



## ***Bacterial colonizations of glass and acryl surfaces immersed in coastal seawater***

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### **Abstract**

Distributions of bacteria and microcolonies developed on glass and acryl surfaces were observed microscopically to study bacterial adhesion to and accumulation on the surfaces immersed in coastal seawater. Adhesion of bacteria to the surfaces seemed to play a dominant role in bacterial colonization of the surfaces during the early phase (< 6 d) of microbial film formations. Adhesion rates tended to increase for the first day of immersion, suggesting that cooperative interactions among initial colonizers. Acryl surface seemed to allow more rapid rates of adhesion and accumulation than glass surface, and thus more colonized bacteria. In both surfaces, specific accumulation rates tended to decrease to near zero rates as bacterial film developed. Biovolumes of adhered bacteria changed from large biovolumes ( $0.31\text{-}0.39 \mu\text{m}^3$ ) at 1 d of colonization to smaller ones ( $0.24\text{-}0.27 \mu\text{m}^3$ ) at 6 d, although biovolumes of adhered bacteria were still larger than those of bulk-phase water bacteria ( $0.13\text{-}0.24 \mu\text{m}^3$ ). Bacterial abundances, growth rates and fractions of hydrophobic bacteria of the bulk-phase water did not significantly correlate with adhesion rates of bacteria.

ural bacteria (Cooksey and Wigglesworth-Cooksey, 1995). The surface colonization by bacteria soon leads to the formation of microbial films. The formation of microbial films on diverse surfaces attracts many practical interests (Denyer *et al.*, 1993). Thus, many studies on this subject have been performed (Little, 1984; Melo, 1992; Geesey *et al.*, 1994). Information on bacterial adhesion rates and growth rates after the adhesion are very important to understand and regulate bacterial surface colonization. However, there are limited quantitative studies on adhesion and accumulation rates of natural marine bacteria to surfaces (Fera *et al.*, 1989; Zheng *et al.*, 1994).

Adhesion of bacteria to surfaces is primarily determined by the interactions between surface properties (e.g. hydrophobicity vs hydrophilicity and roughness vs smoothness) of substrate and bacterial surface characteristics under constant conditions of physico-chemical environmental factors (Fera *et al.*, 1989; Rosenberg *et al.*, 1991). Many studies employed diverse surfaces to analyze the effects of substrate surface properties on bacterial surface colonizations. However, no measurements of bacterial surface characteristics have been made for natural marine bacteria. In this study, we asked (1) what are the adhesion and accumulation rates of natural marine bacteria? (2) Do bacterial adhesion rates and accumulation rates on glass and acryl

### **Introduction**

Surfaces exposed in marine waters adsorb dissolved organic matter from the surrounding waters, and the conditioned surfaces are invariably colonized by marine nat-

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surfaces dynamically change during the development of microbial films? (3) Are bacterial parameters of the bulk-phase water including bacterial surface characteristics related to bacterial attachment to the surfaces?

## Materials and Methods

### 1. Study area and colonization experiments

Studies on bacterial surface colonization were done in Kyunggi bay for 3 times: the 1st experiment was conducted on April 28th, the 2nd on July 18th, and the 3rd on August 10th in 1995. In April, an experiment was carried out in a glass container containing ca. 40 l of seawater from Kyunggi bay, and settlements of particles were prevented by intermittent shaking. The 2nd and 3rd experiments were made *in situ* in Incheon harbor. In *in situ* experiment, slide holders were tied to a buoy to maintain a constant (ca. 1 m) depth. Slide holders, which were made of acryl, could hold up to 10 slides in vertical position. On the 2nd day of the 3rd experiment, another set of slide holders was immersed in 1.0  $\mu\text{m}$  filtrate of seawater in a glass container which was shaken intermittently. To provide surfaces, glass or acryl slides were used. Glass or acryl slides were washed copiously with 10% HCl and Milli-Q distilled water. Slide holders with slides were immersed in a container in laboratory or *in situ* for up to 6 days. Duplicate slides or single slide were removed from slide holders periodically to examine microscopically. Also, surrounding waters were sampled for measurements of bacterial abundance, bacterial production, and bacterial surface characteristics (see below). The recovered slides were washed gently with 0.2  $\mu\text{m}$  filtered seawater to remove unattached bacteria, and put in 0.2  $\mu\text{m}$  filtered formalin solution (final conc. of 2%) to fix bacteria. The fixed slides were stored in refrigerator until examination. The slides were stained with DAPI, and examined under an epifluorescence microscope (Wolfaardt *et al.*, 1991). The number of microscopic fields (area of 6400  $\mu\text{m}^2$ ) examined were from 15 to 30.

### 2. Kinetics of colonization

Estimations of bacterial adhesion rates and microcolony accumulation rates of a particular size were made on the basis of distributions of attached bacterial numbers and microcolony numbers as follows: Numbers of microcolonies containing unicells or more and numbers of bacteria in microcolonies of a particular size were

recorded for each slides sampled. Adhesion rate ( $A_t$ ) at time  $t$  can be determined from the difference of total colony numbers at time  $t_{-1}$  and  $t$  divided by the incubation time interval.

$$A_t = (C_{t+1} - C_t) / (t_{t+1} - t_t),$$

where  $C_t$  is the total number of microcolonies containing unicells or more at time  $t$ , and  $C_{t-1}$  is the total number of microcolonies containing unicells or more at time  $t-1$ . In other words,  $C_t$  is the sum of numbers of microcolonies of unicells ( $C_1$ ), two-cells ( $C_2$ ), four-cells ( $C_4$ ), eight-cells ( $C_8$ ), and sixteen-cells ( $C_{16}$ ) at time  $t$ . Significant numbers of nonexponential colony sizes (e.g.,  $C_3$ ,  $C_5$ ,  $C_7$ , etc.) did occur. We followed the classification of such colonies according to Malone and Caldwell (1983). In our experiments lasting for 6 d, microcolonies containing more than 16 cells were not observed. Specific accumulation rate of a microcolony ( $\mu_s$ ) of a particular size ( $s$ ) at time  $t$  can be calculated from the difference between sum of microcolony numbers at time  $t_{-1}$  and that at  $t$ , where sizes of microcolonies used for the calculations are greater than  $s$ . Here, the calculated specific accumulation rate represents the net result of the microcolony growth and removal rate. The detailed equations for calculation of the specific accumulation rate of a microcolony of a particular size ( $s$ ) at a given time ( $t$ ) are shown as follows:

$$\mu_s = (\ln N_{s,t+1} - \ln N_{s,t}) / (T_{t+1} - T_t),$$

$$N_{s,t+1} = [C_{s+1,t+1} + C_{s+2,t+1} + C_{s+4,t+1} + C_{s+8,t+1} + \dots] - (C_{s+1,t} + C_{s+2,t} + C_{s+4,t} + \dots),$$

$$\text{and } N_{s,t} = [C_{s,t}]$$

where  $(T_{t+1} - T_t)$  is an incubation time interval and  $C_s$  is the number of microcolony of a particular size( $s$ ) at time  $t$ . For example, for  $s = 2$  the number of microcolony ( $C_2$ ) at time  $t$  is  $C_{2,t}$ ; likewise  $C_{3,t}$ ,  $C_{4,t}$ , and  $C_{8,t}$  represents  $C_3$ ,  $C_4$ , and  $C_{8,t}$ , respectively.

### 3. Characterization of bacteria and substrate surfaces

Use of n-hexadecane was employed to characterize bacterial cell surface (Rosenberg *et al.*, 1991). To 10 ml of seawater samples, 0.8 ml of n-hexadecane was added and vortexed for 3 min in maximum power. Bacterial abundance in aqueous phase was measured (Porter and Feig, 1980) and subtracted from total bacterial abundance to calculate fraction of hydrophobic bacteria. The use of 0.8 ml n-hexadecane addition to 10 ml sample was empirically determined in this study to give optimal concentration of n-hexadecane (not shown). To measure

wettability of substrate, aqueous methanol solutions were used (Gerhart *et al.*, 1992).

#### 4. Other analyses

Bacterial production was measured by  $^3\text{H}$ -thymidine method (Fuhrman and Azam, 1982) modified by Wicks and Robarts (1987). Specific growth rates of the bulk-phase water bacteria were calculated by using data of bacterial production and abundance. In the calculation of specific growth rates, exponential growth in bacteria was assumed. Bacterial abundance were measured from DAPI-stained bacteria under an epifluorescence microscope (Porter and Feig, 1980). Biovolumes of bacteria were measured by the method of Ammerman *et al.* (1984). Temperature was measured with a thermometer.

## Results

Colonizations of marine natural bacteria on glass and acryl surfaces for < 6 d were in general in an exponential mode (Figs 1-4A), and sometimes showed saturation even after 50 h incubation (Fig. 3A). In one experiment (Fig. 2A), colonization of bacteria on glass surface was negligible up to 75 h incubation, but afterwards it was in an exponential mode. Numbers of total-microcolonies corresponded to 56-87% (Fig. 2A), 78-89% (Fig. 3A) and 57-77% of total bacterial numbers (Fig. 4A) after 1 d incubation. Microcolonies containing unicells comprised a dominant fraction (> 55%) of total microcolony numbers throughout this study. Thus, changes of unicell-microcolony numbers closely resembled those of total number of bacteria (Figs 1-4A & 1-4B). Also, unicell-microcolonies dominated total bacterial counts (> 50%)

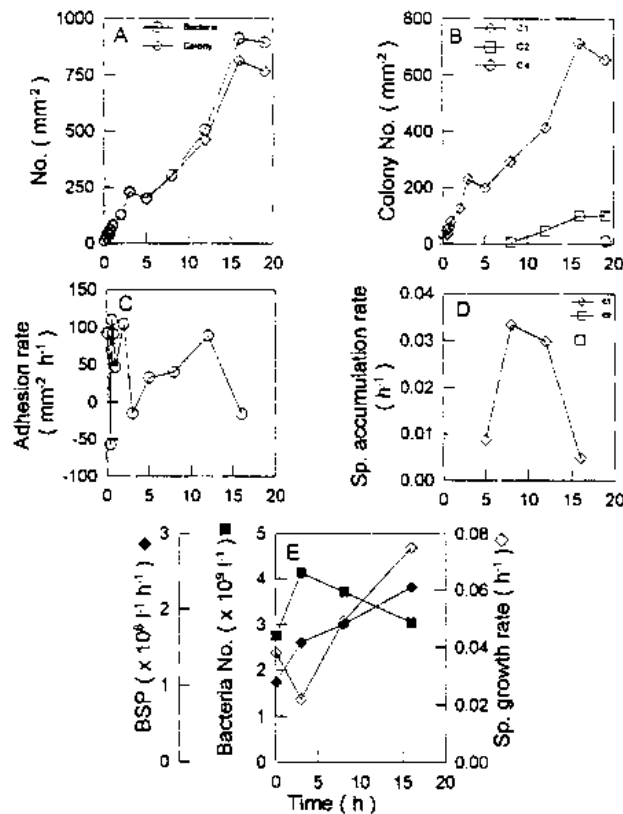


Fig. 1. Time-course changes of (A) bacterial abundance and microcolony numbers, (B) numbers of microcolony containing unicell (C<sub>1</sub>), two-cells (C<sub>2</sub>) and four-cells (C<sub>4</sub>), (C) adhesion rates, (D) specific accumulation rates of microcolonies, and (E) bacterial production (BSP), bacterial abundance, and specific bacterial growth rates of the bulk-phase water. Glass surface was used for bacterial colonization. Experiment was done in a container on April 28th in 1995 in Kyunggi bay.

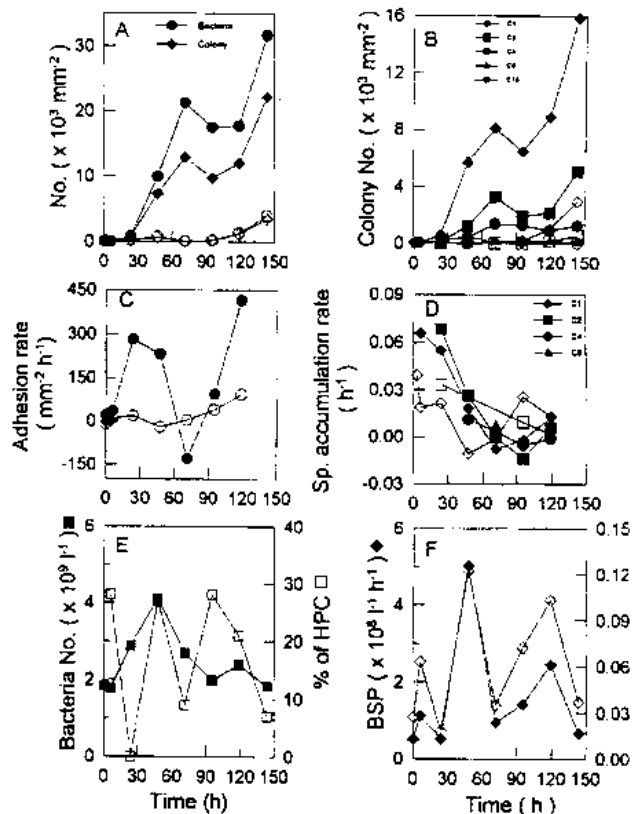


Fig. 2. Time-course changes of (A) bacterial abundance and microcolony numbers, (B) numbers of microcolony containing unicell (C<sub>1</sub>), two-cells (C<sub>2</sub>), four-cells (C<sub>4</sub>), eight-cells (C<sub>8</sub>), and sixteen-cells (C<sub>16</sub>), (C) adhesion rates, (D) specific accumulation rates of microcolonies, and (E) bacterial abundance and fraction of hydrophobic bacteria in bacterial community (% of HPC) of the bulk-phase water, and (F) bacterial production (BSP) and specific bacterial growth rates of the bulk-phase water. Glass (open symbols) and acryl surfaces (closed symbols) were used for bacterial colonization (A to D). Experiment was commenced *in situ* on July 18th in 1995 in Kyunggi bay.

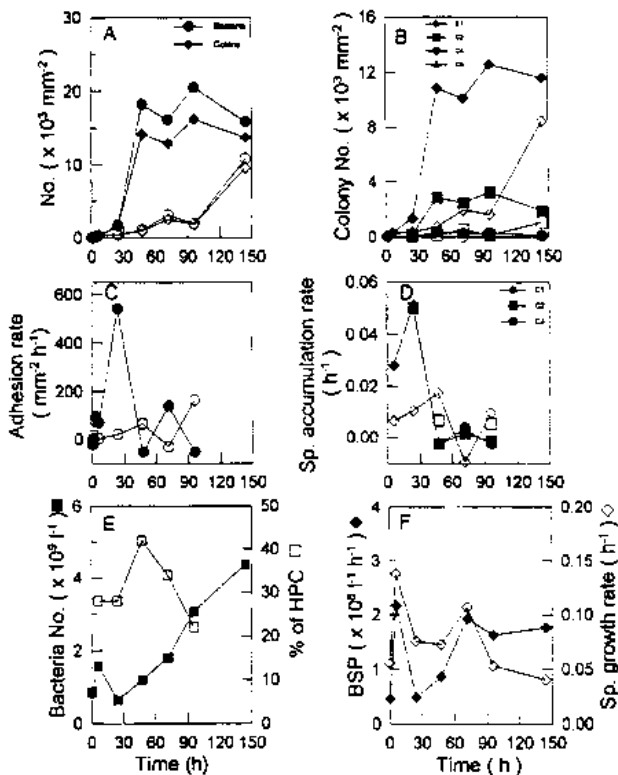


Fig. 3. Same as Figure 2. Experiment was commenced *in situ* on August 10th in 1995 in Kyunggi bay.

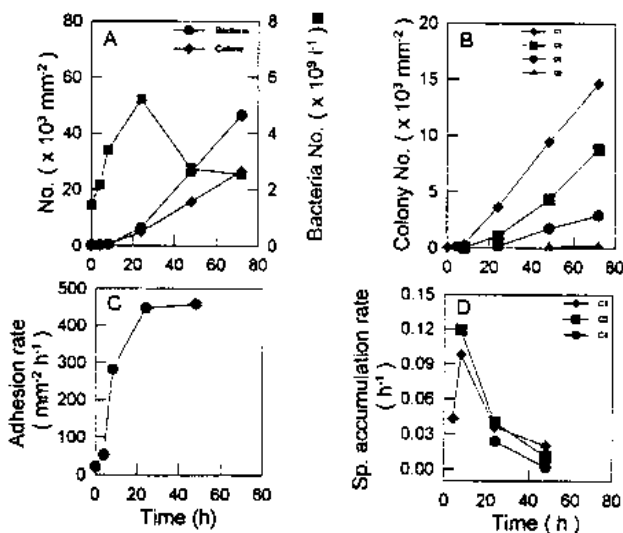


Fig. 4. Time-course changes of (A) bacterial abundance (●) and microcolony numbers (◆) on acryl surface and bacterial abundance of the bulk-phase water (■), (B) numbers of microcolony containing unicell (C<sub>1</sub>), two-cells (C<sub>2</sub>), four-cells (C<sub>4</sub>), and eight-cells (C<sub>8</sub>), (C) adhesion rates, (D) specific accumulation rates of microcolonies. Experiment was done in a container containing 1.0  $\mu$  filtrate of seawater on August 10th in 1995 in Kyunggi bay.

in most experiments. Only in two cases, i.e. 71-95 h incubation on acryl surface in July (Fig. 2) and after 40 h incubation on acryl surface in August (Fig. 4), unicell-microcolonies comprised 31-38% of total bacterial numbers. Our results suggested that adhesion was the major process in bacterial surface colonization during the early phase of microbial film formations. Eight-cell microcolonies (C<sub>8</sub>) were observed when total number of microcolonies were more than  $10 \times 10^3$  mm $^{-2}$  (Figs 2-4B). Bacterial abundance per mm $^2$  after 6 d incubation ranged from  $4.0 \times 10^3$  to  $10.7 \times 10^3$  on glass and from  $15.8 \times 10^3$  to  $31.7 \times 10^3$  on acryl surface. Numbers of bacteria colonized on glass surface were 2-8 fold lower than those on acryl (Figs 2A & 3A), consistent with the result that glass surface was more hydrophilic than acryl surface: glass and acryl surfaces showed standardized harmonic mean of 59 and 25, respectively, by aqueous methanol solution test. The high density of bacteria on acryl surface was due to faster adhesion rates on acryl than on glass surface (see below).

Adhesion rates of bacteria seemed to be quite variable during the colonization of surfaces (Figs 1-4C). Apparently, adhesion rates dramatically decreased in accordance with decreases in total bacterial numbers or total microcolony numbers, and increased again with increases in total bacterial numbers or total microcolony numbers (Figs 2-4). Negative values of adhesion rates were sometimes observed when total microcolony numbers substantially decreased (Fig. 2C). Adhesion rates were faster in most cases on acryl substrata than glass (Figs 2C & 3C). Adhesion rates on glass surface from all experiments ranged from negative values (i.e. detachment of bacteria) to 162 bacteria mm $^{-2}$  h $^{-1}$ , and those on acryl from negative values to 543 bacteria mm $^{-2}$  h $^{-1}$ .

Specific accumulation rate of a microcolony of a particular size also changed during the incubation, and tended to decrease with time (Figs 1-4D). The specific accumulation rates among microcolonies of different sizes at a certain time were not usually similar. Negative values of specific accumulation rates (i.e. net removal of bacteria) were observed when total bacterial numbers decreased substantially.

Mean biovolumes of adhered bacteria on acryl surface were large ( $0.31$ - $0.39$   $\mu$ m $^3$ ) after 1 d of immersion and decreased after 3-6 d to  $0.24$ - $0.27$   $\mu$ m $^3$ , which was still larger than those of the bulk-phase water bacteria ( $0.13$ - $0.24$   $\mu$ m $^3$ , Fig. 5).

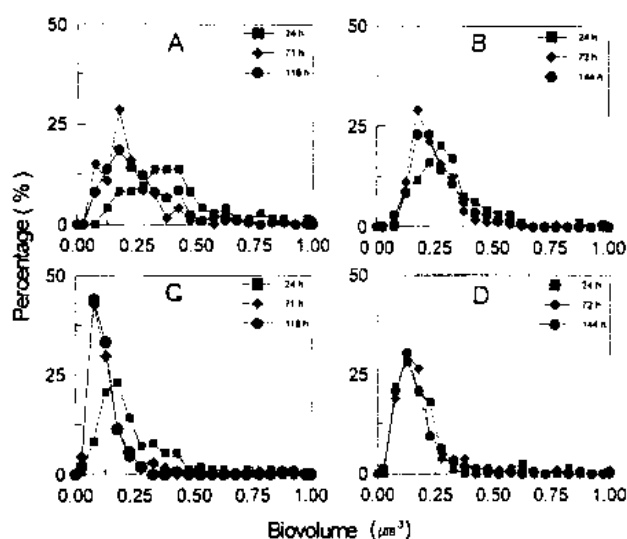


Fig. 5. Time-course changes of (A & B) biovolumes of adhered bacteria on acryl surface, and (C & D) biovolumes of bulk-phase water bacteria. Experiments were commenced on July 18th (A & C) and August 10th (B & D) in 1995.

Specific growth rates of bacteria in surrounding waters ranged from 0.02–0.14 h<sup>-1</sup> (Figs 1E, 2F & 3F). Bacterial growth rates in the bulk-phase water were greater than or comparable to initial accumulation rates of bacteria on glass surface (Figs 1–3), whereas bacterial growth rates in the bulk-phase water were slower than or comparable to initial accumulation rates of bacteria on acryl. The bacterial growth rates of the bulk-phase water were not related to adhesion rates of bacteria (not shown). Neither bacterial abundances nor water temperature did correlate with adhesion rates of bacteria (not shown). Further, fractions of hydrophobic bacteria in bacterial community did not show significant correlation with adhesion rates of bacteria (not shown). Interestingly, fractions of hydrophobic bacteria in bacterial community showed a significant correlation with bacterial growth rates in the bulk-phase water ( $r^2 = 0.80$ ,  $p < 0.01$ ,  $n = 14$ ) for the 2nd experiment (Fig. 2) and pooled data ( $r^2 = 0.73$ ,  $p < 0.01$ ,  $n = 24$ ) of the 2nd and 3rd experiments. Fractions of hydrophobic bacteria ranged from 0% to 28% and from 22% to 42% in the 2nd experiment and 3rd experiment (Figs 2 & 3), respectively, indicating that hydrophilic bacteria seemed to dominate bacterial community in the bulk-phase water examined.

## Discussion

A noteworthy result from this study is that bacterial

surface colonization seems to be dominated by adhesion of bacteria rather than growth of adhered bacteria on the surfaces during the early phase (< 6 d) of bacterial surface colonization. With time, growth of adhered bacteria seems to become a significant contributor to colonization of surfaces, as indicated by observations that differences between numbers of total bacteria and total microcolonies started to diverge after 1 d and then remained relatively constant afterwards (Figs 1–4A). Interestingly, in the very early phase (< 1 d) of bacterial surface colonization, adhesion rates seem to keep increasing. This trend was, especially, clearly observed on acryl surface. Since we intermittently shook the containers and no continuously increasing flow conditions were observed during the *in situ* study, the increasing rates of attachment within 1 d of bacterial colonization could not be due to increasing hydrodynamic conditions which would increase the transport of bacteria to surfaces. The increased adhesion rates during the very early phase (< 1 d) of bacterial surface colonization (Figs. 2–4) suggest that adhesion of initial colonizers to surfaces increased attachments of next colonizers (Vandevivere and Kirchman, 1993). Thus, positive cooperative interactions among initial colonizers might be responsible for the increased adhesion of the next colonizers.

Specific accumulation rates of microcolonies changed widely during the microbial film formations, often with initial increases and subsequent decreases to near zero rates. This trend in changes of accumulation rates of microcolonies could be related to nutrient depletion (Ford *et al.*, 1989; Zheng *et al.*, 1994) or accumulation of metabolic wastes on surfaces during the development of microbial films. Numbers of colonized bacteria on surfaces in our study often showed temporary saturation after 2–6 d immersion. Although reasons for it are unclear, the temporary saturation of bacteria for some periods of incubation seems to widely occurs (Fera *et al.*, 1989; Wolfaardt *et al.*, 1991) before bacterial density increases again. Our estimates of accumulation rates are the net results of bacterial growth on the surface and removal rates of attached bacteria. Thus, with measurements of *in situ* bacterial growth (e.g. <sup>3</sup>H-thymidine incorporation method) in microbial films we might estimate removal rates of bacteria from the surface. Growth rates of adhered bacteria were not measured in this study, but biovolumes of attached bacteria were always greater than those of the bulk-phase water bacteria (Fig. 5). Recently, Gasol *et al.* (1995) reported that large bacteria were more active and grew faster than small bacteria. It seemed thus that adhered bacteria would maintain

active growth and metabolisms during the development of microbial films in this study.

Quantitative studies on adhesion and accumulation rates of natural marine bacteria are not many. Adhesion rates of 20-800 bacteria  $\text{mm}^{-2} \text{h}^{-1}$  were reported for aluminium, stainless steel and polycarbonate filter surfaces for the first 1 h of incubation in seawater (Fera *et al.*, 1989). Zheng *et al.* (1994) reported attachment rates of ca. 50-140 bacteria  $\text{mm}^{-2} \text{h}^{-1}$  to Pyrex surface. Our adhesion rates of bacteria to acryl surface for the first 1 d immersion ranged from negligible to 543 bacteria  $\text{mm}^{-2} \text{h}^{-1}$ , and are comparable to the reported ranges. Areal density of bacteria on aluminium plate submerged in a seawater flow system was ca.  $1 \times 10^3$  bacteria  $\text{mm}^{-2}$  at 3 d and  $1 \times 10^3$  bacteria  $\text{mm}^{-2}$  after 6 d, respectively (Fera *et al.*, 1989). In a study using a two-stage continuous culture, Zheng *et al.* (1994) showed that natural marine bacteria could accumulate on Pyrex surfaces ca.  $1-16 \times 10^2$  bacteria  $\text{mm}^{-2}$  at 0.5 d depending on laminar flow rates. Areal density of bacteria on metals (copper alloys, titanium and stainless steel) exposed in an Arctic river for 3 d and 6 d showed ranges of  $1-4 \times 10^3$  bacteria  $\text{mm}^{-2}$  and  $4-11 \times 10^3$  bacteria  $\text{mm}^{-2}$ , respectively (Ford *et al.*, 1989). In Woods Hole harbor a minimum estimate of  $1.2 \times 10^6$  bacteria  $\text{mm}^{-2}$  on glass was observed in summer after 168 h immersion by scanning electron microscopy (Dexter *et al.*, 1975). Our results of areal bacteria density ( $4-10.7 \times 10^3$  and  $15.8-46.4 \times 10^3$  bacteria  $\text{mm}^{-2}$  after 6 d on glass and acryl surface, respectively) are generally within the ranges reported for natural bacteria colonized on diverse substrates. A huge difference between ours and Woods Hole harbor samples might be in part due to more eutrophic conditions in the latter. Adhesion rates changed obviously in a close relation with changes of total bacteria (or microcolony) numbers on the surfaces during the early (< 6 d) development of microbial film formations in our study. Adhesion rates did not either correlate with bacterial abundance, or bacterial growth rates of the bulk-phase water. Zheng *et al.* (1994) suggested that variation of bacterial abundance from  $1.7 \times 10^6$  to  $8.5 \times 10^6$  bacteria  $\text{ml}^{-1}$  did not cause significant effects on variations of attachment rates. Bacterial growth rates of the bulk-phase water might be related to bacterial cell surface characteristics as shown in this study, but bacterial surface hydrophobicity did not seem to be related to attachment rates. This suggests that substratum-surface properties might change with time during microbial film formations, and dynamic interactions of substrate surface's properties and bacterial surface characteristics would determine the attachment

rates of bacteria for a given moment. A recent study showed that most isolates of adhered bacteria were hydrophilic (Sonak and Bhosle, 1995). Especially, for the 1st day of immersion 78% of bacterial isolates were hydrophilic (Sonak and Bhosle, 1995). Since hydrophilic bacteria were dominant in the bulk-phase water, it might be possible that cooperative interactions among hydrophilic initial colonizers during the very early phase of colonization might contribute to surface hydrophilicity.

Interestingly, fractions of hydrophobic bacteria in bacterial community showed significant relationships with growth rates of bacteria in the bulk-phase water. This information is new as far as we know. It seems that fraction of hydrophobic bacteria in bacterial community increases when bacterial community grows faster. But, we should note that majority of bacterial community was composed of hydrophilic bacteria in this study. Since hydrophobicity is thought to be involved to bacterial attachment (Van Loosdrecht *et al.*, 1990; Rosenberg *et al.*, 1991) and survival advantage of starved bacteria (Kjelleberg and Hermansson, 1984), relative increases of hydrophobic bacteria in growing bacterial community rather surprising. Oligotrophic bacteria are reported to be more adhering to surfaces than copiotrophic bacteria (Kjelleberg *et al.*, 1985). Possibly, growth conditions of seawater in this study might favored growths of hydrophobic and oligotrophic bacteria. Further studies on hydrophobicity of growing marine natural bacteria needs to be made. Also, microbial ecological aspects of surface colonization by natural microbes, i.e. microbial interactions, exoenzyme activities, material cycles and energy flow in microbial films are deserved to future studies (Denyer *et al.*, 1993; Melo, 1992; Freeman and Lock, 1995) to understand the processes of and regulate the formations of biofouling.

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## References

- Ammerman, J. W., J. A. Fuhrman, A. Hagstrom, and F. Azam. 1984. Bacterioplankton growth in seawater: I. Growth kinetics and cellular characteristics in seawater cultures. *Mar. Ecol. Prog. Ser.* 18: 31-39.

- Cooksey, K. E. and B. Wigglesworth-Cooksey. 1995. Adhesion of bacteria and diatoms to surfaces in the sea: a review. *Aq. Microb. Ecol.* 9: 87-96.
- Denyer, S. P., S. P. Gorman, and M. Sussman. 1993. *Microbial biofilms: formation and control*. Blackwell Scientific Publications.
- Dexter, S. C., J. D. Sullivan, Jr., J. Williams III, and S. W. Watson. 1975. Influence of substrate wettability on the attachment of marine bacteria to various surfaces. *Appl. Environ. Microbiol.* 30: 298-308.
- Fera, P., M. A. Siebel, W. G. Characklis, and D. Prieur. 1989. Seasonal variations in bacterial colonization of stainless steel, aluminum and polycarbonate surfaces in a seawater flow system. *Biofouling* 1: 251-261.
- Ford, T. E., M. Waich, R. Mitchell, M. J. Kaufman, J. R. Vestal, S. A. Dimer, and M. A. Lock. 1989. Microbial film formation on metals in an enriched arctic river. *Biofouling* 1: 301-311.
- Freeman, C. and M. A. Lock. 1995. The biofilm polysaccharide matrix: A buffer against changing organic substrate supply? *Limnol. Oceanogr.* 40: 273-278.
- Fuhrman, J. A. and F. Azam. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface seawaters: evaluation and field results. *Mar. Biol.* 66: 109-120.
- Gasol, J. M., P. A. del Giorgio, R. Massana, and C. M. Duarte. 1995. Active versus inactive bacteria: size-dependence in a coastal marine plankton community. *Mar. Ecol. Prog. Ser.* 128: 91-97.
- Geesey, G. G., Z. Lewandowski, and H-C. Flemming. 1994. *Biofouling and biocorrosion in industrial water systems*. Lewis Publishers.
- Gerhart, D. J., D. Rittschof, I. R. Hooper, K. Eisenman, A. E. Meyer, R. E. Baier, and C. Young. 1992. Rapid and inexpensive quantification of the combined polar components of surface wettability: application to biofouling. *Biofouling* 5: 251-259.
- Kjelleberg, S. and M. Hermansson. 1984. Starvation-induced effects on bacterial surface characteristics. *Appl. Environ. Microbiol.* 48: 497-503.
- Kjelleberg, S., K. C. Marshall, and M. Hermansson. 1985. Oligotrophic and copiotrophic marine bacteria-observations related to attachment. *FEMS Microbiol. Ecol.* 31: 89-96.
- Little, B. J. 1984. Succession in microfouling, p. 63-67. In J. D. Costlow and R. C. Tipper (eds.), *Marine biodeterioration: an interdisciplinary study*. US Naval Institute Press.
- Malone, J. A. and D. E. Caldwell. 1983. Evaluation of surface colonization kinetics in continuous culture. *Microb. Ecol.* 9: 299-305.
- Melo, L. F. 1992. *Biofilms-science and technology*. Kluwer Academic Publishers.
- Porter, K. G. and Y. S. Feig. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* 25: 943-948.
- Rosenberg, M., M. Barki, R. Bar-Ness, S. Goldberg, and R. J. Doyle. 1991. Microbial adhesion to hydrocarbons (MATH). *Biofouling* 4: 121-128.
- Sonak, S. and N. Bhosle. 1995. Observations on biofilm bacteria isolated from aluminium panels immersed in estuarine waters. *Biofouling* 8: 243-254.
- Vandevivere, P. and D. L. Kirchman. 1993. Attachment stimulates exopolysaccharide synthesis by a bacterium. *Appl. Environ. Microbiol.* 59: 3280-3286.
- Van Loosdrecht, M. C. M., J. Lyklema, W. Norde, and A. J. B. Zehnder. 1990. Influence of interfaces on microbial activity. *Microbiol. Rev.* 54: 75-87.
- Wicks, R. J. and R. D. Robarts. 1987. The extraction and purification of DNA labeled with [methyl-<sup>3</sup>H]thymidine in aquatic bacterial production studies. *J. Plankton Res.* 9: 1159-1166.
- Wolfaardt, G. M., R. E. M. Archibald, and T. E. Cloete. 1991. The use of DAPI in the quantification of sessile bacteria on submerged surfaces. *Biofouling* 4: 265-274.
- Zheng, D., G. T. Taylor, and G. Gyananath. 1994. Influence of laminar flow velocity and nutrient concentration on attachment of marine bacterioplankton. *Biofouling* 8: 107-120.