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# Bacterial Alginate Produced by a Mutant of *Azotobacter vinelandii*

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**The optimum conditions in shaken flasks for production of bacterial alginate by mutant C-14 of *Azotobacter vinelandii* NCIB 9068 and a comparison of the properties of bacterial and algal alginates were investigated. The largest amount of bacterial alginate was obtained in about 110 h by a culture grown on optimum medium at 34°C and 170 rpm shaking speed. The viscosity of the culture broth was 18,400 cps and the alginate concentration reached 6.22 g/liter. The viscosity of the purified bacterial alginate was as high as 11,200 cps at a low concentration (0.6%). A greater than fivefold concentration of algal alginate was required to reach the same viscosity at a low shear rate. A solution of bacterial alginate was more pseudoplastic than that of algal alginate was. No significant differences were observed in other properties of bacterial and algal alginates such as gel formation with calcium ion, thermostability, and effect of temperature, pH, and sodium chloride on viscosity.**

Alginates are important polysaccharides with many applications in the food, pharmaceutical, textiles, and paper industries (14). They are usually obtained from brown algae consisting of D-mannuronic and L-guluronic acids (6, 7). The products vary in quality and are short in supply. Thus, an effort has been made during the past 20 years to find alternative alginates from microorganisms. Since Linker and Jones (11) first reported that *Pseudomonas aeruginosa* produced a polysaccharide similar in composition to algal alginate, several strains of *Azotobacter vinelandii* and mutants capable of producing bacterial alginate have been found (2, 4, 6, 8, 10, 13). However, publications comparing properties of bacterial and algal alginates are not available yet.

Chen et al. reported the isolation of a mutant of *A. vinelandii* NCIB 9068 which produced a good quantity of bacterial alginate when grown on sucrose and minerals (1). In this study, we report the optimum conditions in shaken flasks for production of bacterial alginate. A comparison of properties of bacterial and algal alginates is also presented.

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## MATERIALS AND METHODS

**Microorganism.** *A. vinelandii* NCIB 9068 was improved by mutation with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and mutant C-14 was selected for this study (1).

**Media and growth.** Burk nitrogen-free medium fortified with 2% sucrose and 1.5% agar was used as the medium for slant cultures (15). Organisms from a slant culture were grown in a 500-ml Hinton flask, maintained at various temperatures on a rotary shaker at various shaking speeds, on 100 ml of optimum medium (1). The optimum medium contained 2% sucrose, 0.2% $CH_3COONa$, 0.12% $K_2HPO_4$, 0.03% $KH_2PO_4$, 0.015% $CaSO_4 \cdot 2H_2O$, 0.01% $MgSO_4 \cdot 7H_2O$, 1.5 mg of $FeSO_4 \cdot 7H_2O$ per 100 ml, and 0.075 mg of $Na_2MoO_4 \cdot 2H_2O$ per 100 ml. The pH of the medium was adjusted to 6.5.

**Analytical procedures.** The viscosity of the culture broth was measured with a Brookfield synchrolectic viscometer (model LVF, spindle no. 3; Brookfield Engineering Laboratories, Inc.) at 25°C and 6 rpm.

The cell concentration was measured as follows: 20 g of culture broth was diluted with 100 ml of distilled water and 6 ml of 20% EDTA disodium salt and then centrifuged at 15,000 × *g* for 30 min. The cell precipitate was washed with 120 ml of distilled water and then centrifuged. The cell precipitate obtained was finally dried to a constant weight at 105°C.

The alginate concentration was determined as follows: 20 g of culture broth and 1 ml of 20% sodium chloride were mixed well. The alginate and cells of the mixture were precipitated with 40 ml of 95% isopropanol. The collected precipitate was then dehydrated with a small amount of isopropanol. After centrifugation, the precipitate was dried to a constant weight at 105°C. The alginate weight was obtained by subtracting the dried cell weight from the dried precipitate weight mentioned above. The concentration of cells and alginate was expressed in grams per liter.

Residual sucrose in the culture broth was hydrolyzed with acid, and the resulting reducing sugars were measured according to the dinitrosalicylic acid method (12).

## RESULTS

**Optimum conditions for production of bacterial alginate.** Production of bacterial alginate by mutant C-14 of *A. vinelandii* NCIB 9068 was carried out in a flask on a rotary shaker at various shaking speeds (110, 140, 170, and 200 rpm). The effect of shaking speed on production of bacterial alginate in optimum medium (1) is shown in Fig. 1. The viscosity and alginate concentration of the culture broth were highest when the shaking speed was 170 rpm.

The effect of cultivation temperature (25, 28, 31, 34, and 37°C) on production of bacterial alginate was investigated. The results indicated that the highest viscosity and alginate concentration were obtained when mutant C-14 was cultivated at 34°C, which was also the optimum temperature for cell growth (data not shown).

To compare the bacterial alginate production of *A. vinelandii* NCIB 9068 and its mutants (C-14, B-5, B-9, and B-11), fermentations were conducted in optimum medium at 34°C and 170 rpm for 3 days. Of the various strains, mutant C-14 produced culture broth having highest viscosity and alginate concentration (Fig. 2).

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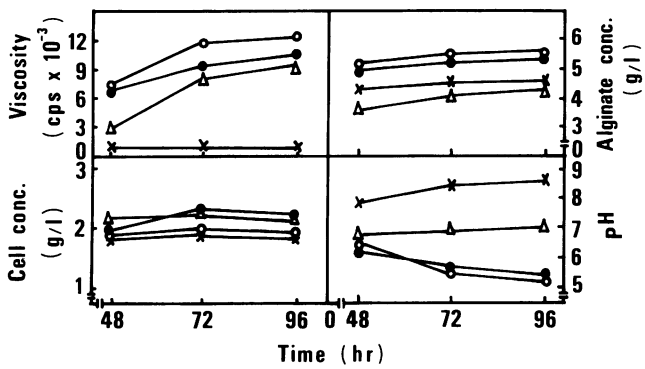


FIG. 1. Effect of shaking speed on production of bacterial alginate. Mutant C-14 was cultivated in optimum medium at 34°C. Symbols: △, 110 rpm; ●, 140 rpm; ○, 170 rpm; ×, 200 rpm.

Figure 3 shows the time course of bacterial alginate production by mutant C-14 under the optimum conditions described above. During the time course, the viscosity, alginate concentration, cell growth, pH, and residual sucrose of the culture broth were determined. Cell growth reached the stationary phase at around 48 h (Fig. 3). The pH of the culture broth rose sharply at an early stage of cultivation and then declined slowly. At the late logarithmic phase of cell growth, the viscosity and alginate concentration increased very rapidly and reached the maximum, which was 18,400 cps and 6.22 g/liter, respectively, at around 110 h. Residual sucrose dropped to 0.2% at the late stage of fermentation. The yield of bacterial alginate produced based on sucrose added was 31%.

**Comparison of properties of bacterial and algal alginates.** The purified bacterial alginate obtained from mutant C-14 was used for determination of chemical composition. The results indicated that it contained D-mannuronic acid, L-guluronic acid, and O-acetyl group (W.-P. Chen, manuscript in preparation). Crude bacterial alginate was obtained by precipitating the culture broth with isopropanol without removal of cells. The two commercial algal alginates I and II were purchased from Grinsted Products Co. and Wako Pure Chemical Industries, Ltd., respectively. The following results show comparison of the properties of purified and crude bacterial alginates as well as of the two algal alginates mentioned above.

The effect of alginate concentration on viscosity of alginate solutions is shown in Fig. 4. The viscosity of the purified

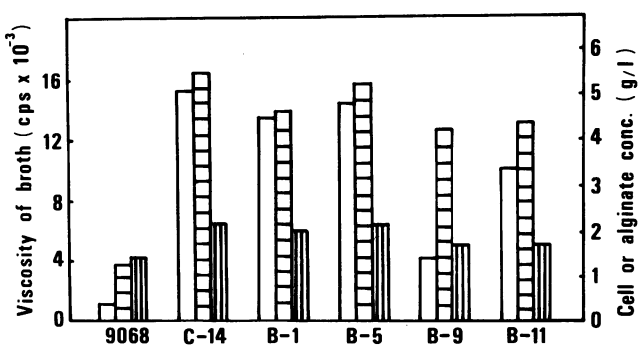


FIG. 2. Production of bacterial alginate by mutants of *A. vinelandii* NCIB 9068. The mutants were cultivated in optimum medium at 34°C and 170 rpm for 3 days. Symbols: □, viscosity of broth; ▤, alginate concentration; ▥, cell concentration.

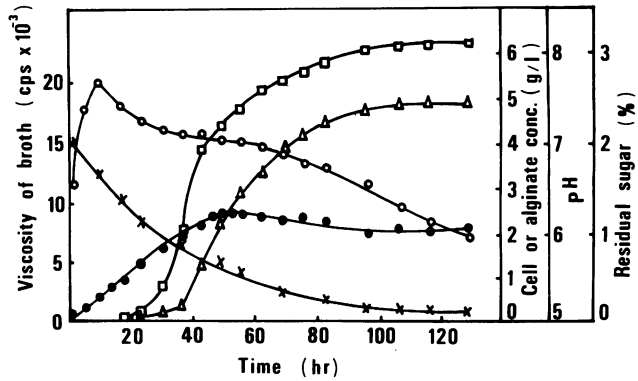


FIG. 3. Effect of fermentation time on production of bacterial alginate. Mutant C-14 was cultivated in optimum medium at 34°C and 170 rpm for different times. Symbols: △, viscosity of broth; □, alginate concentration; ○, pH; ●, cell concentration; ×, residual sugar.

bacterial alginate was as high as 11,200 cps at a low concentration (0.6%). A greater than fivefold concentration of algal alginates was required to reach the same viscosity at a low shear rate.

Figure 5 shows the viscosity of alginate solutions at various shear rates. The viscosity of bacterial alginates decreased as the shear rate increased. However, the viscosity of algal alginates was not affected by changing shear rate. The results indicated that the solution of bacterial alginate was more pseudoplastic than was that of algal alginates.

The viscosity of bacterial and algal alginate solutions was determined at different temperatures (30 to 90°C). The results showed that the viscosity of bacterial and algal alginates decreased gradually when the temperature rose. However, the bacterial alginate had a slightly higher viscosity than the algal alginate at 90°C (data not shown). The alginate solutions were heated at different temperatures for 30 min and then cooled to room temperature; the residual viscosity was determined. When the temperature was below 75°C, the effect of heat treatment on viscosity was not

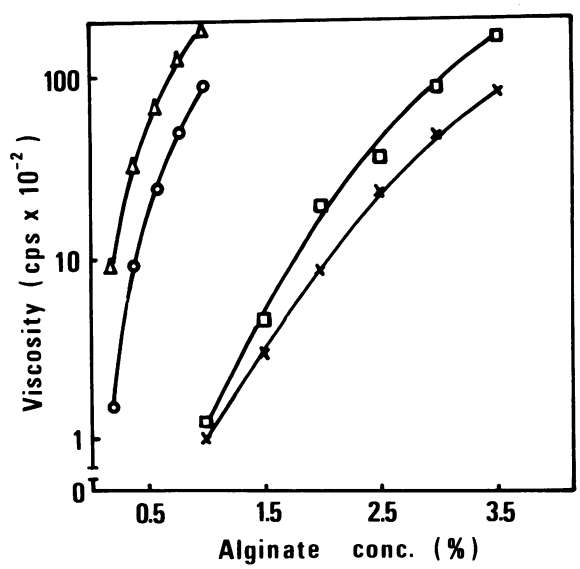


FIG. 4. Effect of alginate concentration on viscosity of bacterial and algal alginates. Bacterial alginate: △, pure; ○, crude. Algal alginate: ×, I; □, II.

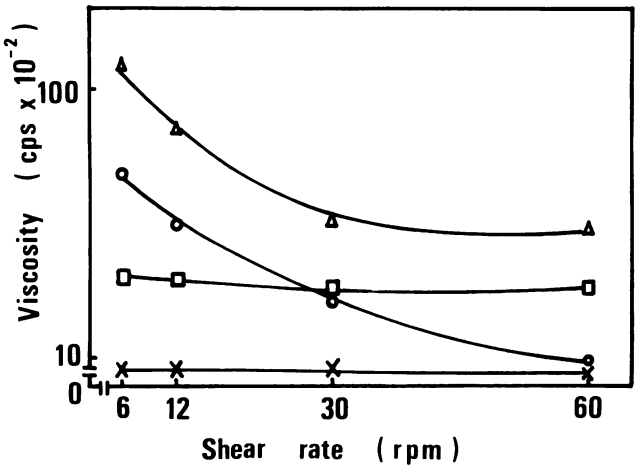


FIG. 5. Effect of shear rate on viscosity of bacterial and algal alginates. Bacterial alginate (0.6%): △, pure; ○, crude. Algal alginate (2%): ×, I; □, II.

significant for either bacterial or algal alginates. However, the residual viscosity dropped very sharply when the temperature was higher than 75°C. The viscosity was almost destroyed when the alginate was heated at 121°C for 30 min (data not shown).

The effects of pH (4 to 11) and sodium chloride (1 to 5%) on viscosity of bacterial and algal alginate solutions were investigated. The effects were not significant for any of the alginate solutions (data not shown). No significant differences between bacterial and algal alginates were observed in gel formation with calcium ion (data not shown).

## DISCUSSION

Mutant C-14 of *A. vinelandii* was cultivated in flasks on a rotary shaker at various speeds (110 to 200 rpm). Viscosity and alginate concentration of the culture broth were highest when the shaking speed was 170 rpm. A lower or higher shaking speed was unfavorable for bacterial alginate production. The result was similar to those reported by Horan et al. (9) and Deavin et al. (3). Horan et al. indicated that the amount of bacterial alginate produced was dependent on the dissolved oxygen tension (DOT) of the culture broth increasing to a plateau between 1 and 5% DOT and then decreasing at higher DOT values. Deavin et al. reported that an increase in DOT of culture broth resulted in an increase in respiration rate of microorganisms. Thus, the rate of bacterial alginate biosynthesis decreased at high DOT because most of the carbon source (sucrose) was burnt off as carbon dioxide. Oxygen limitation has proved to be disadvantageous for bacterial alginate formation. Under low DOT, abundant poly-β-hydroxybutyrate was accumulated in cells of *A. vinelandii* up to 32% of the dry cell weight (9). The results obtained in these studies could provide useful information for bacterial alginate production by fermenters.

A temperature of 30°C was usually used for the production of bacterial alginate by *A. vinelandii* (Table 1). In this study, however, 34°C was the optimum temperature. Moreover, viscosity and alginate concentration of culture broth obtained at 37°C were higher than those obtained at 25°C. The results indicated that a relatively high temperature was favorable for bacterial alginate production by mutant C-14.

A comparison of bacterial alginate production from this study and others is shown in Table 1. The bacterial alginate concentration in this study was higher than in others. However, it is impossible to compare the viscosity of the culture broth due to the absence of such data in other studies. When compared with our previous study (1), the viscosity and alginate concentration increased by 43 and 12%, respectively. The yield of bacterial alginate produced based on sucrose added was 31%, which is also higher than that (25%) reported by Deavin et al. (3).

With regard to the alginate properties, a greater than fivefold concentration of algal alginate was required to reach the same viscosity as bacterial alginate at a low shear rate. Thus, the cost of alginate to various industries could be

TABLE 1. Comparison of bacterial alginate production from results of this study and others

| Location | Strain used | Sucrose concn (g/liter) | Alginate concn in broth (g/liter) | Fermentation time (h) | pH | Temp (°C) | Reference |
|---|---|---|---|---|---|---|---|
| National Chung Hsing University (ROC)[a] | Mutant C-14 of *A. vinelandii* NCIB 9068 | 20 | 6.22 | 110 | 6.5 | 34 | |
| National Chung Hsing University (ROC) | Mutant C-14 of *A. vinelandii* NCIB 9068 | 20 | 5.54 | 84 | 6.5 | 28 | 1 |
| University of Hull (England) | Mutant SM52B of *A. vinelandii* NCIB 9068 | 20 | 5.00 | 90 | 7.2 | 30 | 8 |
| Tate & Lyle Ltd., England | *A. vinelandii* NCIB 9068 | 40 | 3.00 | 70 | 7.3–7.5 | 30 | 10 |
| Norwegian Institute of Seaweed Research | Strain E of *A. vinelandii* | 20 | 2.40 | 100 | | 30 | 6 |
| Nihon University, Japan | Strain N-II-1-2 of *A. vinelandii* | 20 | 1.50 | 120 | 7.2 | 30 | 13 |
| National Research Council of Canada | *A. vinelandii* 534 | 20 | 0.95 | 96–120 | 7.2 | 25 | 4 |
| University of Vermont | *A. vinelandii* ATCC 12837 | 15 | 0.74 | 120 | 7.0 | 31 | 2 |

[a] ROC, Republic of China.

reduced dramatically if bacterial alginate were chosen. The results also indicated that a solution of bacterial alginate was more pseudoplastic than was that of algal alginate. Thus, the bacterial alginate could result in a pleasing texture in the mouth and excellent flavor release when the product is used in foods. Pseudoplasticity could also provide advantages in processing, such as pumping and filling. However, it is still unknown whether the pseudoplasticity of bacterial alginate is advantageous to industries other than the food industry. Further investigation is needed. No significant differences were observed in other properties of bacterial and algal alginates. Thus, we concluded that the properties of bacterial alginates were superior to those of algal alginates from the above-mentioned results. However, we did not compare the properties with those of other bacterial alginates because of the absence of such data in the literature.

## ACKNOWLEDGMENTS

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