Production of Elastase and Other Exoproducts by Environmental Isolates of *Pseudomonas aeruginosa*

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***Pseudomonas aeruginosa* isolates from environmental sources and bacteremic patients were compared for their levels of elastolytic activity. No significant differences were found. The incidence of production of toxin A, phospholipase C, alkaline protease, and elastase among the environmental strains was also as high as that previously reported for clinical isolates.**

Pseudomonas aeruginosa produces a large number of products which have the potential to contribute to pathogenesis (12, 14). These include two proteases, elastase and alkaline protease, toxin A, and phospholipase C. *P. aeruginosa*, however, is an opportunistic pathogen generally able to cause infection only in an injured or compromised host (19). Janda and co-workers (8, 9) reported that environmental isolates may differ from virulent clinical isolates in their ability to produce elastase, the major protease of *P. aeruginosa* and a product believed to contribute to virulence (14, 21). We were therefore interested in investigating whether the production of virulence-related enzymes was a special property of those strains which cause infection or a general property of *P. aeruginosa*. We report that strains isolated from their natural environment, soil and water, are similar to those isolated from bacteremic patients in both their incidence and yields of elastase production. The environmental strains were also found to be similar to previously studied clinical isolates in their incidence of production of other exoproducts.

Bacterial strains. Environmental isolates of *P. aeruginosa* were kindly provided by J. Y. Homma, Kitasato Institute, Tokyo, Japan, and by M. Glass, Washington Department of Social and Health Services, Seattle. Strains from J. Y. Homma constituted 30 isolates from rivers in the Toyama Prefecture in Japan, collected and described by Kodama et al. (10). Serotyping was used to establish that these isolates were different strains (10). The Washington strains constituted soil and water isolates from the Mount St. Helens volcano area. Pyocin typing by the method of Farmer and Herman (3) was used to establish that these isolates were different strains. Forty-one isolates from bacteremic patients at Walter Reed Army Hospital, Washington, D.C., were obtained from A. Cross. These strains had previously been serotyped and pyocin typed.

Assays for extracellular products. All of the assays used have been described previously. Elastolytic activity was assayed on elastin nutrient agar (15). Zones of elastolytic activity were measured after 48 h of incubation at 37°C. Elastase production was further confirmed by radial immunoprecipitation of antibody to purified elastase placed in wells adjacent to colonies grown on brain heart infusion agar (15, 16). Alkaline protease production was evaluated by a similar immunoprecipitation assay with antibody to purified

alkaline protease (5). Casein digestion was assayed on modified brain heart infusion agar containing skim milk (18). Phospholipase C was assayed as described by Berka et al. (1) by the *p*-nitrophenylphosphorylcholine assay (11) of culture supernatants of cells grown in low-phosphate medium. Production of toxin A was screened by radial immunoprecipitation of specific antibody by colonies grown on modified Trypticase (BBL Microbiology Systems) soy agar (16). Those strains which did not show toxin A production in this assay were grown in modified Trypticase soy broth (16) for 24 h, after which supernatants were collected, concentrated 20-fold by ammonium sulfate precipitation, and assayed for toxin A activity (6).

Comparison of elastase activity of environmental and bacteremic isolates. Both the environmental and bacteremic isolates showed similar levels of elastolytic activity. Mean zone sizes on elastin nutrient agar were not significantly different (Table 1; $P > 0.1$ by Student's *t* test). The incidence of elastase production in the two groups of strains was also similar at 89 and 87%, respectively ($P = 0.1$ by χ^2).

In vitro production of extracellular enzymes by environmental isolates. The incidence of production of four exoenzymes by 44 environmental isolates randomly chosen from the total of 66 is summarized in Table 2. Most strains produced both alkaline protease and elastase. Interestingly, the percentage of elastase-positive strains was the same when tested by enzymatic activity (Table 1) or by antigen production (Table 2). Two strains did not produce detectable levels of either of these two specific proteases but were able to produce a small zone of casein hydrolysis (data not shown), and both of these strains produced toxin A. All strains produced detectable levels of phospholipase C activity. Toxin A production was detected in 50% of strains by immunoprecipitation assay (data not shown), and an additional 14 strains had detectable levels of toxin A when assayed for enzymatic activity, for a total of 82% with detectable toxin A.

Comparison of incidence of exoproduct production in environmental and clinical isolates. Previous studies of clinical isolates of *P. aeruginosa* have generally shown a high incidence of production of exoenzymes (1, 2, 4, 7-9, 13, 17, 20). Our data suggest that the incidence of production of these products in isolates from the natural environment of *P. aeruginosa*, soil and water, is similarly high. We know of no other studies which examined nonclinical isolates (other than a few laboratory reference strains) for toxin A or phospholipase C production.

Previous studies by two groups have suggested a lower

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TABLE 1. Elastase production by isolates of *P. aeruginosa* from the environment and from bacteremic patients

Type of isolate	Mean zone size (mm) $\pm$ SEM ^a	No. positive/no. tested (%)
Environmental	14.2 $\pm$ 3.5	59/66 (89)
Bacteremic	12.6 $\pm$ 3.8	41/47 (87)

^a Zone of elastolysis after 48 h of growth.

incidence of elastase or protease in environmental strains than in clinical isolates (4, 8, 9). Hedberg et al. (4) found elastase production in 57% of 21 isolates taken from the hospital environment compared with 65 to 85% of clinical isolates; however, this difference was not statistically significant ($P = 0.25$ by χ^2 [our calculation]). In contrast, Janda et al. (8, 9) found significant differences in protease and elastase production between a group of environmental isolates from soil and water and clinical isolates. In their studies, isolates from bacteremic patients had higher levels of elastase and were more likely to be proteolytic or elastolytic than isolates from superficial or local infections (8, 9). Their 22 environmental isolates showed a much lower incidence of proteolytic activity (54% compared with 98% for clinical isolates), and none were elastolytic. Our findings of similar incidences and similar levels of elastase activity in bacteremic and environmental strains are in agreement with those of Hedberg et al. (4) but contrast markedly with those of Janda et al. (8, 9). The higher incidences of protease production we observed could possibly be accounted for by our use of more sensitive assays; however, the difference in levels of elastase measured by a similar semiquantitative plate assay may reflect differences in the strains tested. In the studies of Janda et al. (8, 9), no attempt to ascertain that the isolates used were distinct strains was reported. Their 22 environmental isolates were all unpigmented and may represent an atypical sampling of natural isolates.

All the environmental strains we tested had phospholipase C activity, as has been reported for clinical isolates (1). In the semiquantitative assay used, approximately half of the strains had higher activity than the control strain, PAO (data not shown). A similar variation in the phospholipase C level has been observed in clinical isolates (1).

Toxin A production was also a property of most environmental isolates. Similar incidences of toxin A production, 87 and 90%, have been reported previously in studies of clinical isolates (2, 17). Comparison of the environmental isolates studied by us and a group of 85 clinical isolates previously examined in our laboratory by the same assays (2) showed no significant difference between the two groups with regard to incidence of toxin production ($P = 0.5$ by χ^2).

TABLE 2. Production of extracellular enzymes by environmental isolates of *P. aeruginosa*

Enzyme	% Positive	No. of isolates positive/no. tested
Elastase ^a	89	39/44 ^b
Alkaline protease ^a	89	39/44
Toxin A ^c	82	37/44
Phospholipase C ^d	100	44/44

^a Assayed by radial immunoprecipitation.^b Of the five elastase-negative strains tested, three produced alkaline protease and all produced toxin A.^c Determined by immunoprecipitation and enzymatic assay.^d Determined by enzymatic assay.

Elastase, alkaline protease, and toxin A have clearly been associated with virulence of *P. aeruginosa* in animal model studies (14), and phospholipase C is also likely to play a role (12). However, our data suggest that there is no significant difference between strains isolated from nature in their ability to produce proteases, toxin A, and phospholipase C. Thus, it is possible that pathogenic potential is a general property of *P. aeruginosa* from all sources.

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