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Studies on a Neutral Exopolysaccharide of *Lactobacillus fermentum* TDS030603

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Abstract: A neutral exopolysaccharide produced in large amounts by *Lactobacillus fermentum* TDS030603 was purified from culture supernatants and its structure determined by monosaccharide analysis, methylation analysis and ^{1}H-NMR spectroscopy. Optimum conditions for the production of this exopolysaccharide by the above bacterial strain, when cultured using cheese whey medium or MRS broth, were also investigated. The exopolysaccharide was found to be composed of D-galactose and D-glucose in the molar ratio of 1:2.5 and to consist of one major as well as one minor saccharide, both of which contained a tetrasaccharide repeating unit consisting of two α and two β anomers. The major saccharide contained non-reducing glucopyranose, O-3 substituted glucopyranose, O-6 substituted galactopyranose and O-2,3 disubstituted glucopyranose. The minor saccharide consisted of non-reducing galactopyranose, O-3 substituted glucopyranose, O-6 substituted galactopyranose and O-2,3 disubstituted glucopyranose. The results suggest that these exopolysaccharides are novel saccharides that differ from previously reported products of *Lactobacillus fermentum* strains. Their presence may contribute to the ropy properties of the medium.

Key words: lactic acid bacteria, exopolysaccharide, *Lactobacillus fermentum* TDS030603

The production of exopolysaccharides (EPS) is an important feature of some strains of dairy lactic acid bacteria. These polysaccharides originate from the cell surface capsules or the extracellular slime.[1)] Since some lactic acid bacteria are food grade micro-organisms which carry a GRAS (Generally Recognized as Safe) status, the use, as natural additives, of strains that secrete polysaccharides has received great attention in the food industry. Although microbial exopolysaccharides produce undesired ropiness,[2,3)] these polymers are often used to improve the texture of fermented dairy products.[4)]

In addition to these important roles relating to the texture and consistency of fermented foods, some of these polysaccharides have been reported to have beneficial health properties such as anti-tumor, anti-ulcer and immune-stimulating activities.[5–7)]

In this study, we have clarified the chemical features of the neutral exopolysaccharide produced by *Lactobacillus fermentum, a* strain TDS030603, obtained from the collection of lactic acid bacteria in our laboratory. This polysaccharide, which produces a high degree of ropiness, is a candidate for use as an additive to increase the viscosity of food. The present study was also undertaken to determine the growth and exopolysaccharide production by the above strain in both cheese whey-based medium as well as the MRS broth. As it's well known that cheese whey is an available biomass product, the high production of this polysaccharide in cheese whey will lead us to the availability of this strain for industrial use.

MATERIALS AND METHODS

Lactobacillus fermentum TSD030603 was from a collection of lactic acid bacterial strains isolated from traditional fermented milks at Obihiro University of Agriculture and Veterinary Medicine. High degrees of viscosity and stickiness were observed in cultures of this strain, which were stored at −80°C in de Man, Rogosa and Sharpe (MRS) broth plus 20% (v/v) glycerol until use. Before use the cultures were propagated twice in MRS medium (5 mL) at 30°C for 48 h; then the transfer inoculums of 2% (v/v) of the culture were grown in fresh medium.

This strain was identified using bacterial identification kit API50 CHL (Bio-Merieux, France) and confirmed by amplification of its ribosomal subunit (16S rRNA) molecule.

Culture conditions for polysaccharide production. The bacterial strain was cultivated in both MRS and modified cheese whey-based media. The MRS medium was used to determine the optimal temperatures for maximum yield of polysaccharide, whereas the whey-based media containing various nutrients were prepared and

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Abbreviations: MRS-, de Mann Rogosa and Sharpe; EPS-, exopolysaccharide; GRAS-, generally recognized as safe; DEAE-, diethylaminoethyl; TFA-, trifluroacetic acid; TLC-, thin layer chromatography; HPLC-, high performance liquid chromatography; PA-, pyridylamination; Mn^{2+}-, manganese ion.

used to investigate essential nutrients which could enhance exopolysaccharide production by the strain. The strain was cultured with MRS medium at three different temperatures (30, 37 and 40°C) for 72 h. The crude and purified products were lyophilized and weighed. The whey-based media were prepared as follows. Cheese whey powder was dissolved in ion exchanged water (10 g per 100 mL) and the solution was autoclaved at 121°C for 15 min, followed by deproteinization by ultracentrifugation at 12,000 rpm for 20 min. Then 1% tryptone, 0.4% yeast extract and 0.8% beef extract with other nutrients, as shown in Table 2, were added to the supernatant, followed by autoclaving at 121°C for 20 min.

Isolation and purification of polysaccharide from culture with MRS broth. After incubation for 72 h, the extracellular polysaccharide was obtained from the culture with MRS broth as follows. After stirring for 1 h, cells and precipitated proteins were removed by centrifugation (2×30 min, 15,000 × *g*, 4°C), and the polysaccharide-containing fraction in the supernatants was precipitated with an equal volume of ice-cold ethanol. Aqueous suspensions of the precipitated materials were extensively dialyzed against running tap water. The precipitates formed were re-dissolved in hot water and re-precipitated by adding 40% (v/v) acetone to remove contaminants. The polysaccharide fraction was again dialyzed with water and then lyophilized and labeled as crude polysaccharide.

Further purification of this crude polysaccharide was performed using an anion exchange column (1.5×20 cm) of DEAE Sephadex A-50, equilibrated with 50 mM Tris-hydroxyamino-methane hydrochloric acid buffer, pH 8.7. The lyophilizate of the crude polysaccharide fraction (50 mg) was dissolved in the buffer (2 mL), applied to the column and eluted with the same buffer. The unadsorbed components were eluted with 200 mL of buffer, after which elution was continued using a linear gradient of NaCl from 0 to 0.5 M in a total volume of 400 mL. Elution was done at a flow rate of 15 mL/h and fractions of 5 mL were collected. Protein and sugar in the eluent fractions were monitored by absorbance at 280 nm and at 490 nm, respectively, with the phenol-sulfuric acid method.[8] Peak fractions from the anion exchange chromatogram were pooled, dialyzed with water and lyophilized. Later the lyophilyzate was further separated by gel filtration using a TOYOPEARL HW-75F column (2.6 cm×100 cm), to separate this polysaccharide from mannan contaminants from the MRS broth. The fractions were monitored for sugars as above and the peak fractions were pooled, dialyzed with water, lyophilised, and labeled as purified polysaccharide.

Isolation and purification of polysaccharide from culture with whey based medium. The *Lactobacillus fermentum* TDS030603 strain was pre-incubated with the whey-based medium at 30°C for 24 h, after which 5% (v/v) of the culture solution was inoculated into the medium, followed by incubation at 25°C for 24 h. An equal volume of ethanol was added to 3 L of the culture medium and the solution was stored at 5°C for 24 h. It was then centrifuged at 12,000 rpm for 20 min; the precipitate was collected and dissolved in 1 L of ion-exchanged water, followed by neutralization with 1 M NaOH. The solution was dialyzed with water and the retentate was diluted to 3 L with water. After heating at 70°C for 30 min with stirring, the solution was centrifuged at 5000 rpm for 15 min. The supernatant was freeze-dried and designated as crude polysaccharide. Further purification of the polysaccharide was performed by anion exchange chromatography as described above. The fractions containing unadsorbed material were pooled, dialysed with water and lyophilized.

Monosaccharide composition of polysaccharide. For monosaccharide analysis, three procedures were used: **I** Polysaccharides were hydrolyzed with 2 N trifluoroacetic acid (TFA) at 100°C for 5 h; excess acid was removed by co-distillation with distilled water (3×), followed by lyophilisation. The lyophilized hydrolysate of polysaccharide was analyzed by thin layer chromatography (TLC) using silica gel aluminum plates. The developing solvent system was acetone/2-propanol/0.1 M lactic acid (2:2:1 v/v/v) in a one-dimensional run. The components were detected by spraying with 5% sulfuric acid in ethanol and heat on a burner for 5 min. The standards used were D-glucose, D-galactose and D-mannose. **II** The monosaccharide compositions were obtained by HPLC of the polysaccharide TFA hydrolyzate. The lyophilized hydrolyzate of the polysaccharide was subjected to pyridylamination by the method of Hase,[9] followed by lyophilization and HPLC analysis. The HPLC analyses were done on an Inertsil ODS-3V column (4.6×250, GL Science) using UV 310 nm as detector. Elution was performed isocratically with 0.1 M ammonium acetate pH 5.0 at a flow rate of 0.5mL/min. **III** The polysaccharide (1.5 mg) was dissolved in 0.6 mL of distilled water in a 1.5 mL test tube. 3.3 M TFA, 0.9 mL was then added and the polysaccharide was hydrolyzed by heating at 100°C for 3 h, followed by lypholization. The monosaccharide composition of the hydrolysate was obtained by comparison of the peak area with the standards following ion-exchange chromatography on a Dionex DX-500 (Japan Dionex, Tokyo, Japan) CarboPac PA-1 column. The components were eluted with 5 mM NaOH at the flow rate of 1 mL/min and detected with a pulsed amperometry detector.

Methylation analysis. The polysaccharide was methylated using the Hakomori[10] method and the methylate was further purified by silica gel chromatograpahy on a column of Wakogel S-1. The alditol acetate derivative of partially methylated sugar was prepared from the permethylated polysaccharide using the method of Stellner.[11] The partially methylated alditol acetate was analyzed by gas chromatography-mass spectrometry using a Hewlett Packard HP5890 gas chromatography/HP5972MSD mass spectrometer. A DB-17 column (Agilent Technologies Co.) with a temperature program of 150°C for 1 min, 150–200°C at 15°C/min was used. Each partially methylated alditol acetate was identified by comparison of its retention time during GC with a standard prepared from the polysaccharide produced by *Streptococcus thermophilus* OR 901[12] and also by its mass fragmentation pattern.

NMR spectroscopy. Prior to NMR spectroscopic analysis, samples were exchanged in 99.9% D_2O (Merck) and lyophilized, then later dissolved in 100.00% D_2O (Sigma). 1D ^1H NMR spectra were recorded in D_2O at 500 MHz with a Jeol ECP-500 FT-NMR spectrometer,

with the probe temperature of 70°C. Chemical shifts were expressed in ppm by reference to internal acetone (δ 2.225).

Viscosity measurement of polysaccharide aqueous solution. A Rheometrics Dynamic Analyzer RDA II (RAA-FRT-DY-FC Model) with a conic cylinder (diameter of 13 mm, 12.5 mm and length 32 mm) was used to measure viscosity. The lyophilizate of polysaccharide was dissolved in hot water to form a 1% (w/v) solution of polysaccharide. The shear rates were within 100 s^{-1} and the test temperature used was 20°C. The flow type of viscosity against shear rates was determined.

RESULTS AND DISCUSSION

Optimal culture conditions for exopolysaccharide production.

I. Exopolysaccharide production by culture using MRS broth

To determine the optimal culture conditions, temperature was one of the parameters investigated. Table 1 shows that the yields of crude and purified exopolysaccharide fractions obtained were higher at 30 and 37°C than at 40°C. There was no significant difference between the yields obtained at 30 and 37°C. The yields were more than 500 mg/L and 100 mg/L of crude and purified exopolysaccharide, respectively, in MRS medium. It was previously reported that the total yield of polysaccharide produced by lactic acid bacteria depends on the composition of the medium and the growth conditions, i.e. temperature, pH and incubation time.[13] The present study showed that both 30 and 37°C were suitable for the growth and exopolysaccharide production by this strain.

Table 1. The effect of temperature on growth and exopolysaccharide production by *Lactobacillus fermentum* TDS030603.

Temperature	Crude exopolysaccharide production (mg/L)	Exopolysaccharide after purification (mg/L)
30°C	568.6±0.1	151.2±0.1
37°C	566.5±0.1	150.6±0.1
40°C	524.9±0.1	139.6±0.1

The strain was cultured aerobically in MRS for 72 h, at temperatures of 30, 37 and 40°C. The weights of crude and purified exopolysaccharide were measured (mg/L).

II. Culture condition using whey-mediated broth

Partially deproteinised cheese whey-mediated broths incorporating various nutrients were tested for the growth and exopolysaccharide production of *Lactobacillus fermentum* TDS030603. Table 2 lists the nutrients in the whey-mediated broth tested in this study. The addition of several nutrients was tested for both the growth and exopolysaccharide production of this strain because it was thought that some of the nutrients were lacking in deproteinized cheese whey medium. The additional nutrients were components which contained in MRS broth, since the strain grew and produced polysaccharide in MRS. The test was performed for the purpose of the utilization of cheese whey as a medium for the growth and exopolysaccharide production of this strain for future industrial use. The growth of this strain and an increase in the viscosity of the medium were observed with broth G as with the MRS broth. With the other broths A–F, only small growth of the strain was observed during incubation for 72 h, while the viscosity of the media did not increase. These results showed that *Lactobacillus fermentum* TDS030603 requires $MnSO_4 \cdot 4H_2O$ for growth in whey-mediated broth. In general, phosphate, Mn^{2+} and citrate are essential for the growth and production of exopolysaccharide by lactic acid bacterial strains.[14,15] It can be concluded that the exopolysaccharide production of *Lactobacillus fermentum* TDS030603 in the cheese whey medium is favored by the presence of macro-elements of Mn^{2+}.

Purification and chemical analysis of the exopolysaccharide.

The crude polysaccharide fractions separated from cheese whey-mediated broth and MRS broth were purified by anion exchange chromatography as shown in Fig. 1 and successive dialysis with water. The unadsorbed fractions were designated as neutral polysaccharides and further analyzed as described below. The adsorbed fractions were not investigated in this study. A preliminary experiment showed that the hydrolysate of polysaccharide from whey-mediated broth consisted of galactose and glucose, while that from MRS broth contained mannose as well as glucose and galactose. The MRS broth contains a large amount of yeast extract and strongly interferes with the isolation procedure of macro-molecules such as exopolysaccharide, as was also reported in a previous study.[16] The

Table 2. The influence of nutrients on growth and polysaccharide production by *Lactobacillus fermentum* TDS030603.

Nutrients	Medium composition						
	A	B	C	D	E	F	G*
Deprotienized whey	+	+	+	+	+	+	+
Tryptone	1.0%	1.0%	1.0%	1.0%	1.0%	1.0 %	1.0 %
Yeast extract	0.4%	0.4%	0.4%	0.4%	0.4%	0.4 %	0.4 %
Beef extract	0.8%	0.8%	0.8%	0.8%	0.8%	0.8 %	0.8 %
Sodium acetate	−	0.5%	0.5%	0.5%	0.5%	0.5 %	0.5 %
$K_2HPO_4 \cdot 3H_2O$	−	−	0.2%	0.2%	0.2%	0.2 %	0.2 %
Diammonium hydrogen citrate	−	−	−	0.2%	0.2%	0.2 %	0.2 %
Tween 80	−	−	−	−	0.1%	0.1 %	0.1 %
$MgSO_4 \cdot 7H_2O$	−	−	−	−	−	0.02%	0.02 %
$MnSO_4 \cdot 4H_2O$	−	−	−	−	−	−	0.004%

The strain was cultured in cheese whey-based media of varying composition, prepared by the addition of various nutrients. The medium denoted as G* was the only medium that supported the growth and exopolysaccharide production by *Lactobacillus fermentum* TDS030603.

mannan polysaccharide was separated from MRS broth by a similar separation procedure in the control experiment. As it was assumed that the mannose originated from mannan in the MRS broth, this neutral polysaccharide fraction was subjected to gel chromatography on TOYOPEARL HW-75 F. The saccharide was separated into two components, as shown in Fig. 2. The higher molecular fraction,

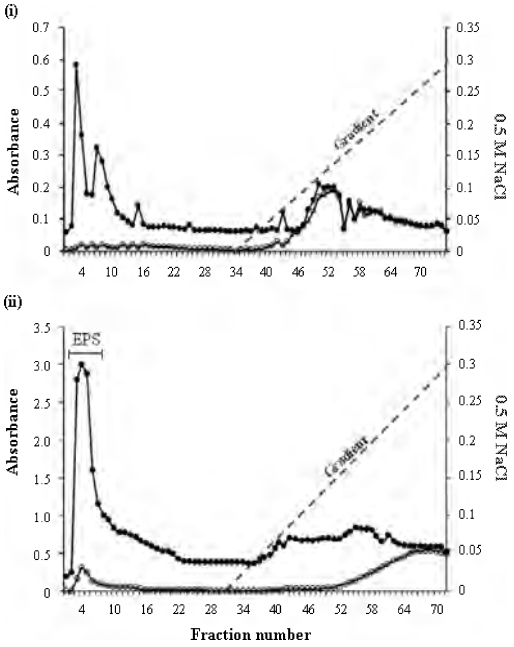

Fig. 1. Anion exchange chromatogram of exopolysaccharide isolated from cultures of *Lactobacillus fermentum* TDS 030603 in **(i)** Cheese whey-based broth and **(ii)** MRS broth. DEAE Sephadex A-50 (2.0 cm×20 cm) column equilibrated with 50 mM Tris-hydroxyaminomethane hydrochloric acid buffer of pH 8.7 was used.

The sample was washed at the flow rate of 15 mL/h with the same buffer and later eluted with a linear gradient of NaCl from 0 to 0.5 M. The absorbance of each fraction was determined for : hexose (●) at 490 nm with phenol-H_2SO_4 and protein (○) at 280 nm.

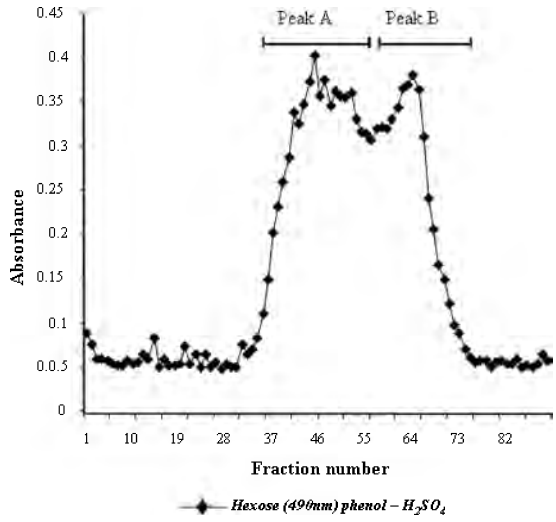

Fig. 2. Gel filtration chromatogram of exopolysaccharide separated from MRS broth, after DEAE-Sephadex A-50 anion exchange chromatography.

Gel filtration was achieved on a TOYOPEARL HW-75F (2.6 cm ×100 cm) column, equilibrated and eluted with distilled water at flow rate of 15 mL/h. The absorbance of each fraction was determined at 490 nm with the phenol-sulfuric acid method.

Peak A, was designated as exopolysaccharide of *Lactobacillus fermentum* TDS030603 and was further characterized in this study. The second component, Peak B, was assumed to contain mannan contaminants from the MRS broth ingredients. As the ^{1}H-NMR spectra of polysaccharide from whey-mediated broth and MRS broth were very similar (Fig. 3), further analyses were focused on the saccharide from whey-mediated broth.

Quantitative monosaccharide analysis using procedures **II** and **III**, revealed the presence of D-galactose and D-glucose in a molar ratio of 1:2.5 (Fig. 4). The results suggested the exopolysaccharide was composed of D-glucose and D-galactose.

The identification of each partially methylated alditol acetate prepared from the polysaccharide revealed that the following derivatives were found in a molar ratio of 1: 0.25:0.88:1.01:0.88, respectively, 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetylglucitol , 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetylgalactitol, 2,4,6-tri-*O*-methyl-1,3,5-tri-*O*-acetyl glucitol, 2,3,4-tri-*O*-methyl-1,5,6-tri-*O*-acetylgalactitol and 4,6-di-*O*-methyl-1,2,3,5-tetra-*O*-acetylglucitol (Fig. 5, Table 3). The peak between peak A and B was not derived from the polysaccharide as was shown by the mass spectrum of this peak. This peak must have originated from a contaminant in the reagents which had been used in the preparation process. The peak ratio of peaks C–E was not equal in the chromatogram in Fig. 5; it was suggested that this small variation was due to the loss of the components during the preparation process. However, the components in peak A–E were deduced to have been derived from non-reducing glucose, non-reducing galactose, *O*-3 substituted glucose, *O*-6 substituted galactose and *O*-2,3-disubstituted glucose. The presence of two non-reducing

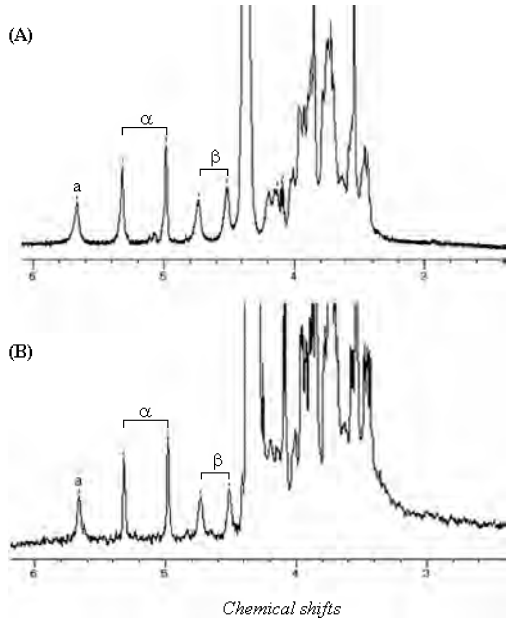

Fig. 3. 500-MHz ^{1}H-NMR spectra of exopolysaccharide produced by *Lactobacillus fermentum* TDS030603 recorded in D_2O at 70°C.

Chemical shifts are expressed in ppm by reference to internal acetone (δ=2.225). (A) Exopolysaccharide separated from MRS broth. (B) Exopolysaccharide separated from cheese whey-mediated broth. Both have two α and two β anomers. [a]Down-field shift signal arising from substitution of OH group of glucose residue.

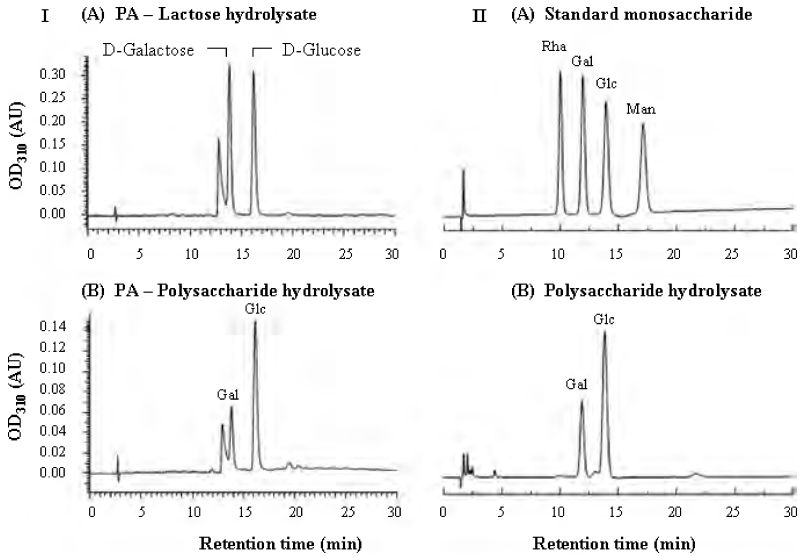

Fig. 4. **I**. HPLC chromatograms of pyridylamination (PA) derivatives : (A) lactose hydrolysate (standard), (B) polysaccharide hydrolysate. **II**. Ion exchange chromatograms of (A) standard monosaccharide; rhamnose (Rha), galactose (Gal), glucose (Glc) and mannose (Man), (B) polysaccharide hydrolysate.

The HPLC was performed on an Inertsil ODS 3V column (4.6×250, GL Science) and detected at UV 310 nm. The chromatogram was achieved using a Dionex DX-500 system with a CarbPac PA-1 column and detected with a pulsed amperometry detector.

Table 3. Retention times and mass spectra primary fragments of methyl alditol acetates.

Peak[a]	Positions of methylation	Retention time (min)	Primary fragments in the mass spectra
A	2,3,4,6-Glc[b]	6.10	43,87,101,117,129,145,161,205
B	2,3,4,6-Gal	6.45	43,87,101,117,129,145,161,205
C	2,4,6-Glc	7.71	43,101,117,129,161,233
D	2,3,4-Gal	8.70	43,87,99,101,117,129,161,189,233
E	4,6-Glc	9.72	43,101,129,161,261

[a]Refer to Fig. 5. [b]2,3,4,6-Glc=1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glutiol, *etc*.

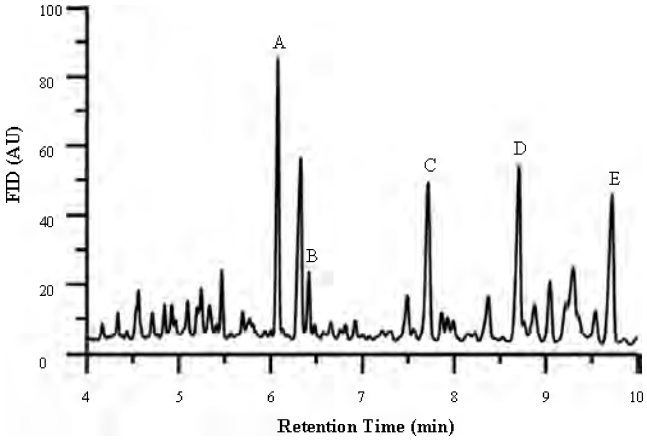

Fig. 5. Gas liquid chromatogram of the partially methylated alditol acetates derived from polysaccharide produced by *Lactobacillus fermentum* TDS030603.

GLC-MS measurements were performed on the column DB 17, using a temperature program of 150–200°C at 15°C/min.

monosaccharides and branched repeating units showed that the fraction consisted of two branched polysaccharides, one major and one minor, each of which had a tetrasaccharide repeating unit. The results suggested that the major saccharide has a non-reducing glucose whereas the minor one has a non-reducing galactose residue.

The ^{1}H-NMR spectrum of the polysaccharide had anomeric shifts at δ 5.319, 4.981, 4.735 and 4.514. It was assumed that the shift at δ 5.664 arose from the H-2 or H-3 of disubstituted glucose residue. The unusual down-field shift would appear to be due to the disubstitution of H-2 and H-3 of this residue. It was concluded that the shifts at 5.319 and 4.981 corresponded to H-1 of α-glycosides, while the shifts at 4.735 and 4.514 were caused by H-1 of β-glycosides.

From these observations, it was concluded that the major polysaccharide contains a tetrasaccharide repeating unit, which consisting of three glucose and one galactose residue, whereas the minor polysaccharide contains a different tetrasaccharide repeating unit, consisting of two galactose and two glucose residues, with both repeating units consisting of two α and two β anomers (Fig. 6). Even though it was difficult to estimate the precise molar ratio of major to minor polysaccharide by the ratio of each of partially methylated alditol acetate and monosaccharide analysis; due to a lot of derivitization and hydrolysis steps involved in the preparation process, it was assumed that the molar ratio of major to minor could be between 4:1 and 6:1.

A solution of the exopolysaccharide (1% w/v) had a high viscosity, as revealed by rheological analysis. The decrease of viscosity of the exopolysaccharide aqueous solution with increasing shear rate also clearly demonstrated the non-Newtonian behavior (shear thinning) of the solution (Fig. 7).

There have been a number of previous reports of the

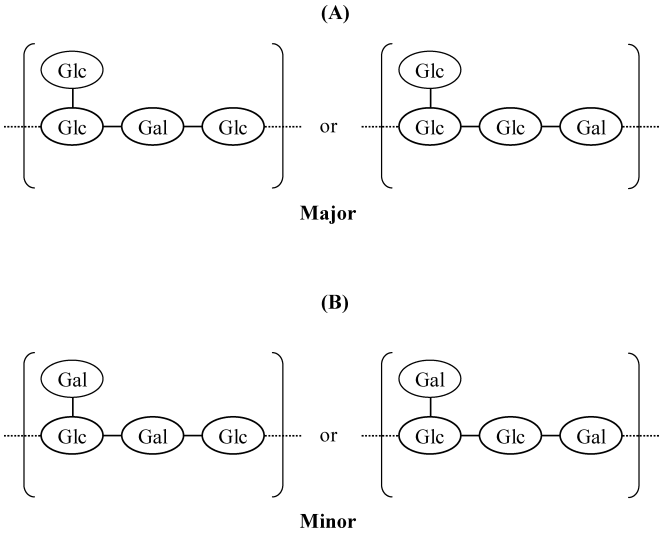

Fig. 6. The proposed structures of the repeating units.

(A) Major saccharide repeating unit, (B) minor saccharide repeating unit.

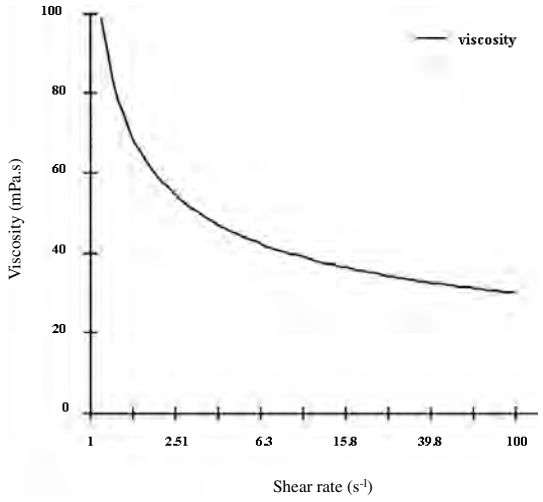

Fig. 7. Flow curve of *Lactobacillus fermentum* TDS030603 exopolysaccharide (1% w/v) solution.

Viscosity was measured against shear rate up to 100 s^{-1}. As increasing shear was applied; the viscosity of the polysaccharide solution dropped providing shear thinning behavior. When shear stress was removed, the viscosity instantly recovered to its original value.

production of exopolysaccharide by dairy lactic acid bacteria, including *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactococcus lactis* subsp. *cremoris* and others.[17–27] One report described a strain of *Lactobacillus fermentum* isolated from Burkino Faso fermented milk producing an exopolysaccharide which was composed almost wholly of D-galactose (99.18% galactose and 0.80% glucose).[28] The chemical structure of our polysaccharide, produced by *Lactobacillus fermentum* TDS030603, is clearly different from the structures of those produced by other lactic acid bacterial strains, including the above-mentioned *Lactobacillus fermentum* strain.

This study showed that our strain requires Mn^{2+} for its growth in cheese whey-mediated broth. In a preliminary experiment with the cheese whey medium, the yield of crude polysaccharide was 4.30 g/L. From this high production of exopolysaccharide it can be expected that this strain can be used for the production of highly viscous biomaterial for the food industry.

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Lactobacillus fermentum TDS030603 株の生産する中性多糖の構造研究

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Lactobacillus fermentum TDS030603 株の生産する中性多糖を，培養上清から精製し，単糖組成分析，メチル化分析および^{1}H-NMR で構造解析した．チーズホエーを原料とする培地ならびに MRS 培地で培養した場合の，同菌株による多糖生産の至適条件を検討した．多糖は 1 : 2.5 の割合で単糖として D-ガラクトースと D-グルコースを含み，おのおの 2 単位ずつの α-，ならびに β-アノマーからなる 4 糖繰り返し単位をもったメジャー成分とマイナー成分を含むことが示された．メジャー成分は，非還元末端グルコピラノース，3 位置換グルコピラノース，6 位置換ガラクトピラノースおよび 2 位，3 位置換グルコピラノースから成る一方，マイナー成分は，非還元末端ガラクトピラノース，3 位置換グルコピラノース，6 位置換ガラクトピラノースおよび 2 位，3 位置換グルコピラノースから成ることが示された．これは以前報告されている *Lactobacillus fermentum* の別の株の生産する多糖とは異なり，新規の多糖である．同多糖は培養物の粘性の付与に利用することが期待される．