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Enrichment and Association of Bacteria and Particulates in Salt Marsh Surface Water

R. W. HARVEY AND L. Y. YOUNG*

Environmental Engineering and Science, Department of Civil Engineering, Stanford University, Stanford, California 94305

Elevated counts of bacteria were found during outgoing tides in surface microlayers (~300 μm) of Sippewissett salt marsh, Falmouth, Massachusetts, and Palo Alto salt marsh, Palo Alto, California. At both sampling sites, the degrees by which bacteria were concentrated into the surface microlayer were linearly dependent upon surface concentration of particulate material. A significant percentage of bacteria in the microlayer were found to be attached to particulate material, while bacterial populations in the subsurface water were largely planktonic. Proportions of the bacterial populations which could be grown on seawater nutrient agar were also greater in the microlayer than in the subsurface waters and were positively correlated with the fraction of bacteria attached to particulate matter. Data from these studies suggest that particulates in the microlayer waters of the salt marsh influenced the observed increase in both the readily grown and the total numbers of bacteria.

Although elevated counts of bacteria at the surface microlayer of the open ocean have been reported by many authors (18, 24, 25, 28), there is evidence that these surface layer bacteria (bacterioneuston) are, in general, less active than the subsurface populations. For example, Dietz et al. (6) reported elevated bacterial counts in the marine surface microlayer but lower adenosine triphosphate concentrations, although it is unclear what portion of these measurements corresponded solely to bacterial cells. Calculated heterotrophic potential and activity per colony-forming unit were only 33% and 10%, respectively, of that for the subsurface. Marumo et al. (18) noted the surface film microbiota of Pacific equatorial waters to be in poor physiological condition, which he largely attributed to intense solar radiation.

There have been few reports on heterotrophic activity in salt marsh microlayers. However, it is reported that a significant portion of the net planktonic productivity of the salt marsh takes place within this surface layer (8). Preliminary studies done on the Palo Alto salt marsh demonstrated that, in contrast to open ocean systems, significant elevations of heterotrophic bacteria that readily grew on seawater nutrient agar plates did occur in the surface layer (15). This was most pronounced during periods of high and outgoing tides. There also appeared to be a positive correlation between the number of these plate count bacteria in the surface microlayer and the number of particles at the surface.

The large amounts of organic particulate material in the salt marsh microlayer are likely to have significant ecological consequences for microbial populations there. Taguchi and Nakajima (27) found particulate organic carbon in the surface layer of a Japanese inlet to be enriched by a maximum of 17.6. Valiela et al. (29) reported that particulate carbon exported to coastal waters from Great Sippewissett Marsh accounted for some 40% of the net annual production of the dominant macrophyte, *Spartina alterniflora*. It has been reported that the litter of this plant is capable of directly sorbing onto the microlayer (20). Microscopic observations of surface particulates from the marsh indicate a number of algal fragments as well (15). This report examines the relationship between particulate material and populations of surface layer bacteria in a salt marsh.

MATERIALS AND METHODS

Surface microlayer and subsurface waters were sampled at regular intervals from two tidal creek sample sites, one located in the Sippewissett salt marsh, Falmouth, Mass., and the other in the Palo Alto salt marsh, Palo Alto, Calif. These two salt marshes exhibit significant differences in vegetation. The Sippewissett salt marsh is characterized by a single dominant macrophyte, *S. alterniflora*, whereas the Palo Alto salt marsh contains three codominant forms of vegetation: a pickleweed *Salicornia virginia*, a salt grass *Distichlis spicata*, and a cord grass *Spartina foliosa*. Both the Sippewissett samples (taken 22 July 1977) and Palo Alto samples (taken 2 May 1978) were obtained over a portion of the outgoing tide, when surface slicks were observed. Samples taken 22 July 1977 from

the Sippewissett marsh were obtained from a tidal creek which served as a tributary to the main channel. The Palo Alto samples were taken from the main tidal creek at the intersection of a boardwalk approximately 60 m from the outlet to San Francisco Bay. Sampling commenced 15 min before high tide and continued every 20 min. In addition, samples were taken from the main Sippewissett marsh channel near its confluence with Buzzards Bay on 30 June 1977, when no microlayer was observed. The lack of a visible microlayer was attributed to high winds and water turbulence.

Each surface layer sample and corresponding subsurface sample was assayed for plate count, total bacterial concentrations, and percentages of bacterial cells attached to particulate material. In addition, Palo Alto samples were assayed for particulate concentration and size distribution. Surface microlayer samples were collected with an 0.3 m^2, 18-mesh polyethylene screen, by a method described by Garrett (9). With this procedure, it is estimated that the upper 150 to 300 μm of water surface is collected. Plastic gloves were worn to prevent sample contamination. Subsurface samples were taken at 0.2-m depth. All samples were collected in sterilized glass sample bottles and kept on ice until processed. Subsamples to be used for total counts of bacteria and particulates were fixed in the field with 0.2% (final volume) glutaraldehyde.

Plate count bacteria were determined with standard spread plate procedures. Appropriate sample dilutions were plated out on seawater nutrient agar within 8 h of collection and incubated at 30°C for 48 h.

Total bacterial counts and numbers of bacteria attached to particulate material were assayed within 48 h of collection by the acridine orange epifluorescent technique described by Hobbie et al. (13). Counts were made on Nuclepore filters stained with irgalen black and observed under a Leitz Ortholux epifluorescence microscope for Sippewissett salt marsh samples and an Olympus Vannox epifluorescence microscope for Palo Alto marsh samples. Because of their distinctive shapes and color of fluorescence, bacterial cells were easily distinguished from the particulates to which they were attached. All bacterial counts were made to within ±10% at the 90% confidence level by published schedules of precision (19).

Particulate concentrations and size distributions were determined for samples from the Palo Alto site with a model TA II counter (Coulter Electronics, Inc., Hialeah, Fla.). A 200-μm aperture, responding to particle diameters between 2.5 and 100 μm, was used for all counts and was not sensitive to bacterial-sized objects less than 2.5 μm in diameter.

RESULTS

Bacterial counts. Total counts and plate count data for the surface layer and subsurface waters are summarized in Table 1 for the two salt marshes studied. In all samples, the numbers of plate count bacteria were several orders of magnitude lower than the total counts. This is partly attributable to the procedure itself; we recognize that growth on the seawater nutrient

TABLE 1. *Bacteria in the surface and subsurface waters of Sippewissett and Palo Alto salt marshes*

Salt marsh (n = 10 samples)	Total no. (per ml)[a]			Plate counts (per ml)[b]			Bacteria associated with particles (%)[a]	
	Surface (range)	Subsurface (range)	Surface concn factor (S_1/S_2)[c]	Surface (range)	Subsurface (range)	Surface concn factor (S_1/S_2)[c]	Surface (range)	Subsurface (range)
Sippewissett	$6.59^d \pm 2.42 \times 10^7$ ($2.59–11.2 \times 10^7$)	$1.99 \pm 0.44 \times 10^7$ ($1.34–2.85 \pm 10^7$)	3.42	$4.54^d \pm 4.75 \times 10^5$ ($0.42–13.9 \times 10^5$)	$1.43 \pm 0.48 \times 10^4$ ($0.80–2.20 \times 10^4$)	30.0	$24.3^d \pm 10.6$ (11.2–42.4)	1.27 ± 0.91 (0.26–2.93)
Palo Alto	$15.5 \pm 7.67 \times 10^6$ ($7.73–35.4 \times 10^6$)	$9.20 \pm 0.62 \times 10^6$ ($8.31–10.4 \times 10^6$)	1.66	$5.23 \pm 5.96 \times 10^4$ ($0.23–19.1 \times 10^4$)	$0.75 \pm 0.59 \times 10^4$ ($0.18–2.30 \times 10^4$)	7.44	43.8 ± 28.2 (8.50–87.2)	4.60 ± 2.20 (2.30–10.8)

[a] Determined by acridine orange epifluorescence. Counts on individual samples made to ± 10% at the 90% confidence level.
[b] Standard serial dilution and spread plate procedure on seawater nutrient agar.
[c] Abbreviations: S_1, surface; S_2, subsurface.
[d] Data presented as mean ± standard deviation for the entire sample population.

agar detects a selected population of bacteria, e.g., those able to grow rapidly on the media provided. Discussion of these data, therefore, takes this into consideration, and comparisons are made only on those data gathered with the same procedure.

For the Sippewissett samples, surface microlayer concentrations for both total and plate count bacteria varied with the tidal cycle, ranging from ~3 to 11 × 10^7 per ml and 0.42 to 14 × 10^5 per ml, respectively (Table 1). These values were significantly higher than those from corresponding subsurface samples. Calculated surface concentration factors show total counts to be more than 3 times higher in surface waters whereas plate counts were 30 times higher.

Data from the Palo Alto site also exhibited a high degree of variability in surface layer counts, less variability in the subsurface samples, and a larger population in the surface layer for both total and plate count bacteria. The levels of the surface layer bacteria (both total and plate count) steadily decreased with time after high-tide, corresponding to the apparent dispersion of the compressed microlayer. At both sites the surface concentration factor was significantly larger for the plate count data than for the total count (30 and 7.4 compared with 3.4 and 1.7, respectively).

Bacteria and particulates. Percentages of bacteria found attached to particulate matter for the two salt marshes are also shown in Table 1. These percentages, determined by acridine orange epifluorescent technique, were significantly higher in the surface microlayer than in the subsurface for both sites. The level of particle-bound bacteria was found to be approximately 20 times higher at the surface for Sippewissett and 10 times higher for Palo Alto.

Only ~1 to 5% of the bacteria in the subsurface waters were particle bound. Higher percentages of bacteria were particle bound in both surface and subsurface waters at Palo Alto than at Sippewissett.

The surface concentration factors enumerated in Table 1 and shown in Fig. 1 are computed as the ratio of bacterial numbers in the surface to those in the subsurface. As illustrated in Fig. 1, the surface concentration factor is positively correlated with the percent bacteria bound to particles at the surface. The correlation coefficient r was 0.91 for the Sippewissett site (Fig. 1A) and 0.89 for the Palo Alto site. The Sippewissett data included samples taken on a day when little or no elevation in surface counts of bacteria was found to occur and few of them were attached to particles (lower end of the curve). On the other hand, surface samples corresponding to high concentration factors contained significant

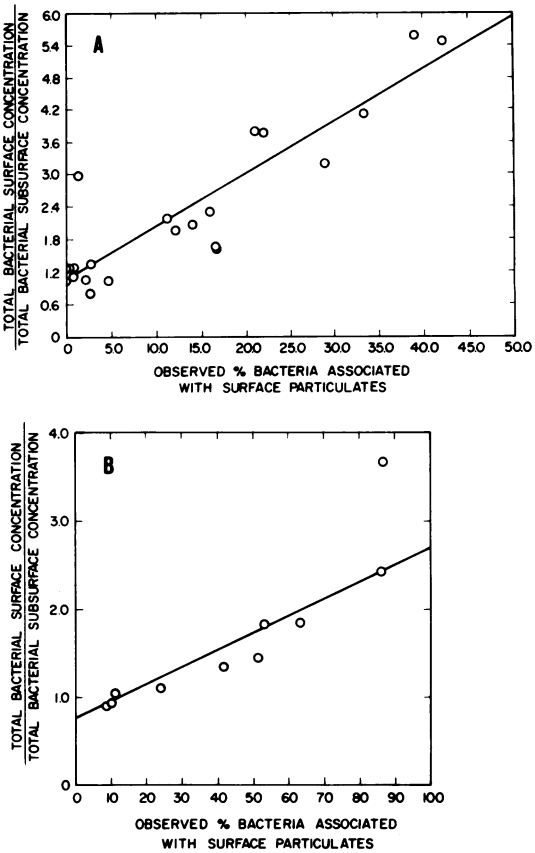

FIG. 1. *Surface concentration factors, shown as the ratio of total bacteria in the surface to total bacteria in the subsurface versus observed percentage of bacteria associated with particles at the surface. (A) Sippewissett marsh, n = 23, r = 0.91. (B) Palo Alto marsh, n = 10, r = 0.89.*

numbers of particle-bound cells. Therefore, as the fraction of particle-bound bacteria increases, the surface enrichment of bacteria also increases.

Figure 2 illustrates the relationship between the surface enrichment of plate count and total bacteria, and that for particles. As illustrated in the figure, the slope of the line relating surface enrichment of plate count bacteria (m = 0.31) is significantly steeper than that for total count bacteria, suggesting that as the enrichment of particles in the surface increases, there is a greater influence on the surface enrichment of readily grown plate count bacteria compared with that for total count bacteria.

The ratio of plate count bacteria to total counts in the surface layer for the Palo Alto samples and the percent of bacteria attached to particles for each sample are illustrated in Fig. 3. These plate count-to-total count ratios were

highest in samples with the highest proportion of particle-bound cells, also suggesting that association with particles positively influences the numbers of readily grown plate count bacteria.

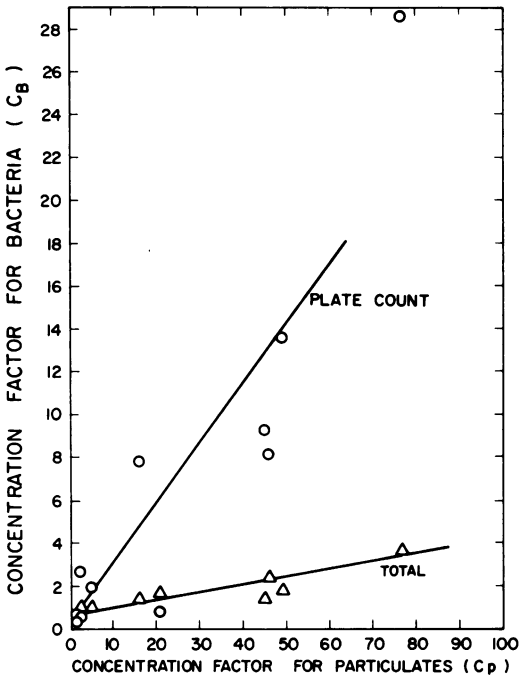

FIG. 2. *Surface concentration factor for bacteria versus that for particulates for readily grown plate count bacteria* (○) *and for total count bacteria* (△). *n = 10 for each set of samples.* C_P = *surface concentration of particulates/subsurface concentration of particulates.* C_B = *surface concentration of bacteria/subsurface concentration of bacteria.*

DISCUSSION

The data in Table 1 indicate that plate count and total numbers of bacteria are markedly elevated in the surface microlayer habitat (18, 24, 25, 28). We believe this is the first such report specifically involving the salt marsh environment. The higher concentration factors calculated for plate count populations than for total counts (Table 1) suggest that the proportion of the surface population that is readily grown and, thus, metabolically active is greater than that in the subsurface. Although the plate count procedure is limited in the numbers and types of active bacteria detectable, it was quite useful for the purpose of assaying relative differences between surface layer and subsurface populations. No attempt was made to determine all the metabolically active bacteria. When detection of such organisms is desired, other techniques, such as heterotrophic uptake (30), microautoradiography (14), or the use of INT [2-(*p*-iodophenyl-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride] dye, (31) facilitate the determination of organisms which cannot be cultured on standard nutrient media.

The linear relationship between the surface concentration factors for bacteria in the surface and the percentages of surface layer bacteria attached to particles (Fig. 1A and B) was found at both salt marshes and, hence, was not site specific. The facts that the percentage of particle-bound bacteria increased as the surface con-

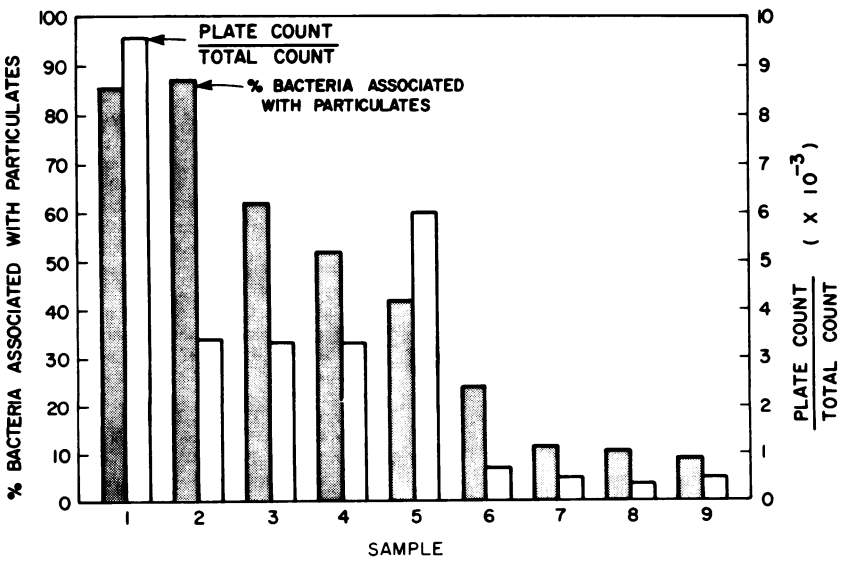

FIG. 3. *Ten samples taken at Palo Alto marsh showing for each the ratio of plate count to total count bacteria and percentage of bacteria associated with particulates.*

centration factors increased and that a higher proportion of all surface-collected bacteria were particle bound than were the bacteria in the subsurface water (Table 1) suggest that particulates can influence microbial activity in the microlayer environment. High numbers of particles can add a significant amount of available surface area for passive and active attachment of bacterial cells. As the surface enrichment of particles increased, however, a greater increase in the enrichment of readily grown bacteria than in total cells was observed (Fig. 2). This, therefore, suggests that particulates can have additional effects on bacteria other than just as a site for adsorption. Known stresses in the surface waters, such as solar radiation (18, 28), may be attenuated by particulates, and predator-prey interactions are observed to be lessened by the microorganism attaching to colloidal particles (22). Recently, Roper and Marshall (23) showed that the numbers of bacteria surviving in the presence of sediment particulates were 2 to 3 orders of magnitude greater than in seawater alone.

Air-water interfaces have long been observed to collect higher concentrations of microorganisms (1–4, 12). DiSalvo (7) reported that the number of bacteria attaching to a glass surface in San Francisco Bay was 1,000 times higher in the surface layer than in subsurface waters, despite the fact that the total numbers in the microlayer were only about 10 times higher. That the bacterioneustons in the surface waters have greater attachment capabilities may be one factor contributing to a significantly greater percentage of particle-bound surface bacteria than of bacteria in the subsurface (Table 1). In addition, it is thought that the solid-water interface is conducive to bacterial proliferation (5, 16, 17). Others report that microorganisms attached to particles have a higher metabolic activity than the planktonic counterparts (11, 21, 26). Recently, Kirchman and Mitchell, using [^{14}C]glucose incorporation, found uptake values of particle-bound bacteria to be an order of magnitude higher than for free bacteria (Annu. Meet. Am. Soc. Limnol. and Oceanogr., 42nd, Stony Brook, N.Y., 1979). Furthermore, investigators working in a Georgia salt marsh have reported 80% of the heterotrophic activity to be associated with >3 μm detritus (10). These reports are corroborated by our data, which show surface enrichment of plate count bacteria (Fig. 2) and a higher proportion of readily grown plate count bacteria in the total population associated with higher percentages of particle-bound bacteria (Fig. 3). In addition, our recent studies indicate a positive correlation between the proportion of respiring bacteria determined by an INT dye procedure (31) and the proportion of particle-bound bacteria (Harvey and Young, manuscript in preparation). Particulates in the microlayer, therefore, may indeed be promoting bacterial activity.

In addition to ameliorating stress factors at the surface, particulates may themselves be a source of nutrients for the bacterioneuston. Although our present results cannot address this, the data do point out that in salt marsh habitats, the surface microlayer contains a higher proportion of bacteria which are readily grown than does the subsurface water, that a significant fraction of them is attached to particles, and that a greater proportion of particle-bound bacteria are also at the surface. These large numbers of both particles and particle-bound bacteria found at the surface layer may have an effect upon nutrient turnover and transport in the salt marsh habitat and raise an issue which warrants further study.

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