INTERNATIONAL JOURNAL of SYSTEMATIC BACTERIOLOGY
October 1971, pp. 323-325

Vol. 21, No. 4
Printed in U.S.A.

Serological Investigations of *Planococcus* Strains

PER OEDING

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Boháček et al. have proposed that the flagellated, gram-positive cocci be included in the genus *Planococcus* Migula, particularly on the basis of their per cent guanine plus cytosine content. A serological examination of these organisms (10 strains) by the author revealed no antigenic relationship to staphylococci or micrococci, thus substantiating the conclusion of Boháček et al. that they do not belong to the genus *Micrococcus*. Antigenically, the group of strains was rather heterogeneous.

Most authors have placed the flagellated, gram-positive cocci of marine origin in the genus *Micrococcus* Cohn. Boháček et al. (2–4) and Kocur et al. (9) demonstrated that these bacteria differ considerably from micrococci and staphylococci in their per cent guanine plus cytosine (GC) content and suggested that they be included in the genus *Planococcus* Migula, 1894. Schleifer and Kandler (13) found that the 10 strains studied by Boháček et al. and Kocur et al. were uniform with respect to the type of murein present in their cell walls and that this murein was different from the mureins found in the cell walls of members of the genera *Micrococcus* and *Staphylococcus*. Their results thus supported the taxonomic suggestion of Boháček et al. (2, 3).

The purpose of the study reported here was to examine serologically the 10 strains of flagellated cocci used by the above-mentioned authors to determine (i) whether they are serologically related to staphylococci and micrococci and (ii) what serological similarities or dissimilarities exist within the group.

MATERIALS AND METHODS

Bacterial strains. The 10 strains of flagellated, marine cocci were kindly furnished by M. Kocur, Czechoslovak Collection of Microorganisms (CCM), Brno. Their cultural, morphological, and biochemical characteristics have been described by Boháček et al. (9).

Sera. Preimmune sera and immune sera were obtained from the Gade Institute's breed of New Zealand White rabbits. Immune sera were produced by intravenous injections of formalin-killed bacteria (12).

Methods. Agglutination was carried out on slides by using live bacteria (12) grown on nutrient agar at 30 C for 18 hr.

The double diffusion in agar technique was the same as that used by Haukenes and Oeding (7). Thick suspensions of live 18-hr cultures were tested against undiluted immune sera. Polysaccharide A (6), polysaccharide 263 (8), and protein A (5) from *Staphylococcus aureus*, polysaccharide B from *S. epidermidis*, and polysaccharide AC from *Micrococcus* (11) were included as references.

Cell walls were prepared from the two flagellated strains 316 and 1849.

RESULTS

Agglutination in rabbit preimmune sera and in S. aureus, S. epidermidis, and Micrococcus immune sera. All strains were tested by agglutination in four preimmune sera, in immune sera against four selected *S. aureus* and four *S. epidermidis* strains, and against nine *Micrococcus* strains representing all of Baird-Parker's (1) eight biochemical types. All the strains agglutinated in titers 1:1 to 1:10 in one or more of the immune sera. Two strains agglutinated in dilution 1:20 of one immune serum each. Although somewhat less frequently, the strains also agglutinated in the preimmune sera in titers 1:1 to 1:10, never higher. CCM 2388 was slightly unstable, and the reactions were therefore difficult to evaluate.

Double diffusion in agar gel with S. aureus, S. epidermidis, and Micrococcus antigen preparations as references. No strain produced any precipitin line corresponding to polysaccharide A (β-glucosaminyl ribitol teichoic acid), polysaccharide 263 (α-glucosaminyl ribitol teichoic acid), polysaccharide B (α-glucosyl glycerol teichoic acid), polysaccharide AC, or protein A. CCM 1849 and CCM 2069 gave a common, sharp precipitin line against *S. aureus* serum Wood 46. This line was identified with a pre-

cipitin line which we have earlier registered in the Wood 46 bacteria-immune serum system and designated the x-line.

Agglutination and double diffusion in agar gel with immune sera against flagellated strains. Immune sera were produced against the flagellated strains CCM 316 (= ATCC 14404 = NCMB 1493) and CCM 1849. The former represented the group of four strains having a per cent GC content of 48 to 52 and was designated as the neotype strain of *Planococcus citreus* (4, 9). The latter was one of the remaining six strains which had a per cent GC content of 39 to 42. The agglutination test showed that CCM 316 differed clearly from the other strains; CCM 1849 and CCM 2069 appeared to be identical or very similar (Table 1). Other agglutinations were observed in undiluted sera only and could not be distinguished from the reactions in preimmune sera. The antigenicity of the two strains selected for immunization was rather weak, the homologous agglutinin titers being only 1:80 and 1:160, respectively.

Double diffusion in agar gel between live 18-hr-old cells of the 10 flagellated strains and the two immune sera revealed four separate precipitin lines which were designated a, b, c, and x (*see* above and Table 2). The lines a, b, and c were straight, whereas the line x was curved with the convex side towards the serum well. Lines b and c were rather faint. The precipitin reactions confirmed the difference between CCM 316 and CCM 1849 and the similarity between CCM 1849 and CCM 2069. Further, an antigenic relationship was demonstrated between CCM 316 and several strains, a relationship which was not revealed by the agglutination reactions.

The cell walls prepared from CCM 316 and CCM 1849 produced none of the precipitin lines observed with bacterial suspensions of the same strains with the exception of a very faint x-line with cell walls from CCM 1849.

TABLE 1. *Agglutination*[a]

Strains	Titers in immune serum	
	CCM 316	CCM 1849
CCM 316	80	1
CCM 1849	1	160
CCM 2069	1	160
7 strains	1(−)[b]	−

[a] Reciprocal values.

[b] −, No agglutination in undiluted serum.

TABLE 2. *Double diffusion in agar gel*

Strains	Immune sera CCM 316 and CCM 1849			
	Precipitin lines			
	a	b	c	x
CCM 316	+	+	−	−
CCM 1849	?	−	+	+
CCM 2069	−	−	+	+
CCM 2104	+	−	−	−
CCM 2387	+	−	−	−
CCM 2388	+	−	−	−
CCM 2389	−	−	−	−
CCM 2414	−	−	−	−
CCM 2415	+	+	−	−
CCM 2416	+	−	−	−

DISCUSSION

With the exception of the x-line (*see* below) no serological relationship was demonstrated between the 10 strains of flagellated marine cocci and staphylococci or micrococci. As immune sera representing all the eight micrococcal types of Baird-Parker (1) were tested by agglutination, strains belonging to the genus *Micrococcus* might be expected to show some degree of cross-reaction (Hasselgren and Oeding, *unpublished data*). However, none of the strains agglutinated at higher titers in the immune sera than in the preimmune sera. In addition, no strain was shown by agar-gel diffusion to have the α- or β-glucosaminyl ribitol teichoic acid or protein A characteristic of *S. aureus*, the α-glucosyl glycerol teichoic acid present in *S. epidermidis*, or the teichoic acid of *Micrococcus* represented by the polysaccharide AC preparation. The reservation must be made that a serological reference system for the β-glucosyl glycerol teichoic acid of *S. epidermidis* was not available, and that micrococci have other types of teichoic acids and polysaccharides in addition to the one tested here. Previous investigations (Oeding and Hasselgren, unpublished data; reference 11) do, however, indicate that the teichoic acid represented in the polysaccharide AC reference system is the most common in micrococci.

The x-line which two of the flagellated strains share with *S. aureus* strain Wood 46 is interesting, but the distribution of this antigen being unknown, it can hardly be given taxonomic importance.

The serological examination of the 10 flagellated strains thus substantiates the conclusion drawn by Boháček et al. (2–4) and Kocur et al. (9) that these strains do not belong to the genus *Micrococcus*.

The serological investigations performed with immune sera against two flagellated strains show that the 10 strains constitute a heterogeneous group with regard to their antigens. The agglutinin reactions very clearly showed that CCM 316 is different from the other strains and that CCM 1849 and CCM 2069 are similar or identical. The precipitin tests confirmed the similarity of the two latter strains and revealed a relationship between CCM 316 and several other strains. CCM 316 belonged to one group and CCM 1849 and CCM 2069 to the other group of Boháček and Kocur (4) according to the per cent GC content. This is in accord with the results given above, but otherwise no correlation was found between the biochemical and serological investigations.

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