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Isolation and Characterization of Dextrans Produced by *Leuconostoc* sp. strain JYY4 from Fermented Kimchi

Ji-Joong Gu[1] · Yoo-Jin Ha[2] · Sun-Kyun Yoo[3,†]

[1]*Department of Oriental Health Care, Jonngbu University*
[2]*Division of Food and Nutrition, Chungnam National University*
[3,†]*Department of Food and Biotechnology, Joongbu University*
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Abstract : Dextran is a generic term for a bacterial exopolysaccharide synthesized from sucrose and composed of chains of D-glucose units connected by α-1,6-linkages by using dextransucrases. Dextran could be used as vicosifying, stabilizing, emulsifying, gelling, bulking, dietary fiber, prebiotics, and water holding agents. We isolated new strain capable of producing dextran from Korean traditional kimchi and identified as Leuconostoc sp. strain JYY4. Batch fermentation was conducted in bioreactor with a working volume of 3 L. The media was MMY and 15% (w/v) sucrose. Mineral medium consisted of 3.0 g KH_2PO_4, 0.01 g $FeSO_4$, H_2O, 0.01 g $MnSO_4$, $4H_2O$, 0.2 g $MgSO_4$ $7H_2O$, 0.01 g NaCl, 0.05 g $CaCl_2$ per 1 liter deionized water. The pH of media was initially adjusted to 6.0. The inoculation rate was 1.0% (v/v) of the working volume. Temperature was maintained at 28°C. The agitation rate was 100 rpm. The production pattern of dextran was associated with the cell growth. After 24 hr dextran reached its highest concentration of 59.4 g/L. The sucrose was consumed completely after 40 hr. Growth reached stationery phase when sucrose became limiting, regardless of the presence of fructose or mannitol. When the specific growth rate was 0.54 hr-1, utilization averaged 5.8 g/L-hr. The yield and productivity of dextran were 80% and 2.0 g/L-hr, respectively. Dextrans produced by were separated to two different size by an alcohol fraction method. The size of high molecular weight dextran (45% alcohol, v/v), less soluble dextran, was between MW 500,000 and 2,000,000. Soluble dextran (55% alcohol, v/v) was between 70,000 and 150,000. The molecular weight average of total dextran (70% alcohol, v/v) was between 150,000 to 500,000. The enzymatic hydrolyzates of total dextran of ATCC 13146 showed branched dextrans by Penicillium dextranase contained of glucose, isomaltose, isomaltotriose, and isomaltooligosaccharides greater than DP4 (degree of polymerization) that had branch points. Compounds greater than DP4 were branched isomaltooligosaacharides. Hydrolysates by the Lipomyces dextranase produced the same composition of oligosaccharides as those by Penicillin dextranase.

Keywords : *dextran, Leuconostroc, fermentaion, kimchi, dextranase*

†Corresponding author
(E-mail: skyoo@joongbu.ac.kr)

1. Introduction

Consumer demand for functional foods such as GRAS (generally recognized as safe) is rapidly increasing [1,2]. Dextran is a generic term for a bacterial exopolysaccharide synthesized from sucrose and composed of chains of D- glucose units connected by α-1,6-linkages by using dextransucrases. Dextran can be branched out by α-1,2, α-1,3, or α-1,4-linkages, to varying degrees, depending on the strains [3]. Dextrans are produced by microorganisms such as *Streptococcus, Lactobacillus, Leuconostoc mesenteroides,* and *Weissella* [4-7].

Dextransucrases can only be produced by sucrose induction, with the exception of the constitutive mutants which are produced from *Leuconostoc* sp [8]. In addition to catalyzing the synthesis of dextrans from sucrose, dextransucrase can also catalyze the transfer of glucose to sucrose to other carbohydrates which are present or have been added to the reaction digest [9-11]. The well-known dextran produced by L. mesenteroides B-512F is widely used as blood-plasma substitute due to the low antigenicity, high water solubility and high biological stability in the human bloodstream [12]. Dextrans have also been applied in food (e.g. emulsifying and thickening agents) and chemical (e.g. molecular sieve) industries [13-15]. The application of dextrans expands to the eye and skin care products for their excellent biocompatibility, moisturizing properties and stability, and to bakery products to improve softness, crumb texture and loaf volume [16].

As the public increase health consciousness, the production of new types of oligosaccharides has been attracted with strong commercial interest [17]. Non-digestible oligosccharides, such as non-linear type of isomaltooligosaccharides or branched isomaltooligosaccharides, show great potential for improving intestinal microflora in human and livestock [18,19]. They encourage the growth of the Bifidobacteria and discourage the growth of harmful bacteria [20]. The commercial processes for isomaltooligosaccharides with high purity has been developed for 20 years, yet their full potential has hardly been realized due to isomaltooligosaccharides' complex structure and difficult purification [21-25]. Isomaltooligosaccharides has been commercially produced from starch by a series of enzyme reactions [26]. However, significant concentrations of digestible sugars such as glucose, maltose, and maltooligosaccharides remain in the final product [22-24].

Accordingly, in this paper we isolated and identified the strain capable of producing dextran from Korean fermented kimchi. With this strain we also performed a batch fermentation to produce dextran and characterized the molecular weight and hydrolysates of dextran.

2. Materials and Methods

2.1. Materials

Different size of dextrans such as T 5, T 10, T 40, T 70, T 500, T 2000 was purchased from Pharmacia (Piscataway, NJ, USA). *Penicillium* dextranase was obtained from Sigma (Seoul, Korea). Dextranase of *L. starkeyi* was produced by the method of Yoo et al. [27]. The identification of products, oligosaccharides, was determinated by comparing with a standard isomaltooligosaccharides mixtures (BioEurope, Toulouse). Sugars such as sucrose, glucose, fructose, and mannitol were supplied from Sigma Company (Seoul, Korea).

2.2. Isolation and identification of microorganism

The strain capable of producing dextran was isolated from the fermented kimchi at local market. One loopful of broth at the bottom of

kimchi was transferred to MRS (Difco. NJ. USA) medium and incubated at 30°C for 24 h. The grown culture was streaked on MRS agar medium containing 2% sucrose and incubated at 30°C for 24 h. Colonies showing slime material like dextran were selected and transferred to new MRS agar plates containing 2% sucrose. Finally one colony was selected and identified. For identification of strain, the sequence of isolated and separated 16S rRNA was analyzed by a ABI PRISM 310 Genetic Analyser (Applied Biosystems, Carsbad, CA). The homology was evaluated by comparing BLAST program of the DDBJ/ NCBI/GenBank database. Phylogenetic tree was determined by a neighbor-joining method.

2.3. Dextran production

Fermentation to produce dextran was conducted in a 5-L Bio Flo II Fermentor (New Brunswick Scientific Co.) with a working volume of 3 L. The media was MMY and 15% (w/v) sucrose. The pH of media was initially adjusted to 6.0 by adding NaOH and then maintained using a pH controller. The inocula were grown in 250 mL shake flasks containing 50 mL of MMY plus 2% (w/v) sucrose. The inoculation rate was 1.0% (v/v) of the working volume. Temperature was maintained at 28°C. The agitation rate was 100 rpm. For inoculum strain culture was maintained on a mineral medium agar slant supplemented by 0.5% (w/v) of yeast extract and 3% (w/v) of sucrose. Mineral medium consisted of 3.0 g KH_2PO_4, 0.01 g $FeSO_4$ H_2O, 0.01 g $MnSO_4$ $4H_2O$, 0.2 g $MgSO_4$ $7H_2O$, 0.01 g NaCl, 0.05 g $CaCl_2$ per 1 liter deionized water. The medium pH was adjusted to 6.5 prior to sterilization.

2.4. Dextran recovery

After harvesting, cells were removed by centrifugation at 10,400 x g for 20 min (Dupont Sorvall RC5C, Newtown, CT) at 4°C. The pH of the supernatant solutions was adjusted to 7.0. The dextrans were precipitated by an addition of ethanol (95%). Ethanol was slowly added to the supernatant solution with stirring at room temperature until the volume of ethanol reached two thirds of that of the original solution. The solution was then allowed to stand for 2 h and then centrifuged at 3,040 x g for 20 min. The recovered dextrans were dissolved in deionized water and reprecipitated with ethanol. The dextrans were either lyophilized or vacuum-dried, and stored for analysis. The quantification of dextran was assayed by the phenol-sulfuric acid methods. refererences.

2.5. Molecular weight determination

Dextran size was determined using gelpermeation chromatography with Ultrahydrogel Linear column (Millipore, MA). Deionized water was the eluent and the temperature was ambient The rate of elution was 1.0 mL / min. The detector was a 410 Differential Refractometer (Millipore Corporation, MA). Dextran sizes were calculated from a standard curve made using the following standards (0.1%, w/v); T 5, T 10, T 40, T 70, T 500, T 2000 (Pharmacia, Piscataway, NJ). Sample concentration was 0.5% (w/v) and it was passed through a 0.2 mm filter prior to use.

2.6. Dextran and oligosaccharide hydrolysis

Dextrans were hydrolyzed by two dextranases produced by L. starkeyi and Penicillium (Sigma Chemical Company, MO). Dexytans, 0.5% (w/v), were dissolved in 2 mL of acetate buffer containing 100 mM sodium acetate, 20 mM $CaCl_2$, and 0.02% (w/v) sodium azide. The solution was adjusted to pH 5.2 and then Penicillium dextranase (0.3 IU / mL) or L. starkeyi (0.5 IU / mL) dextranase was added and incubated with at 35°C for 24 to 48 h. Hydrolyzates were

analyzed by either TLC or HPLC.

2.7. Sugar and oligosaccharide analysis

The amount of sucrose, fructose, and mannitol were determined by HPLC using an ion exchange / size exclusion column (Sugarpak: Millipore, MA, USA) under the following conditions: 90°C column temperature, 0.5 ml/min eluent (deionized water) flow rate, and 50 l injection volume. Sorbitol, 5% (w/v), as an internal standard was added to cell free supernatant before injection. The hydrolysates of dextran was evaluated by a thin layer chromatography (TLC): Whatman K5 0.25 mm silica gel plates. As standard, isomaltooligosaccharide standard (DP 1 to 10: BioEurope, Toulouse, Germany) was loaded on TLC plates. The developing solvent comprised of a mixture of 85 methylacetate, 20 ethylacetate, 50 propanol, and 85 water in a volume proportion. After air drying, the spots on TLC were sprayed with a solvent reagent: 5% (v/v) H_2SO_4 and 0.3% (w/v) naphthol in ethanol. Heating at 100°C for 20 min developed the spots.

3. Results and Discussion

3.1. Isolation of microorganism producing dextran

To isolate the strain capable of producing dextran, the broth of fermented kimchi that smelled pleasantly sour was transferred to the bottom of Petri dish. One loopful of broth was streaked on MRS medium containing sucrose as a substrate. More than 20 colonies typically showing dextran production was selected. Each colony was transferred to MMY containing 2% sucrose and incubated. After fermentation, dextran was recovered using 70% (v/v) alcohol. The yield and productivity of dextran was evaluated, respectively. Finally one strain was selected and identified tentatively as *Leuconostoc citreum*. Accordingly we named this strain as *Leuconostoc* sp. strain JYY4. Fig. 1 and 2. In recent days, *Leuconostoc ctireum* NM105 was isolated from homemade manchurian sauerkraut [28]. The dextran produced with this strain appeared a sheet-like smooth glittering and highly branched surface. *Leuconostoc* strain isolated from Vitis vinifera L. (Grape) purchased from local market using enrichment media technique showed only α-(1→6) glycosidic linkage [29].

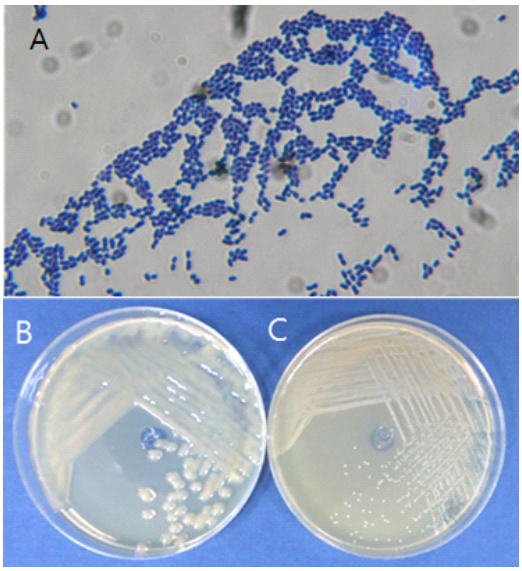

Fig. 1. Morphology and colonies of *Leuconostocs* sp. strain JYY4 isolated from fermented kimchi. Microscopic bacterial cells (A), colonies grown at TSA agar medium plus 3% sucrose (w/v) for 24 h, and colonies grown at TSA medium.

3.2. Dextran fermentation and recovery

Dextran was produced under the following conditions; sucrose 15% (w/v), pH 6.5, 1 vvm of aeration, 100 rpm of agitation, and 28°C. The pH was controlled during the fermentation. The production of dextran by *Leuconostoc* sp. strain JYY4 was linked with the growth. After 24 h dextran reached its highest concentration of 59.4 g/L. The sucrose

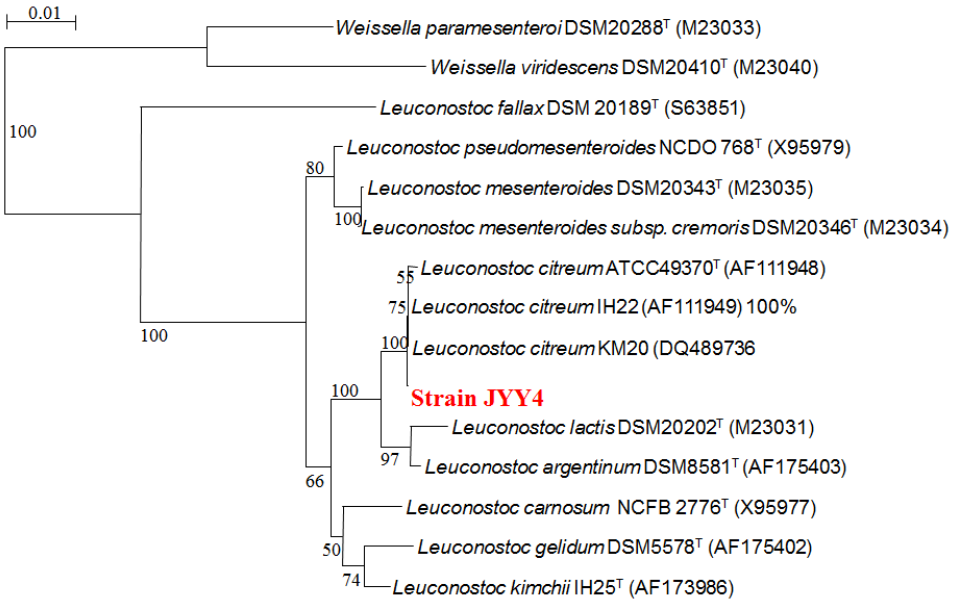

Fig. 2. Phylogenetic tree showing the phylogenetic relationships of *Leuconostoc* sp. strain JYY4 and representative species of the genera *Leuconostoc*. Phylogenetic tree based on rRNA gene sequences showing the position of strain. Numbers at branches are bootstrap values inferred from the BLAST program.

was consumed completely after 40 h. Growth reached stationery phase when sucrose became limiting, regardless of the presence of fructose or mannitol. When the specific growth rate was 0.54 h^{-1}, utilization averaged 5.8 g/L-h. The yield and productivity of dextran were 80% and 2.0 g/L-hr, respectively. In general, the yield of dextran is varied along the changes of the type of bacteria, culture conditions and processing methods. The dextran yield of *L. mesenteroides* BD170 culturedin tomato juice with 15% sucrose was 32 g/L [30]. *W. cibaria* CMGDEX3 cultured in MRS medium with 15% sucrose was 2.4 g/L [15]. Comparing with previous report, *Leuconostoc* sp. strain JYY4 might be a relatively high-production yield and productivity.

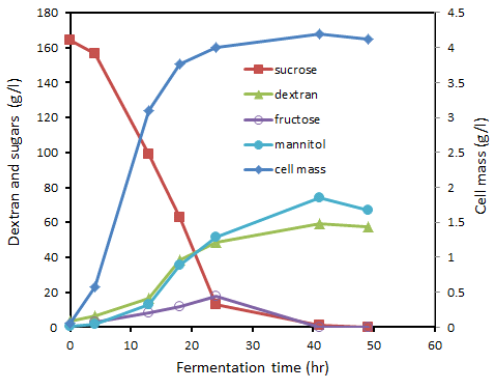

Fig. 3. Dextran production by Leuconostoc strain JYY4 on MMY containing 15% sucrose. Fermentation was performed in pH controlled fermenter. Fermentation conditions were 100 rpm, pH 6.0, and 28°C.

Leuconostoc sp. strain JYY4 produced two types of dextrans and their precipitation was dependant of alcohol concentration. We classified the dextrans into soluble and less soluble dextran; less soluble dextran precipitated at 45% (v/v) alcohol, soluble dextran at 55% (v/v) alcohol, and total dextran at 70% (v/v) alcohol. Less soluble dextran was gummy and brittle in texture. It did not dissolve in water at room temperature, but dissolved after 10 min in boiling water (dextran, 2%). The solution was clear. Soluble dextran was sticky and dissolved at the room temperature. The solution was hazy. The composition of dextran produced by fermentation was 35.5% soluble and 64.5% less soluble dextran.

3.3. Molecular weight of dextran

The range of molecular weights of the dextrans were determined by gel permeation chromatography, Ultrahydrogel Linear column (Millipore). High molecular weight dextran (45% alcohol, v/v), less soluble dextran, eluted from this column between T 500 and T 2000, a commercial linear dextran (Table 1). Soluble dextran (55% alcohol, v/v) eluted at between T 70 and T 150 dextran. The molecular weight average of total dextran (70% alcohol, v/v) was between 150,000 to 500,000. The molecular weight of dextran produced by *Leuconostoc ctireum* NM105 was estimated to be 1.01×10^8 Da [28]. *L. citreum* SK24.002 was 4.62×10^7 Da [15]. The molecular weight of dextran produced in our experiment is relatively lower than their dextran.

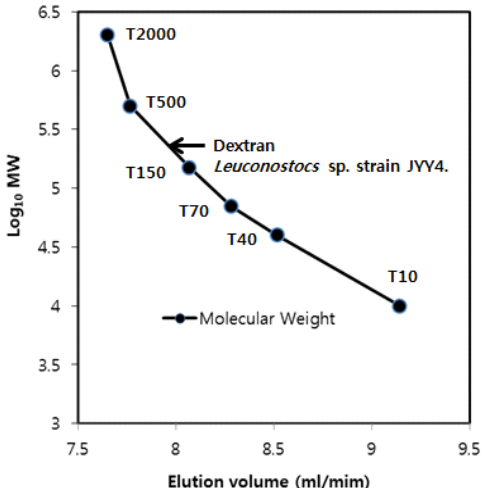

Fig. 4. Molecular weight of dextran corresponding to elution volume of GPC (gel permeation chromatography). T10 to T2,000 is the average molecular weight of dextran standard.

3.4. Dextran hydrolysates.

Dextran produced by *Leuconostocs* sp. strain JYY4 and T 500 as linear type of dextran were hydrolyzed by dextranase produced by a *Penicillium* sp. or *L. starkeyi* ATCC 74054. Dextran produced by *Leuconostocs* sp. strain JYY4 were all more resistant to dextranase than T 500 (Table 1). The degree of hydrolysis of dextran by *Lipomyces* dextranase

Table 1. Hydrolysis of dextran produced by *Leuconostocs* sp. strain JYY4.

Dextrans	% Hydrolysis	
	Penicillium dextrnase	*Lipomyces* dextranase
Dextran (JYY4)	11.96	36.39
Less soluble (45%)	3.94	36.73
Soluble (50%)	6.12	21.52
T 500	38.37	55.49

Dextranase (0.5 IU) produced by *Penicillium* (Sigma) and *Lipomyces* was reacted with 0.5% (w/v) dextran solution at condition of pH 5.2, 100 mM sodium acetate, 35°C, and 48 h reaction.

was greater than that of *Penicillium* dextranase. *Lipomyces* dextranase released mostly glucose (36.7% hydrolysis) from less soluble dextran (Lane A1, 2, and 3). This dextran was resistant to *Penicillin* dextranase (3.9% hydrolysis).

A few oligosaccharides were detected from hydrolysates of less soluble dextran (data not shown). By comparing those of linear standard isomaltooligosaccharides {α-D-(1-6)-linked D-glucopyranosyl residues}, the hydrolyzates of *Leuconostocs* sp. strain JYY4 might contain α-D-(1-2)-glucose or (1-3)-glucose branched linkages on α-D- (1-6)-glucose main chain (Fig. 4).

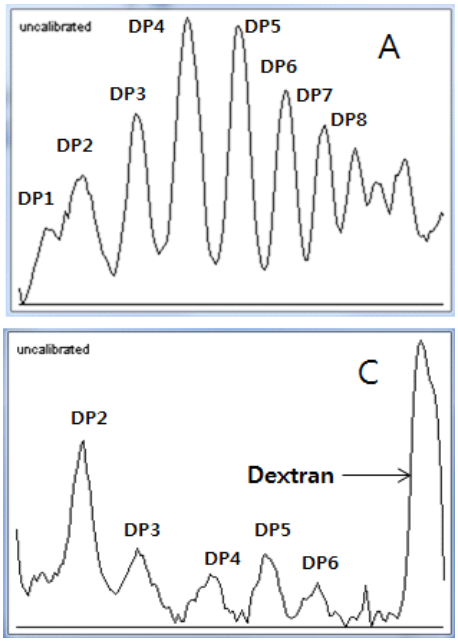

Fig. 4. Chromatogram of NIH Image Program against spots of TLC. Chromatogram A corresponds to standard isomaltooligosccharides (Fig. 5. lane S). Chromatogram B corresponds to hydrolysates of dextran (Fig. 5. lane A2). DP means the degree of polymerization of glucose.

The enzymatic hydrolyzates of *Leuconostocs* sp. strain JYY4 dextran (A1, 2, and 3) and T500 (B1, 2, and 3) showed different patterns. Hydrolysates of JYY4 dextran (Lane A2) showed branched dextran by *Penicillium* dextranase (Fig. 5). It contained of glucose, isomaltose, isomaltotriose, and isomaltooligosaccharides greater than DP4 that had branch points. It means that compounds greater than DP 4 were branched isomaltooligosaacharides. Hydrolysates by the *Lipomyces* dextranase produced the same composition of oligosaccharides as those by *Penicillin* dextranase (A3). The hydolysates of T500 dextran (Lane B2 and 3) consisted of isomaltooligosaccharides that correspond to standard isomaltooligosaccharides (S).

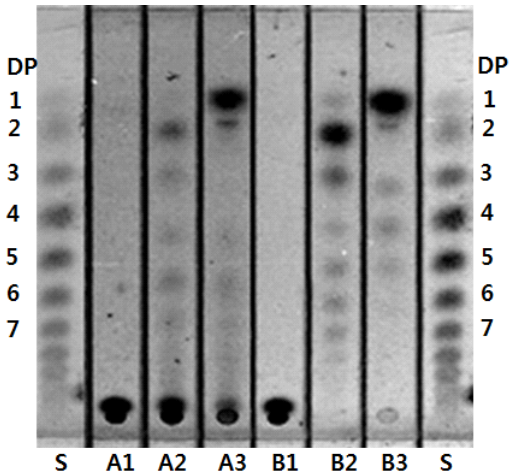

Fig. 5. The enzymatic hydrolyzates of dextran of *Leuconostoc* strain JYY4 (A1, 2, and 3) and T500 (B1, 2, and 3). S represents linear type of isomaltooligosccharides as a standard. The hydrolysis using the dextranase was performed with 0.5% (w/v) dextran solution at condition of pH 5.2, 100 mM sodium acetate, 35°C, and 48 h reaction. DP means the degree of polymerization of glucose.

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References

1. J. Cerning, Exocellular polysaccharides produced by lactic acid bacteria, *FEMS. Microbiol. Rev.,* **87**, 113 (1990).
2. A. H. Ensminger, M. E. Ensminger, J. E. Konlande and J. R. K. Robson, "Food Nutrition Encyclopedia", 2th ed. CRC Press, Boca Raton, New York, U.S.A. (1994).
3. J. F. Robyt, "Encyclopedia of Polymer Science and Technology", p. 752, 4th ed. John Wiley & Sons, New York, U.S.A. (1986).
4. N. H. Maina, M. Tenkanen, H. Maaheimo, R. Juvonen, L. Virkki, NMR spectro-scopic analysis of exopolysaccharides produced by *Leuconostoc citreum* and *Weissella* confuse, *Carbohyd. Res.*, **343**, 1446 (2008).
5. M. Naessens, A. Cerdobbel, W. Soetaert, E. J. Vandamme, *Leuconostoc* dextran-sucrase and dextran: production, properties and applications, *J. Chem. Technol. Biotechnol,.* **80**, 845 (2005).
6. N. H. Main, L. Virkki, H. Pynn¨onen, H. Maaheimo, M. Tenkanen, Structural analysis of enzyme resistant isomaltooligosaccharides reveals the elongation of-(1,3)-linked branches in *Weissella* confuse dextran, *Biomacromolecules.,* **12**, 409 (2011).
7. J. F. Robyt, S. Y. Lee, J. H. Lee, Y. M. Kim, Dextran molecular size and degree ofbranching as a function of sucrose concentration, pH, and temperature of reaction of *Leuconostoc mesenteroides* B-512 FMCM dextransucrase, *Carbohyd. Res.*, **338**, 1183 (2003).
8. D. Kim, J. F Robyt, Selection of *Leuconostoc mesenteroides* mutants constitutive for glucansucrases, *Enzyme. Microbiol. Technol.,* **16**, 1010 (1994).
9. D. Kim, K. H. Park, J. F. Robyt, Acarbose effect for dextran synthesis, acceptor and disproportionation reactions of *Leuconostoc mesenteroides* B-512FMCM dextransucrase, *J. Microbiol. Biotechnol.*, **8**, 287 (1998).
10. D. Kim, Y. M. Kim, M. R. Park, D. H. Park, Modification of Acetobacter xylinum bacterial cellulose using dextransucrase and alternansucrase, *J. Microbio. Biotechnol.*, **9**, 704 (1999).
11. D. Kim, Y. M. Kim, M. R. Park, H. J. Ryu, D. H. Park, J. F. Robyt Enzymatic modification of cellulose using *Leuconostoc mesenteroides* B-742CBM dextransucrase, *J. Microbiol. Biotechnol.*, **9**, 529 (1999).
12. D. de Belder, "Medical application of dextran and its derivatives". In Polysaccharides in medicinal applications. New York, NY: Marcel Dekker.(1996).
13. A. Aman, N. N. Siddiqui, S. A. U. Qader, Characterization and potential applications of high molecular weight dextran produced by *Leuconostoc mesenteroides* AA1, *Carbohydrate. Polymers.*, **87**, 910 (2012).
14. P. Duboc, B. Mollet, Applications of exopolysaccharides in the dairy industry, *International. Dairy. Journal.*, **11**, 759 (2011).
15. J. Han, F. Hang, B. Guo, Z. Liu, C. You, Z. Wu, Dextran synthesized by *Leuconostoc mesenteroides* BD1710 in tomato juice supplemented with sucrose, Carbohydrate. Polymers., **112**, 556 (2014).
16. N. H. Maina, L. Pitkanen, S. Heikkinen, P. Tuomainen, L. Virkki, M. Tenkanen, Challenges in analysis of high-molar mass dextrans: Comparison of HPSEC AsFlFFF and DOSY NMR spectroscopy, *Carbohydrate. Polymers.*, **99**, 199 (2014).
17. C. M. Hasler, Functional foods: the western perspective, *Nutr. Rev.*, **54**, 6 (1996).

18. Z. Djouzi, C. Andrieux, V. Pelenc, S. Somarriba, F. Popot, P. F. Monsan, D. Szylit, Degradation and fermentation of a-gluco oligosaccharides by bacteria strains from -human colon: in vitro and in vivo studies in gnotobiotic rats, *J. Appl. Bacteriol.*, **79**, 117 (1995).
19. M. Hirayama, H. Hidaka, Production and utilization of microbial fructans, pp. 273. In: Science and Technology of Fructans. Susuki M, Chatterton NJ (eds). CRC Press, New York, NY, USA(1993).
20. D. G. Hoover, *Bifidobacteria*: activity and potential benefits, *Food. Technol.,* **47**, 120 (1993).
21. F. D. Day, S. K. Yoo, Natural glucans: production and prospects. pp. 292. In: Biopolymers from polysaccharides and agroproteins, ACS symposium series 786. Gross RA, Scholz C(eds). American Chemical Society, Washington, DC, MD, USA(2001).
22. T. Kurki, M. Tsuda, T. Imananka, Continuous production of pannose by immobilised neopulluanase, *Ferment. J. Bioeng.*, **73**, 198 (1992).
23. M. R. Remaud, M. Willemot, P. Sarcabal, G. P. Montalk, P. Monsan, Glucansucrases: molecular engineering and oligosaccharides synthesis, *J. Mol. Catal.*, **10**, 117 (2000).
24. H. T. Takata, S. Kuriki, S. Okada, Y. Takesada, M. Iizuka, N. Minamiura, T. Imanaka, Action of neopullulanase: neopullanase catalyzes both hydrolysis and transcosylation at a a-(1→4)-and a-(1→6)-glucosidic linkages, *J. Biol. Chem.*, **267**, 18447 (1992).
25. S. K. Yoo, D. H. Kim, D. F. Day, Highly branched glucooligosaccharide and maniitol production by mixed culture fermentation of *leuconostoc mesenteroides* and *lipomyces starkeyi*, *J. Microbiol. Biotechnol.*, **11**, 700 (2001).
26. Y. K. Kim, M. J. Kim, C. S. Park, K. H. Park, Modification of Sorbitol by transglycosylation using *Bacillus stearothermophilus* Maltogenic Amylase, *Food. Sci. Biotechnol.*, **11**, 401 (2002).
27. Y. H. Chang, J. H. Yeom, K. H. Jung, B. C. Chang, J. H. Shin and S. K. Yoo, Optimization of an extrancellular dextranase production from *Lipomyces starkey*i KCTC 17343 and analysis of its dextran hydrolysates, *J. life. Science.,* **19**, 457 (2009).
28. Y. Yang, Q. Peng, Y. Guo, Y. Han, H. xiao, Z. Zhou, Isolation and characterization of dextran produced by *Leuconostoc citreum* NM105 from manchurian sauerkraut, *Carbohydrate. Polymers.,* **133**, 365 (2015).
29. F. Sarwat, S. A. U. Qader, A. Aman, N. Ahmed, Production & Characterization of a Unique Dextran from an Indifenous *Leuconostoc mesenteroides* CMG713, *Int. J. Biol. Sci.,* 4(6):379-386.doi:10.7150/ijbs.4.379 (2008).
30. R. Z. Ahmed, K. Siddiqui, M. Arman, N. Ahmed, Characterization of high molecular weight dextran produced by Weissella cibaria CMGDEX3, *Carbohydrate. Polymers.,* **90**, 441 (2012).