The Reason for Loss of Agglutinability of *Pseudomonas aeruginosa* Cells Heated to 60°C

PINGHUI V. LIU

Department of Microbiology, University of Louisville School of Medicine, Louisville, Kentucky 40292

Received 14 April 1988/Accepted 21 June 1988

The loss of agglutinability of live *Pseudomonas aeruginosa* cells by mild heating to 60 to 80°C is due to denaturation of surface slime, which can no longer combine with antibodies but is still attached to the cell surface and thus prevents access of antibodies to the cell wall. Prolonged boiling or autoclaving would not only denature the slime but also detach it from cell surface and thus make the cells accessible to antibodies directed to cell wall antigens. Heated cells are, however, no longer agglutinable by antibodies directed to slime antigens. After prolonged boiling or autoclaving, a large amount of polysaccharides appeared in the supernatant, and concomitantly, total cell volume as measured by turbidity of the cell suspension was significantly reduced.

*Pseudomonas aeruginosa* cells exhibit a peculiar reaction to heating in that, when live-cell suspensions are exposed to mild heating to 60 to 80°C, they lose their agglutinability by antisera (2). However, agglutinability of these cells can be restored by further, intense heating, such as prolonged boiling or autoclaving (2). Similar loss of agglutinability can be observed after exposure to chemicals, such as alcohol and Formalin (2). The surface of live *P. aeruginosa* cells is known to be covered with a thick layer of slime, which contains protein as well as polysaccharides (4, 5). It is likely that the effects of heat and chemicals are on the heat-labile components of this slime layer. The present study was conducted to obtain explanations for the cause of nonagglutinability of *P. aeruginosa* cells exposed to mild heating.

Slime-specific antiserum was prepared against a nonmotile rough strain of *P. aeruginosa* designated 117 (ATCC 23993). A rabbit was given two intracutaneous injections, 0.1 ml each, of live broth culture of this strain, and antiserum was harvested within 2 weeks after the injections. This antiserum was specific for slime and contained neither antibodies to flagella nor antibodies to the classical O antigens. This antiserum was used to screen a large number of live cells of the usual smooth strains of *P. aeruginosa* for agglutination. A strain designated PA-26 was found to be agglutinated strongly by this antiserum only when live cells were used. Autoclaved cells of this strain were not agglutinated by antiserum to strain 117. The autoclaved cells of strain PA-26 were then used to screen many antisera produced with autoclaved *P. aeruginosa* cells. An antiserum produced against strain PS-113 (ATCC 43732) was found to agglutinate strongly the autoclaved cells of strain PA-26. These two antisera were used to demonstrate changes in the agglutinability of PA-26 cells associated with heating and release of polysaccharide antigens from the cells.

Strain PA-26 was streaked on four plates of Trypticase soy agar (BBL Microbiology Systems) in such a way as to cover the whole surface, and plates were incubated for 18 h at 37°C. The growth on these plates were washed off separately with 10 ml of physiological saline (0.15 M NaCl) and centrifuged at 10,000 × g for 1 h. The supernatants were discarded, and sedimented cell samples each were resuspended in 10 ml of saline. One sample was left standing at room temperature for 2 h as a live cell control. The second sample was heated in a 60°C water bath for 1 h, the third sample was boiled for 2 h, and the fourth sample was autoclaved at 121°C for 0.5 h. At the end of a 2-h period, all four samples were centrifuged again at 10,000 × g for 1 h. The supernatants were collected for measurement of total sugars with the phenol-sulfuric acid method (3) by using dextran as the standard. Cells were suspended in 10 ml of saline, and a 1/10 dilution of these suspensions was used to measure turbidity at 575 nm (Coleman Junior Spectrophotometer) for comparison of cell volume. These cells were also assayed for agglutination with antisera. All of these data are summarized in Table 1.

As evident in Table 1, live cells of PA-26 were agglutinated by both antisera, one directed to the slime of strain 117 and the other directed to the cell wall of PS-113. Live cells left standing at room temperature released about 20 μg of polysaccharides per ml after 2 h. Heating of this cell suspension to 60°C for 1 h resulted in the release of three to four times the amount of polysaccharide released by live cells, and the turbidity of the cell suspension was reduced only slightly. The cell suspension became completely nonagglutinable by either antiserum. When the cell suspension was boiled for 2 h, it released 9 to 10 times the amount of polysaccharide released by live cells, and the turbidity of the cell suspension was reduced to about 75% of that of live cells. This cell suspension became agglutinable by antiserum against PS-113 but not by that against 117. When the cell suspension was autoclaved for 0.5 h, it released 15 to 17 times the amount of polysaccharide released by live cells. The turbidity of the cell suspension was reduced to only about 60% of that of live cells. The reduction in cell volume was quite apparent when the cell mass deposited at the bottom of the centrifuge tube was inspected and compared with that of live cells. The autoclaved cells were also agglutinable by antiserum against PS-113 but not by that against 117.

Results of the present study indicate that the loss of agglutinability of *P. aeruginosa* cells by mild heating to 60 to 80°C is due to the denaturation of some antigens in slimes (most likely proteins). This process, however, is not strong enough to completely destroy the slime complex and make it detach from the cell surface. This denatured slime antigen blocks the access of antibodies to the cell surface and
renders the cell nonagglutinable. Extreme heating by boiling or autoclaving causes denaturation and detachment of the slime complex from cells and renders the cell surface accessible to antibodies directed to the classical O antigens. However, these cells are no longer agglutinable by antisera directed to slime. Similar loss of agglutinability of live P. aeruginosa cells can be demonstrated by exposure of cells to chemicals that denature proteins, such as alcohol or Formalin (2), and these cells can also be rendered agglutinable again by autoclaving.

In the serotyping of P. aeruginosa, antisera are usually produced with autoclaved cells, but live cells are usually used as antigens because of the ease with which they can be prepared (1). In most cases, this practice is satisfactory, but in a critical study it should be remembered that live cells can be agglutinated by antisera directed to slime as well as those directed to O antigens, whereas autoclaved cells are agglutinated only by antisera directed to O antigens.

This work was supported in part by a grant from the Graduate School of the University of Louisville.

**LITERATURE CITED**


---

**TABLE 1. Changes in the agglutinability of *P. aeruginosa* cells (PA-26) after heating and associated with loss of polysaccharides and cell volumes**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Agglutination by antisera against:</th>
<th>Polysaccharides (μg/ml) in supernatant&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Turbidity of cell suspension (&lt;i&gt;A&lt;/i&gt;&lt;sub&gt;575&lt;/sub&gt;)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live</td>
<td>+ +</td>
<td>19.8</td>
<td>0.289</td>
</tr>
<tr>
<td>Heated to 60°C for 1 h</td>
<td>0 0</td>
<td>76.6</td>
<td>0.260</td>
</tr>
<tr>
<td>Boiled for 2 h</td>
<td>0 +</td>
<td>190.0</td>
<td>0.210</td>
</tr>
<tr>
<td>Autoclaved for 0.5 h</td>
<td>0 +</td>
<td>330.0</td>
<td>0.170</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average of three experiments.

<sup>b</sup> Turbidity was measured with a 1/10 dilution of the original saline suspension that contained cell mass (from one agar plate) suspended in 10 ml of saline. Strain 117 was a nonmotile, rough strain which contained neither flagella nor the classical O antigens. Its antisera contained antibodies to surface slime. PS-113 was a smooth-celled strain whose O antigen was identical with that of PA-26 but possessed different slime antigen. Antiserum was made with autoclaved cells.