KOREAN J. FOOD SCI. TECHNOL.
Vol. 21, No. 5, pp. 619~623 (1989)

# Cultural Condition of *Pseudomonas mendocina* for Polysaccharide Production

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**Abstract**

The cultural condition of *Pseudomonas mendocina* for polysaccharide production was examined. The optimal medium contains following composition per liter of distilled water: Sucrose 23.75g, $(NH_4)_2SO_4$ 1.57g, Yeast extract 0.5g, $KH_2PO_4$ 2.0g, $MgSO_4 \cdot 7H_2O$ 1.0g, $CaCO_3$ 2.5g. The optimum temperature and pH were 30 °C and 6.5. At the condition, *Ps mendocina* produced 5.98 g/*l* of polysaccharide. The culture viscosity after 3 days was 191 mPa.s at 70 $sec^{-1}$. The product yield ($Y_{p/s}$) and specific productivity ($Q_p$) were 25.18% and 32.83 mg/g-cell/h.

Key word: *Pseudomonas mendocina*, polysaccharide, cultural condition

## Introduction

Polysaccharides are now extensively used in food and other industries[1]. Even though their production has been originated mainly from plants and animals[2], microorganisms are drawn attention as another sources of great potentiality[3-7]. When selecting a microorganism for polysaccharide production, the properties of the culture broth or the polysaccharides are normally examined from the standpoint of gelling, viscosifying, emulsifying etc. depending upon their end use[1,8].

In a series of screening program searching a novel polysaccharide, a baterium was found to have the property rendering the culture broth highly viscous[9]. The bacterium was identified as *Pseudomonas mendocina* 5704 and the rheological properties was also reported previously[9]. This paper reports the cultural condition of the bacterium for polysaccharide production.

## Materials and Methods

### Microorganism and Cultivation

*Pseudomans mendocina* 5704 isolated from soil was used in this study. The bacterium was grown in YM broth(Difco) and used as starter culture. The inoculum was 5%. The fermentation medium contains glucose (25 g/*l*), peptone(2.0 g/*l*), $MgSO_4 \cdot 7H_2$(1g/*l*), $KH_2PO_4$(1 g/*l*), Yeast extract (0.5 g/*l*), $CaCO_3$ (2.5 g/*l*). Culture was performed in 250 m*l* erlenmeyer flask (working volume: 50 m*l*) on rotary shaker (120 rpm) or Bioflo C-32 fermentor (NBS, U.S.A. Working volume: 1,200 m*l*, agitation: 400 rpm, aeration: 1 vvm).

### Analysis

Analytical method was as previously described[9,10]. Viscosity of culture broth was determined by Brabender viscotron (Model 80241, System E-17, West Germany)

## Results and Discusstion

### Cultivation temperature

Table 1 shows the effect of temperature on the polysaccharide production by *Ps. mendocina* 5704 in flask culture. The cell growth at 40 °C was negligible. The optimum temperature for the cell growth and polysaccharide production was 30 °C. At the temperature, 18.16% of glucose added was converted to polysaccharide. This is similar to the temperature reported for dextran production[11]. Yamaguchi and saito[12] also reported that the temperature for *Alcaligenes faecalis* was 30 °C.

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Table 1. Effect of temperature on the polysaccharide production by *Ps. mendocina* 5704 after 72 hours of incubation

| Temperature [°C] | DCW [g/L] | P [g/L] | Yield [%] | $Q_p$ [mg/g/h] | $\eta_{app}$ [mPa.s] | pH |
|---|---|---|---|---|---|---|
| 25 | 1.30 | 4.00 | 16.00 | 42.74 | 78 | 4.2 |
| 30 | 1.65 | 4.54 | 18.16 | 38.22 | 313 | 5.6 |
| 35 | 1.35 | 3.70 | 14.80 | 38.07 | 234 | 7.0 |
| 40 | — | — | — | — | — | — |

Viscosity was measured at 70 $sec^{-1}$. Medium: Glucose 25 g/L, Peptone 2.06 g/L $KH_2PO_4$ 1 g/L, $MgSO_4 \cdot 7H_2O$ 1 g/L, Yeast extract 0.5 g/L, $CaCO_3$ 2.5 g/L
Initial pH 6.5, DCW: dry cell weight, $Q_p$: specific productivity. $\eta_{app}$: culture viscosity, P: crude polysaccharide

The specific productivity ($Q_p$) was not affected by varying the cultivation temperature. The culture broth grown at lower temperature was thinner than those at higher temperature. The apparent viscosities of culture broths at 30 and 35°C were 313 and 234 mPa.s. at 70 $sec.^{-1}$. The changes in pH of culture broth during fermentation at 25°C was recognized.

### Effect of C/N ratio

Table 2 shows the effect of C/N ratio on the polysaccharide production from glucose by *Ps. mendocina* 5704 in flask culture. The optimum C/N ratio for the polysaccharide production was 30. The polysaccharide production did not increase by using the C/N ratio higher than 30, below which drastic decrease was observed. The polysaccharide production at the optimum C/N ratio was 4.43 *g/l*. Williams and Wimpenny(13) reported that reduction of nitrogen level favored the polysaccharide production. Corpe(14) recommended C/N ratio of 10 for *Chromobacterium violaceum*.

Table 2. Effect of C/N ratio on the polysaccharide production by *Ps. mendocina* 5704 at 30°C after 72 hours of incubation

| C/N ratio | 10 | 30 | 50 | 100 | 200 |
|---|---|---|---|---|---|
| P[g/L] | 1.42 | 4.43 | 3.15 | 3.44 | 3.71 |
| Yield[%] | 5.68 | 17.72 | 12.60 | 13.76 | 14.84 |
| pH | 5.3 | 5.4 | 6.0 | 5.6 | 5.8 |

Table 3. Effect of sugar sources on the polysaccharide production by *Ps. mendocina* 5704 at 30°C after 72 hours of incubation

| Sugar source | DCW [g/L] | P [g/L] | Yield [%] | $Q_p$ [mg/g/h] | $\eta_{app}$ [mPa.s] | pH |
|---|---|---|---|---|---|---|
| Glucose | 1.65 | 4.54 | 18.16 | 38.22 | 273 | 6.0 |
| Lactose | 0.54 | — | — | — | 28 | 8.1 |
| Maltose | 2.51 | 5.16 | 21.73 | 28.55 | 145 | 5.9 |
| Sucrose | 2.31 | 5.50 | 23.16 | 33.07 | 313 | 5.8 |
| Fructose | 2.47 | 1.96 | 7.84 | 11.02 | 45 | 6.9 |
| Xylose | 2.74 | 5.46 | 21.84 | 27.68 | 250 | 5.9 |

Sugar source: Glucose, Fructose, Xylose 25 g/L, Sucrose, Lactose, Maltose 23.75 g/L.

### Effect of carbon source

Table 3 shows the effect of carbon sources on the polysaccharide production by *Ps. mendocina* 5704 in flask culture. *Ps. mendocina* 5704 could utilize all the carbon sources used except lactose. The dry cell weights (DCW) of culture broths when using maltose, sucrose, fructose and xylose were 2.51, 2.31, 2.47 and 2.74 g/L. Copious amount of polysaccharide was formed from all the sugars except lactose and fructose. Optimum carbon source for polysaccharide production was sucrose. At the condition, 5.50 g/L of polysaccharide was produced. The product yield ($Y_{p/s}$) and $Q_p$ were 23.16% and 33.07 mg/g/h. Ueda *et al.*(15) found that sucrose was good for *Aeromonas hydrophila*.

It was also notable that similar amount of polysaccharide was produced from xylose since xylose was known to be easily available and cheap carbon source(16,17). The culture broths from glucose, maltose, sucrose and xylose were very thick. The viscosities of culture broths from the respective carbon source were 273, 145 and 313 and 250 at 70 $sec^{-1}$. The pH of culture broth dropped slightly in the medium containing glucose, maltose, sucrose and xylose.

### Effect of sucrose concentration

Table 4 shows the effect of sucrose concentra-

Table 4. Effect of sucrose concentration on the polysaccharide production by *Ps. mendocina* 5704 after 72 hours of incubation at 30°C

| Concentration [g/L] | DCW [g/L] | P [g/L] | Yield [%] | $Q_p$ [mg/g/h] | $\eta_{app}$ [mPa.s] |
|---|---|---|---|---|---|
| 10 | 1.59 | 1.48 | 14.80 | 12.97 | 56 |
| 15 | 2.37 | 3.14 | 20.93 | 18.42 | 66 |
| 20 | 3.14 | 4.74 | 23.70 | 20.95 | 114 |
| 23.75 | 3.36 | 5.44 | 22.91 | 22.47 | 227 |
| 30 | 3.02 | 5.50 | 18.33 | 25.29 | 20 |
| 40 | 2.87 | 3.18 | 7.95 | 15.39 | 45 |

Nitrogen source: $(NH_4)_2SO_4$, C/N ratio: 30.

tion on the polysaccharide production in flask culture. The DCW increased with the increase of the initial sucrose concentration to the level of 23.75 g/L. The increased polysaccharide production could be achieved until the concentration increased to the level of 23.75-30 g/L. However, the maximum $Y_{p/s}$ was achieved at 30 g/L of sucrose concentration. The $Q_p$ also increased as the concentration increased to the level of 30 g/L. The culture broth was viscous when grown in the medium containing 20-23.75 g/L of sucrose. Bender *et al.*[18] reported that product concentration could be enhanced by increasing the level of substrate but the efficiency was deteriorated for *Pullraria pullulans*.

**Effect of nitrogen source**

Table 5 shows the polysaccharide production from sucrose by *Ps. mendocina* 5704 when grown in the medium containing different nitrogen sources in flask culture. The optimum nitrogen source for polysaccharide production was ammonium sulfate. The amount of polysaccharide formed and $Y_{p/s}$ were 5.71 g/*l* and 24.06%. The other nitrogen sources were found not to be proper. *Bacillus polymyxa*[17] produced polysaccharide only when organic nitrogen such as peptone was used. However, Yamaguchi and Saito[12] recommended $NaNO_3$ for *Alcaligenes faecalis*. The culture broths were also very viscous except that containing urea as nitrogen source. Significant change in pH of culture broth was not recognized.

Table 5. Effect of nitrogen source on the polysaccharide production by *Ps. mendocina* 5704 at 30°C after 72 hours of incubation

| Nitrogen source | P [g/*l*] | Yield [%] | $\eta_{app}$ [mPa.s] | pH |
|---|---|---|---|---|
| $NH_4Cl$ | 2.64 | 11.11 | 128 | 5.7 |
| Urea | 3.27 | 13.77 | 7 | 6.3 |
| $(NH_4)_2SO_4$ | 5.71 | 24.06 | 112 | 6.2 |
| $(NH_4)_2HPO_4$ | 2.38 | 10.02 | 134 | 6.3 |
| $NH_4NO_3$ | 2.59 | 10.91 | 106 | 5.9 |
| Peptone | 3.71 | 15.63 | 162 | 6.6 |

Table 6. Effect of $KH_2PO_4$ concentration on the polysaccharide production by *Ps. mendocina* 5704 at 30°C after 72 hours of incubation

| Concentration [g/*l*] | DCW [g/*l*] | P [g/*l*] | Yield [%] | $Q_p$ [mg/g/h] | $\eta_{app}$ [mPa.s] |
|---|---|---|---|---|---|
| 1 | 3.09 | 5.59 | 23.54 | 25.13 | 227 |
| 2 | 2.53 | 5.98 | 25.18 | 32.83 | 191 |
| 3 | 1.54 | 3.06 | 12.88 | 27.60 | 15 |
| 4 | 0.84 | 1.65 | 6.95 | 27.17 | 16 |
| 5 | — | — | — | — | 3 |

**Effect of inorganic salt**

Inorganic salts such as $KH_2PO_4$ and $MgSO_4$ are known to affect the polysaccharide production[20,21].

Table 6 shows the effect of $KH_2PO_4$ concentration on the polysaccharide production from sucrose in flask culture. The DCW decreased with increase of $KH_2PO_4$ concentration. The decrease in polysaccharide production and viscosity of culture broth was noticeable when more than 2 g/L of $KH_2PO_4$ was used. The optimum concentration was 2 g/L. At the condition, 5.98 g/L of polysaccharide was produced. The $Y_{p/s}$ and $Q_p$ at the concentration were 25.18% and 32.83 mg/g/h.

Table 7 shows the effect of $MgSO_4$ concentration on the polysaccharide production from sucrose flask culture. Cell growth was promoted by increasing the $MgSO_4$ concentration, whereas the polysaccharide production was reduced. The DCW and polysaccharide production were 4.59 and 3.33 g/L when 5 g/L of $MgSO_4$ was added.

Table 7. Effect of $MgSO_4$ concentration on the polysaccharide production by *Ps. mendocina* 5704 at 30°C after 72 hours of incubation

| Concentration [g/l] | DCW [g/l] | P [g/l] | Yield [%] | $Q_p$ [mg/g/h] | $\eta_{app}$ [mPa.s] | pH |
|---|---|---|---|---|---|---|
| 1 | 3.32 | 5.01 | 21.10 | 20.96 | 198 | 5.9 |
| 2 | 3.97 | 3.59 | 15.12 | 12.56 | 191 | 5.9 |
| 3 | 4.12 | 3.48 | 14.65 | 11.73 | 147 | 5.5 |
| 4 | 4.29 | 3.11 | 13.10 | 10.06 | 138 | 5.4 |
| 5 | 4.59 | 3.33 | 14.02 | 10.07 | 126 | 5.3 |

Table 8. Time course of polysaccharide production by *Pseudomonas mendocina* 5704 at 30°C in jar fermentor

| Elapsed fermentation time [hrs] | Polymer [g/l] | Glucose [g/l] | K | $\eta$ [mPa.s] |
|---|---|---|---|---|
| 16 | 3.5 | 17.9 | 143 | 0.68 |
| 22 | 3.6 | 14.4 | 448 | 0.58 |
| 40 | 4.4 | 9.6 | 922 | 0.53 |
| 72 | 4.4 | 3.7 | 1023 | 0.54 |
| 86 | 4.4 | 2.5 | 824 | 0.58 |

Agitation: 400 rpm, Aeration: 1 vvm, Working volume: 1.2 *l*, Medium: see Table 1, K: consistency coefficient, n: flow behaviour index, pH.

The $Y_{p/s}$ and $Q_p$ at the concentration were 14.02% and 10.07 mg/g-cell/h, respectively. The apparent viscosity of culture broth was reduced as the $MgSO_4$ concentration in culture medium increased, showing 126 mPa.s. at 70 $sec^{-1}$ when using 5 g/L of $MgSO_4$. It was, therefore, not necessary to add more than 1 g/L of $MgSO_4$ for polysaccharide production.

#### Fermentation profile of polysaccharide

Table 8 shows the fermentation pattern of *Ps. mendocina* 5704 polysaccharide production in jar fermentor. Most of glucose was utilized after 72 hours of fermentation. Polysaccharide synthesis ceased at 40 hours of elapsed fermentation time and maintained 4.4 g/L level. Higher consistency index was observed between 40-72 hours and then decreased. The culture broth behaved like pseudoplastic fluid after 16 hours of fermentation.

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(Received Apr. 12, 1989)

## *Pseudomonas mendocina* 에 의한 Polysaccharide 생산

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토양으로부터 분리 동정된 *Pseudomonas mendocina* 의 다당류 생산조건을 검토하였다. 최적 배지조성은 Sucrose 23.75 g/L, $(NH_4)_2SO_4$ 1.57 g/L, Yeast extract 0.5 g/L, $KH_2PO_4$ 2.0 g/L, $MgSO_4$. $7H_2O$ 1.0 g/L, $CaCO_3$ 2.5 g/L 이었으며, 최적 pH 및 배양온도는 6.5 및 30℃이었다. 이 조건에서 다당류의 생산량은 5.98 g/L 이며 대당 수율은 25.18%, 비생산속도는 32.83 mg/g-cell/h 이었다.