Chemical Composition and Serological Analysis of the Cell Wall of *Peptostreptococcus*

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**ABSTRACT**

BAHN, ARTHUR N. (Northwestern University, Chicago, Ill.), PATRICK C. Y. KUNG, AND JAMES A. HAYASHI. Chemical composition and serological analysis of the cell wall of *Peptostreptococcus*. J. Bacteriol. 91:1672-1676. 1966.—Chemical and serological analyses were made of the cell wall of *Peptostreptococcus* to characterize taxonomically this genus of anaerobic streptococci. Cell wall hydrolysates of *P. putridus* strains 06 and 85, *P. intermedius* strains 11 and 87, and *P. elsdenii* strain B-159 were prepared, and the cell wall sugars were measured quantitatively by paper chromatography. Strain 85 contained only glucose, whereas strain 06 contained 93% glucose and 7% mannose. Strain 87 contained only rhamnose, and strain 11 contained approximately equal amounts of glucose and rhamnose. Strain B-159 differed from all the strains in having a low (3.1%) content of total carbohydrate, consisting of rhamnose, galactose, and glucose. Quantitative amino acid analyses showed that the major amino compounds present in the cell wall were glutamic and aspartic acids, alanine, lysine, muramic acid, glucosamine, and galactosamine. Strains 06 and 85 possessed this complement of amino compounds, but strains 11 and 87 had relatively little aspartic acid. Strain B-159 was markedly different in having a high content of glycine and diaminopimelic acid, with only traces of lysine; it was the only strain in which teichoic acid was found. Serological analyses were made with the use of cell wall extracts as antigenic material and with homologous antisera, as well as streptococcal group antisera for groups A through S. The only strong agglutination was obtained between strain 87 antigen and group C antisera; weak agglutination was obtained with 87 against N, O, and K, and between strain 11 and groups E and F. All other antisera gave negative reactions. It is concluded that strain B-159 does not belong to the genus *Peptostreptococcus*, that strains 06 and 85 are members of *P. putridus*, and that strains 11 and 87 may be members of two different genera.

*Peptostreptococcus* is a genus of anaerobic streptococci, first described by Prévot (16), which has been associated with human infection; yet, this genus has received little study because the isolation, cultivation, and identification of the obligately anaerobic streptococci are technically difficult. Some species are found as members of the normal flora in the respiratory and gastrointestinal tracts, genitourinary system, oral cavity, and the skin (6, 17). The anaerobic streptococci which produce putrefactive wound infections have been recognized by *Bergey’s Manual of Determinative Bacteriology* as consisting of eight species of anaerobic streptococci on the basis of morphological and physiological criteria as suggested by Prévot (16). This classification is generally considered to be unsatisfactory, because data on cultural conditions, prevalence, biochemical activities, and antigenic conditions are scarce (14).

Chemical analysis of the cell wall is a method used to verify classification of those bacteria which have been classified by conventional methods (3-5). One bacterium can often be differentiated from another by the distinct pattern of the sugar, amino sugar, and amino acid components of the bacterial cell wall mucopentide (21). The precise analysis of these substances may constitute a decisive contribution to the reclassification of bacteria which were formerly classified improperly.

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MATERIALS AND METHODS

Cultures. Ten strains of obligately anaerobic cocci in chains were isolated from patients with osteomyelitis of the mandible and deep submandibular abscesses. They were classified provisionally on the basis of criteria of Bergey's Manual of Determinative Bacteriology into two species: P. putridus and P. intermedius. Two strains of each species were chosen as representative strains, 06 and 85 of P. putridus and strains 87 and 11 of P. intermedius. These were compared with the only available reference strain, which was obtained from M. F. Bryant, U.S. Department of Agriculture, Beltsville, Md. This strain, B-159, was isolated originally from the rumen.

Cultivation of organism and preparation of cell wall extracts. The five strains were grown in modified Todd-Hewitt medium as described by Hess and Slade (11). The cells were incubated at 37°C for 18 hr in an atmosphere of 95% N2 and 5% CO2, harvested, and lyophilized. The cell wall extracts were prepared by the method of Cummins and Harris (4, 5) and were lyophilized.

Serological procedures. Homologous antisera were prepared in rabbits by use of the cell wall fractions. A 10-ml amount of cell walls was suspended in 10 ml of distilled water to which 0.4 ml of 12% sodium azide was added as a preservative. The rabbits were immunized intravenously by the following procedure. The first three daily injections (0.5 ml each) were followed by a 4-day rest period; then the rabbits were given three daily injections (2 ml each) followed by a rest period, and a final series of three daily injections (2 ml each). At 4 days after the last injection, the animals were bled and the serum was collected.

Antisera against streptococcal groups A to M were obtained from the Communicable Disease Center, Chamblee, Ga.; groups N to Q were from the Wellcome Research Laboratories, London, England; group R was from the Streptococcus Reference Laboratory, London, England; and antiserum against group S was obtained from H. D. Slade, Northwestern University Medical School, Chicago, Ill.

The agglutination method of Cummins and Harris (4) was used to detect antigens in the cell wall. The cell walls were tested against antiserum by adding equal volumes (0.5 ml) of antiserum and antigen (added on top of the serum) to agglutination tubes and incubating the tubes in a water bath at 55°C for 2 hr. The tests were read for agglutination immediately after removal from the water bath and after overnight incubation at room temperature.

Chromatographic procedures. The general procedures for qualitative paper chromatography for the detection of sugars and amino acids were done according to Slade and Slamp (19). Hydrolysates of the cell walls were made in sulfuric acid for sugar detection and in hydrochloric acid for detection of amino acids and amino sugars in the manner described by those investigators.

Quantitative paper chromatographic methods for sugars were applied to hydrolysates prepared as described (19). The hydrolysates were chromatographed on a one-dimensional, descending system, with the use of 1-butanol-acetic acid-water (150:30:50) on Whatman no. 1 paper (46.5 by 28.5 cm). The paper was marked into four strips; the inside strips were spotted with the hydrolysate, and the outside strips were spotted with standard sugars. After development, the outside strips were cut off and sprayed with aniline phthalate spray. The center strips were separated from each other and one strip, the reference strip, was sprayed for comparison with the outside strips containing authentic sugars and to indicate the locations of the sugars on the unsprayed center strip. The unsprayed strip was cut into sections corresponding to the location on the reference strip of each sugar. The individual sections were placed in small test tubes, 2 ml of water was added, and the sugar was eluted by shaking the tubes for 30 min. Portions of the eluted sugar solutions were analyzed for their sugar content by the colorimetric, phenol-sulfuric acid method of Dubois et al. (7).

Samples for amino acid and amino sugar analysis were hydrolyzed in hydrochloric acid (15, 19) and were then analyzed as described by Moore et al. (15) on the Beckman-Spinco amino acid analyzer, model 120.

The presence of teichoic acid was established by the method of Baddiley and Davison (1).

RESULTS

The agglutination tests of the cell wall antigen with homologous antisera demonstrated that there was no cross-reaction between the species of Peptostreptococcus (Table 1). There was a reciprocal cross-reaction between both strains of P. putridus, indicating serological similarity. This was not observed between strains 87 and 11 of P. intermedius; antiserum against strain 87 reacted with antigens from both strains 87 and 11, whereas antiserum from strain 11 reacted only with its homologous antigen. Strain B-159 P. elsdenii agglutinated more strongly than the other strains, but only with its homologous antigen.

Cell wall antigens were tested with streptococcal grouping antisera to determine the relationship of the genus Peptostreptococcus to the genus Streptococcus. The serological relationship between the two genera is generally weak. The only reactions observed were a weak (1:40) agglutination of antigen for strain 11 by antisera for groups E and F, whereas strain 87 agglutinated strongly (1:160) with antiserum for group C and to a lesser degree with antisera for groups N, O, and K. The strongest serological relationship was with streptococcal group C. None of the other strains reacted with any of the streptococcal grouping antisera.

The results of qualitative and quantitative sugar analyses of the cell walls are summarized in Table 2. Strains 06 and 85, the P. putridus
TABLE 1. **Agglutination test of cell wall extracts from strains of Peptostreptococcus with homologous antisera**

<table>
<thead>
<tr>
<th>Antigens from strains</th>
<th>Titer with antisera for strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>06</td>
</tr>
<tr>
<td>06</td>
<td>1:1,280</td>
</tr>
<tr>
<td>85</td>
<td>1:640</td>
</tr>
<tr>
<td>11</td>
<td>—</td>
</tr>
<tr>
<td>87</td>
<td>—</td>
</tr>
<tr>
<td>B-159</td>
<td>—</td>
</tr>
</tbody>
</table>

* Dash indicates no titer with undiluted serum.

**Table 2. Amounts of sugars in cell walls of different strains of Peptostreptococcus**

<table>
<thead>
<tr>
<th>Strain of Peptostreptococcus</th>
<th>Sugars detected qualitatively</th>
<th>Amount of sugar per 100 mg of cell wall</th>
<th>Total sugar in cell wall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>3,730</td>
<td>4.0%</td>
</tr>
<tr>
<td></td>
<td>Mannose</td>
<td>266</td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>Glucose</td>
<td>6,527</td>
<td>6.5%</td>
</tr>
<tr>
<td>85</td>
<td>Glucose</td>
<td>5,195</td>
<td>11.0%</td>
</tr>
<tr>
<td></td>
<td>Rhamnose</td>
<td>5,728</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Rhamnose</td>
<td>8,924</td>
<td>9.0%</td>
</tr>
<tr>
<td>87</td>
<td>Glucose</td>
<td>1,624</td>
<td>3.1%</td>
</tr>
<tr>
<td>B-159</td>
<td>Glucose</td>
<td>1,025</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhamnose</td>
<td>436</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Strains, both contain predominantly glucose, since the mannose present in strain 06 represents only about 7% of the total carbohydrate in the cell wall. The *P. intermedius* strains are characterized by the presence of rhamnose, but in strain 87 it is the only sugar, whereas in strain 11 only 52% of the total carbohydrate is rhamnose. Strain B-159 differs from all the other strains tested, qualitatively in the presence of galactose as 15% of the total carbohydrate, but also in the relatively small amount (3.1%) of total carbohydrate in the cell wall.

Qualitative paper chromatographic analyses of amino acids and amino sugars showed that all strains possessed as major constituents, alanine, glutamic acid, lysine, glycine, serine, muramic acid, glucosamine, and galactosamine. In addition, strain B-159 possessed diaminopimelic acid and tyrosine. In all strains except 87 and 11, aspartic acid was also found.

The results of quantitative amino acid analyses (Table 3) reinforced the qualitative analyses and further emphasized some of the strain differences noted already in the study. Strains 06 and 85 showed large amounts of glutamic acid, aspartic acid, alanine, and lysine. Strains 11 and 87 showed the same pattern except for much less aspartic acid, a lack which might account for the relatively higher amount of alanine found in these strains. Strain B-159 again showed a major difference from the other four strains in that it contained a larger amount of diaminopimelic acid and only a trace of lysine. Furthermore, B-159 possessed a substantially higher amount of tyrosine, threonine, serine, valine, isoleucine, leucine, phenylalanine, and arginine than all the other strains.

The differences in amino sugar content (Table 3) are less marked among the strains studied. Strain B-159, however, is again notable in that it has the lowest total amino sugar content. The differences between the *P. putridus* and the *P. intermedius* strains may or may not be significant as group characteristics, but there is a higher galactosamine and a lower glucosamine content in the *P. intermedius* group compared to the *P. putridus* strains.

Teichoic acid was found only in strain B-159, showing again that B-159 is probably not in the same genus as the other strains studied here.

**Discussion**

The representative strains of *Peptostreptococcus* were tested by the cell wall agglutination method, a more sensitive serological test for cell wall antigens than the precipitin test, which utilizes soluble polysaccharide antigenic extracts from whole cells. The results suggest that this genus, except for strain 87, is not related to the genus *Streptococcus*. Strains 06, 85, and B-159 did not react with grouping antisera to groups A through H, J, K, M, O, P, Q, R, and S. Strain 11 showed a low (1:40) titer with antiserum for groups E and F. Strain 87 gave 1:40 agglutinin titers with groups K and O; however, the limits of reliability of the test were passed when a 1:80 titer with antiserum for group N was noted and a 1:60 titer with group C antiserum. The latter reaction is the only strong serological relationship to any of the groups of *Streptococcus*.

The chemical analyses indicated that strain B-159, which is a ruminal bacterium, does not belong to the same genus as the other four strains. It was known (2, 9) that strain B-159 was anaerobic, but also gram-negative, unlike the other strains studied here. It differed from the other strains since its cell wall lacked lysine as a major amino acid; instead, it contained large amounts of diaminopimelic acid, as well as a relatively large amount of glycine. Its content of minor amino acids was also different in that it contained substantially larger amounts than did the other four.
strains of eight amino acids: threonine, serine, valine, isoleucine, leucine, phenylalanine, tyrosine, and arginine. It alone of all strains contained teichoic acid in the cell wall. Teichoic acid has been shown to be a constituent of the determinant antigen of group N streptococci and lactobacilli (1, 8). It was concluded, therefore, that strain B-159 does not belong to the genus Peptostreptococcus and should be considered a member of a separate genus.

It is of interest to consider the classification of the other four strains in light of the statement by Cummins and Harris (4) that the amino acids present in the cell wall seem to be characteristic of the genus and the sugars and the amino sugars seem to characterize the species within the genus. All four strains contained, as major amino acids in the cell wall, glutamic acid, alanine, and lysine. Strains 06 and 85 of *P. putridus* also contained sufficient amounts of aspartic acid to designate that amino acid as a major one. Strains 11 and 87, however, contained, respectively, 45 and 7% the amounts of aspartic acid found in strains 06 and 85. It appears that strains 11 and 87 should not be classified in the same genus with the *P. putridus* strains, although in at least one other genus, *Streptococcus*, it has been shown that aspartic acid appears as a major constituent in the cell walls of some strains and not in others (19). Since the cell wall agglutination tests showed no relationship between strains 11 and 87 and strains 06 and 85, it appears at the present time that strains 11 and 87 are not members of the same genus as strains 06 and 85. The nonreciprocal reactivity shown between strains 11 and 87 in the cell wall agglutination test seems to indicate some similarity but not identity of the two strains.

The carbohydrate analyses showed that strain 87 contained rhamnose and strain 11 contained rhamnose and glucose. These data, and the amino acid data mentioned above, are consistent with strains 11 and 87 being separate species, possibly in the same genus, which is not the same as that of strains 06 and 85.

Strains 06 and 85 of *P. putridus* contain in the cell walls the same kinds and amounts of amino acids and amino sugars, as well as the same sugar, glucose, although strain 06 does also contain a trace of mannose. The small amount of mannose does not appear to play a significant role in antigenic specificity. In light of the chemical analyses, the nutritional and physiological similarities, and the reciprocal cross-reactivity in the cell wall agglutination test, it is concluded that the two

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### TABLE 3. Results* of amino acid and amino sugar analyses of Peptostreptococcus cell walls

<table>
<thead>
<tr>
<th>Component</th>
<th>06</th>
<th>85</th>
<th>11</th>
<th>87</th>
<th>B-159</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amino acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1.98</td>
<td>1.88</td>
<td>1.56</td>
<td>1.01</td>
<td>1.38</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>1.62</td>
<td>1.60</td>
<td>0.074</td>
<td>0.011</td>
<td>1.27</td>
</tr>
<tr>
<td>Alanine</td>
<td>3.14</td>
<td>3.16</td>
<td>6.29</td>
<td>5.96</td>
<td>2.85</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.50</td>
<td>1.58</td>
<td>1.70</td>
<td>1.69</td>
<td>0.439</td>
</tr>
<tr>
<td>Diaminopimelic acid</td>
<td>0.068</td>
<td>0.088</td>
<td>0.047</td>
<td>0.040</td>
<td>1.08</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.183</td>
<td>0.114</td>
<td>0.117</td>
<td>0.195</td>
<td>1.12</td>
</tr>
<tr>
<td>Phosphoserine</td>
<td>0.029</td>
<td>0.033</td>
<td>0.057</td>
<td>0.052</td>
<td>TR</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.103†</td>
<td>0.006</td>
<td>0.068</td>
<td>0.081</td>
<td>0.588</td>
</tr>
<tr>
<td>Serine</td>
<td>0.046</td>
<td>0.026</td>
<td>0.068</td>
<td>0.087</td>
<td>0.377</td>
</tr>
<tr>
<td>Proline</td>
<td>TR</td>
<td>0.014</td>
<td>0.027</td>
<td>0.040</td>
<td>0.037</td>
</tr>
<tr>
<td>Valine</td>
<td>0.114</td>
<td>0.028</td>
<td>0.036</td>
<td>0.091</td>
<td>0.457</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.095</td>
<td>0.107</td>
<td>TR</td>
<td>—</td>
<td>0.036†</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.048</td>
<td>0.025</td>
<td>0.017</td>
<td>0.039</td>
<td>0.235</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.061</td>
<td>0.025</td>
<td>0.022</td>
<td>0.055</td>
<td>0.325</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.265†</td>
<td>0.052†</td>
<td>TR</td>
<td>—</td>
<td>0.477†</td>
</tr>
<tr>
<td>Ornithine</td>
<td>0.124</td>
<td>0.183</td>
<td>0.089</td>
<td>0.013†</td>
<td>0.090</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.029†</td>
<td>0.011†</td>
<td>0.020†</td>
<td>0.016</td>
<td>0.062</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.076†</td>
<td>0.054†</td>
<td>0.027†</td>
<td>TR</td>
<td>0.217</td>
</tr>
<tr>
<td><strong>Amino sugars</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muramic acid</td>
<td>0.921</td>
<td>0.823</td>
<td>0.822</td>
<td>0.932</td>
<td>0.592</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>1.99</td>
<td>1.65</td>
<td>1.20</td>
<td>1.25</td>
<td>1.65</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>1.34</td>
<td>0.966</td>
<td>1.50</td>
<td>2.72</td>
<td>0.587</td>
</tr>
</tbody>
</table>

*Expressed as micromoles of component per 5.3 mg of cell wall. TR = trace; — = absent.
† Estimated.
strains 06 and 85 represent one species and can be classified as \textit{P. putridus}.

Stone (20), using biochemical and serological reactions, could not correlate the two methods in any workable taxonomic scheme, although he suggested from the results of precipitin tests that anaerobic streptococci may have some relationship to groups A, B, and C of \textit{Streptococcus}. Mergenhagen and Scherp (14) studied patterns of nutritional requirements between anaerobic streptococci and other members of the genus \textit{Streptococcus}. They defined three groups, one typified by \textit{P. putridus}, the second by \textit{P. foetidus}, and the third resembling oral strains of \textit{S. salivarius}. The results of this investigation confirm only Mergenhagen’s classification of \textit{P. putridus} (14).

It is known that the genus- and species-specific antigens may not reside on the cell wall. For example, in group D streptococci the species-specific antigen is a glucosyl glycerol teichoic acid, and appears to be associated closely with the protoplasm membrane (18). The rupture of the cell, preparatory to the cell wall agglutination test, may expose those antigens which are not in the external layer of the cell wall. The exposed antigens may then be able to combine with specific agglutinins. The rupture method of antigenic preparation of cell walls appears to be useful in those microorganisms, for example, the “untypable” streptococci, where methods of acid hydrolysis in antigen preparation have not proved successful (12).

The serological analyses of cell wall antigens by agglutination combined with analyses of chemical constituents of the cell wall appears to be a more definite method of classifying anaerobic streptococci.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


