

Production of Cold-Induced Novel Extracellular Biopolymer by *Pseudomonas fluorescens* BM07 and Environmental Applications of Its Colloidal Form

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Pseudomonas fluorescens BM07 was induced to excrete an extracellular biopolymer by decreasing the cultivation temperature down to as low as 10 °C. Maximum production of the cold induced biopolymer was obtained when cells were grown aerobically at 10 °C and its production was prohibited at 30 °C. A reverse correlation between temperature and biopolymer production was shown. All the factors examined including carbon and nitrogen sources, temperature, pH seemed to influence biopolymer production efficiency. The best production yield of 2.5 g/L was obtained when the cells were grown on M1 medium containing 70 mM sucrose and 0.2% (wt/v) casamino acid. Compositional analysis of protein fraction as the major constituent (99.5% w/w) revealed that it was composed mainly of hydrophobic (42%) and less frequently of charged (32%), polar (29%), acidic (17%) and basic (12%) amino acids. Analysis by HPAEC-PAD chromatography showed that carbohydrate moiety comprised a small fraction of biopolymer (~0.5% w/w), composed of glucosamine and galactosamine in a ratio of 9:1. Change of the above culture parameters generally did not affect the amino acid and monosaccharide compositions. However, the use of gluconic acid and glycerol as carbon source resulted in

the formation of a biopolymer which contained glucose in addition to the two amino-sugars. BM07 biopolymer showed high ion binding capacity with particular preference to uptake cadmium and mercury (70% and ~45% respectively). In addition, the flocculating efficiency of the biopolymer was considerable for kaolin suspension and correspondingly increasing with growth phase. In the light of the results obtained here, the extracellular biopolymer was involved in the aggregation of bacteria into flocs.

References

1. Beech I, Hanjagsit L, Kalaji, M, Neal A.L, Zinkevich V (1999) Chemical and structural characterization of exopolymers produced by *Pseudomonas* sp. NCIMB 2021 in continuous culture. *Microbiology* 145: 1491-1497.
2. Chan R, Lam JS, lam K, Costerton JW (1984) Influence of culture conditions on expression of the mucoid mode of growth of *Pseudomonas aeruginosa*. *J. Clin. Microbiol.* 19:8-16.
3. Decho AW (1990). Microbial exopolymer secretion in ocean environments: Their roles in food webs and marine processes. *Oceanogr Mar Biol Annu Rev* 28:73-153.
4. Gehrke T, Telegdi J, Thierry D, Sand W (1998) Importance of extracellular polymeric substances from *Thiobacillus ferrooxidans* for bioleaching. *Appl Environ Microbiol* 64(7):2743-2747.
5. Guvan, JRW (1975) Mucoid strains of *Pseudomonas aeruginosa*: The influence of culture medium on the stability of mucous production. *J Med Microbiol.* 8: 513-526.
6. Geesey GG (1982) Microbial exopolymers: ecological and economic consideration. *ASM News* 48:9-14.
7. Higgins MJ, Noak JT (1997). Characterization of exocellular protein and its role in bioflocculation. *J. Environ Eng* 5: 479-485.
8. Kuran, R, Toeda K, Takeda K, Suzuki T (1986a) Culture condition for production of microbial flocculation by *Rhodococcus erythropolis*. *Agric Biol Chem* 50: 2309-2313.
9. Kurane R, Hatamochi K, Kakuno T, Kiyohara M, Kawaguchi K, Mizuno Y,

- Hirano M, Taniguchi Y (1994) purification and characterization of lipid bioflocculant produced by *Rhodococcus erythropolis*. *Biosci Biotech Biochem* 58(11):1977-1982.
10. Watanabe M, Suzuki Y, Sasaki K, Nakashimada, Y, Nishio N (1999) Flocculating property of extracellular polymeric substances derived from a marine photosynthetic bacterium, *Rhodovulum* sp. *J Biosci Bioeng* 87(5): 625-629.