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MARINE BIOTECHNOLOGY

Changes of Biofilm-Forming Bacterial Community on Acrylic Surface Emerged in Seawater

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Abstract: Early phase of biofilm formation and seasonal fluctuation of film-forming bacteria on acrylic surface in seawater were investigated. During the initial period of biofilm formation, bacterial numbers increased rapidly after 3 days of adaptation. The bacterial community analyzed by the T-RFLP method changed simultaneously with the change of bacterial number and divided into four major clusters. The composition of culturable bacteria changed severely during biofilm formation. Among the isolates, genus *Erythrobacter longus* was always detected.

Key words: biofilm, acrylic surface, community composition, T-RFLP.

INTRODUCTION

The ocean is typically known as a poor nutrient environment for bacterial metabolism and growth. The interfacial effect of naturally occurring surfaces, however, causes the collection and concentration of nutrients by charge–charge or hydrophobic interactions (Beveridge et al., 1997), thereby providing microorganisms with important advantages (Dang and Lovell, 2000). Bacterial colonization on abiotic materials such as suspended particles, metal surfaces, and concrete or biotic surfaces is thought to be one of the microbial survival strategies. Biofilm is also a major cause of biocorrosion and/or biofouling on construction or ship hulls (Flemming, 2002), eventually doing severe economic damage. Therefore, understanding biofilm formation on abiotic surfaces is as important as biofilm formation on biotic surfaces, both of which have an intimate relationship with pathogenicity. However, 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 and O'Toole, 2000). In the present study we analyze the relationship between changes of bacterial number and community structure during the biofilm formation on acrylic surfaces in seawater.

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MATERIALS AND METHODS

The 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 KORDI allowed the experimental surfaces to be suspended in the water. The average water depth beneath the buoys was approximately 7 m.

Acid-cleaned acrylic coupons (75 × 25 × 1 mm) were submerged to a depth of approximately 75 cm. The

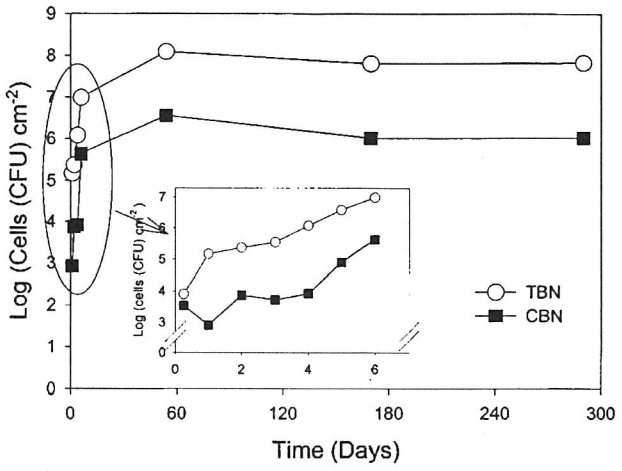

Figure 1. Fluctuations of the total bacterial number (TBN) and culturable bacterial number (CBN) on acrylic surface during the biofilm formation.

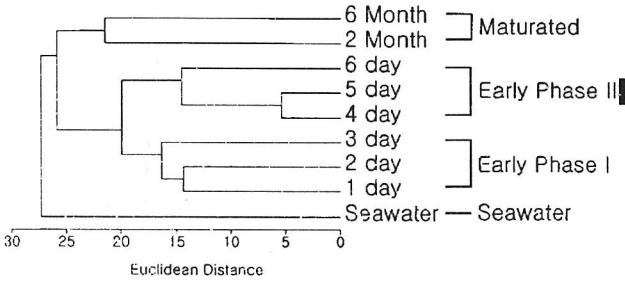

Figure 2. Relatedness of biofilm-forming bacterial community based on Euclidean distance. Comparison of T-RFLP fingerprinting was conducted using the WPGMA method.

coupons were oriented parallel to the surface of the water. The coupons were withdrawn periodically and rinsed with filter-sterilized seawater. After rinsing, coupons for measurement of total bacterial number (TBN) were fixed with 1% formalin and analyzed according to Lee et al. (1999). Biofilm for analyzing culturable bacterial number (CBN) was scrapped from coupons using a razor blade, diluted with sterilized seawater, and spread on ZoBell 2216e agar media. Terminal restriction fragment length polymorphism (T-RFLP) of bacterial and archaeal 16S rDNA was analyzed (Lee et al., 2003) using genomic DNA extracted from coupons. Partial sequences of 16S rDNA from bacterial isolates were also analyzed for the evaluation of culturable diversity of bacteria of the biofilm matrix.

Results and Discussion

Bacteria attached on to the acrylic surface on the first day but the numbers fluctuated after 3–4 days (Figure 1). TBN on the acrylic surface increased slightly to 3.49×10^5 cells cm^{-2} between 1 and 3 days after a rapid increase to 1.48×10^5 cells cm^{-2} on the first day. TBN showed exponential growth after the third day and reached 9.73×10^6 cells cm^{-2} by the sixth day. TBN increased further with growth of biofilm, reaching 1.28×10^8 cells cm^{-2} at day 54. CBN showed a similar pattern, increasing to 3.63×10^6 CFU cm^{-2}. However, the numbers decreased slightly and remained steady until 10 months after the start of the investigation.

The detected fragment size ranged from 37 to 813 bases and the detected number of T-RFs ranged from 19 to 56 in bacteria. T-RF sizes of 60, 82, 92, 203, 343, 357, 370, 374, 548, 571, and 573 bp were detected at all times. T-RF of 60 bp showed the highest proportion, ranging from 13.8% to 33.4% in archaeal T-RFs. The proportion of T-RFs of 210, 367, 374, 376, and 564 bp decreased and some of them disappeared during the early phase. After maturation the number of detected T-RFs decreased with time. Comparison of T-RFLP fingerprinting was conducted using WPGMA method. Bacterial communities divided into four major clusters: seawater, the initial stage of the early phase (early phase I), the late stage of the early phase (early phase II), and the maturated phase (Figure 2). Differentiation of the early phase into two stages is well matched with the 3–4-day lag period before the sudden increase of bacterial number (Figure 1). An increase of bacterial number might progress after adaptation of the major bacterial group in the biofilm matrix during the early phase. The bacterial community in seawater was clearly differentiated with that of the biofilm matrix.

Partial sequences of 16S rDNA of 116 bacterial isolates from the biofilm matrix were analyzed and they were assigned to 24 genera except 7 unidentified strains. During the initial phase *α-Proteobacteria* appeared to be the dominant group. This result is similar to that of the report of Dang and Lovell (2000) who conducted their study at the seawater surface. Among the isolates, *Bacillus* strains were always detected. Contrary to the *Bacillus* and *α-Proteobacteria* strains, the strains belonging to the *γ-Proteobacteria* group were detected after maturation of the biofilm matrix. Among the isolates, *Erythrobacter longus* was detected at all sampling times and it might be the major constituent of culturable bacteria in the biofilm matrix of the present investigation. T-RFs of isolates did not match well with T-RFs of the whole community. There are great differences between culturable diversity and total community structure as is already known (Fuhrman et al., 1993).

In summary, the number of biofilm-forming bacteria increased rapidly after 3–4 days of lag period. The growth of biofilm and consequent increase of metabolic activity might progress after the settlement of a specific bacterial group on the surface during the initial phase of biofilm formation in seawater. After maturation, bacterial number and activities showed seasonal fluctuation and genetic diversity decreased in spite of biofilm growth.

Acknowledgment

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