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Heterotrophic Bacteria of the Freshwater Neuston and Their Ability to Act as Plasmid Recipients under Nutrient Deprived Conditions

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Abstract. Significantly higher numbers of Gram-negative heterotrophic bacteria were present at the air–water interface (neuston) of freshwater lakes than in the bulk water. Neuston bacteria were distinguished as a population distinct from bacteria in the bulk water by a higher incidence of pigmented colony types and significantly greater levels of multiple resistance to antibiotics and heavy metals. The incidence of plasmids in 236 neuston and 229 bulk water strains were similar (14 and 16.2%, respectively). Nine of 168 plasmid-free strains and 2 of 14 plasmid carrying strains, isolated from both bulk water and neuston, acted as recipients of plasmid R68.45 in plate matings with a *Pseudomonas aeruginosa* donor strain PAO4032 at 21°C, but at frequencies below that of matings with a restriction-minus recipient strain of *P. aeruginosa,* strain PAO1168. In a model system composed of nutrient-free synthetic lake water, plasmid R68.45 was shown to transfer between *P. aeruginosa* strains at frequencies between 10^{-3} and 10^{-5}. Transconjugants were detected about 100 times more frequently at the interface than in the bulk water, which in part reflected a greater enrichment of the donor at this site. None of the aquatic isolates were able to act as recipients of plasmid R68.45 in this model system with strain PAO4032 as donor. The results suggest that under nutrient deprived conditions, the spread of plasmid R68.45 and similar plasmids by lateral transfer into this particular aquatic population would be a rare event.

Introduction

The air–water interface constitutes a surface with physical and chemical properties very different from those of the bulk water [17, 21], which in marine areas has been shown to harbor a unique bacterial flora [11]. It has been

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proposed that the distinguishing characteristics of the surface microflora of marine environments (significantly higher incidences of pigmented and multiple antibiotic resistant bacteria) may reflect mechanisms that protect cells against lethal solar radiation and accumulated antimicrobial substances in the surface film [11]. Moreover, the higher frequency of plasmids in interface bacteria may be the result of these populations accumulating plasmid genes that provide functions that enhance microbial survival at the air–water interface [11]. If this were the case, then effective mechanisms for the acquisition of plasmids may exist within the bacterial flora of the neuston and these organisms could, therefore, act as reservoirs for introduced plasmids.

Plasmid transfer between bacteria is known to occur in aquatic environments. Plasmid transfer between bacteria has been observed within the epilithon of rivers [1], within submerged chambers [22], and between *Escherichia coli* strains in both wastewater [8] and sediment [25]. Surfaces and interfaces may be important sites of microbial conjugation in aquatic locations, because microbial populations are often denser on surfaces [18], and the majority of conjugative plasmids, particularly those that encode rigid pili are more efficiently, if not exclusively, transferred between cells on surfaces [5].

If the air–water interface in freshwater systems harbors heterotrophic bacteria distinct from those of the bulk water, then this population must be characterized before meaningful studies on the influence of the interface on plasmid transfer between cells localized at the surface film can be accomplished. Accordingly, the existence of unique heterotrophic bacterial populations at the air–water interface of freshwater systems was first investigated, and the Gram-negative isolates were tentatively assigned to one of seven groups. Subsequently, the conjugal transfer of the broad host-range IncP group plasmid R68.45 from *P. aeruginosa* to representative aquatic isolates was measured at the air–water interface of a model aquatic system. This model aquatic system utilized synthetic lake water to overcome variations in the qualities of natural lake waters [19].

Methods

Isolation and Characterization of Freshwater Bacteria

Two Lake Michigan stations (Garden Peninsular and Benton Harbor) and a Lake Superior station at Marquette were sampled during June and July of 1988. Three local lakes (Whitmore, Independence, and Geddes) that differ in usages and development were examined at intervals from October 1987 to August 1988.

Near-shore, air–water interface samples were obtained with 10 Teflon sheets [15]. Each Teflon sheet had a surface area of 63.6 cm^2 and lifted an average of 25 μl of air–water interface film, giving an average sample depth of about 4 μm. Bulk water samples were taken at a depth of 0.5 m with a bottle sampler (Grab Sampler, Fisher) at the same site. Dilutions were plated onto a standard plate count (SP) agar (which contained, per liter: 5 g Bacto Tryptone, 2.5 g Bacto Yeast Extract, 1 g glucose, 15 g Bacto Agar, final pH 7.0) [23] and onto SP agar containing 5 μg/ml of either novobiocin (Nb), nalidixic acid (Nx), ampicillin (Ap), streptomycin (Sm), chloramphenicol (Cm), tetracycline (Tc), carbenicillin (Cb), or kanamycin (Km), and onto SP agar containing 20 μg/ml of either $ZnCl_2$ (Zn), $CuSO_4$ (Cu), $CoCl_2$ (Co), $KCrO_4$ (Cr), $HgCl_2$ (Hg), or $NiCl_2$ (Ni). The inclusion of antibiotics and heavy metals allowed for the identification of subpopulations that

would be overlooked on SP agar. Plates were counted and the pigmentation of colonies evaluated as described [11] after incubation for 3 days at 21°C. Isolates, representing different colony and/or pigmentation types in a sample, were purified on SP agar and tested for oxidase, the oxidation/fermentation of glucose and motility, and then stored in 50% glycerol at −70°C.

All isolates were examined for plasmids by the method of Kado and Liu [13]. Minimal inhibitory concentrations (MIC) for the antibiotics and metal ions given above were determined by replica plating onto SP agar containing either 5, 15, 50, or 100 μg/ml of an antibiotic, or 20, 50, 100, or 200 μg/ml of a heavy metal salt.

Bacterial Strains and Plasmids

The broad host-range IncP-1 group plasmid R68.45 (Tc^r, Cb^r, Km^r, Tra^+) [10] was used throughout these studies. *P. aeruginosa* strain PAO4032 (*catA1, met-9020, nar-9011, mtu-9002, tyu9030*) was obtained from R. Olsen, University of Michigan, and used as the standard donor of plasmid R68.45 throughout these experiments. The restriction-negative *P. aeruginosa* strain PAO1168 (*leu-38, hsdR15,* $FP2^+$) was provided by B. W. Holloway (Monash University, Australia) and used as the recipient. All other bacteria used as recipients were isolated from freshwater lakes in Michigan as described above. Cultures were routinely grown on SP agar, or in SP broth at 21°C.

Conjugation

All conjugation experiments were done at 21°C. Plate conjugation was carried out on SP agar by spreading a 100 μl volume of a mixed suspension of the donor and the recipient strains over an area of about 5 cm^2, followed by incubation for 18 hours. M9 minimal media containing methionine or leucine (100 μg/ml) were used for the selection of the donor strain PAO4032 and the recipient strain, PAO1168, respectively. Transconjugants of strain PAO1168 were selected on M9 media containing leucine, Tc (25 μg/ml), Cb (125 μg/ml), and/or Km (50 μg/ml). Aquatic isolates were recovered from mating mixtures either on M9 medium or on SP agar containing an antibiotic or heavy metal to select against the donor strain PAO4032. Transconjugants of water isolates were selected at three times the MIC of the recipient for Tc, Cb (or Ap), and/or Km. All putative transconjugants were reexamined for resistance to Tc, Cm, and Km, and for the presence of plasmid R68.45.

Conjugation experiments were done in synthetic lake water (SLW), the composition of which was based on the inorganic components of Lake Michigan water present at greater than 10^{-7} M [24]. SLW contained per liter of double distilled water: $NaHCO_3$, 19.7 mg; $KHCO_3$, 3.2 mg; $CaCO_3$, 86 mg; $MgCO_3$, 38 mg; HCl, 0.31 ml of a 1 M solution; H_2SO_4, 0.5 ml of a 0.5 M solution. After autoclaving, the salts were dissolved by sparging with filter-sterilized CO_2 to give a pH less than 5.5, followed by sparging overnight with filter-sterilized air. After equilibration with air, the pH was 8.2 and the conductivity 200 to 220 μmhos, similar in both respects to Lake Michigan [24] and to the local lakes examined (unpublished data). Plate counts showed that the bacteria used in these studies remained 100% viable in SLW for the duration of the test period, and survival in SLW was comparable with survival in ^{60}Cobalt sterilized water samples from lakes Superior, Geddes, and Whitmore (data not shown).

Twice washed donor and recipient bacteria were mixed in SLW in glass pots (15.9 cm^2 by 3.5 cm deep, Kimax Brand, Fisher) to give a final concentration of about 1×10^7/ml of each. After an 18 hour incubation at 21°C, the surface films were obtained with Teflon sheets (4.5 cm in diameter) and bulk samples taken at a depth of 1.5 cm. Volumes (100 μl) of each sample were plated as described above for the enumeration of donors, recipients, and transconjugants. Organic surface films composed of oleic acid, yeast extract, and peptone [16] were used for some experiments.

Nalidixic acid was not used to control for conjugation on the isolation plates because mutants more resistant to nalidixic acid than the donor strain, PAO4032, were rarely obtained from the aquatic isolates. Therefore, controls were set-up in the following way: Separate suspensions (1×10^7/ml) of donors and recipients were incubated in SLW in parallel with the mating test. Surface

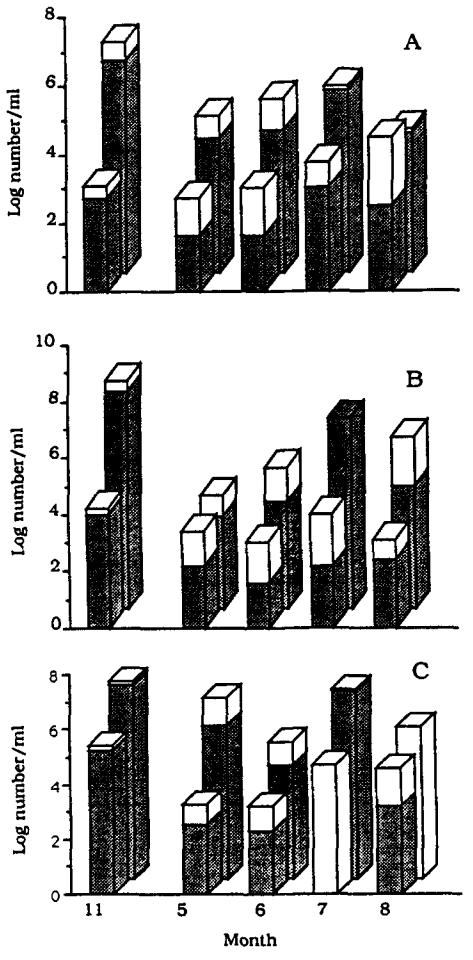

Fig. 1. Distribution of heterotrophic bacteria at the air–water interface and in the bulk waters of three freshwater lakes. Open bars show the numbers (log_{10}) of bacteria per ml of paired surface and bulk samples obtained from November 1987 (month 11) to August 1988 (month 8); shaded areas represent the numbers (log_{10})/ml of pigmented colony types in that population. The front column represents the bulk sample and the back column the surface sample. **A** Independence; **B** Whitmore; **C** Gedde lakes.

Table 1. Differentiation of heterotrophic bacterial populations of the air–water interface and the bulk water by primary isolation on media containing single antibiotics or heavy metals

Station	Site[a]	Percentage resistant to:[b]							
		Nx	Nb	Ap	Sm	Cm	Tc	Cb	Km
Michigan	S	67	7.1	24	36	12	0	9.4	82
(Benton Harbor)	B	0.7	5.9	1.7	3.1	0.8	0	1.4	2.6
Independence	S	0	7.0	24	0	14	0	11	0
	B	0	0	0	0	0	0	63	30
Geddes	S	0	100	57	63	57	66	82	1.3
	B	0	100	0	0	0	0	0	3.6

[a] S, surface; B, bulk samples
[b] Viable counts on SP agar containing 5 μg/ml antibiotic or 20 μg/ml metal ions as a percentage of the total viable counts on SP agar

and bulk water samples obtained as described were counted. Volumes (50 μl) of the surface and the bulk water samples of the donor and of the potential recipient were then mixed immediately on the plates used for the selection of transconjugants. Transconjugant colonies arising from plasmid transfer on these plates usually appeared 6 or more hours later than transconjugants already present in the mating mixture. The number of transconjugants on these control plates was then subtracted from the transconjugant counts obtained from the mating mixture. Frequencies were defined as number of transconjugants per donor cell in the same sample volume and also as number of transconjugants per donor cell × recipient cell to reflect relative differences in the localization of the donor strain, PAO4032, relative to the recipient strain. Antagonism between mating pairs during the transfer period was shown by the differences in viable counts of donors and recipients when these were incubated separately (controls) and when they were incubated together (mating mixture).

Results

Distribution of Bacteria in Freshwater Lakes

The concentrations of bacteria at the air–water interfaces of freshwater lakes were significantly higher than those in the bulk waters in 19 of 21 paired samples. The $\log_{10}$ mean number (± SD) of heterotrophic bacteria per ml in the interface samples was 5.9 (± 1.2) compared with 3.5 (± 0.7) for bulk samples ($P < 0.0001$ for differences between means by Student's t test). Seasonal fluctuations in the numbers of bacteria recovered from the interface of the three local lakes sampled over a period of one year were generally similar (Fig. 1). The fluctuations in the concentrations of bacteria in the bulk generally mirrored those of the surface. Pigmented colonies were isolated on SP agar from all but one of the 21 paired samples. The proportion of colonies recovered from the interface that were pigmented was significantly higher than those isolated from the bulk waters, giving an overall median value for the ratio of bulk to interface of 4.4 $\log_{10}$. The existence of distinct bacterial populations at the interface and in bulk water was also supported by the evidence presented in Table 1. The three examples demonstrated that distinct differences existed in the proportions of the total plate counts of paired surface and bulk samples that were able to grow on SP agar containing 5 μg/ml antibiotic or 20 μg/ml heavy metal ions on primary isolation.

Table 1. Extended

Percentage resistant to:[b]					
Zn	Cu	Co	Cr	Hg	Ni
12	8.0	17	21	0	14
3.8	6.5	35	2.5	0	2.1
32	18	20	0	0	35
84	100	7.4	0	0	100
3.7	2.5	2.4	1.8	0.3	2.4
100	93	100	73	87	100

Table 2. Group assignment of air–water interface and bulk water isolates

Group	Oxidase	Glucose[a]	Motility	Pigmentation[b]					Number of isolates	
				W	Y	B	R	L	Surface	Bulk
1	+	O	+	+	+	+	+	+	73	51
2	+	O	+	+	−	−	−	−	32	36
3	+	O	−	−	+	+	+	−	25	27
4	+	F	+/−	+	+	+	−	−	23	20
5	+	F	(−)[c]	−	−	−	−	+	0	6
6	−	F	+/−	+	+	+	+	−	17	9
7	− (+)	(O)	−	+	−	(+)	−	−	66	80

[a] O, oxidation; F, fermentation of glucose
[b] W, white or colorless; Y, yellow; B, brown; R, red; L, blue or black pigmentation [11] on SP agar at 21°C
[c] ( ), variable, weak, delayed, or uncertain property

Characterization of Isolates

Isolates representative of the Gram-negative bacteria recovered on SP agar plates were selected on the basis of pigmentation and colony morphology and assigned to 1 of 7 groups as described above (Table 2). Group 1 bacteria were the second most frequently isolated bacteria and were recovered from all but one sample. These isolates seem to most closely resemble pseudomonads and 33% produced fluorescent pigments on King's medium [14]. Group 2 isolates often grew in flat, effuse colonies on SP agar and were alcaligenes-like in character. The highly pigmented bacteria of Group 3 were differentiated from both Group 1 and Group 2 bacteria by the absence of motility and appeared to resemble flavobacteria. Group 4 isolates had the characteristics of vibrios and aeromonads. Bacteria in Group 5 had the intense pigmentation resembling chromobacteria. Motile and non-motile bacteria that were oxidase-negative and fermented glucose rapidly were assigned to Group 6. These bacteria were similar to the Enterobacteriaceae. Group 7 was composed of non-motile organisms that were either oxidase-negative, or gave a weak delayed reaction, and either did not utilize glucose, or oxidized it slowly. Group 7 bacteria resembled acinetobacters and were the most commonly isolated bacteria.

Because less than five different colony/pigmentation types were usually present in each paired sample, representatives of each colony/pigmentation type could be selected and placed in a group as described above. Estimates of the relative contribution of each group to the neuston and bulk water showed that bacteria of all groups, with the possible exception of groups 5 and 6, appeared to be evenly distributed between bulk and surface sites (Table 2).

Antibiotic and Heavy Metal Resistance

Multiple drug and heavy metal resistance were determined from measurements of the MICs on SP agar containing 0–100 μg/ml antibiotic or 0–200 μg/ml

Table 3. Multiple antibiotic and heavy metal resistance and incidence of plasmids in aquatic isolates

Group	Site[a]	N[b]	Resistance (mean ± SD)[c]	Plasmids (%)[d]
1	S	73	7.1 ± 3.2	12.3
	B	51	4.7 ± 2.7 ($P < 0.0001$)[e]	21.6
2	S	32	6.8 ± 2.7	9.4
	B	36	3.2 ± 2.3 ($P < 0.0001$)	13.9
3	S	25	3.5 ± 3.4	36.0
	B	27	2.6 ± 2.1 ($P > 0.1$)	22.2
4	S	23	5.3 ± 2.4	17.4
	B	20	2.6 ± 2.0 ($P < 0.0002$)	15.0
5	S	0	–	–
	B	6	5.0 ± 3.9	<15.0[f]
6	S	17	4.4 ± 2.8	17.7
	B	9	2.3 ± 1.5 ($P > 0.2$)	<10.0
7	S	66	4.1 ± 2.7	15.2
	B	80	2.3 ± 2.1 ($P < 0.0001$)	15.0

[a] S, surface; B, bulk samples
[b] Total number of isolates examined
[c] Mean number (±SD) of 8 antibiotics and 6 heavy metals to which isolates of each group were resistant (see text)
[d] Percentage of isolates harboring plasmids
[e] P values for differences between population means of surface and bulk isolates by Student's t test
[f] Lower limits of detection based on total number of isolates

metals. A bacterial isolate was considered antibiotic-resistant if growth occurred at 50 μg/ml. However, because of an overall greater sensitivity to Nx, Tc, and Km, bacteria were considered to be resistant to these three antibiotics if growth occurred at 15 μg/ml. Resistance to heavy metals was set at 100 μg/ml, except for Hg and Co (20 μg/ml). With the exception of Group 3 isolates, significant differences in the multiple resistance of surface and bulk isolates were apparent ($P < 0.01 - \ll 0.0001$ by Student's t test for individual groups; Table 3). The pseudomonas-like isolates of Group1 were the most resistant, a finding similar to that of others for freshwater bacteria [12].

Incidence of Plasmids

The incidence of plasmids was generally similar in surface and bulk isolates of all groups (Table 3), averaging 14 and 16.2% for surface and bulk, respectively. A similar percentage of freshwater bacteria isolated from some sediments were found to harbor plasmids [6]. Isolates recovered from primary isolation plates containing antibiotic (5 μg/ml) or heavy metal (20 μg/ml) plates did not exhibit a statistically higher incidence of plasmids (14.7 and 18.7% for the surface and the bulk isolates, respectively). Two-thirds of the detected plasmids were 45-kb or greater in size and 41% of the strains harbored an average of 3 plasmids.

Table 4. Transfer of plasmid R68.45 from *P. aeruginosa* strain PA04032 to *P. aeruginosa* strain PA01168

Surface film[a]	Site	Transconjugants/ donor (ratio)[b]	Transconjugants/ donor × recipient (ratio)
Present	Surface	4.1×10^{-3} (137)	2.2×10^{-13} (2.9)
	Bulk	3.0×10^{-5}	7.7×10^{-14}
Absent	Surface	5.6×10^{-3} (82)	1.8×10^{-13} (7.5)
	Bulk	6.8×10^{-5}	2.4×10^{-14}
NA[c]	Plate	2.0×10^{-2}	ND[d]

[a] Monolayer of oleic acid plus peptone/yeast extract deposited on surface [16]
[b] Ratio of plasmid transfer frequency at the interface/transfer frequency in the bulk
[c] NA, not applicable
[d] ND, not done

Plasmid Transfer

Conjugal transfer frequencies of plasmid R68.45 from *P. aeruginosa* strain PAO4032 to *P. aeruginosa* strain, PAO1168, were obtained using the SLW model. Conjugation occurred at about 100-fold higher frequencies/donor at the air–water interface compared with the bulk. With the presence of a protein/lipid surface film, transfer frequencies at the interface increased almost twofold compared with the bulk (Table 4). Because the recipient and the donor were enriched at the interface, with the donor localizing more efficiently than the recipient, transfer frequencies on the surface were not as markedly elevated when expressed as transconjugants per (donor × recipient). This was particularly noticeable when a surface film was present, with which the recipient strain more readily associated. Plasmid transfer frequencies on SP plates at 21°C were significantly higher (Table 4).

One hundred and sixty-eight aquatic isolates that were plasmid-free and could grow on M9 minimal medium, or had suitable antibiotic and/or heavy metal resistant patterns for selection against the donor on SP agar, were chosen for further study. All groups, recovered from both bulk and surface samples at all stations, were represented in these isolates. Two surface and two bulk isolates from each group that carried plasmids were also included. Of these, nine plasmid-free strains and two strains with indigenous plasmids yielded transconjugants in plate matings with strain PAO4032 at 21°C; transfer frequencies per donor were of the order of 10^{-4} to 10^{-7}. These strains represented Group 1 bacteria (6 isolates), groups 3 and 4 (1 isolate each), and Group 7 (3 isolates). Plasmid transfer to these strains was not observed in the SLW model, however, in the presence or absence of surface films. The limits of detection of plasmid transfer in these experiments were about 1.5×10^{-5}/donor to 1.3×10^{-10}/donor, depending upon the distribution of the donor and recipient bacteria between the surface and the bulk phases and upon antagonism between the mating pair. In three cases, obvious antagonism of the aquatic isolate by strain

PAO4032 was apparent, with the result that no recipients were recovered from water matings after 24 hours.

Discussion

The Gram-negative heterotrophic bacteria of the freshwater neuston show striking similarities to the bacteria found at the marine air–water interface [11] with the exception of the incidence of plasmid carriage. Namely, compared with bacteria in the bulk waters, the air–water interface bacteria were present in higher numbers, a higher proportion of the types isolated were pigmented, and there was a higher incidence of multiple resistance to antibiotics and heavy metals. These similarities suggest that similar selective pressures are operational at the surfaces of both marine and freshwater habitats. The possible nature of these selective pressures has been discussed [11]. In our study a distinctive surface population was maintained at all three stations despite mixing and heavy recreational use at two of these (Geddes and Whitmore). Changes in the composition of the bulk water populations over time, however, did not parallel those at the surface, and distinctly different patterns of resistance to antimicrobials of primary isolates were noted. Both observations indicate that the interface and bulk bacteria constitute separate populations and that the population differences are maintained despite neuston bacteria continually being lost to and acquired from the bulk waters by, for example, bubble transport [3, 4, 17].

As may be expected, only a fraction of the heterotrophic bacteria were recovered on SP agar. Comparative counts on SP agar and on Collins medium [7], for example, showed that approximately 5 to 10 times more bacteria were recovered on the latter from some sites (data not shown). Despite this limitation, SP agar was selected because pigmentation was markedly better developed with this medium than with Collins medium. This allowed preliminary differentiation between surface and bulk populations on the basis of pigmentation and enabled us to select representative colony/pigmentation types for further examination. The assignment of these more readily isolated bacteria to groups according to established criteria [20] provided a reasonable basis for their differentiation and a basis for the comparison of these heterotrophic bacteria as potential recipients of introduced plasmids.

Only 7% of aquatic isolates acted as recipients of plasmid R68.45 in plate matings with strain *P. aeruginosa* PAO4032, which is less than that observed by others [9]. The majority of those that did act as recipients, however, were pseudomonas-like isolates assigned to Group 1, which is consistent with the observations of Genthner et al. [9]. Although conjugation between the two strains of *P. aeruginosa* in water was established and occurred at a higher frequency at the air–water interface, no transfer of plasmid R68.45 from *P. aeruginosa* to any aquatic isolate was observed in the model despite the use of bacterial suspensions more than 100-fold above the highest concentration found in the bulk waters of the natural habitats studied. The mating temperature of 21°C was probably not the limiting factor in plasmid transfer to aquatic isolates in water. This temperature did not prevent plasmid transfer on SP agar, and

the transfer of plasmids, including R68.45, is known to occur at this lower environmental temperature [2, 22]. The apparent absence of plasmid transfer to aquatic isolates in water does not necessarily mean that this cannot occur, although the plate mating results suggest that few isolates will act as effective recipients.

The absence of significant levels of added nutrients in our model system was probably a major reason for reduced plasmid transfer to the aquatic isolates. Bacteria, following their introduction into the nutrient-free model mating system are likely to undergo a nutrient starvation response [18], a probable consequence of which is reduced activity of conjugal transfer systems. The higher frequency of plasmid transfer at the surface may be the result of the surface potentiation of conjugation [5] mediated by rigid IncP group pili. However, a more likely explanation of the higher transfer frequency at the interface is the accumulation of cells at higher concentrations at the surface. A surface film of oleic acid, protein, and yeast extract had a small effect on conjugation, and plasmid transfer should be expected to occur more efficiently in the presence of such nutrients. The transfer of plasmids between bacteria of the epilithon [1], for example, probably occurs at high nutrient concentrations on the surface of rock. Presumably the surface film provided insufficient nutrients to maintain the bacteria in the growth phase. It would be of interest, therefore, to study the effects of higher nutrient levels on conjugation frequencies in this model, because it is possible that plasmids transfer during periods of nutrient pulses and bacterial growth. This would have important consequences, since once a plasmid has established in an aquatic population under such conditions, it may subsequently proliferate through the population.

The unique physical and chemical characteristics at the air–water interface result in very different conditions from those of the bulk water and, therefore, in the development of a distinct bacterial flora in both marine [11, 17] and freshwater systems. Given that the interface of marine and freshwater systems differs markedly from the bulk waters [11] in physical characteristics and bacterial flora, it would seem prudent to pursue the investigation of the neuston to include less easily isolated bacteria, as well as the possible proliferation of conjugative plasmids and spread of introduced genetic elements through bacterial populations during periods of higher nutrient concentrations.

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