

## Novel Exopolysaccharide from Marine Biofilm-Forming Microorganisms

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The vast majority of microorganisms are living and growing in aggregated form such as biofilm. Biofilms develope adherent to a soil surface at soil-water interfaces, but can also be found at water-oil, water-air and solid-air surfaces. Biofilms are accumulations of microorganisms, extracelluar polymeric substrates (EPS), multivalent cations, biogenic and inorganic particles as well as colloidal and dissolved compounds. The common feature of this mode of existance is that microorganisms are embedded in a matrix of EPS (Characklis *et al.*, 1987). EPS are mainly responsible for the structural and functional integrity of biofilms and are concentered as key compounds that determine the physicochemical and biological properties of biofilms (Wingender *et al.*, 1999).

Most marine environments contain only dilute substances that can be used for metabolism and growth. In contrast, natural surfaces tend to collect and concentrate nutrients by charge-charge or hydrophobic interactions (Beveridgee et al., 1997). Bacterial colonization on abiotic materials such as suspended particles, metal surfaces and concrete or on biotic surfaces was thought to be one of the microbial survival strategies because it provides microorganisms with important advantages, including 1) increased access nutrients, 2) protection against toxins and antibiotics, 3) maintenance of extracellular enzyme activities and 4) shelter from predation (Dang and Rovell, 2000). For these reasons, surfaces in contact with water are rapidly colonized by microroganisms. During the process of colonization on particular surfaces, microroganisms overproduce EPS (Geesey and White, 1990). These polymers, especially exopolysaccharide, are the materials which construct the biofilm matrix, serving as a multipurpose functional element for adhesion, immobilization of cells on the colonized surface, protection, recognition and faciliting spatial arrangement of different species with the biofilm (Allison et al., 1998). Among the microbial EPS, interest has focused particularly on selected bacterial extracellular polysaccharides which are commercial interest in biotechnology for various industrial and biomedical applications. As to their commercial exploitation, bacterial extracellular polysaccharides such as xanthan, gellan, celluose, hyaluronic acid, and several other β -D-glucans have found various applications, whereas newly discovered polysaccharides and chemical modifications of established polysaccharides offer the potential of novel applications in future (Becker et al., 1998; Sutherland, 1998).

So, we performed 1) isolation of bacteria from various natural biofilms and sampling slides that had been exposed to natural seawater and, 2) identification of bacterial strains, 3) estimation of exopolysaccharide-producing ability, 4) characterization of the exopolysaccharide produced by selected strains, and 5) presentation of usage from isolated exopolysaccharide.

To find novel exopolysaccharide, 620 marine bacterial strains of mucoid type were isolated from natural biofilm samples collected in coastal regions of South Sea, Korea. Strain 00SS11568 was selected as a producer for viscous exopolysaccharide, named as p-11568. The isolate was identified as

Alteromonas sp. based on 16S rDNA, morphological, and biochemical properties. The p-11568 was found to have average molecular masses of  $4.4 \times 10^5$ . The sugar compositions revealed a heteropolysc-charidic nature, consisted of glucose, galactose and galactosamine in a molar ratio 1: 1.3: 0.9, and galacturonic acid as miner sugar. The absorbance bands of the exopolysaccharide determinated by FT-IR spectrum analysis. The effects of salt, pH, temperature, inorganic compounds, and C, N-source were tested to get the optimal composition of medium for the production of p-11568. Maximum polysaccharide production(19.2 g/l) was obtained when grown in M-11568 medium. The rheological properties of p-11568 was investigated. p-11568 solution showed a characteristic for non-Newtonian behavior fluid properties. In 1% aqueous dispersions of p-11568, consistency index and flow behavior index were 4,409 cp and 0.42. p-11568 was pseudoplastic fluid by Power-Low model. Rheological properties of p-11568 were showed to be influenced by the concentration of salt, pH, temperature, ionic compounds. Especially, p-11568 has a rheological property among Xanthan gum and Gellan gum.

And other exopolysacchrides from biofilm-forming microorganisms, such as *Idiomarina sp.* 00SS11484, *Alteromonas* sp. 01CJ12700, *Chroococcus* sp. 00SS-CY36 also revealed novel sugar composition and rheological properties.

Therefore, screening of exopolysacchrides from biofilm-forming microorganism has potential to discover novel polysaccharides and chemical modifications of established polysaccharides will offer the potential of novel applications in future.

## References

- 1. Allison, D.G., B. Ruiz, C. San-Jose, A. Jaspe, and P. Gilbert, 1998. Extracelluar products as mediators of the formation and detachment of *Pseudomonas fluoresence* biofilms. *FEMS. Microbiol. Ecol.* 167;179-184.
- 2. Becker, A., F. Katzen, A. Puhler, and L. Ielpi, 1998. Xanthan gum biosynthesis and application: a biochemical/genetic perspective. *Appl. Microbiol. Biotechnol.*, 50;145-152.
- 3. Beveridgee, T. J., S.A. Markin, J.L. Kadurugamuwa, and Z. Li, 1997. Inreactions between biofilms and the environments. *FEMS. Microbiol. Rev.*, 20;290-303.
- 4. Characklis, W.G. and Winderer P.A., 1987. In: Characklis W.G., Wilderer P.A. (eds) Structure and function of biofilm. *Wiley, Chichester*, pp 369-371.
- 5. Dang, H. and C.R. Rovell, 2000. Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction and sequence analysis of 16S rRNA gene. *Appl. Environ. Microbiol.* 66;467-475.
- 6. Geesey, G.G. and D.C. White, 1990. Determination of bacterial growth and activity at solid-liquid interfaces. *Ann. Rev. Microbiol.* 44;579-602.
- 7. Sutherland, I.W., 1998. Novel and established applications of microbial polysaccharides. *Trends Biotechnol.*, 16;214-219.
- 8. Wingender, J., T.R. Neu, and H.C. Flemming, 1999. In: Wingender J, T.R. Neu, and H.C. Flemming (eds) Microbial extracelluar polymeric substrates. *Springer*. pp 1-15.

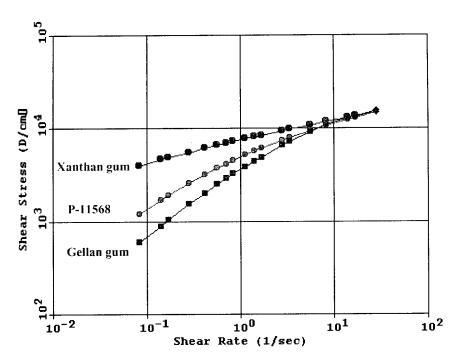


Fig. 1. Relationship between shear stress and shear rate of p-11568 according to Xanthan gum and Gellan gum.