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Isolation of a Polysaccharide Producing Bacterium and Properties of Its Polysaccharide

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다당류 생산세균의 분리동정 및 그 물질의 특성

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초 록

토양으로부터 점질성을 나타내 주는 물질 생산세균을 분리하였으며 이 균을 동정한 결과 *Enterobacter agglomerans*로 동정되었다. 이 균이 생산하는 다당류는 glucose와 galactose로 구성된 β-glucan polymer로 추정되었다. 이 다당류는 1% 농도에서 264 mPa.s.의 점도를 나타내며 항복치는 4.89Pa이었다. 한편 이 다당류 용액은 열안정성은 없으며 pH 및 NaCl에 대한 안정성은 있었다.

Introduction

Polysaccharides are produced by various spectra of microorganisms[1] and bearing an importance as substitutes for traditional polysaccharides derived from tree exudates, seed and seaweed extracts etc[2]. Furthermore, some microbial polysaccharides have many desirable properties superior to those of traditional ones[3]. In this sense, many scientist have been trying to find a new and novel polysaccharide producer. In a screening program to search polysaccharide-synthesizing microorganisms, a bacterium which produced copious amount of polymer was isolated. In this paper, the properties of the bacterium and physicochemical characteristics of the polysaccharide will be discussed.

1988년 4월 11일 수리
Corrponding author : J.Y. Yoo

Materials and Methods

1. Isolaion of bacterium and production of polysaccharide

The bacterium was isolated on PCA agar plate at 30°C from soil collected at Boen, Chungnam. The bacterium was grown in glucose-peptone medium[4] at 30°C using Bioflo C-32 (NBS, U.S.A., working volume 1.2L, agitation: 400rpm, aeration: 1vvm). Polysaccharide was harvested by isopropanol(66%) precipitation of cell-free supernatant of 3 days old culture broth[4].

2. Analysis and viscometry

Analysis was as reported by Yoo et al.[4]. Viscometry was conducted with Brabender viscotron(Model 80241, System E-17, West Germany) and capillary viscometer[5]. IR spectrum was obtained with IR spectrometer(Shimadzu Model

IR 435, Japan).

3. Indentification of bacterium

The bacterium was identified according to the Bergey's Mannual of Systematic Bacteriology[6]. Electron photomicrograph was with TEM(Hitachi Model H-700, Japan).

Results and Discussion

1. Identification of microorganism

The polysaccharide-synthesizing bacterium isolated was identified. The colonial, morphological and biochemical characteristics were as in Fig. 1 and Table 1.

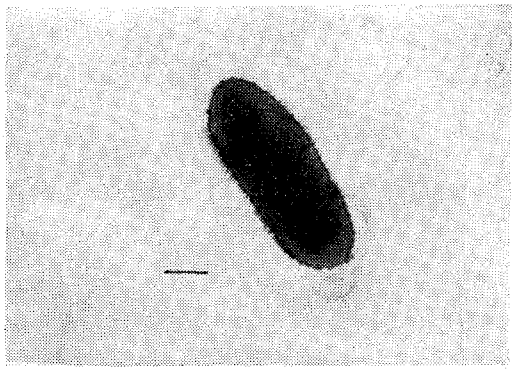

Fig. 1. Transmission electron photomicrograph of isolate U-1(Bar: 0.5μm).

Strain U-1 had circular, entire, convex and echinulate and transparent colony on nutrient agar and irregular, entire, convex, echinulate, mucoid and gigant colony on YM agar. The strain was Gram negative, facultatively anaerobic, motile, short rod(0.90~1.12×2.95~3.37 μm) with peritrichous flagella. It was catalase positive, oxidase negative, had fermentative metabolism and grew well on McConkey agar. The strain U-1 showed negative reaction in growth at 41°C, gelatin liquefaction, arginine dihydrolase, lysine decarboxylase, methyl red test, urease, starch hydrolysis, indole, hydrogen-sulfide formation and DNase. Positive reaction was observed in acid and gas production from

Table 1. Morphological and biochemical characteristics of strain U-1

Characteristics	Record	Characteristics	Record
Gram staining	−ve	Acid and gas from Glucose	+ve
Cell size	0.9~1.12×2.95~3.37μm	Glalactose	+ve
Motility	+ve	Raffinose	+ve
Flagella	Lateral	Trehalose	+ve
Colony	Transparent	Xylose	+ve
Growth	Facultative anaerobic	Maltose	+ve
Metabolism	Fermentative	Lactose	+ve
Catalase	+ve	Fructose	+ve
Oxidase	−ve	Sucrose	+ve
DNase	−ve	Mannose	+ve
Methyl red	−ve	Rhamnose	+ve
Voges-Proskauer	+ve	Mannitol	+ve
Nitrate reduction	+ve	Ribose	+ve
Arginine dihydrolase	+ve	Arabinose	+ve
Lysine decarboxylase	−ve	Dulcitol	+ve
Ornithine decarboxylase	+ve	Citrate utilization	−ve
Gelatin hydrolysis	−ve	Malonate utilization	+ve
Hydrogen sulfide(TSI)	−ve	Starch hydrolysis	−ve
Indole	−ve	Urease	−ve

glucose, acid from galactose, raffinose, trehalose, xylose, maltose, lactose, fructose, sucrose, mannose, rhamnose, mannitol, ribose, arabinose, salicin and ornithine decarboxylase. Voges-Proskauer test, nitrate reduction, citrate and alanine utilization were positive. From these properties, U-1 could be identified as a related strain of *Enterobacter agglomerans*.

2. Rheology of U-1 polymer

The U-1 polysaccharide was harvested from glucose medium and partially purified by repeated alcohol precipitation and subjected to viscometry.

The capillary viscometry(Fig. 2) showed that the intrinsic viscosity of U-1 polysaccharide was 30.02 dl/g. The viscosity of the polysaccharide was lower than xanthan gum but higher than sodium alginate, guar gum and locust bean gum[7]. Kikumoto et al.[8] reported that the intrinsic viscosity of shizophyllan was 12~13 dl/g. The relative viscosity at 0.003% concentration of U-1 polysaccharide was 1.08.

Fig. 3 shows the viscosities of aqueous solution of U-1 polysaccharide at various shear rates and concentrations. The polysaccharide solution showed pseudoplastic behaviour. The viscosity rose up drastically when the concentration increased from 0.5% to 0.75% and then showed steady increase. The viscosities of 0.5, 0.75, 1.0, 1.25 and 1.5% solution of U-1 polysaccharide were 37, 145, 264, 466 and 651 mPa.s. at 42 sec^{-1}. This is more highly viscous than tragacanth and karaya gums. The solution showed a yield point. The yield stress of 1% aqueous solution was 4.89 Pa. The flow behaviour indices at the respective concentration were 0.67, 0.53, 0.45, 0.37 and 0.33 respectively and consistency coefficients were 135, 854, 1, 979, 4,576, and 7,462 mPa.s. at each concentration. The concentration dependency was slightly reduced with the rise of the temperature. The a-values in double logarithmic plot[9] were 3.63, 2.83, 2.80 and 3.37 and K_1-values were 1.95, 1.79, 1.04 and 0.78 Pa.s. at 25, 45, 65 and 85°C.

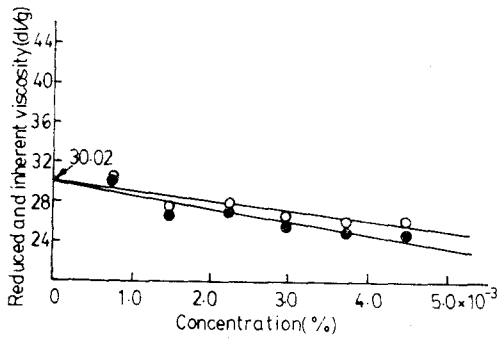

Fig. 2. Reduced and inherent viscosity of polysaccharide produced by *Enterobacter agglomerans* U-1(○ : Reduced viscosity and ● : Inherent viscosity).

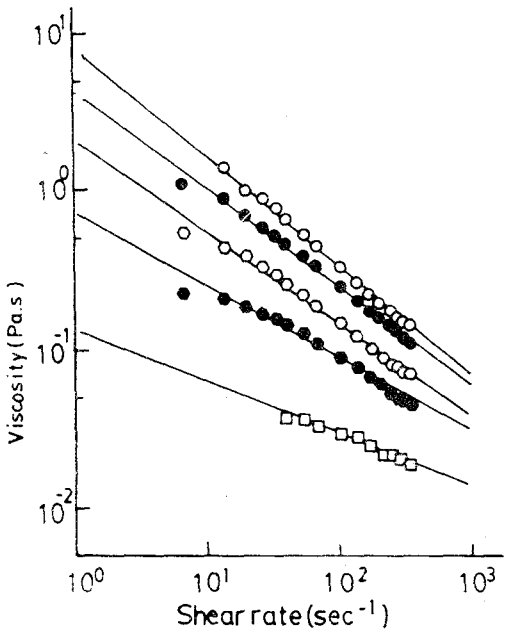

Fig. 3. Concentration dependence of polysaccharide produced by *Enterobacter agglomerans* U-1(○ : 1.5%, ● : 1.25%, ◇ : 1.0%, ◆ : 0.75% and □ : 0.5%).

3. pH dependence of polysaccharide solution

Fig. 4 shows the viscosities of 0.75% polysaccharide solution at various pHs. The viscosity of U-1 polysaccharide solution were relatively stable over a wide range of pH values. The apparent viscosities of 0.75% U-1 polysaccharide solution at pH 3, 5, 7, 9 and 11 were 59, 75,

71,65 and 71 mPa.s. at 210 sec^{-1}, respectively. The polysaccharide produced by *Alcaligenes* sp. was reported as showing low viscosity between 6~9 and below and above which it started to increase[10], however, xanthan gum was reported to be stable over wide range of pH value[11].

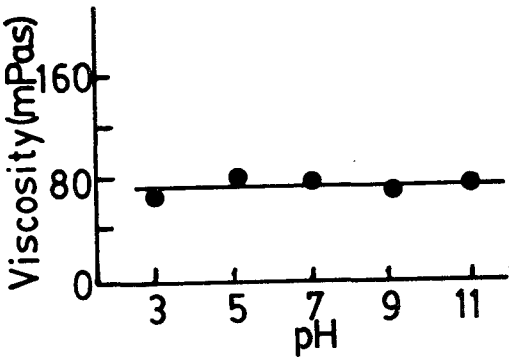

Fig. 4. Effect of pH on the viscosity of 0.75% U-1 polysaccharide solution.

4. Effect of NaCl on the viscosity

Fig. 5 shows the viscosity of 0.75% U-1 polysaccharide solution as affected by the addition of NaCl. The viscosity seemed not much affected by adding NaCl. The solution was not NaCl-precipitatable. The apparent viscosities of the solution at 210sec^{-1} were 71,60,70 and 80 mPa.s for the polysaccharide solution containing 0,0.5,1.0 and 1.5% NaCl. The apparent viscosity of some polysaccharide solution was reported to be enhanced by the addition of Na Cl[10]. When autoclaving at 121°C for 15min., the viscosity was reduced to 18~26 mPa.s. So the polysaccharide was not thermogellable but was thermally unstable. There was no noticeable effect of NaCl addition on the thermal stability.

5. Temperature dependence of polysaccharide solution

Fig. 6 shows typical curve of viscosities versus shear rate of 1% U-1 polysaccharide solution at different temperature. The solution became less viscous as the temperature increased,

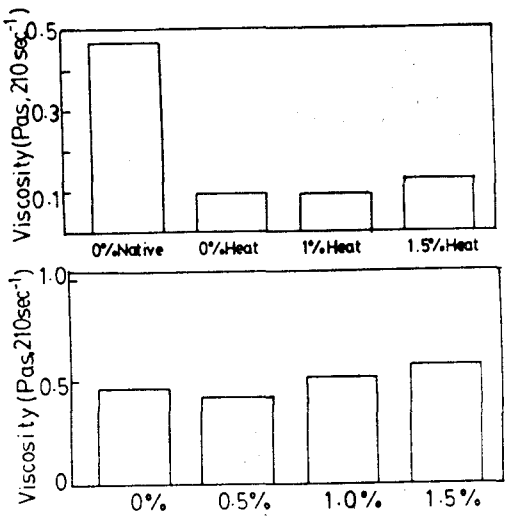

Fig. 5. Effect of NaCl on the viscosity of 10.75 % U-1 polysaccharide solution(Upper: Autoclaving at 121°C for 15 minutes and Lower: Native).

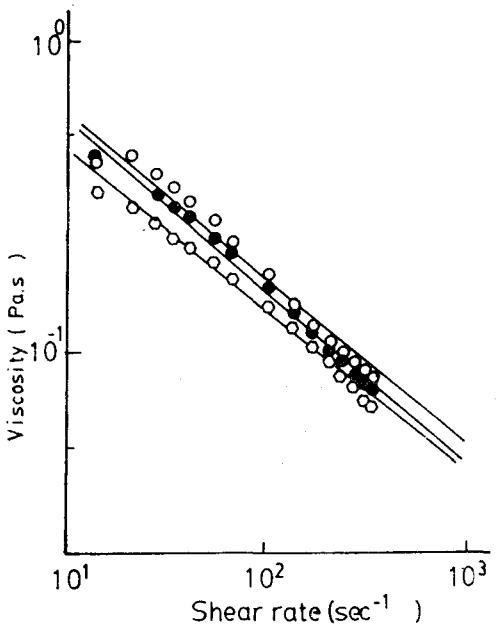

Fig. 6. Temperature dependence of 1% polysaccharide solution produced by *Enterobacter agglomerans* U-1(○ : 25°C, ● : 45°C and ◇: 75°C).

if not significant. The apparent viscosities of the solution at 42sec^{-1} were 307, 272 and 220 mPa.s at 25, 45 and 75°C. Fig. 7 shows the loss in pseudoplasticity of various concentration of U-1 polysaccharide solution. The sensitivity

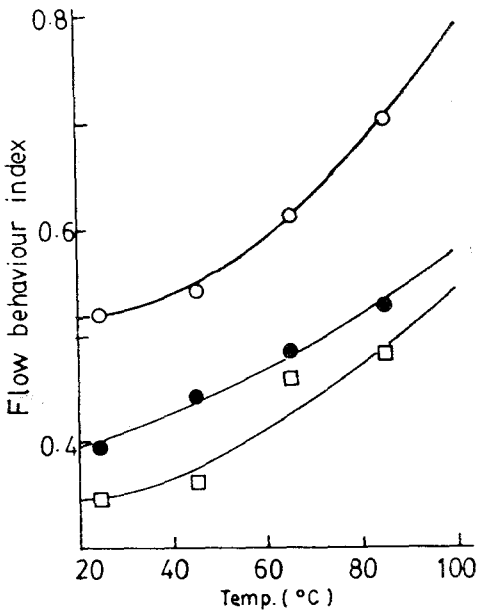

Fig. 7. Flow behaviour index of polysaccharide solution produced by *Enterobacter agglomerans* U-1(○ : 0.75%, ● : 1.25% and □ : 1.5%).

of flow behaviour index to temperature was higher when the concentration was lower. When increasing the temperature from 25°C to 85°C, the flow behaviour index of 0.75% solution increased from 0.52 to 0.70, whereas that of 1.5% solution increased from 0.35 to 0.48. Arrehenius plotting of consistency coefficient of 0.75% solution is shown in Fig. 8. The temperature dependence of consistency coefficients was sensitive at the lower concentration. The activation energy of flow of 0.75% solution were 4.505kcal/mole.

6. Analysis of U-1 polysaccharide

Sugar components of U-1 polysaccharide was analyzed by HPLC. The acid hydrolyzate of the polysaccharide was composed of glucose(1.0 mole) and galactose (1.1mole) (Fig. 9). IR spectrometric analysis implies that the polysaccharide is a glucan polymer containing betaglycosidic bond and O-acetyl group in the structure (Fig. 10).

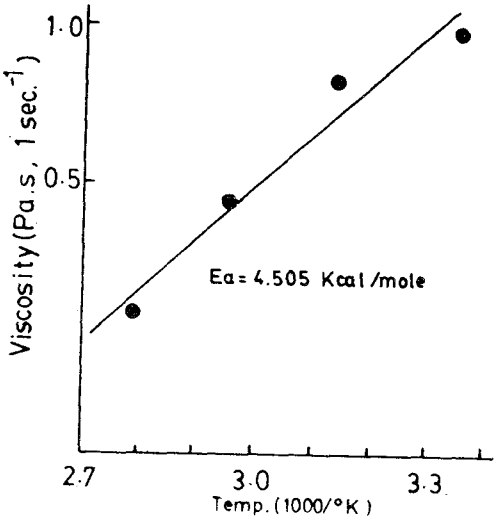

Fig. 8. Arrehenius plot of viscosity of 0.75% polysaccharide solution produced by *Enterobacter agglomerans* U-1.

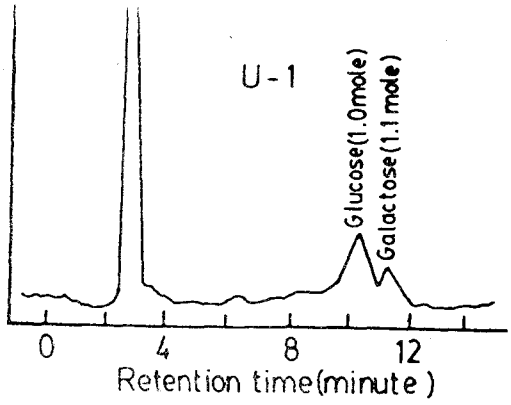

Fig. 9. High performance liquid chromatogram of U-1 polysaccharide hydrolyzate.

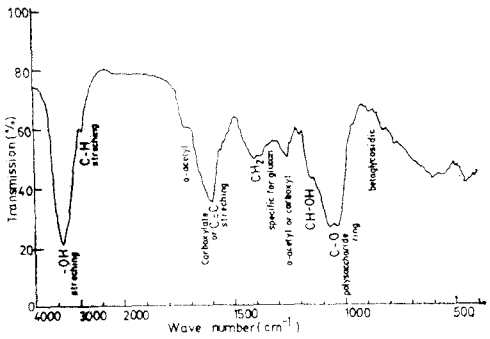

Fig. 10. IR spectrum of polysaccharide by *Enterobacter agglomerans* U-1.

Abstract

A bacterium synthesizing extracellular polysaccharide was isolated from soil and identified as *Enterobacter agglomerans*. The polysaccharide was found to be glucan polymer containing glucose and galactose in a molar ratio of 1 : 1.1. The aqueous solution was very viscous. The viscosity of 1% solution was 264 mPa.s. at 42 sec^{-1} and yield stress was 4.89 Pa. The polysaccharide solution did not have thermal stability but pH and salt stability.

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