Note

Localization of a Biopolymer Produced by *Rhodococcus erythropolis* Grown on *n*-Pentadecane[†]

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Rhodococcus erythropolis S-1 was isolated from soil^{1,2)} and found to produce a biopolymer, named NOC-1, which flocculated kaolin clay in the presence of cations.³⁾ NOC-1 was composed of protein and formed micelles in culture broth.⁴⁾ The optimum culture conditions for the polymer production were studied, and results indicated that saccharides were a better carbon source than *n*-paraffines.⁵⁾ In this experiment, the effects of carbon sources on biopolymer localization were examined.

S-1 was cultivated at 30°C for 4–5 days in 100 ml of medium. The medium contained 1% carbon source (either D-glucose or *n*-pentadecane), 0.05% yeast extract, 0.05% urea, 0.5% K₂HPO₄, 0.2% KH₂PO₄, 0.01% NaCl, 0.02% MgSO₄ · 7H₂O, and distilled water (pH 7.2).

When S-1 was cultivated in the medium containing *n*-pentadecane, cells formed fibrous flocs that floated at the surface. Contrastingly, when glucose was used as the carbon source, the cells were dispersed. The dry cell yields from 100 ml of culture broth containing either glucose or *n*-pentadecane were 0.31–0.37 g and 0.38–0.44 g, respectively, thereby indicating that *n*-pentadecane was a more favorable growth substrate.

Flocculation tests were done using kaolin as a suspended solid. One-tenth ml of 1% aluminium sulfate solution and 0.2 ml of culture broth dialysate were added to 5 ml of 10% kaolin suspension, then vortexed and left to settle for 1 min. No flocculating activity was found in the *n*-pentadecane medium, while high activity was observed in the glucose medium (Fig. 1). The cells from 0.2 ml of culture were added to the test kaolin suspension instead of the culture broth dialysates and flocculation tests were

Fig. 1. Flocculating Activity of Culture Broth.

(a) control kaolin suspension. Culture broth dialysates from (b) glucose and (c) *n*-pentadecane medium were added to the kaolin suspension.

Fig. 2. Flocculating Activity of Cells.

(a) control kaolin suspension. Cells from cultures containing (b) glucose and (c) *n*-pentadecane were added to the kaolin suspension.

done. It was observed that cells grown on *n*-pentadecane had a higher flocculating activity than those grown on glucose (Fig. 2). It is assumed that the biopolymer (NOC-1) produced was not dispersed in the culture broth but located on the cell surface, when the microorganism was grown on *n*-pentadecane.

The biopolymer released in the culture broth was obtained by the method previously described.⁴⁾ Ammonium sulfate (40 g) and *n*-butanol (100 ml) were added to the culture broth (100 ml) and mixed vigorously. The mixture was centrifuged, and a thin biopolymer layer was formed at the water and *n*-butanol interface. The biopolymer layer thus prepared was suspended in distilled water and dialyzed against water. The yield of the biopolymer from 100 ml of the glucose medium was 20–50 mg, while none was obtained from the *n*-pentadecane medium.

The cell surface biopolymer was measured as follows.

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[†] Microbial Flocculant Part IV.

Fig. 3. SDS-PAGE of the Biopolymers.

Samples were incubated at 100°C for 2 min in the presence of 1% SDS and 1% 2-mercaptoethanol before electrophoresis through a 5–20% (w/v) acrylamide gel. After electrophoresis, the gel was stained with Coomassie Brilliant Blue R 250. Lane a, molecular weight marker proteins (MW-Marker, oriental): 1, MW 74,400; 2, MW 49,600; 3, MW 37,200; 4, MW 24,800; 5, MW 12,400. Lane b, biopolymer prepared from culture broth; lane c, cell surface biopolymer.

Cells grown in 100 ml of the *n*-pentadecane or glucose medium were washed four times with a 50% (v/v) pyridine solution (30 ml). The cell flocs formed in *n*-pentadecane medium were completely broken by this washing treatment.

Each washed solution (120 ml) was neutralized by the addition of acetic acid, and then *n*-butanol (120 ml) and saturated ammonium sulfate solution (120 ml) were added. The mixture was centrifuged and a thin biopolymer layer formed at the boundary phase was collected. Approximately 5 mg of biopolymer was obtained from the cells grown on *n*-pentadecane, while less than 1 mg of it was obtained from the cells grown on glucose.

The biopolymer prepared from the surface of the cells grown on *n*-pentadecane and that from the culture broth of the glucose medium were analyzed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoretic patterns of both polymers coincided (Fig. 3).

It is believed that a majority of the produced biopolymer (NOC-1) dispersed in the culture broth when the microorganism was grown on glucose, and that most of the biopolymer remained on the surface of the cells when grown on *n*-pentadecane. The biopolymer, located on the cell surface, is assumed to bind the adjoining cells and lead to floc formation.

References

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