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Distribution of *Pseudomonas aeruginosa* in a Riverine Ecosystem†

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The distribution of *Pseudomonas aeruginosa* in navigation pool 8 of the upper Mississippi River was investigated by acetamide broth enrichment of water, sediment, and swab (solid-water interface) samples. Among the 152 *P. aeruginosa* isolates, serological type 1 was most prevalent (34.2%), and a small number (13.2%) showed carbenicillin resistance. Pigmentation was variable, with only 44.7% elaborating typical blue-green pigment. *P. aeruginosa* was most commonly isolated from sediment, with solid-water interfaces (aufwuchs samples) also exhibiting high frequencies of isolation. Current velocity, oxygen and nutrient availability, surface tension, dessication, and negative phototropism were important factors in the riverine distribution of this epibacterium.

Numerous studies document the ubiquity of *Pseudomonas aeruginosa* in hospital environments (2, 6, 14, 17). In the natural environment, *P. aeruginosa* has been labeled a surface water contaminant (17, 26), causing eye, ear, nose, and throat infections of swimmers (16, 17). In both situations, the bacterium is truly aquatic (17) and does not long survive dessication (9, 24, 29). However, the precise distribution of *P. aeruginosa* in a freshwater habitat has never been determined. Accordingly, it was our objective to ascertain the relative distribution of *P. aeruginosa* in a natural body of water. Strain homogeneity was investigated by serotype, pyocin type, antibiotype, and pigment determinations.

Pool 8 of the Mississippi River extends from lock and dam 8 at river mile 679.2 (ca. 1,093 km), Genoa, Wis., to lock and dam 7 at river mile 702.5 (ca. 1,131 km), Dresbach, Minn. It has a total water surface area of 8,420 hectares (20,810 acres), which includes a navigation channel and extensive backwater sloughs and potholes. The pool is heavily used for commercial navigation, commercial fishing, sport fishing, hunting, trapping, swimming, water skiing, power boating, and sailing.

Water, sediment, fish, aquatic macrophyte surfaces, and aufwuchs (surface growth on submerged objects) were sampled for the presence of *P. aeruginosa* during September and October 1975. Surface (≤5 cm) water samples were collected by means of grab sampling (skimming the surface) with Whirl-pak bags (Nasco), whereas a modification of the device described by Edwards (8) was used for subsurface (0.5 to 1.0 m) water sampling. Our modification consisted of gluing a Vacutainer holder (no. 4893; Becton, Dickinson & Co.) to a 1-m length of 18-mm (0.75-in)-diameter PVC pipe. The holder was fitted with a sterile luer adapter (no. 5731; Becton, Dickinson & Co.) before sampling, and a sterile 10-ml Vacutainer tube (no. 4670; Becton, Dickinson & Co.) was then driven onto the adapter needle with a plunger (rubber-tipped dowel rod), resulting in collection of the sample. A Petite Ponar grab dredge (Wildlife Supply Co.) was used for acquiring sediment samples. Fecal samples from small fish were collected by removal of intestines; large fish were swabbed in the anal region for slime removal before anal insertion of a second sterile swab. Collection of fish slime, plant surface, and aufwuchs specimens was by means of sterile cotton swabs. Current velocities were measured with a Price current meter (Kahl Scientific Instrument Corp.).

All samples, water, sediment, and swabs were enriched for *P. aeruginosa* rather than enumerated on selective, differential media. Previous work with membrane filtration and Cetrimide agar (Difco Laboratories) had shown that *P. aeruginosa* densities in pool 8 were consistently less than 1.4/100 ml (Grimes, unpublished data). In addition, a qualitative and quantitative inventory of bacteria present in pool 8 did not detect *P. aeruginosa* when 310 water samples collected during the summer of 1975 were spread plated

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onto plate count agar (Difco) and 1,522 colonies were randomly picked for identification (Grimes, unpublished data). Accordingly, samples were inoculated into 10-ml tubes of acetamide enrichment broth (15, 31), incubated at 35°C for up to 7 days, and checked daily with UV light (peak intensity at 366 nm). Fluorescing tubes were streaked onto MacConkey agar (Difco) plates for isolation. Colonies that fluoresced (3) were confirmed as *P. aeruginosa* if they were gram-negative, oxidase-positive rods; capable of growth at 42°C in brain heart infusion broth (Difco); motile in SIM medium (Difco), indole and hydrogen sulfide negative in SIM medium; and typical for cultural morphology and odor (22). Confirmed isolates and positive and negative controls were serotyped by slide agglutination with rabbit antiserum (Parke, Davis & Co.) to each of the seven Fisher immunotypes (11). Pyocin (aeruginocin) typing was done by a modification (7) of the method of Zabransky and Day (32, 34), using the 27 indicators of Farmer and Herman (10). All confirmed isolates and four *P. aeruginosa* controls were subjected to disk diffusion antibiotic sensitivity testing (22); the Mueller-Hinton agar (Difco; control no. 614247) contained 107 mg of calcium and 34 mg of magnesium per liter of medium (Difco, personal communication) as suggested by Reller et al. (27). The antibiotics chosen were those being used by area hospitals to control *P. aeruginosa* infections; the disks (BBL Microbiology Systems) contained carbenicillin (100 μg), gentamicin (10 μg), polymyxin B (300 U), or tobramycin (10 μg). Pigment formed in the Mueller-Hinton agar sensitivity plates was visually described.

A total of 152 of 316 samples (48.1%) collected were found to contain *P. aeruginosa*. Serological type 1 was predominant (34.2%), followed by types 4, 2, 7, 3, 5, and 6, respectively (Table 1). Since several studies (4, 7, 20, 33) have shown serotype 1 to predominate in hospital environments, we offer the hypothesis that the relative incidence of *P. aeruginosa* serotypes in clinical infections simply parallels the incidence in the natural environment.

An analysis of pyocin typing data was equivocal. It showed that isolates with similar susceptible indicator strains were clustered in certain serological types (e.g., 65% of serotype 1 was pyocinogenic toward indicator strain 20) and that *P. aeruginosa* strains in pool 8 were not homogeneous. Since the utility of pyocin typing as an epidemiological tool is well established (6, 7, 10, 20, 34), its value to our study was to further document strain diversity of *P. aeruginosa* in pool 8.

Antibiotic sensitivity testing revealed that only 20 isolates (13.2%) were resistant to carbenicillin (Table 1) and that 1 (0.6%) was resistant to polymyxin B; none was resistant to gentamicin or tobramycin, and none was multiply resistant. The sample distribution is shown in Table 2. Resistance to carbenicillin is common among clinical isolates (5), and Lowbury and Jones (25) have shown that the great majority of *P. aeruginosa* are only moderately sensitive to this penicillin derivative. Presumably, antibiotic concentrations in pool 8 were not high enough to give selective advantage to antibiotic-resistant strains.

Visible pigmentation on Mueller-Hinton agar was limited to 91 of the 152 isolates. Of these, 68 (44.7%) elaborated typical blue-green (pyocyanin and pyoverdin) pigmentation, 14 (9.2%) produced red (pyorubin) pigment, and 9 (5.9%) produced a yellow-green (pyoverdin) pigment. None of the isolates produced pyomelanin, and 61 (40.1%) were nonpigmented. It should again be pointed out that all cultures initially fluoresced on MacConkey agar, due to our method

TABLE 1. Distribution of *P. aeruginosa* isolates and carbenicillin resistance according to serological type

Serological type	No. of isolates	No. resistant to carbenicillin
1	52	9
2	17	1
3	15	4
4	25	3
5	11	1
6	1	0
7	16	2
Nontypable	12	0
Autoagglutinating	2	0
Rough	1	0

TABLE 2. Sample sources, relative incidence, and carbenicillin resistance of *P. aeruginosa* in pool 8

Source	No. of samples	No. (%) positive for *P. aeruginosa*	No. (%) resistant to carbenicillin
Water	77	26 (33.8)	6 (23.1)
Sediment	31	21 (67.7)	1 (4.8)
Fish[a]	112	52 (46.4)	9 (17.3)
Plants	47	22 (46.8)	0
Aufwuchs[b]	49	31 (63.3)	4 (12.9)
Total	316	152 (48.1)	20 (13.2)

[a] A total of 56 fish were sampled; each was sampled for slime and feces.

[b] This group encompasses swab samples from surfaces of rocks, clams, snails, detritus, aquatic invertebrates, turtles, outboard lower units, plastic and metal containers, creosoted dock pilings, and surface oil slicks.

of isolation. The large number of nonpigmented strains observed is consistent with other studies (12, 22) but may be somewhat misleading since pigmentation is medium dependent (1, 18, 19) and some strains degrade pigments produced (1). We did not extract Mueller-Hinton agar plates for nonvisible pigment (22), and the plates were incubated for 18 h, which is not sufficient time for all chromogenic strains to elaborate visible pigment. However, our results demonstrate that a large proportion of natural isolates may be nonpigmented. Consequently, the use of pigmentation as the sole means of differentiation on a primary isolation medium could significantly underestimate real density.

The distribution of *P. aeruginosa* according to sample type (Table 2) revealed significant differences (P by chi-square test = 0.997), with sediment samples containing the largest percentage of samples positive for *P. aeruginosa* (67.7%), followed in decreasing order by aufwuchs, plants, fish, and water samples. Hoadley (17), in at least two different studies, observed that *P. aeruginosa* disappears from surface waters when introduced as a fecal contaminant. Based on our findings and on field and laboratory survival studies (9, 17), this observation might be explained by sedimentation rather than by die-off. Interestingly, Lighthart (23) has found that the occurrence of *Pseudomonas*-like bacteria increases with depth, the greatest number being found in sediment samples. These findings, together with the results of our study, indicate that sediment may be the primary reservoir for *P. aeruginosa* in natural aquatic environments. Another observation was that *P. aeruginosa* was frequent at all solid-water interfaces, including submerged plant surfaces and aufwuchs; its lowest incidence was in water itself and on aerial plant surfaces (Tables 2 and 3).

A comparison of isolate distribution according to current velocity showed that subsurface lotic water (current velocity $\geq$ 0.1 m/s) and surface lentic water (current velocity $<$ 0.1 m/s) had higher incidences (P by chi-square test = 0.756) of *P. aeruginosa* than did surface lotic and subsurface lentic water, respectively (Table 3). In lentic areas, high surface tension and water buoyancy cause surface retention of organic nutrients. Also, placid water promotes a greater oxygen content at the surface. These factors probably explain the greater concentration of *P. aeruginosa* at the surface of lentic areas. In support of this hypothesis, Smith and Doetsch (30) have observed flagellated *Pseudomonas fluorescens* to outgrow and greatly outnumber (by a factor of 10) nonflagellated mutants of the same strain when the two types are grown together in nonaerated, stationary culture.

Subsurface lotic water had a higher concentration of *P. aeruginosa* than did corresponding surface water (Table 3). A possible explanation for this is negative phototropism (30). The organisms might have been positioning themselves in deeper water to escape photooxidation since, theoretically, no nutrient or oxygen benefit could be gained from being at the surface of lotic habitats. Current velocity did not significantly affect any other distribution (Table 3).

Plant parts above water had a significantly lower incidence (P by chi-square test = 0.962) of *P. aeruginosa* than did submerged plant surfaces (Table 3), a finding consistent with the suscepti-

TABLE 3. Distribution of *P. aeruginosa* according to current velocity of sampling area

Source	Lotic[a]		Lentic[b]	
	No. of samples	No. (%) positive	No. of samples	No. (%) positive
Water				
Surface[c]	14	3 (21.4)	20	9 (45.0)
Subsurface[d]	15	7 (46.7)	28	7 (25.0)
Total	29	10 (34.5)	48	16 (33.3)
Sediment	11	8 (72.7)	20	13 (65.0)
Plants				
Aerial	14	5 (35.7)	24	10 (41.7)
Submerged	5	4 (80.0)	4	3 (75.0)
Whole	19	9 (47.4)	28	13 (46.4)
Aufwuchs	11	7 (63.6)	38	24 (63.2)

[a] Current velocity of $\geq$0.1 m/s.
[b] Current velocity of $<$0.1 m/s.
[c] Top 5 cm of the water column.
[d] Collections from 0.5 to 1.0 m.

bility of *P. aeruginosa* to drying (9, 29) and with our hypothesis that *P. aeruginosa* is frequent at solid-water interfaces. No attempt was made to associate *P. aeruginosa* incidence with pathology of the plants collected.

In fish, 28 (53.8%) of the 52 isolates originated from surface slime, with 21 (40.3%) coming from fecal material. Only 13 (23.2%) of the 56 fish sampled carried *P. aeruginosa* in both slime and feces. This high incidence in slime further supported our observation that *P. aeruginosa* was frequent at solid-water interfaces; technically, these bacteria could also be classified as aufwuchs. Piscivorous fish (n = 31) produced 36 (69.2%) of 52 positive samples (either slime or fecal material), and 16 positive samples (30.8%) were from nonpiscivorous species (n = 25). No attempt was made to associate *P. aeruginosa* incidence with pathology of the fish collected.

In summary, we found *P. aeruginosa*, an opportunisitic pathogen of wide host range, to be distributed throughout pool 8 of the upper Mississippi River. Unlike clinical isolates, the incidence of antibiotic resistance was very low. The origin of *P. aeruginosa* could not be ascertained from our data, since there were several potential point and nonpoint sources (13). However, based on lack of homogeneity among serotypes, pyocin types, and pigment types found, it is doubtful that there was any one major allochthonous source of *P. aeruginosa*. It is highly probable that *P. aeruginosa* is an autochthonous member of the bacterial community in pool 8. Algal blooms have been reported to stimulate *P. aeruginosa* (17), and pool 8 supports large cyanobacteria blooms in late summer and early fall (River Studies Center, unpublished data). Regardless of source, allochthonous or autochthonous, our data support the hypothesis that riverine *P. aeruginosa* is an epibacterium (28) that is more frequent in sediment and at solid-water interfaces than in the water column itself. Consequently, surveillance of natural waters for *P. aeruginosa* should not be limited to water samples alone, a conclusion reached by LaLiberte and Grimes (21) for fecal coliforms.

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