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A Yellow Pigmented Soil Bacterium Producing a Polysaccharide of High Viscosity

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고점도 다당류생산 미생물의 분리 및 특성

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Abstract

A strictly aerobic bacterium forming yellow pigment and a highly viscous polysaccharide was isolated. The bacterium was identified as *Pseudomonas mendocia*. The polysaccharide was presumed to be β-glucan with o-acetyl group in its structure and the constituent sugar components were glucose and rhamnose in the molar ratio of 2.2:1.0. The intrinsic viscosity was 64.73 dl/g. The apparent viscosity of 1% aqueous solution was 428 mPa.s. at 42 $sec.^{-1}$ and the yield stress of the solution was 8.89 Pa. The polysaccharide did not have thermal stability but show pH and salt stability.

Key words: *Pseudomonas* sp., polysaccharide, analysis, rheology

Introduction

Efforts have been devoted to searching a new polysaccharide with novel properties[1-4]. Especially, microbial polysaccharides were the subject of research because of their advantages in production[5] and their novel rheological properties[6] superior to that of traditional ones. Many microbial polysaccharides were highly evaluated due to their gelling properties or the high viscosity. However only several polysaccharides including dextran[7], xanthan[8] and gellan gum[9] were commercialized[1,10-12]. Nevertheless, polysaccharides with new composition and useful properties have been continuously discovered and this fact implies the possibility to findnew polysaccharide of commercial potentiality from different habitat.

In this paper, we reports on the properties of a soil strain that produces extracellular polysaccharide.

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Materials and Methods

Isolation, Culture of the Bacterium and Recovery of Polysaccharide

The bacterium used in this study was isolated on Plate count agar (Difco) at 30°C from soil collected from Uijeongbu area, Kyonggido. The bacterium was grown for 3 days in the medium containing glucose (25 g/*l*), peptone (2.0g/*l*), $MgSO_4 \cdot 7H_2O$(1.0g/*l*), KH_2PO_4(1.0g/*l*), yeast extract (0.5 g/*l*) and $CaCO_3$(2.5 g/*l*) in jar fermentor (NBS, U.S.A., working volume: 1.2*l*, agitation; 400 rpm, aeration: 1 vvm). The polysaccharide was harvested from 3 days old colture broth by isopropanol precipitation as described by Yoo *et al.*[13]

Analysis

Sugar analysis was as previously reported[13]. The viscometry was conducted by Capillary viscometer[15] and Brabender Viscotron (Model 80241, West Germany). IR spectrum of KBr-Polymer pellet was obtained with Shimatsu Model IR 435 Spectrometer (Japan). pH was measured with

Orion Model 520 pH meter.

Identification

The bacterium was identified according to the Bergey's Manual of Systematic Bacteriology[15] and transmission electron photomicrograph was obtained by Hitachi Model-700 TEM (Japan).

Results and Discussion

Properties of bacterium

The strain 5704 was identified as *Pseudomonas mendocina* by the colonial, morphological and biochemical characteristics as in Fig. 1 and Table 1. The strain 5704 formed circular, entire, pulvinate, filliform and yellow colony on nutrient agar and it formed mucoid, irregular, entire, convex and echinulate colony on YM agar.

Fig. 1. Transmission electron photomicrograph of 5704 isolate. (Bar: 0.5 μm)

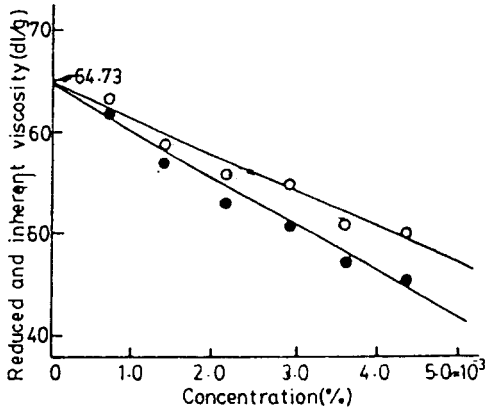

Fig. 2. Reduced and inherent viscosity of polysaccharide produced by *Pseudomonas mendocina* 5704. (○: Reduced viscosity, ●: Inherent viscosity)

Rheology of 5704 polyssaccharide

Fig. 2 shows the concentration dependency of specific and reduced viscosity. The intrinsic viscosity of 5704 polysaccharide derived from the figure was 64.73 dl/g. It is the value lower than that of xanthan gum but higher than that of guar gum or locust bean gum[16,17]. The intrinsic viscosity of fucogalactan sythesized by *Rhodotorula glutinis* was reported as 3.2 dl/g[18]. The relative viscosity of 5704 polysaccharide at 0.003% concen-

Table 1. Morphological and biochemical characteristics of strain 5704

Characteristics	Record	Characteristics	Record
Gram staining	−	DNase	−
Cell size	1.04-1.18× 3.58-4.2 μm	LDC	−
		ODC	
Motility	+	Argininedi-hydrolase	+
Flagella	lateral	Acid and gas from	
Colony	yellow	Glucose	+
Growth	Strictly aerobic	Fructose	+
Metabolism	Oxidative	Galactose	+
Catalase	+	Xylose	+
Oxidase	+	Mannose	−
PHB	Not formed	Rhamnose	+
Levan	Not formed	Mannitol	−
Growth factor	Not required	Ribose	−
Growth at 41°C at 12% NaCl	+ −	Arabinose	+
		Adonitol	+
Fluorescent Pigment	Not formed	Inositol	+
		Malonate	+
Hydrolysis of		Lactose	−
Starch	−	Sucrose	+
Gelatin	−	Trehalose	−
Urease	−	Raffinose	−
Indole	−	Utillization of	
Methylred	−	Alanine	+
Voges-Proskauer	+	Dulcitol	−
Nitrate reduction	−	Salicin	−

LDC: lysinedecarboxylase, ODC: ornithine decarboxylase

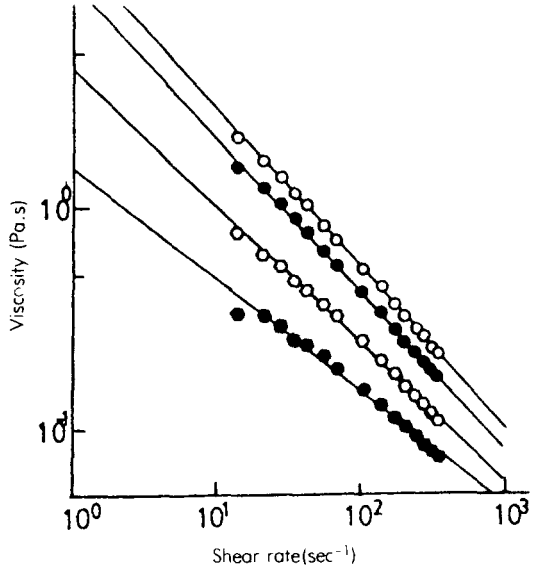

Fig. 3. Concentration dependency of viscosity of polysaccharide produced by *Pseudomonas mendocina* 5704. (●: 0.75%, ○: 1.0% ●: 1.25% ○: 1.5%)

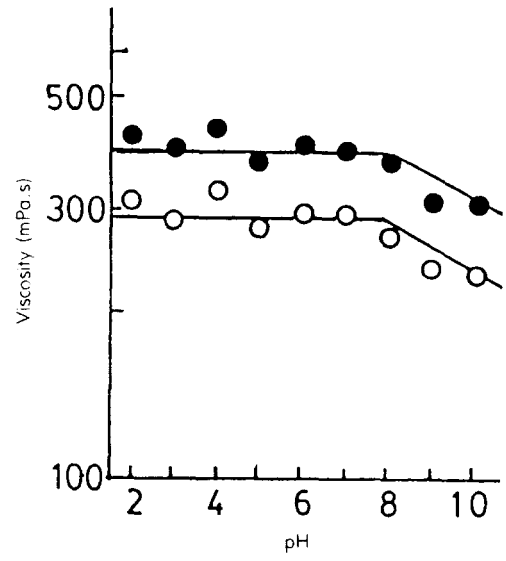

Fig. 4. Effect of pH on the visocity of 1% 5704 polysaccharide solution., ●: 42 sec^{-1}, ○: 70 sec^{-1}

tration was 1.16.

Fig. 3. shows the apparent viscosity vs. shear rate and concentration. The viscosity of 5704 polysaccharide was very high, showed shear-thinning property and was comparable with xanthan gum. The viscosity of the polysaccharide solution was 428 mPa.s at 42 sec.$^{-1}$. The flow behaviour index of 1% 5704 polysaccharide solution was 0.38. The yield point value for 5704 polysaccharide solution was 8.89 Pa. The polymer solution showed a steady rise in viscosity with the increase in concentration. The viscosities of 0.75, 1.0, 1.25 and 1.5% at 42 sec.$^{-1}$ were 248, 428, 801 and 1052 mPa.s. The flow behaviour indices showing pseudoplasticity were 0.47, 0.38, 0.30 and 0.29 at the respective concentration and the consistency coefficients were 1.75, 4.22, 10.70 and 14.83 Pa.s. The concentration dependency of 5704 polysaccharide solution was found so high and increased slightly with the rise of temperature. When fitting the concentration vs. viscosity by double logarithmic plotting[19], the a-values at 25, 45, 65 and 85 °C were 3.14, 3.39, 3.47 and 3.45 and K_1 values were 4.44, 2.90, 1.72 and 0.72 Pa.s.

pH dependency of polysaccharide solution

Fig. 4 shows the change in viscosity of 1% 5704 polysaccharide solution with pH change of aqueous solution. The viscosity profile versus pH revealed that it was stable at the pH between 2-8 and then decreased as the pH became more alkaline. The viscosities remained near 400 mPa.s at 42 sec.$^{-1}$. Bodie *et al.* reported that polysaccharide produced by *Arthrobacter* sp. was stable between 4-10 and below pH 4, the viscosity decreased[20]. A polysaccharide produced by *Alcaligenes* sp. was reported to show gelling property at extremly low pH[21].

Effect of NaCl on the viscosity of polysaccharide solution

Fig. 5 shows the viscosity profile of 1% 5704 polysaccharide solution at various shear rate as affected by NaCl concentration. There was only slight increase in viscosity over wide range of shear rate even when 1% NaCl was added to the polysaccharide solution. The viscosity of the solution containing 0, 0.4, 0.8 and 1.0% NaCl were 439, 428, 409 and 498 mPa.s at 42 sec.$^{-1}$. The effect of NaCl concentration on the thermal stability is

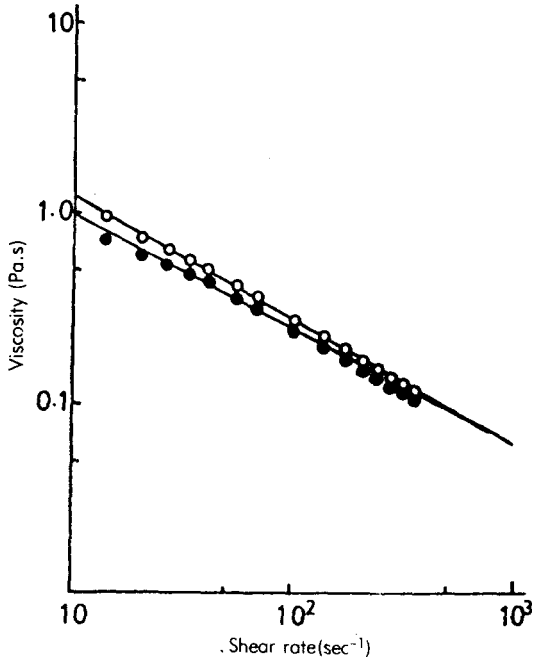

Fig. 5. Effect of NaCl on viscosity of polysaccharide solution produced by *Pseudomonas mendocina* 5704. (○: 1% NaCl, ●: Native, 1% solution)

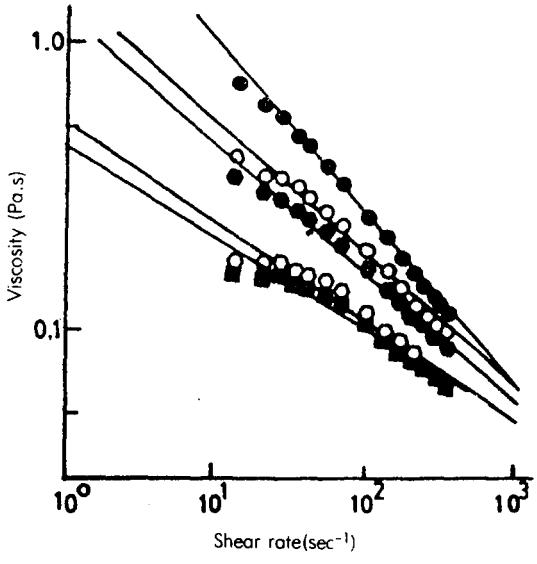

Fig. 6. Effect of NaCl on thermal reduction of viscosity of polysaccharide solution produced by *Pseudomonas mendocina* 5704. (121°C, 15 min. ○: 0%, ■: 0.4%, ●: 0.8%, ○: 1.%, ●: Control)

shown in Fig. 6. The solution containing 0.4% NaCl showed similar viscosity loss to that of the solution without NaCl, whereas the effect on thermal stability could be recognized when the NaCl concentration increased up to 1.0% when autoclaving, therefore 66% of original viscosity was remained comparing 35% loss for the solution without NaCl. The viscosity was reduced from 438 mPa.s to 151, 140, 217 and 289 mPa.s after autoclaving when 0, 0.4, 0.8 and 1.0% NaCl was added. The addition of salt in polysaccharide solution affects the property and thus increases[20-22] or somtimes yields a gel system[23] even though they are dependent on the kinds of salt.

Temperature dependency of polysaccharide solution

Fig. 7 shows the temperature dependency of viscosity of 1% 5704 polysaccharide solution at various shear rate. The viscosity change caused

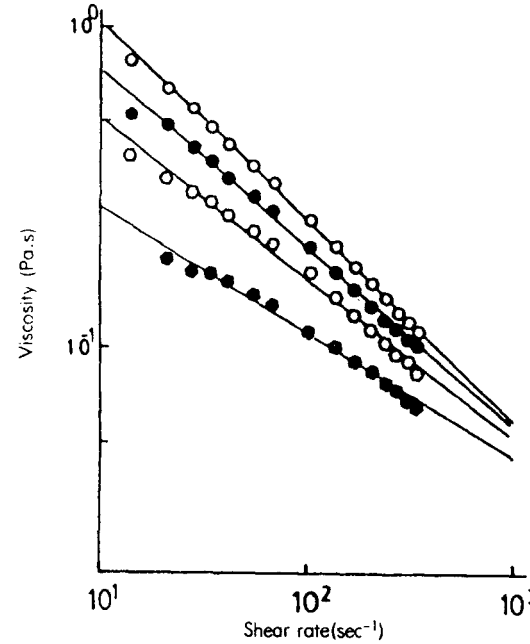

Fig. 7. Temperature dependence of 1% polysaccharide solution produced by *Pseudomonas mendocina* 5704. (○: 25°C, ●: 45°C, ○: 65°C, ●: 85°C)

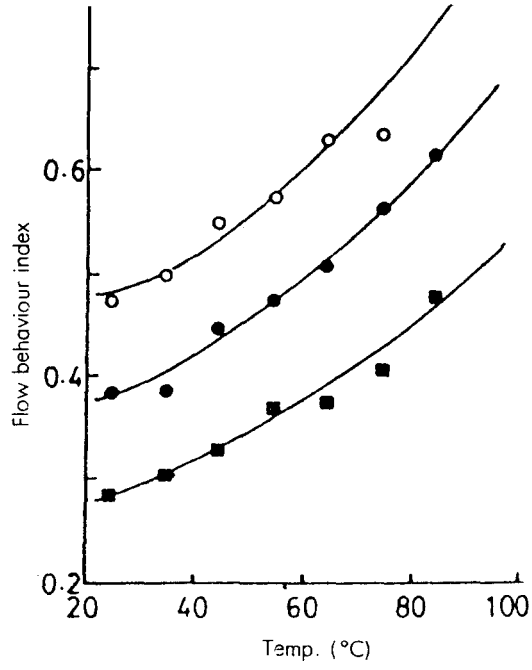

Fig. 8. Flow behaviour index of polysaccharide solution produced by *Pseudomonas mendocina* 5704.(■: 1.5%, ●: 1.0%, ○: 0.75%)

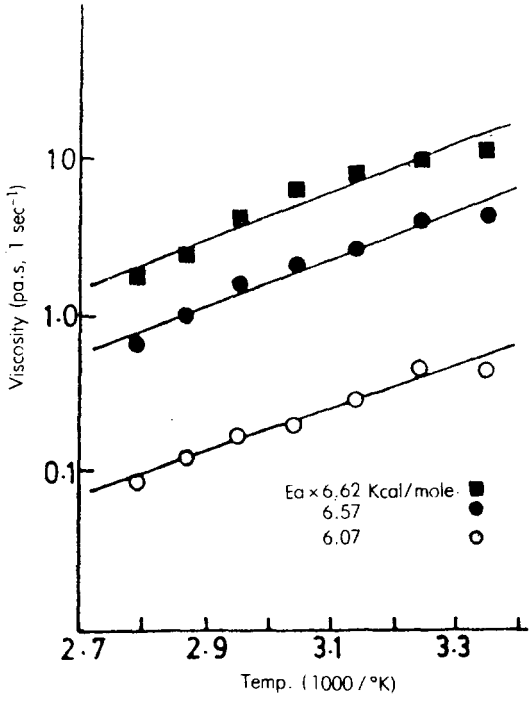

Fig. 9. Arrehenius plot of viscosity of 5704 polysaccharide solution.(■: 1.25%, ●: 1.0%, ○: 0.5%)

by temperature rise was relatively big. The apparent viscosities at 42 $sec.^{-1}$ of the solution were 428, 335, 261 and 158 mPa.s at 25, 45, 65 and 85 °C. The viscosity of most gums except xanthan and PS-7[24] decreased with the rise in temperature of system. Fig. 8 shows the change in pseudoplasticity at the different temperatures. The flow behaviours indices of 1.5 and 1.0% solutions were increased from 0.29 and 0.38 to 0.48 and 0.61 when temperature increased from 25 °C to 85 °C. The Arrhenius plotting of consistency coefficients is shown in Fig. 9. The activation energy of flow of 1.25, 1.0 and 0.5% were 6.62, 6.57 and 6.07 Kcal/mole, respectively, indicating the increased sensitivity at higher concentration.

Composition of polysaccharide

The sugar component of polysaccharide was analyzed by HPLC. The polysaccharide contains glucose (2.2) and rhamnose (1.0) (Fig. 10). This is similar to that of PS-7, a polymer of *Azotobacter* sp[25].

Fig. 11 shows IR spectrum of 5704 polysaccharide. The spectrum indicates the polymer to contain betaglycosidic bond and o-acetyl group[26].

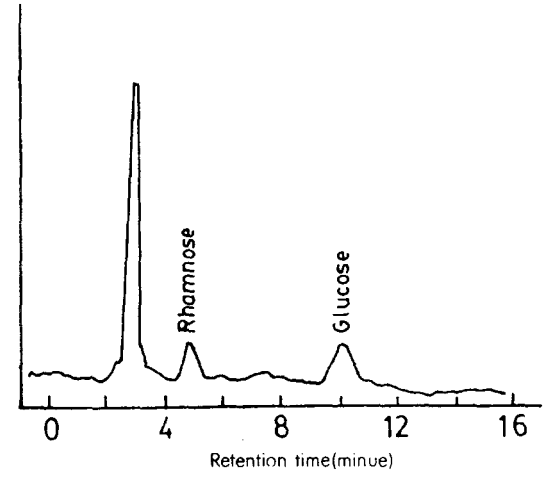

Fig. 10. High performance liquid chromatogram of polysaccharide hydrolyzate, produced by *Pseudomonas mendocina* 5704. (mobile phase: acetonitrile/water, 85/15)

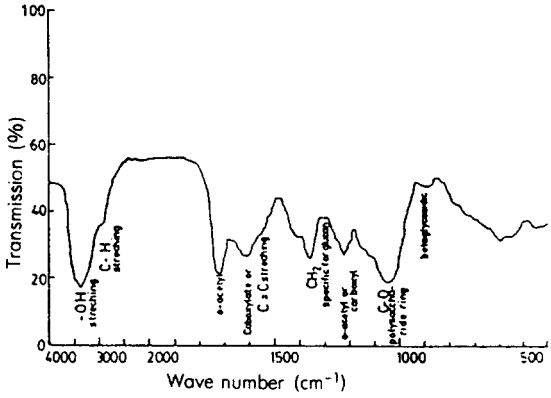

Fig. 11. IR spectrum of polysaccharide produced by *Pseudomonas mendocina* 5704.

요 약

산업용 다당류의 개발을 목적으로 고점도의 다당류생산 미생물을 토양으로부터 분리하였다. 본 세균은 동정한 결과 *Pseudomonas mendocina*로 생각되었다. 본 세균이 생산하는 다당류는 o-acetyl기를 함유한 β-glucan으로 추정되며 구성당으로서는 포도당과 람노오스가 몰비로 2.2 : 1.0의 구성으로 되어 있고 수용액은 의가소성 유체이다. 1% 용액은 428 mPa.s의 점도를 나타내며 항복치는 8.89 Pa이다. 이 용액은 열안정성은 없으며 pH 안정성과 염안정성은 있었다. 유동활성화에너지는 6.57 Kcal이다.

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