Seasonal Progress of Biofilm-Forming Bacteria on Acrylic Surface in Seawater

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Introduction

Usual marine environments contain only dilute substances which can be used for metabolism and growth, and interfacial effect of natural surfaces tends to collect and concentrate nutrients by charge-charge or hydrophobic interactions (Beveridge *et al.*, 1997). Bacterial colonization on abiotic materials such as suspended particles, metal surfaces and concrete or biotic surfaces was thought to be one of the microbial survival strategy because it provides microorganisms with important advantages, including i) increased access to nutrients, ii) protection against toxins and antibiotics, iii) maintenance of extracellular enzyme activities and iv) shelter from predation (Dang and Lovell). So, surfaces contact with water rapidly colonized by bacteria.

However, biofilm can occur in the wrong place and wrong times and cause severe problems due to the corrosion and/or biofouling on construction or ship surfaces. For a sustainable anti-fouling strategy the analysis of the fouling situation, a development of suitable anti-fouling components and an effective and representative monitoring of biofilm development were recommended.

While there is a paucity of information concerning the changes in biofilm structure and organization during the early phase of biofilm formation in seawater (Stickler 1999, Davey & O'Toole 2000).

In this study we analyze the bacterial number, extracellular enzyme activities, metabolic changes and the changes of community composition of biofilm on acrylic surface in seawater during initial period and after maturation.

Materials and Methods

Study was conducted in JangMok Cove, Goje Island, which is located to the south of the Korean Peninsula. A buoy system constructed near the Namhae Institute of the Korea Ocean Research and Development Institute (KORDI) allowed the experimental surfaces to be suspended in the water. The average water depth beneath the buoys was approximately seven meters.

Acid-cleaned acrylic coupons (75 x 25 x 1 mm) were submerged to a depth of approximately 75 cm. The coupons were oriented horizontally to the surface of the water. Sampling Coupons were withdrawn periodically and rinsed with filter-sterilized seawater. Then, the total bacterial number (TBN) using DAPI method, culturable bacteria (VBN) on ZoBell 2216e agar media, extracellular enzyme activity (eea) and bacterial diversity based on diversity of terminal restriction fragments were investigated.

Results and Discussion

Bacteria rapidly attached on acrylic surface within initial 1 day but the number fluctuated during 3 to 4 days. TBN on acrylic surface slightly increased to 3.49 x 10^5 cells cm^{-2} between 1 to 3 days after rapid

increase to 1.48 x 10^5 cells cm^{-2} within 1 day. TBN showed exponential growth after 3rd day and reached to 9.73 x 10^6 cells cm^{-2} in 6th day. TBN increased with growth of biofilm and it reached to 1.28 x 10^8 cells cm^{-2} at 54th day. VBN showed similar pattern with that of TBN and increased to 3.63 x 10^6 CFU cm^{-2}. But the numbers were slightly decrease and maintained till to 10 month after start of investigation.

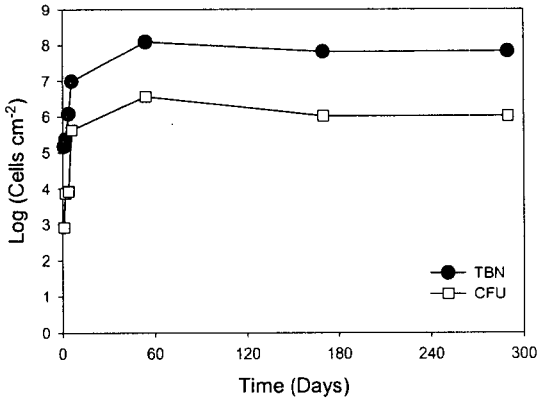

Fig. 1. Fluctuations of the total bacterial number (TBN) and the culturable bacterial number (CFU) during the initial stage of biofilm formation on acrylic surfaces.

EEA also increased exponentially after 3 to 4 days of fluctuation and showed maximum activities after 6 months of experiment. Of them b-D-glucosidase showed lowest activity, it might reflect minor role of carbohydrates as c-sources. The high activity of phosphatase seemed to reflect the limitation of inorganic phosphate in the inner space of biofilm matrix.

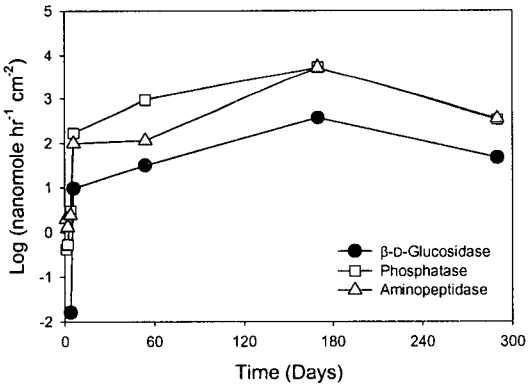

Fig. 2. Changes of extracellular enzyme activity during the early phase of biofilm formation.

The detected fragment size ranged from 37~813 base and the detected number of T-RFs ranged 19~56 in bacteria. T-RFs size of 60, 82, 92, 203, 343, 357, 370, 374, 548, 571 and 573 bp detected all times. T-RF having 60 bp showed the highest proportion, ranged 13.8 to 33.4% in archaeal T-RFs. The proportion of T-RFs showing 210, 367, 374, 376 and 564 base were decreased and some of them were disappeared during the early phase. Comparison of T-RFLP fingerprinting was conducted with WPGMA method. Bacterial communities divided into 3 major clusters, first one was the initial stage of early phase, second one was late stage of early phase and finally after maturation of biofilm. After maturation number of detected T-RFs was decreased with time.

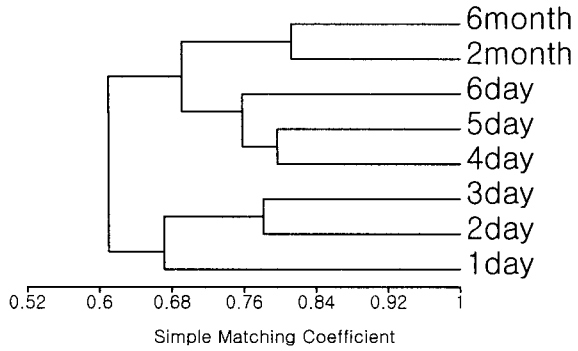

Fig. 3. Dendrogram of community relatedness of biofilm-forming bacteria based on Simple Matching Coefficient. Comparison of T-RFLP fingerprinting was conducted using the WPGMA method.

The number and enzyme activities of biofilm-forming bacteria increased rapidly after 3 to 4 days of lag period. The growth of biofilm and consequent increase of metabolic activity might progressed after the settlement of specific bacterial group on surface after the changes of reversible attachment to irreversible one during initial phase. After maturation bacterial number and activities fluctuated regardless of film thickness increase.

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References

1. Beveridge, J.J., S.A. Makin, J.L. Kadurugamuwa & Z. Lee, 1997. *FEMS Microbiol Rev.* 20, 291-303
2. Dang H. and C. R. Lovell, 2000. *Appl. Environ. Microbiol.* 66, 467-475
3. Davey ME, O'Toole GA, 2000. *Microbiol Molecular Biol Rev.* 64, 847-867
4. Giovannoni S.J. 1991. p 177-201 *In* (Stackebrandt E. & M. Goodfellow eds.) Nucleic acid techniques in bacterial systematics. John Wiley & Sons, N.Y.
5. Hoppe, H. G. 1993. p 423-431 *In* (Kemp P.F., B.F. Sherr, E.B. Sherr and J.J. Cole eds.) Handbook of Methods in Aquatic Microbial Ecology. Lewis Pub. Boca Raton, Florida, USA.
6. Lee H.S., K.K. Kwon, J.H. Lee and H.K. Lee. 1999. *J. Microbiol.* 37, 263-266
7. Stickler D. 1999. *Curr. Opinion Microbiol.* 2, 270-275