A POLYSACCHARIDE PRODUCED BY *Lactococcus lactis* subsp. *lactis* YZ1 ISOLATED FROM TRADITIONAL INDONESIAN FERMENTED MILK, "DADIH".

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ABSTRACT

Lactococcus lactis subsp. *lactis* YZ1 was isolated from M17 agar in which diluted Dadih was poured and incubated at 30°C for 48 h. Taxonomix properties of the isolate were examined according to Bergey's Manual of Systematic Bacteriology and Manual for Identification of Medical Bacteria. The isolation of polysaccharide from the precipitant was performed on an ion-exchange chromatography. The result showed that the polysaccharides produced by *Lactococcus lactis* subsp. *lactis* YZ1 were neutral sugar (unadsorbrd fraction) and glycoconjugated (absorbed fraction). The neutral sugar had molecular weight of 10,000 and 200,000 with an α-glycoside linkage. The monosaccharide composition was mannose, glucose and galactose with a molar ratio of 1 : 1,5 : 4,9.

INTRODUCTION

It is known that many microorganisms produce exopolysaccharides which are located outside the cell wall. Exocellular polysaccharides usually originate from a capsule on bacterial cell. Several polysaccharides produced by lactic acid bacteria have been studied by many researchers (1, 3, 7, 8, 9).

A strain of *Streptococcus salivarius* subsp. *thermophilus* OR 901, grown on partially deproteinized whey, produced an exocellular polysaccharide which had a heptasaccharide repeating unit consisting of two rhamnose and five galactose residues (2). The polysaccharide produced by the slime forming *Lactococcus lactis* subsp. *cremoris* strain LAPT 3001, isolated from Sweedish ropy sour milk "Langfil", consists of rhamnose, glucose, galactose, glycerol and phosphorus (11). The slime-material of *Lactococcus lactis* subsp. *cremoris* L416 is a glycoprotein that contains protein (47%), hexoses (9.3%) 6-deoxyhexoses (20%), and sialic acid (2.8%) (6).

In this study we describe a chemical property of an exocellular polysaccharide produced by *Lactococcus lactis* subs. *lactis* YZ1 isolated from "Dadih", a traditional fermented milk in Sumatera, Indonesia.

MATERIALS AND METHODS

Identification of *Lactococcus lactis* subsp. *Lactis* YZ1

The strain was isolated from M17 agar in which diluted Dadih was poured and incubated at 30°C for 48 h. Taxonomic properties of the isolate were examined according to Bergey's Manual of Systematic Bacteriology (10) and Manual for Identification of Medical Bacteria (5). The bacterial identification was performed by several tests including gram staining, catalase test, oxidase test, litmus milk test, OF test, motility test, growth temperature test, gas production from glucose test, anaerobic growth test, growth at pH 4.8 and 9.6, growth at NaCl 6.5%, nitrate reduction, carbohydrate fermentation and arginine hydrolysis (BBL Minitek System).

Observation of Encapsulated Strains from Dadih

Encapsulated strains were isolated from Dadih on M17 agar medium and incubated at 30°C for 48 h. Strains were inoculated into partially deproteinized whey containing 1%

peptone and incubated at 30°C for 42 h, followed by observation of the formation of a capsule with a microscope under indian ink staining.

Preparation of Polysaccharide

The culture of an encapsulated strain of *Lactococcus lactis* subsp. *lactis* YZ1 isolated from Dadih in sterilized Brigg's broth[14] was incubated at 30°C for 18 h. It was inoculated into partially deproteinized whey containing 1% peptone and incubated at 30°C for 42 h under aerobic condition. After incubation, the bacterial cell were removed by centrifugation at 11,000 rpm. for 20 min at 20°C. The polysaccharide in the supernatant was precipitated by addition of 3 volumes of cold ethanol and then refrigerated (4°C) overnight. The precipitate was separated from the supernatant by centrifugation at 7,000 rpm for 15 min. at 20°C, redissolved in hot water (50°C) and recentrifuged at the same conditions, followed by dialysis with tap water and distilled water overnight. The supernatants were lyophilized.

The isolation of polysaccharide from the precipitant performed on an ion-exchange column (1.5 x 20 cm) of DEAE-Sephadex A-50 (Pharmacia fine chemicals) equilibrated with 50 mM Tris-aminomethane hydrochloric acid buffer, pH 8.7. The lyophilizate was applied on the column and washed with the same buffer. The column was eluted with linear gradient on NaCl from 0 to 1.0 M in a total volume 500 ml. Using phenol sulfuric acid method protein and sugar in the eluent were monitored at absorbance of 280 nm and 490 nm, recpectively.

High Performance Liquid Chromatography (HPLC)

The molecular weight of polysaccharide was determined by HPLC equipped with a TKS-Gel G6000 Pwxl 78 mm ID x 30 cm (TOSOH) column. It was eluted with distilled water and monitored with RI detector. Shodex standard Pullulan Kit P-800 (Showa Denko Ltd. Tokyo. Japan) was used as a standard calibration.

^{1}H-NMR Analysis

^{1}H-NMR spectrum was recorded in D_2O (99.95% atom D, Merck Germany) at 270 MHz, using a Joel JNM-270 spectrometer. Chemical shifts were expressed down field from internal 3-(trimethylsilyl)-1-propane sulfonic acid, sodium salt (TPS) but are actually measured by reference to internal acetone (δ=2.22%).

Methanolysis of Polysaccharide

The methanolysis of polysaccharide was performed in 2% HCl-methanol at 80°C for 20 h. The 2% HCL-methanole was prepared from 5% HCl-methanol (Wako Co., Tokyo. Japan) by dilution with anhydrous methanol. The methanolysate was trimethylsylated with a TMS HT kit (Tokyo Kasei Co. Tokyo. Japan) and subjected to gas liquid chromatography with a Shimadzu 13 B gas chromatograph. The gas chromatograph was equipped with capillary column DB-17 (0.32 mm x 30 m) (Shimadzu. Tokyo. Japan) and programmed from 150 to 250°C at 3°C/min.

RESULTS AND DISCUSSION

Identification of Lactic Acid Bacteria

The isolated bacteria from Dadih was a nonspore-forming, facultative anaerobic, catalase-negative, oxidase-negative, gram-positive ovoid cell, 1.0 μm in diameter in short chains. It grew well at 30°C. Acid was produced from glucose, lactose, galactose, maltose, mannose, salicin and trehalose, but not from raffinose, rhamnose, sorbitol or sucrose. These characteristics were in agreement with *Lactococcus lactis* subsp. *lactis* according to Bergey's Manual of Systematic Bacteriology (10) and Manual for Identification of Medical Bacteria (5), so the strain has been designated as *Lactococcus lactis* supsp. *lactis* YZ1.

Chemical Characteristics of Polysaccharide Produced by *Lactococcus lactis* subsp. *lactis* YZ1

The precipitates with ethanol from the culture of *Lactococcus lactis* subsp. *lactis* YZ1 was 974.35 mg/1 culture. Separation of the polysaccharide was accomplished by DEAE-Sephadex A-50 ion exchange chromatography (Fig.1). The sugar fraction was divided into two parts. The components in fraction number 10 - 25 and 60 - 90 were suggested to be a neutral sugar (7.8 mg/l culture) and a glycoconjugate, respectively (Fig.1).

Gel filtration chromatography on HPLC by pululan standard showed that neutral sugar had two molecular weights of around 10,000 and 200,000 (Fig 2). The aromatic shift at δ 5.476 in ^{1}H-NMR spectrum of neutral polysaccharide showed that the glycosidic linkage is only an α-form (Fig. 3).

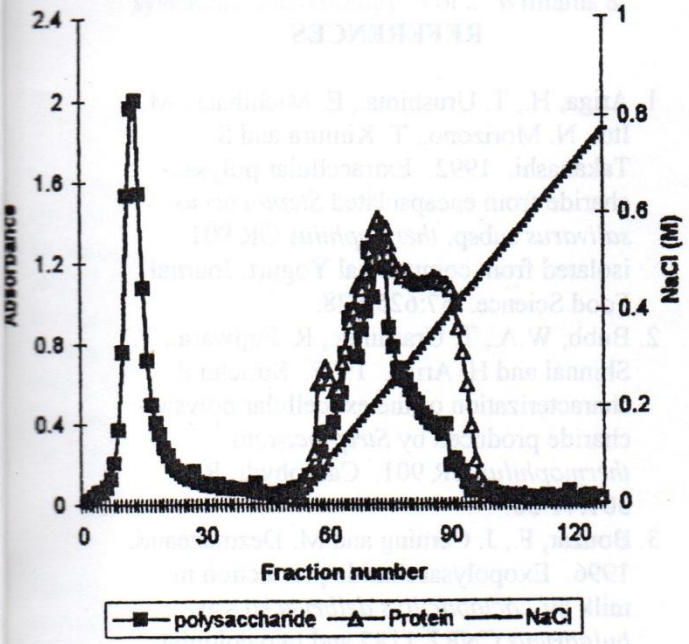


Fig. 1. DEAE-Sephadex A-50 ion exchange chromatogram of polysaccharide produced by *Lactococcus* subsp. *lactis* YZ1 isolated from Dadih

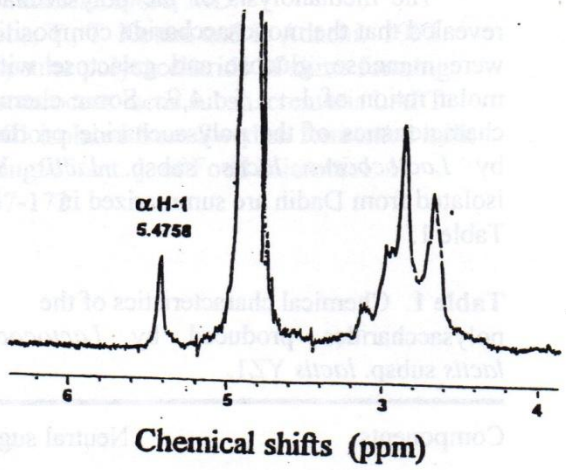
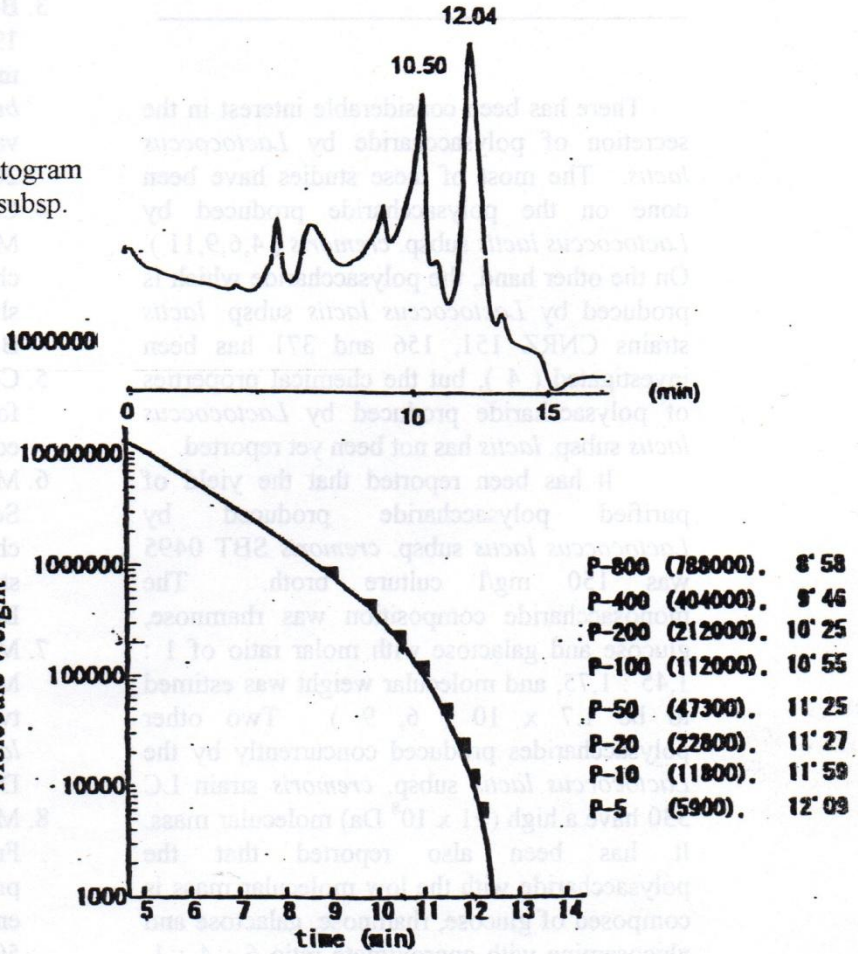


Fig. 3. Spectrum of the neutral sugar at 270.05 MHz ^{1}H-NMR. The spectrum was recorded in D2O (99%).



g. 2. HPLC of polysaccharide and standard curve of molecular weight with shodex standard pullulan Kit P-800.

The methanolysis of the polysaccharide revealed that the monosaccharide compositions were mannose, glucose and galactose with a molar ration of 1 : 1,5 : 4,9. Some chemical characteristics of the polysaccharide produced by *Lactococcus lactis* subsp. *lactis* YZ1 isolated from Dadih are summarized in Table 1.

Table 1. Chemical characteristics of the polysaccharides produced by *Lactococcus lactis* subsp. *lactis* YZ1.

Components	Neutral sugar
Yield (mg/l)	7.8
Molecular weight	10,000 200,000
Monosaccharide composition	Man, Gal, Glc
Molar ratio	1 : 1.5 : 4.9

There has been considerable interest in the secretion of polysaccharide by *Lactococcus lactis*. The most of these studies have been done on the polysaccharide produced by *Lactococcus lactis* subsp. *cremoris* (4,6,9,11). On the other hand, the polysaccharide which is produced by *Lactococcus lactis* subsp. *lactis* strains CNRZ 151, 156 and 371 has been investigated (4), but the chemical properties of polysaccharide produced by *Lactococcus lactis* subsp. *lactis* has not been yet reported.

It has been reported that the yield of purified polysaccharide produced by *Lactococcus lactis* subsp. *cremoris* SBT 0495 was 150 mg/l culture broth. The monosaccharide composition was rhamnose, glucose and galactose with molar ratio of 1 : 1,45 : 1,75, and molecular weight was estimed to be 1.7 x 10 (6, 9). Two other polysaccharides produced concurrently by the *Lactococcus lactis* subsp. *cremoris* strain LC 330 have a high (>1 x 10^8 Da) molecular mass. It has been also reported that the polysaccharide with the low molecular mass is composed of glucose, rhamnose, galactose and glucosamine with approximate ratio 6 : 4 : 1, whereas the one with high molecular mass is composed of rhamnose, galactose and glucose with approximate ratio of 6 : 3 : 2 (7).

Lactococcus lactis subsp. *lactis* strains CNRZ 151, 156 and 371 produced 85, 55 and 30 mg EPS/1, respectively (4). Our results showed that the yield of polysaccharide produced by *Lactococcus lactis* subsp. *lactis* YZ1 were much higher than those.

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