of biopolymer) production was performed using the response surface methodology. The fermentation process temperature range of 18 to 32°C, the fermentation medium pH in the range of 5.1 to 7.9. The yield of LMBP production by the strain was found to be significantly affected by q fermentation temperature and pH. The optimal fermentation conditions were found at the normal point, and the production yield was more than 75% at pH 7.5 and temperature of 23°C.

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Keywords : Weissella, prebiotic, fermentation, low molecular weight biopolymer, response surface methodology

1. Introduction

A World Health Organization (WHO) report of October 2001 defined probiotics as "living microorganisms that, when administered in appropriate amounts, confer a health benefit on the host" [1,2]. In general, probiotics are living microorganisms that improve the intestinal microbiom and promote health when ingested [3,4]. However, recent studies have announced that probiotics are generally safe to consume, but rarely can cause unwanted side effects from bacterial-human interactions [1, 5-6].

Therefore, the importance of prebiotics that can form beneficial microbiomes in the intestine has been emphasized. According to the prebiotics definition, prebiotics have been used to refer to functional foods that are difficult to digest in the digestive tract and improve the health of the host through selective proliferation of certain bacteria by reaching the intestine [7,8].

Recently, the World Prebiotics Association (GPA) defined prebiotics as products or ingredients that help the proliferation of beneficial microorganisms that provide health or functionality[9]. Among prebiotics, oligosaccharides such as LMBP (low molecular weight biopolymer) and resistant starch are the main sources of commonly known prebiotics[10-12]. In particular, fructan and galactan have been found to stimulate the growth of beneficial microorganisms in the gut [13]. Other dietary fibers such as pectin, [14] beta-glucan, and xylo-oligosaccharide are also in the category of prebiotics. [15]

Weissella is a genus of gram-positive bacteria in the family *Lactobacillaceae* that was previously considered a species in the *Leuconostoc paramesenteroides group*[16-17]. These strains have shown probiotic potential and have recently been approved for use in food by the Senate Committee on Food Safety[18].

A study on the prebiotics properties of *Weissella* isolated from kimchi was reported to inhibit the proliferation of foodborne pathogens and adherence to intestinal epithelial cells[19]. A study using a similar strain announced that the biopoymer produced by this strain inhibited the proliferation of the major pathogens of upper respiratory tract infections (URTIs): *Streptococcus pyogenes, Staphylococcus aureus, S. pneumoniae*, and *Moraxella catarrhalis* [20]. Acceptor reaction performed by *Weissella* and *Leuconostoc* strains has been used for the production of prebiotic oligosaccharides [21-22]. It has been known that most of the oligosaccharides produced by these strains had the structure that is difficult to digest by digestive enzymes [23-26].

Therefore, the purpose of this study is to isolate the biopoymer-producing strain from traditional fermented foods, to reveal the structural characteristics of the low-molecular-weight biopoymer produced using the receptor reaction, and to establish an optimal production process.

2. Materials and methods

2.1. Fermentation medium preparation and reagents

The medium for isolating the fermentation strain in the experiment was MRS (Difco, USA). MRS solid medium was prepared by adding 2% (w/v) agar, and was labeled as MRSA (MRS plus 2% agar). When 2% (w/v) of sucrose as the carbon source was added,

the medium was marked as MRSAS. The MM (mineral medium) used in this experiment was prepared by dissolving 3 g K_2HPO_4, 0.2 g $MgSO_4$, 0.05 g $CaCl_2$, 0.1 g NaCl in 1 L of distilled water. When yeast extract was included in the salt medium, it was marked as MMY. When 2% sucrose, a carbon source, was added, it was marked as MMYS. In the case of preparing a solid medium, it was marked with MMYAS as mentioned above.

2.2. Isolation and identification of strains from fermented kimchi

The fermented kimchi used in the experiment was purchased from the market. For strain isolation, a portion of the broth of the sample was smeared on MRSAS plate medium and incubated for 24 to 48 hrs at 30°C incubator. For long-term preservation of the isolated prebiotic fermented strains, the strain solution grown in MRS was diluted in the same amount (750 ul) in a sterile 40% glycerol solution, and then put into a sterile Eppendorf tube and stored in a −45°C freezer. For the identification of the isolated strains, the analysis was performed by Solgent Company (Daejeon, Korea). The Solgent provided the 16s rRNA gene sequencing and provided phylogenic trees. Of those strains, the strain that shows the best fermentation characteristics was finally chosen. The finally selected strain was named as WCYSK01 (*Wessellia cibaria* YSK01).

2.3. The optimization of LMBP fermentation

In order to obtain the optimal conditions for the optimal process for LMBP production using the WCYSK01 strain, a response surface methodology was applied. All experimental plans consisted of a total of 13 treatment combinations, including repeating the center run 5 times under the conditions of two independent variables such as fermentation temperature and pH. The central composite design was carried out according to three important procedures. First, an experiment was performed according to a designed experiment, second, coefficients of a model were obtained, and third, the suitability of the model was determined.

In order to facilitate statistical processing, independent variables were coded as follows. The two variables X_1 and X_2 correspond to temperature and pH respectively. The values of standardization are calculated according to the following formula, and the calculated result value is denoted by Z.

$$Z = (X - X^0)/\Delta X \quad \text{--- (1)}$$

X_0 is the central value of the standardized value. ΔX is the magnitude of the value increasing or decreasing by one unit. As a result of the experiment, the multiple regression equation representing the optimal process condition is expressed as the following equation (2). In addition, the surface reaction equation was expressed as the following equation (3).

$$Y = B_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=j=1}^{k} B_{ij} X_i X_j \quad \text{--- (2)}$$

$$Y = B_0 + B_1 X_1 + B_2 X_2 + B_{11} X_1^2 + B_{22} X_2^2 + B_{12} X_1 X_2 \quad \text{--- (3)}$$

Statistical analysis of the results confirmed after the experiment was performed using Design Expert (Couresy: Stat-ease Inc., Statistics Made Easy, Minneapolis, USA). The values of the independent variables were set at 17.95℃ (−1.41), 20℃ (−1), 25℃ (0), 30℃ (+1), and 32.05℃ (+1.41) for X_1 (℃). X_2 (pH) was set to 5.09(−1.41), 5.5(−1), 6.5(0), 7.5(+1), and 7.91(+1.41) (Table 1).

Table 1. Levels of independent variables such as reaction temperature and ratio amount(water/powdered Jerusalem artichoke tubes) in central composit design.

X_i	Independent variables	Level				
		−1.41	−1	0	+1	+1.41
X_1	Temperature (℃)	17.95	20	25	30	32.05
X_2	pH	5.09	5.5	6.5	7.5	7.91

The optimization of fermentation process performed under the fermentation conditions established from the above experimental design. LMPB fermentation production was performed in MMY medium supplemented with 2% sucrose, 1.0% maltose, and 0.5% yeast extract. The yield of LMBP produced by WCYSK01 was calculated by dividing the total LMBP production by the amount of the input substrate. The total amount of LMBP is the sum of DP3, DP4, DP5, and DP6.

2.4. Batch fermentation for LMBP fermentation

Batch fermentation production of LMPB was performed under the fermentation conditions established from the above experimental results. Using a prototype type fermenter (5L), LMPB fermentation production was performed in MMY medium supplemented with 5% sucrose, 2.5% maltose, and 0.5% yeast extract. The fermentation working volume was 3 L. Seed culture was prepared using 500 mL of MMY medium containing 3% sucrose, 1.5% maltose, and 0.5% yeast extract. Fermentation was carried out for 12 hrs at 100 rpm at 23°C and pH 7.0, which are optimal fermentation conditions. During fermentation, samples were collected at regular intervals and stored in a refrigerator until used for analysis.

After fermentation, centrifugation was performed at 4℃ and 10,000 rpm x 20 min. to remove cells. A double amount of cold ethanol was added to the supernatant, and the supernatant was collected after waiting until the precipitation was completed. Doubled amount of ethanol was added again to the taken supernatant to cause precipitation. This process was repeated 3 times. The supernatant obtained finally was concentrated in a vacuum concentrator and dried in a vacuum dryer.

2.5. Analysis of low molecular weight of biopolymer

The TLC plate used to separate the sugars present in the fermentation broth during the fermentation process was Merck K Sillica gel 60 F_{254} (Seoul, Korea). The sample loading position was marked with a pencil about 1.5 cm from the bottom of the TLC plate. The TLC developing solvent was a mixture of 85 mL methylacetate, 20 mL ethylacetate, 50 mL propanol, and 85 mL water. The spotting interval was 1.5 cm, and was lightly marked with a pencil in advance. Drops were loaded by 1 μL using a micro-pipette. After completion of TLC development, the plate was dried. The dried TLC plate was immersed in 5% H_2SO_4-MeOH plus α-naphthol solution, and then heated in a 105°C dry oven for 10 min. to develop color. The sugars shown in TLC were quantitatively and qualitatively analyzed using a scion image densitometer.

3. Results and Discussion

3.1. Isolation of strains

The strain used in this study was isolated from commercially available fermented kimchi and sour craft produced in the development lab. Among the isolated strains, 6 strains producing biopolymers using sucrose as a substrate were finally selected (Fig. 1). Strains

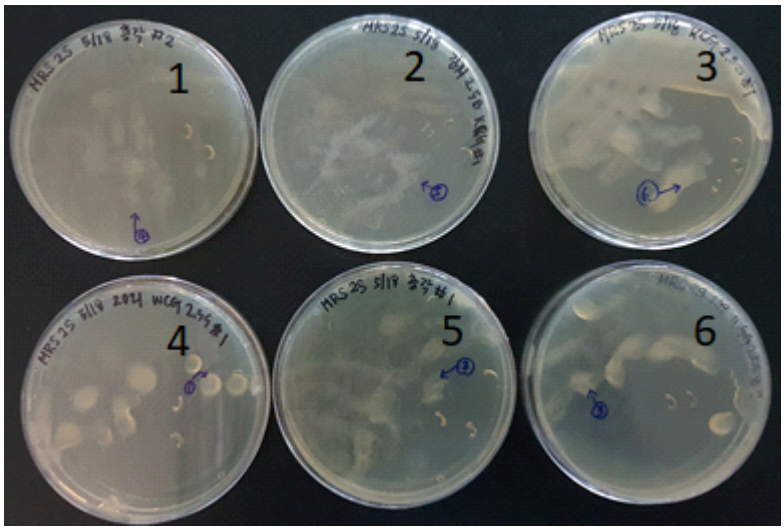

Fig. 1. Colonies of biopolymer-producing strains isolated from sauerkraut and fermented kimchi. All strains showed biopoymer production when the substrate was used as sucrose. After analyzing the ribosomal RNA gene sequencing for 6 strains, the microbial species were classified and identified by comparing the homology with the GeneBank Database. Strains 1,2,3, and 5 were identified as *Leuconostoc* strains 4 and 6 as *Weissella* strains.

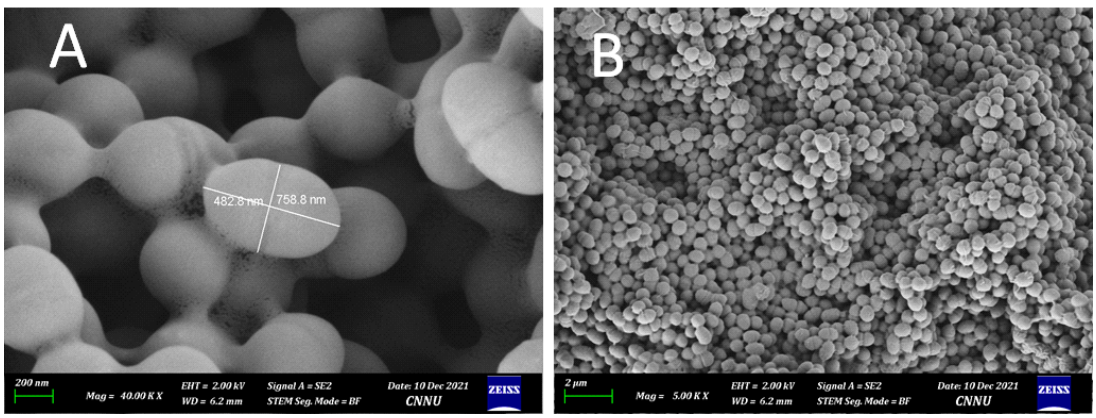

Fig. 2. Cellular morphology of *Weisella cibaria* strain YSK01. seen under an electron microscope. A: 40,000 times electron microscope magnification, B: 5,000 times electron microscope magnification.

1,2,3, and 5 were identified as *Leuconostoc* strains 4 and 6 as *Weissella* strains. Among these strains, the strain 6 producing branched oligosaccharides by receptor reaction using sucrose and maltose as substrates was finally selected. It was named *Weissella cibaria* strain YSK01, and was hereinafter referred to as WCYSK01. The low molecular weight biopolymer produced by this strain was expressed as LMBP. The electron microscope shape of the strain is shown in Fig. 2.

3.2. Structural Characteristics of LMBP Produced by WCYSK01

TLC showing the structural characteristics of LMBP produced by WCYSK01 was presented at Fig. 3. Lanes 1, 2, and 3 correspond to sucrose, maltose, and fructose, respectively. The lane of the Fig. 3A indicates the branched types of isomaltooligosaccharides as standards including DP2 (matose), DP3 (isomaltotriose), DP4 (isomaltotetraose), DP5 (isomaltopentaose), and DP6 (isomaltoheptaose). Lane of Fig. 3B corresponds to oligosaccharides produced. DP 3 to 6 are thought to be synthesized by dextran sucrose enzyme receptor reaction. According to the results of many past studies, glucose is bound to the receptor, maltose, and then glucose is continuously bound linearly. When a certain amount is reached, the synthesis stops. These products are oligosaccharides and their structural form is branched isomaltooligosaccharides. The maltose is considered as the residual amount after the receptor reaction. As a result similar to the results of this study, the oligosaccharides produced by *Weissella* showed a branched structure [24-26].

3.3. Optimization of LMBP fermentation

This experiment was to establish LMBP optimal production conditions using fermentation temperature and pH as process variables. The fermentation temperature range applied to the experiment was about 17.95°C to 32.05°C and the pH range was 5.09 to 7.91 (Table 2.). The yield and output of LMBP production relied significantly on changes in the two variables.

Table 3 shows the suitability of the model established by the response surface analysis method for the ANOVA for the regression equation using the experimental results. Table 3 shows the ANOVA results of the quadratic regression model. According to their results, LMBP production was found to be affected

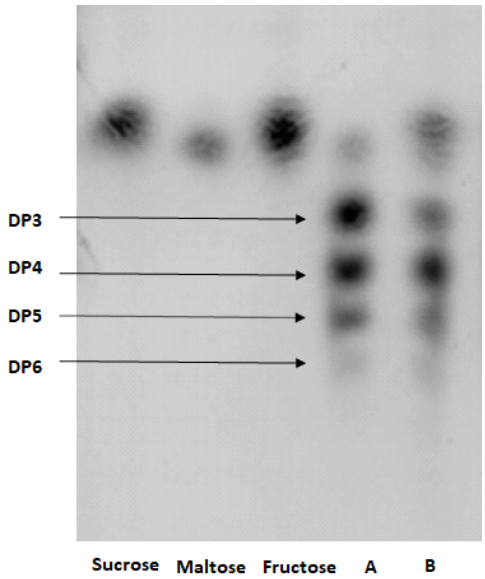

Fig. 3. TLC chromatograms and chemical structures of LMBP (Low molecular weight biopolymer). The produced LMBP were consisted of branched oligosaccharides such as DP3 (panose), DP4 (isomaltotetrose,) DP5(isomaltotetrose), DP6 (isomaltoheptose). A: standards, B: oligosaccharides of LMBP produced by *Weissella cibaria* strain YSK01.

Table. 2. Experimental data of LMBP (low molecular weight biopolymer) production by *Weissella cibaria* strain YSK01

Fermentation	Temperature(°C)	pH	Yield of LMBP (%)
1	−1	−1	21.84
2	1	−1	23.09
3	−1	1	65.68
4	1	1	45.01
5	−1.414	0	41.56
6	1.414	0	14.44
7	0	−1.414	20.08
8	0	1.414	74.76
9	0	0	67.43
10	0	0	64.36
11	0	0	59.96
12	0	0	63.40
13	0	0	65.60

Table 3. Analysis of variance(ANOVA) for response surface quadratic model to the degree of hydrolysis

Source	Squares	DF	Square	Value	Prob > F
Model	5,528.59	5	1105.72	81.08	< 0.0001
X_1	417.29	1	417.29	30.60	0.00
X_2	2,558.94	1	2,558.94	187.63	< 0.0001
X_1^2	2,198.56	1	2,198.56	161.21	< 0.0001
X_2^2	452.55	1	452.55	33.18	0.00
X_1X_2	120.08	1	120.08	8.80	0.02
Residual	95.47	7	13.64		
Lack of Fit	64.40	3	21.47	2.76	0.18
Pure Error	31.07	4	7.77		
Cor Total	5,624.06	12			

by fermentation temperature and pH. The model determination coefficient R^2 shows the degree of correlation between the observed value and the predicted value. In this model, the R^2 value was 0.94. As a result of this experiment, the effects on fermentation temperature and pH were significant in the 1st, 2nd, and cross product terms, and it was found that the factors had an independent or mutual influence. The multiple regression equation determined by the experimental results is shown in Table 4.

Table 4. Estimated coefficient for the filled second oder polynomial representing the relationship between the response and process variables. Polynomial equations calculated by response surface program.

Response	Second oder polynomial equations	R^2
LMBP production	$y= -7.22X_1+17.8X_2 - 17.7X_1^2 - 8.1X_2^2 - 5.48X_1X_2 + 64.15$	0.98

X_1 : pH, X_2 : temperature (℃). LMBP represents a low molecular weight biopolymer.

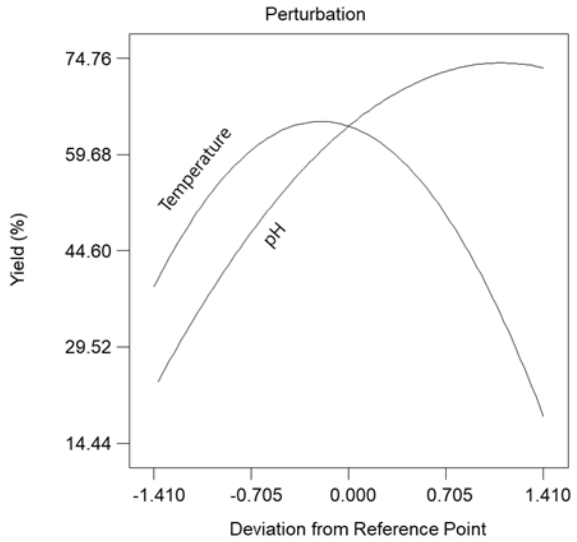

Fig. 4. This graph shows the change in the yield of LMBP production according to the change in fermentation temperature and pH.

3.4. Effect of fermentation temperature and time by WCYSK01 strain on LMBP production

LMBP fermentation production by WCYSK01 schematically shows that fermentation temperature and time are affected within the modeling range of CRD (central composite rotatable design) (Fig. 5). In LMBP production, the production increased as the fermentation temperature increased, but after the peak it did not increase any more and showed a decreasing pattern. The fermentation process pH also showed a similar pattern to the fermentation process temperature. Therefore, it was found that the optimum fermentation conditions exist within the pH and temperature range of the fermentation process determined in the experiment.

3.5. The contour and 3 D surface of LMBP fermentation production profile derived from response surface methodology

The effect of fermentation process pH and temperature on LMBP production yield of WCYSK01 is shown in Fig. In the fermentation process temperature range of 18 to 32°C, the fermentation medium pH in the range of 5.1 to 7.9, the yield of LMBP production by the strain was found to be significantly affected by q fermentation temperature and pH. The yield of LMBP production increased rapidly as the fermentation pH increased from 5.1, rose to

near pH 7.5, and then decreased. This trend was found to be significantly affected by the low temperature of the fermentation process. This trend was different from the fermentation process temperature. As the fermentation temperature increased, the yield increased rapidly and then showed a tendency to decrease. The optimal fermentation conditions were found at the normal point, and the production yield was more than 75% at pH 7.5 and temperature of 23°C.

3.6. Fermentation of LMBP production by WCYSK01 strain in a prototype pilot plant fermentor

The sucrose (50 g/L) and maltose (25 g/L) used as carbon sources decreased with the elapse of fermentation time and showed that they were almost completely consumed within 48 hours. Sucrose decreased rapidly until 24 hours of fermentation and then gradually decreased, while maltose decreased slowly at the beginning and then rapidly decreased until 32 hours. Glucose, a hydrolyzate of sucrose, did not appear during the fermentation process, while fructose gradually increased, but hardly appeared at the end of fermentation. This is probably because glucose is used for LMBP synthesis immediately after sucrose digestion, and fructose is used slowly by the cells. During the substrate change as above, LMBP fermentation production increased linearly from the beginning of fermentation to the end of the log phase. Thereafter, it was increased until the substrate was completely consumed.

This batch fermentation production type can be viewed as a typical growth associated pattern. The final fermentation yield of LMBP was 25.62 g/L. This is about 33.6% with respect to the added sugar. Since only glucose and maltose are involved in LMBP synthesis, the theoretical yield is 72.4% based on the total sugar input. Therefore, it can be evaluated that the fermented LMBP is 73.4% of the theoretical production.

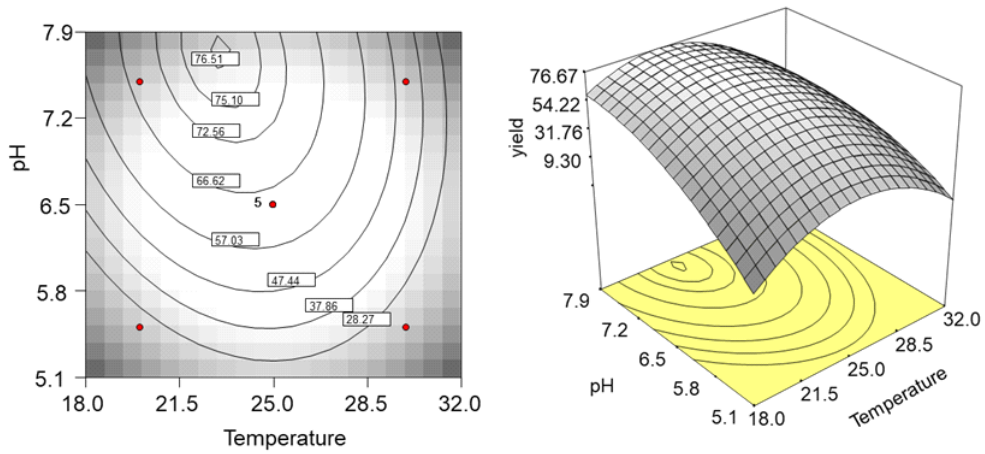

Fig. 5. The contour (left) and 3 D surface (right) of LMBP fermentation production profile derived from response surface methodology. The yield of LMBP produced by WCYSK01 was calculated by dividing the total LMBP production by the amount of the input substrate. The total amount of LMBP is the sum of DP3, DP4, DP5, and DP6.

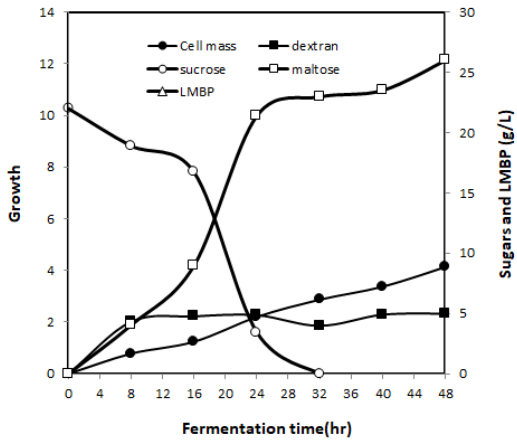

Fig. 6. Kinetics of fermentation showed the change of LMBP production, sucrose utilization, dextran production, and cells mass. Fermentation was conducted on 23.0°C and pH 7.0.

4. Conclusion

WCYSK01, a strain isolated from kimchi, a traditional fermented food, synthesized LMBP using sugar and maltose as substrates. The produced LMBP was composed of branched oligosaccharides such as DP3 to DP6. At optimal fermentation conditions such as pH 7.5 and temperature of 23°C, the production yield was more than 75%. Therefore, the strain isolated in this study is expected to have a very high utility value as an intestinal-improving prebiotic and functional material as a long-lasting food material.

References

1. S. Doron, D. R. Snydman, "Risk and safety of probiotics", *Clin Infect Dis,* Vol.60, pp. 129-234, (2015).
2. G. T. Rijkers, W. M. de. Vos, R. J. Brummer, L. Morelli, G. Corthier, P. Marteau, "Health benefits and health claims of probiotics: Bridging science and marketing", *British Journal of Nutrition,* Vol.106, No.9 pp. 1291-1296, (2011).
3. J. M. Hamilton-Miller, G. R. Gibson, W. Bruck, "Some insights into the derivation and early uses of the word probiotic", *Br. J. Nutr*, Vol.90, No.4 pp. 845-849, (2003).
4. C. Hill, F. Guarner, G. Reid, G. R. Gibson, D. J. Merenstein, B. Pot, L. Morelli, R. B. Canani, H. J. Flint, S. Salminen, P. C. Calder, M. E. Sanders, "Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic", *Gastroenterology & Hepatology,* Vol.11, No.8 pp. 506-514, (2014).
5. S. C. Singhi, S. Kumar, "Probiotics in critically ill children", *F1000Res*, Vol.5, pp. 407, (2016).
6. F. Durchschein, W. Petritsch, H. F. Hammer, "Diet therapy for inflammatory bowel diseases", *The established and the new. World J Gastroenterol,* Vol.22, No.7 pp. 2179-2194, (2016).
7. J. Slavin, "Fiber and prebiotics: mechanisms and health benefits", *Nutrients,* Vol.5, No.4 pp. 1417-1435, (2013).
8. B. Bindels. Laure, M. Delzenne. Nathalie, D. Cani. Patrice, Jens. Walter, "Towards a more comprehensive concept for prebiotics", *Gastroenterology & Hepatology*, Vol.12, No.5 pp. 303-310, (2015).
9. F. Enam, T. J. Mansell, "Prebiotics: tools to manipulate the gut microbiome and metabolome", *Journal of Industrial Microbiology & Biotechnology*, Vol.46, No.9-10 pp. 1445-1459, (2019).
10. S. A. Zaman. S. R. Sarbini. "The potential of resistant starch as a prebiotic", *Critical Reviews in Biotechnology,* Vol.36, No.3 pp. 578-584, (2015).
11. C. K. Rajendran, S. R. Okolie, C. L. Udenigwe, C. C. Mason B, "Structural features underlying prebiotic activity of conventional and potential prebiotic

oligosaccharides in food and health", *Journal of Food Biochemistry*, Vol.41, No.5 pp. 1745-4514, (2017).
12. A. Bird, M. Conlon, C. Christophersen, D. Topping, "Resistant starch, large bowel fermentation and a broader perspective of prebiotics and probiotics", *Beneficial Microbes*, Vol.1, No.4 pp. 423-431, (2010).
13. B. P. Lamsal, "Production, health aspects and potential food uses of dairy prebiotic galactooligosaccharides", *Journal of the Science of Food and Agriculture*, Vol.92, No.10 pp. 2020-2028, (2012).
14. M. P. Arena, G. Caggianiello, D. Fiocco, P. Russo, M. Torelli, G. Spano, V. Capozzi, "Barley β-glucans-containing food enhances probiotic performances of beneficial bacteria", *International Journal of Molecular Sciences*, Vol.15, No.2 pp. 3025-3039, (2014).
15. J. A. Delcour, P. Aman, C. H. Courtin, B. R. Hamaker, K. Verbeke, "Prebiotics, Fermentable Dietary Fiber, and Health Claims", *Advances in Nutrition*, Vol.7, No.1 pp. 1-4, (2016).
16. M. D. Collins, J. Samelis, J. Metaxopoulos, S. Wallbanks, "Taxonomic studies on some *leuconostoc*-like organisms from fermented sausages: Description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species", *Journal of Applied Microbiology*, Vol.75, No.6 pp. 595-603, (1993).
17. Bjorkroth. Johanna, Holzapfel, Wilhelm, "Genera *Leuconostoc, Oenococcus* and *Weissella*", *The Prokaryotes*, pp. 267-319, (2006).
18. Kang. Wook. Lee, Ji. Yeong. Park, Hee. Rok. Jeong, Ho. Jin. Heo, Nam. Soo. Han, Jeong Hwan. Kim, "Probiotic properties of *Weissella* strains isolated from human faeces", *Anaerobe*, Vol.18, No.1 pp. 96-102, (2012).
19. Dilna. Sasidharan. Vasanthakumari, Surya. Harikumar, Divya. Jayakumar. Beena, Ashok. Pandey, Kesavan. Madhavan. Nampoothiri, "Physicochemical Characterization of an Exopolysaccharide Produced by a Newly Isolated *Weissella cibaria*", *Applied Biochemistry and Biotechnology*, Vol.176, No.2 pp. 440-453, (2015).
20. H. S. Yu, H. J. Jang, N. K. Lee, H. D. Paik, "Evaluation of the probiotic characteristics and prophylactic potential of *Weissella cibaria* strains isolated from kimchi", *LWT*, Vol.112, pp. 108-229, (2019).
21. D. Goffin, N. Delzenne, C. Blecker, E. Hanon, C. Deroanne, M. Paquot. "Will isomalto-oligosaccharides, a well-established functional food in Asia, break through the European and American market? The status of knowledge on these prebiotics.", *Food Sci Nutr*, Vol.51, No.5 pp. 394-409, (2011).
22. Q. Shi, M. Juvonen, Y. Hou, I. Kajala, A. Nyyssölä, N. H. Maina, H. Maaheimo, L. Virkki, M. Tenkanen, "Lactose- and cellobiose-derived branched trisaccharides and a sucrose-containing trisaccharide produced by acceptor reactions of *Weissella confusa* dextransucrase", *Food Chem,* Vol.1, No.190 pp. 226-236, (2016).
23. M. Dols, M. R. Simeon, R. M. Willemot, M. R. Vignon, P. F. Monsan, "Structural characterization of the maltose acceptor-products synthesized by *Leuconostoc mesenteroides* NRRL B-1299 dextransucrase", *Carbohydr Res*, Vol.305, No.3-4 pp. 549-59, (1997).
24. Y. Hu, V. Winter, X. Y. Chen, M. G. Gänzle, "Effect of acceptor carbohydrates on oligosaccharide and polysaccharide synthesis by dextransucrase from *Weissella cibaria*", *Food Res Int*, Vol.99, No.1 pp. 603-611, (2017).
25. S. hukla, Q. Shi, N. H. Maina, M. Juvonen, Maijatenkanen, A. Goyal, "*Weissella confusa* Cab3 dextransucrase: properties and in vitro synthesis of dextran and glucooligosaccharides", *Carbohydr Polym*,

Vol.101, No.30 pp. 554-64, (2014).
26. Qiao. Shi, Yaxi. Hou, Minna. Juvonen, Päivi. Tuomainen, Ilkka. Kajala, Shraddha. Shukla, Arun. Goyal, Hannu. Maaheimo, Kati. Katina, Maija. Tenkanen, "Optimization of Isomaltooligosaccharide Size Distribution by Acceptor Reaction of *Weissella confusa* Dextransucrase and Characterization of Novel α-(1→2)-Branched Isomaltooligosaccharides", *J. Agric. Food Chem,* Vol.64, No.16 pp. 3276-3286, (2016).