Correlation of Proteolytic Activity of *Pseudomonas aeruginosa* with Site of Isolation

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*Pseudomonas aeruginosa* isolates were evaluated for protease activity by use of a semiquantitative plate assay. Differences were noted with respect to site of isolation, origin, and colonial morphology.

*Pseudomonas aeruginosa* is a gram-negative bacterium frequently isolated from both natural and clinical sources. In the natural environment, this microorganism can easily be recovered from both soil and water and may also be isolated from the foliage of plants. In hospitalized patients, severe infections with this microbe may develop, especially in patients with burns and wounds, in those suffering from cystic fibrosis, and in immunocompromised individuals (10, 12).

*P. aeruginosa* elaborates a large number of exoenzymes including an exotoxin which has been implicated in its pathogenicity. Though intensive investigation and characterization of a number of these factors has occurred, the pathogenesis of this organism remains poorly understood.

Of the exotoxic enzymes of *P. aeruginosa*, protease was highlighted by Liu (5) as the most likely factor responsible for both the destruction of corneal tissue and hemorrhage in internal organs in systemic disease. A number of other investigators have implicated pseudomonal proteases in virulence for experimentally infected animals (1, 3, 8). The exact nature and number of proteases produced by this bacterium are of considerable controversy. In a recent analysis of clinical strains, Muszynski (6) compared the enzymatic properties of *P. aeruginosa* strains of high and low virulence for mice and found that greater protease production among the former was the only significant difference between the two groups. This investigator’s results suggest that protease plays a substantial role in pseudomonal virulence.

Other studies, however, indicate a less prominent role for proteases in virulence. In studying experimental infections in mice, Kobayashi (2) could not correlate virulence with extracellular products. This finding was supported by a later work in which mutagen-derived protease-deficient mutants of a *P. aeruginosa* strain did not appreciably lose virulence for mice when compared with the parental strain (14). These results leave the role of this enzyme in pathogenesis highly speculative.

We have studied the protease activity of various clinical isolates of *P. aeruginosa*, reasoning that if proteases play important roles in systemic infections, higher enzymatic levels might be found in those strains derived from systemic sources when compared with isolates from non-systemic infections. Protease activity of clinical strains was also compared with that of natural environmental strains recovered from puddles, streams, and soil. This comparative study is the subject for this report.

Based on source, the 54 clinical isolates were divided into four groups, namely, sputum, urogenital, systemic, and facial (Table 1). None of the sputum isolates was derived from cystic fibrosis patients. The 22 environmental isolates were all apyocyanogenic.

Protease activity was assessed with the medium of Sokol (9), which consisted of dialyzed

### Table 1. Comparison of protease production of clinical and natural isolates of *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of strains tested</th>
<th>No. of positive strains (%)</th>
<th>Range (mm)</th>
<th>Avg zone of proteolysis* (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>54</td>
<td>53 (98)</td>
<td>1.0-6.6</td>
<td>3.76</td>
</tr>
<tr>
<td>Sputum</td>
<td>20</td>
<td>19</td>
<td>1.0-6.0</td>
<td>3.10</td>
</tr>
<tr>
<td>Urogenital</td>
<td>16</td>
<td>16</td>
<td>1.5-6.2</td>
<td>3.80</td>
</tr>
<tr>
<td>Systemic</td>
<td>15</td>
<td>15</td>
<td>1.6-6.6</td>
<td>4.23</td>
</tr>
<tr>
<td>Facial</td>
<td>3</td>
<td>3</td>
<td>1.0-5.7</td>
<td>3.80</td>
</tr>
<tr>
<td>Natural</td>
<td>22</td>
<td>12 (54)</td>
<td>0.2-1.8</td>
<td>0.80</td>
</tr>
<tr>
<td>Water</td>
<td>15</td>
<td>12</td>
<td>0.2-1.8</td>
<td>0.80</td>
</tr>
<tr>
<td>Soil</td>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Average zone size of a number of isolated colonies of each strain as measured from the edge of the colony to the periphery of the zone of proteolysis.

*Source of 15 systemic isolates: blood (1), bile (2), aspirates (3), bone (1), ulcer (1), tracheal secretion (1), stool (1), throat (1), wound (2), abscess (1), and lung biopsy (1).
brain heart infusion broth, 3% skim milk, and 1.5% agar. Proteolysis, as evidenced by clearing of the medium around isolated colonies of the test organism after 48 h of incubation (Fig. 1), was measured semiquantitatively on equivalent-depth poured plates. Protease activity was present in 98% of the clinical isolates (one apyocyanogenic strain was negative) and in only 54% of the natural isolates (Table 1). The zone of hydrolysis around colonies of the clinical strains ranged from 1.0 to 6.6 mm. Natural strains by comparison showed a markedly restricted range averaging 0.2 to 1.8 mm. Among the clinical strains, a 30% increase in the average zone of proteolysis was noted among strains from systemic sites as contrasted to sputum isolates. This zonal difference was statistically significant as determined by the t test (t = 2.04; P < 0.05). Urogenital and facial isolates fell between these two groups in protease activity. As a group, the clinical isolates exhibited a four- to fivefold-greater averaged zone size than the natural strains.

A correlation between colonial morphology and proteolytic activity of clinical strains was also observed (Table 2). *P. aeruginosa* strains exhibiting the classical type I morphology (irregular 2- to 3-mm effusely elevated colony with fimbriated and lobated edge) dominated the highly and moderately proteolytic categories; 71% of all strains in these groups showed type I morphology. Weakly proteolytic strains (≤3.0 mm) were colonially much more diverse. Over one-half of the coliform-like (type II) strains and the two dwarf strains (type VI) fell into this group.

The observed results of proteolytic activity support the hypotheses of Liu (5) and Muszynski (6) and suggest an important role for these enzymes in pseudomonal pathogenesis. Although

![Image](http://jcm.asm.org/)

**Fig. 1.** Proteolytic zones around colonies of low (A) and high (B) protease-producing strains of *P. aeruginosa* 48 h after incubation at 37°C.

<table>
<thead>
<tr>
<th>Colony Type</th>
<th>Description</th>
<th>No. of strains with following zone size (mm):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≥5.0 (14)</td>
</tr>
<tr>
<td>I Classic</td>
<td>12 (83)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>II Coliform-like</td>
<td>2 (17)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>III Rough</td>
<td>0</td>
<td>3 (15)</td>
</tr>
<tr>
<td>VI Dwarf</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Growth on nutrient agar at 37°C after 24 h by the method of Wahba and Darrell (13).
* As described by Phillips (7).
* Number within parentheses shows number of strains falling into indicated proteolytic range.
* Percentage of total number of strains is shown within parentheses.

facial isolates had modest proteolytic capacities, the two eye isolates were extremely proteolytic (average of 5.2 mm). This is not surprising, since proteases have been implicated in the destruction of corneal tissue and may contribute to the dermonecrosis in burn patients and the endothelial invasion in ecchyma gangrenosum (4, 11). Elevated protease activity among *P. aeruginosa* isolates may thus enhance invasive potential.

In the clinical environment, there appears to be a distinct selection process favoring protease-producing *P. aeruginosa* strains. As judged by the proteolytic activity of the clinical isolates tested herein, a hierarchy could be discerned. Of the clinical groups, sputum strains had the lowest average proteolytic activity, and since many of the strains isolated from this site may only reflect colonization and not true infection, it is not surprising that this group of isolates might contain a large number of weakly proteolytic strains. *P. aeruginosa* of systemic origin, however, had the highest protease activity, whereas
urogenital and facial isolates cascaded in between. Whether increased protease activity in the systemic isolates as demonstrated by our assay approximates the in vivo situation, where other biological fluids may influence the regulation of protease production, remains to be determined and is currently being studied.

LITERATURE CITED