RED HALOPHILIC BACTERIA—THE IDENTITY OF SOME WELL-KNOWN SPECIES*

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The red halophilic bacteria from salts causing discolourations of salted fish and hides, have been studied for well over seven decades but till lately very little detailed account about their morphology, biochemical activities and taxonomy was available. Petter (1932), Lochhead (1934) and Gibbons (1936) described some of these bacteria in detail. Quite recently, Anderson (1954) and Venkataraman and Sreenivasan (1956) described a number of rod forms, mostly new species. Despite these researches, the position is still not satisfactory especially with regard to the coccus forms. For example, in the sixth edition of Bergey’s Manual only Sarcina littoralis Poulsen is described, whereas in the literature numerous others are enumerated. In the Manual, Micrococcus (Diplococcus) morrhuae Klebahn is mentioned in Appendix III of the family Micrococcaceae, as additional species, but in the proposed seventh edition of the Manual (in the press), M. morrhuae is included in the genus Micrococcus and described in detail. Sarcina morrhuae Farlow and Micrococcus littoralis Kellerman were considered synonymous, while Diplococcus gadidarum Beckwith and M. littoralis gadidarum were considered to be varieties of S. littoralis (Stuart, Frey and James, 1933). Lochhead classified Klebahn’s S. morrhuae and other similar organisms reviewed above as Sarcina littoralis Poulsen. Gibbons, in fact, preferred to designate this as Sarcina (Micrococcus) littoralis, since it was difficult to say whether the organism was a micrococcus or sarcina. In this confusion, the culture designated as M. roseus halophilus (Petrowa) or Tetracoccus roseus halophilus (Wlassowa) has escaped attention.

METHODS

The cultures were studied by methods described in our earlier work (Venkataraman and Sreenivasan, 1956). The media used for growing them and for the study of physiological properties are described by Sreenivasan and Venkataraman (in press). Conventional media used by Gibbons (1936) were also tried. Tests for H₂S production were done according to Anderson’s (1954) procedure.

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DISCUSSION

Four authentic coccus forms and four rod forms have been studied in detail and the results summarized in detail in Table I. It is clear that S. littoralis, S. morrhuae, M. morrhuae and M. roseus halophilus are four different species and not variants of a single species. Morphologically, S. littoralis, though described as a sarcina, does not show typical packets in all media or constantly in a single medium even. It is mostly seen as pairs or single or clusters even in wet mount preparations as well as in stained preparations. This is why probably Gibbons (1936) was inclined to call it a micrococcus, with which we also agree. On the other hand, S. morrhuae always exhibits an arrangement in packets of three planes, consisting of 16, 32 or more cells and tetrads. In appearance while S. littoralis is smooth, S. morrhuae, as it ages, shows rough, 'warty' dry folded brick-red appearance. Both are brilliant red in colour. S. morrhuae slowly digests milk, produces alkaline reaction in sugars and also NH₃ from a salt-prawn broth-starch medium in contrast to S. littoralis. M. morrhuae is orange red to bright red large coccus which does not show tetrad forms. This also digests casein slowly, and agrees with the description given in the seventh edition of the Manual. In our "Rice milk" medium, S. morrhuae shows clearing of the medium due to digestion of casein and M. morrhuae shows "Pits" or deep depressions due to starch digestion. S. littoralis and M. roseus halophilus grow on this medium without these changes. The latter is orange red to deep red as any of the above cultures. This is also a truly obligate halophile since it failed to grow even in 10% NaCl media. Though Petrowa (1935) had stated that this culture is a salt-adapted form of the ordinary mesophilic M. roseus, the culture studied by us could not be acclimatized to low-salt media. This culture was mostly in pairs, or short chains and sometimes in tetrads. On the other hand we had a truly rose-coloured culture isolated from salt-milk-agar tube as a (aerial ?) contaminant. This culture grew slowly and sparsely on 20% salt-milk-agar but grew also on sea-water agar and broth, while M. roseus halophilus failed to grow in liquid media. This latter culture was of further interest in producing indole. M. rhodocrous, a red coccus from sea-water, failed to grow in 20% salt-milk-agar though a slight growth was seen in 10% NaCl.

The following rod forms have been described well in literature—Pseudomonas salinaria, Ps. cutirubra, Bacterium halobium and B. trapanicum. In his excellent paper Lochhead (1934) had mentioned that Ps. cutirubra differs from Ps. salinaria in slight variation in colour and in its greater proteolytic action on milk. This itself is not sufficient to separate them but as seen from Table I there are differences warranting recognition of them as different species. Gelatin hydrolysis and nitrate reduction were positive in Ps. cutirubra but not
### Table I

**Morphological, cultural characteristics and physiological reactions of the red halophilic bacteria**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Cultural and morphological characters</th>
<th>Growth in 10% NaCl</th>
<th>Gelatin hydrolysis</th>
<th>Casein digestion</th>
<th>Nitrate reduction</th>
<th>Starch hydrolysis</th>
<th>Indole production</th>
<th>H₂S</th>
<th>Sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas salinaria</em></td>
<td>Circular, drop-like, entire, 2 mm. red. Deep red, smooth, filiform, moist growth in 2–3 days on salt-milk-agar. Very highly pleomorphic, motile rods. Simple rods rare; gram negative.</td>
<td>−</td>
<td>−</td>
<td>±</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>NC</td>
</tr>
<tr>
<td><em>Ps. cutirubra</em></td>
<td>Colonies as in <em>P. salinaria</em>. On salt-milk-agar, colour is darker, scarlet. On gelatin agar shows a pink-mauve colour. Growth in less than 2 days. Highly pleomorphic, motile rods, more of simple rods with higher NaCl content; gram negative.</td>
<td>−</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>NC</td>
</tr>
<tr>
<td><em>Bacterium halobium</em></td>
<td>Circular, red, entire colonies on salt-milk-agar. Smooth, sometimes viscous growth, drawing out on wire. Good growth in 2–3 days on salt-milk-agar. Rods, long, thin, bent, rounded ends, gram negative, motile.</td>
<td>−</td>
<td>+</td>
<td>±</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>NC</td>
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<tr>
<td>Species</td>
<td>Appearance</td>
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<tr>
<td>Bact. trapanicum</td>
<td>Rose-red, circular, entire; filiform, smooth, growth on salt-milk-agar in 4 days. Motile, simple straight rods, gram negative. Turbidity in broth also. Better growth on Rice milk medium</td>
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<tr>
<td>Sarcina littoralis</td>
<td>Circular, convex, scarlet red, moist, glistening; smooth, butyrous, abundant in 2-3 days on salt-milk-agar. Also grows on Lochhead's yeast-starch agar. Coci in pairs, single, clusters and tetrads; gram positive.</td>
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<td>Sarcina morrhuae</td>
<td>Deep red, circular, entire, raised on salt-milk-agar; rough, dry growth on rice-milk-salt medium. Red, moist, butyrous, growth in 2-3 days on salt-milk-agar; on yeast-starch agar, also, dry &quot;warty&quot; rough growth characteristic. Coci in packets of 16, 32, or more or tetrads; gram positive.</td>
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<tr>
<td>Micrococcus morrhuae</td>
<td>Orange-red circular, entire, raised, on salt-milk-agar. On rice-milk-salt medium, deep depressions characteristic; on starch media colour turns brick-red, dry. Large gram positive cocci, mostly pairs, single, rarely tetrads.</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Alk (Sl)</td>
</tr>
<tr>
<td>Micrococcus roseus halophilus</td>
<td>Rose-red to deep red, circular, entire; smooth, moist, butyrous on salt. milk-agar in 2-3 days, cocci, gram positive, mostly pairs, a few tetrads and single.</td>
<td>−</td>
<td>±</td>
<td>−</td>
<td>±</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>Alk</td>
</tr>
</tbody>
</table>

− = Negative. ± = Partial or slow. NC = No change. Alk = Alkaline.

1 Nomenclature: As furnished by the donors of cultures.
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in *Ps. salinaria*. The former digests casein rapidly producing off-odours and shows beautiful change in colour from red to pink and mauve especially in gelatin media. With 25% NaCl more of simple rods are seen than pleomorphic forms. *Bacterium trapanicum* is of lighter rose-red shade in contrast to *B. halobium*, which also shows a mauve-pink colour in gelatin agar media. *B. trapanicum* is slower in growing than is *B. halobium* on salt-milk-agar. Further differences between the two are evident in the table. All the eight cultures are catalase positive.

Breed (1955) has proposed the creation of a new genus *Halobacterium Elizari-volcani* in family *Pseudomonadaceae* for the polar flagellated, red brine organisms. The position of *B. halobium* and *B. trapanicum* whose flagellation is not clear, is not established but it is desirable to include them also in this new genus. The rod forms described by Anderson (1954) and Venkataraman and Sreenivasan (1956) also should belong to this genus. Though halophilic cocci and sarcina are not separated from their non-halophilic compatriots it would be reasonable to accord them also a generic rank and designated as *Halococcus*, as suggested by Schoop (1934), in view of their obligate halophily.

ACKNOWLEDGEMENTS

Thanks are due to Dr. A. J. Kluyver, for supplying the cultures of *Bact. halobium*, *B. trapanicum S. morrhue* and to Dr. J. M. Shewan, for other cultures. Thanks are also due to Dr. R. S. Breed, for supplying the preprint of the description of *M. morrhue* and for his suggestions on the classification of red halophilic rods.

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