LYSOZYME PRODUCTION BY STAPHYLOCOCCI AND MICROCOCCI

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KASHIBA et al. (1959) showed that coagulase-positive staphylococci formed lysozyme and were also resistant to the leucozyme A of leucocytes. They thought that the pathogenicity of these strains might be related to their resistance to the bactericidal action of leucocytes. Goldbach and Haenel (1964) found that all of 82 coagulase-positive staphylococci from faeces produced lysozyme, but that 15 of 43 coagulase-negative staphylococci from faeces also gave a positive reaction. Jay (1966) examined 49 strains of coagulase-negative staphylococci and found lysozyme production in four, of which three were regarded as possible pathogens. Grossgebauer, Schmidt and Langmaack (1968) reported that all of 156 strains of Staphylococcus albus (epidermidis) were lysozyme-negative, but Hawiger (1968) found that 13 of 35 strains of Staph. epidermidis produced lysozyme.

Coagulase-negative members of the Micrococcaceae are now recognised as primary pathogens in the urinary tract (Torres Pereira, 1962; Mitchell, 1968; Mortensen, 1969). They also often cause indolent bacterial colonisation of artificial internal prostheses, almost always with an accompanying bacteriemia. Ventriculo-atrial shunts for the relief of hydrocephalus are particularly liable to this complication (Carrington, 1959; Holt, 1969a), as are synthetic patches for the repair of cardiac septal and aortic defects, and Starr-Edward prostheses for defective heart valves (Wilson and Stuart, 1965).

A simple test for lysozyme production was applied to recent isolates of staphylococci and micrococci, especially coagulase-negative strains recovered from the urinary tract and from colonised internal prostheses.

MATERIALS AND METHODS

Cultures

All the cultures were Gram-positive, catalase-positive cocci, and had been classified in the scheme of Baird-Parker (1963) by methods that have been reported elsewhere (Holt, 1969a and b).

Coagulase-negative cocci. These included 36 cultures from the urine of patients (30 children and 6 adults) with infection of the urinary tract, 40 cultures from the shunt, blood or ventricles of children with colonised ventriculo-atrial shunts, five biotypes from the blood of two adults with infected prosthetic heart valves, and three obtained in pure culture from surgical wounds; 253 skin strains were obtained from patients and staff of Queen Mary's Hospital for Children, and from healthy adults and children, none of whom had a skin or nasal infection. In addition, 52 strains from various sources classified as Sarcina spp. were tested.

Coagulase-positive cocci. These comprised 100 strains from septic lesions in children or staff in this hospital and 100 strains from the nares of child patients who had no obvious association with each other.

Detection of lysozyme

Cultures were seeded as spots on to the surface of a plate of nutrient agar that contained a thick suspension of a heat-killed lysozyme-sensitive coccus (Jay; Grossgebauer et al.); Hawiger. After incubation for 1-2 days, lysozyme production was indicated by a clear zone of lysis of the semi-opaque medium around the area of bacterial growth.
Most workers have used Micrococcus lysodeikticus (NCTC2665) as a source of lysozyme-susceptible whole cells or cell walls, although Hawiger also used cell walls from S. epidermidis strain Zak. M. lysodeikticus belongs to Baird-Parker's subgroup Micrococcus 7; I tested many members of this and other subgroups of staphylococci and micrococci as possible substrates and found a few that showed good lysis, but none that was more sensitive than M. lysodeikticus NCTC2665; this strain was therefore used throughout.

The suspension of cells was prepared by the method of Hawiger, except that the culture was grown on infusion broth agar (Cruickshank, 1965). This was found to be as satisfactory as the special media suggested by Smolelis and Hartsell (1949) and Grossgebauer et al. The harvested growth was autoclaved for 10 min. at 121°C. The sterilised cell suspension was added to 15 ml melted infusion broth agar (Cruickshank) at a final concentration of about 10⁸ cells per ml. The agar suspension was poured on to a base layer of 10 ml infusion agar, and the plates were dried for 2 hr at 37°C. They kept well for several days in sealed bags at 4°C.

Point-inocula from 18-hr plate cultures of the test strains were made on the surface of the medium, six to eight on each 9-cm petri dish. After incubation for 3 days at 37°C in a sealed bag, the results were recorded. Clear lytic zones extending for more than 3 mm from the growth edge were regarded as indicating strong lysozyme production; weak production was denoted by lytic zones less than 3 mm in width.

In preliminary experiments, incubation under strict anaerobiosis produced exceptionally well-defined zones of lysis but no additional positive reactions were found.

RESULTS

The table summarises the results of lysozyme production by 589 cultures of staphylococci, micrococci and sarcinae classified according to Baird-Parker's scheme. All 200 cultures of Staphylococcus subgroup I (Staph. aureus) gave strongly positive reactions. Among the 176 other staphylococci there were 17 producers of lysozyme (7 strong and 10 weak); an occasional positive reaction was obtained in each subgroup except IV. There were also 19 lysozyme producers (14 strong and 5 weak) among 161 micrococci; most of the cultures giving a positive reaction were members of subgroup 3 (16 of 28 cultures tested), and no positive reactions were found in subgroups 1, 2, 4 and 7. All 52 cultures of Sarcina spp. were lysozyme negative.

Only three of 40 cultures of coagulase-negative staphylococci isolated from colonised ventriculo-atrial shunts—all members of subgroup II—produced lysozyme, a proportion similar to that found among members of the same subgroup isolated from normal skin. Five coagulase-negative staphylococci from the blood of patients with heart-valve prostheses and three from surgical wounds were all lysozyme-negative. Among staphylococci and micrococci of all subgroups from the normal skin only 13 of 253 produced lysozyme.

On the other hand, 20 of 36 staphylococci and micrococci from infections of the urinary tract produced lysozyme, and 11 of them belonged to Micrococcus subgroup 3. Although members of this subgroup from other sites are often lysozyme-positive, the proportion was higher in urinary cultures (11 of 13) than in cultures from normal skin (5 of 15). Lysozyme production also appeared to be more frequent in urinary than in skin strains in Staphylococcus subgroups II, III and VI and in Micrococcus subgroups 5 and 6, though in some cases the numbers of cultures available for comparison were small.

DISCUSSION

The purpose of this study was to investigate the significance of lysozyme production by coagulase-negative members of the Micrococcaceae as an index of pathogenicity, and the opportunity was taken to see whether there was any relation between their production of this enzyme and the classification of the cultures in the Baird-Parker scheme. Many of the coagulase-negative strains from urinary tract infections were lysozyme-positive, particularly the Micrococcus subgroup 3 strains, shown by Mitchell (1968) to have a remarkable predilection for this site; their evocation of leucocytosis both in urine and blood is strong.
## Table

**Lysozyme production by staphylococci, micrococci and sarcinae obtained from lesions and from normal skin, and classified according to Baird-Parker’s scheme**

<table>
<thead>
<tr>
<th>Baird-Parker subgroup</th>
<th>Origin of cultures</th>
<th>Number of cultures tested</th>
<th>Lysozyme production: number of cultures that gave a strong reaction</th>
<th>weak reaction</th>
<th>negative reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI*</td>
<td>Septic skin lesion</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Anterior nares</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
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<tr>
<td>SII</td>
<td>Colonised ventriculo-atrial shunt</td>
<td>40</td>
<td>0</td>
<td>3</td>
<td>37</td>
</tr>
<tr>
<td>Blood†</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Surgical wound</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>6</td>
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<tr>
<td>Normal skin</td>
<td>50</td>
<td>1</td>
<td>2</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>SIII</td>
<td>Blood†</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal skin</td>
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<td>11</td>
<td>11</td>
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<td>19</td>
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<td>14</td>
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<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Normal skin</td>
<td>20</td>
<td>1</td>
<td>2</td>
<td>17</td>
<td>17</td>
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<tr>
<td>M1</td>
<td>Normal skin</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8</td>
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<td>M2</td>
<td>Normal skin</td>
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<td>0</td>
<td>0</td>
<td>20</td>
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<td>M3</td>
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<td>13</td>
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<tr>
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<td>0</td>
<td>6</td>
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<td>2</td>
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<td>0</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>M7</td>
<td>Normal skin</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>48</td>
</tr>
<tr>
<td><strong>Sarcina spp.</strong></td>
<td>Various</td>
<td>52</td>
<td>0</td>
<td>0</td>
<td>52</td>
</tr>
</tbody>
</table>

*S = Staphylococcus; M = Micrococcus.

*SI = Staph. aureus. † Patients with heart-valve prosthesis.
evidence of primary pathogenicity, and there may be a connexion, however indirect, between this and their lysozyme production. Very few coagulase-negative members of the Micrococcaceae from healthy human skin produce lysozyme and most of these belong to Micrococcus subgroup 3. It would be interesting to know whether subjects with urinary-tract infection caused by lysozyme-positive Micrococcus subgroup 3 strains carry this biotype in their resident skin flora. My figures suggest, however, that in this subgroup, and also in a number of other subgroups of staphylococci and micrococci, lysozyme production occurs more often in strains isolated from the urinary tract than in those found on normal skin.

The staphylococci of subgroup II from colonised prostheses were almost invariably lysozyme-negative, and this at first sight removes any prospect of a relation between pathogenicity and lysozyme production. There is, however, a growing tendency among workers in this field not to regard chronic colonisation of ventriculo-atrial shunts by coagulase-negative staphylococci as a consequence of conventional pathogenicity. Even when colonisation and bacteriaemia are well established there may be remarkably few clinical signs, and it has been our continued experience with these cases that little or no leucocytosis occurs in the blood or brain ventricles, despite the presence of enormous numbers of cocci; the first evidence of colonisation usually comes from the blood cultures or from mechanical malfunction of the shunt valves. Removal of the infected prostheses almost invariably results in the disappearance of bacteriaemia, after which a replacement shunt can be implanted with no greater risk of subsequent recolonisation. An entirely different picture is presented on the rare occasions when shunts are invaded by coagulase-positive staphylococci; high pyrexia and leucocytosis occur rapidly, and treatment must be started immediately. It is suggested that indolent colonisation follows insidious invasion by bacteria that provoke negligible response in the host, that these organisms therefore need little protection from phagocytosis, and that their inability to produce lysozyme does not detract from their role as stealthy colonisers of artificial surfaces.

**SUMMARY**

Lysozyme production by members of the Micrococcaceae was tested for by a plate method. All coagulase-positive staphylococci and a number of other staphylococci and micrococci produced lysozyme.

Many coagulase-negative staphylococci and micrococci from infections of the urinary tract produced lysozyme, particularly those belonging to Micrococcus subgroup 3 in Baird-Parker's scheme, but lysozyme production was more frequent in urinary than in skin strains, not only in this subgroup but in several other subgroups of staphylococci and micrococci.

Coagulase-negative staphylococci that colonised internal artificial prostheses rarely produced lysozyme. It is suggested that cocci that cause indolent colonisation of internal prostheses are not pathogenic in the conventional sense.

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**REFERENCES**


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