THE TAXONOMIC STATUS OF THE GENUS
PLANOCOCCUS MIGULA 1894

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ABSTRACT. Seven strains of flagellated marine cocci have been subjected to taxonomic analysis. It is suggested that they be included in the genus Planococcus Migula, 1894. A revised description of the genus Planococcus is proposed. The strain Planococcus citreus CCM 316 (Micrococcus aquivivus ATCC 14404) is proposed as the neotype culture.

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The recommendation of Migula (1894, 1900) to include flagellated cocci either in the genus Planococcus or Planosarcina has hitherto been accepted by a few authors only, e.g. Krasil'nikov (1949). The majority of other authors have included the flagellated cocci in the genus Micrococcus (Hucker and Breed 1957, Leifson 1964). That was because these cocci could be differentiated from the other members of the genus Micrococcus only by their motility. Most authors considered this characteristic to be inadequate for the recognition of a new genus. The findings of Boháček, Kocur and Martinec (1967, 1968) that the flagellated cocci differ considerably in their %GC content in DNA from micrococci has thrown new light on their taxonomic position. The above difference justifies the transfer of the flagellated cocci to a separate genus. It was therefore proposed by Boháček et al. (1967) to include the flagellated cocci with a %GC content ranging from 40—50% in the genus Planococcus Migula.

The purpose of the present paper is to give a revised description of the genus Planococcus.

MATERIAL AND METHODS

In the present paper, seven strains of flagellated cocci of marine origin were investigated (Table 1). Stock cultures were maintained on nutrient agar at 4°C. For morphological, cultural, and physiological studies, cultures 24 hrs old were used. All tests were carried out at 30°C.

Morphological properties and Gram staining were studied according to the methods recommended in the Manual of Microbiological Methods (1957).

For determination of motility, cultures from liquid and solid media were examined under a phase-contrast optical microscope at low (x 100) and high (x 500) magnifications.

Table 1. List of strains used.

	CCM No.	Other numbers and references
<u>M. aquivivus</u>	316	NCMB 1493 ATCC 14404, strain 628 ZoBell and Upham (1944)
<u>M. eucinetus</u>	2387	NCMB 1494 Strain XQ 11, Leifson (1964)
	2388	NCMB 1495 Strain XQ 58, Leifson (1964)
	2389	NCMB 1496 Strain XQ 40, Leifson (1964)
<u>Micrococcus</u> sp.	1849	NCMB 629 Strain 732, Georgala (1957)
	2069	NCMB 628 Strain 450, Georgala (1957)
	2104	NCMB 1491 (Bain, Shewan and Hodgkiss 1957)

Abbreviations used:

ATCC = American Type Culture Collection, Rockville, Md., U.S.A.

CCM = Czechoslovak Collection of Microorganisms, Brno, CSSR.

NCMB = National Collection of Marine Bacteria, Torry Research
Station, Aberdeen, Scotland

For electron microscopy, cells were harvested from liquid media by centrifuging at low speed (2000 g) and were resuspended in freshly distilled, sterile water. From solid media the cells were harvested by suspending them in freshly distilled sterile water. The harvested cells were washed three times by centrifuging in freshly distilled, sterile water in which a final suspension of cells was prepared. Metal-shadowed specimens were prepared on formvar-coated 200 mesh copper grids. A droplet of a cell suspension was placed on a grid air-dried and shadowed with gold-palladium (40:60) at an angle of 20°. Negatively-stained specimens were prepared by mixing an aliquot of the cell suspension with an equal volume of 1% (w/v) aqueous ammonium molybdate or 1% (w/v) aqueous phosphotungstic acid adjusted to pH 7.0. A droplet of this mixture was placed on a carbon-stabilized formvar-coated 200 mesh grid and air-dried. Specimens were examined in a Siemens Elmiskop I using the double condenser system, a 200 μ condenser aperture, a 50 μ objective aperture and an accelerating voltage of 80 kV. Micrographs were recorded on Ilford N50 plates.

Spore formation was studied on medium no. 12 recommended by MacDonald and MacDonald (1962). After 7 days cultivation the cultures were examined microscopically and subjected to heating to various temperatures. Sporosarcina ureae strain CCM 860 with good sporulation was used as control. Most of the biochemical methods were those described by Mortensen and Kocur (1967). Four different media for the study of utilization of carbohydrates were used: (i) MOF medium according to

Leifson (1963), (ii) sea water medium, (iii) medium of Evans, Bradford and Niven (1955), and (iv) medium recommended by the International Subcommittee (1965). Production of indole, hydrogen sulfide, phenylalanine deaminase, and growth on Simmons' citrate agar were studied by means of methods recommended by Ewing (1960). Aesculin hydrolysis and nitrite reduction were studied by means of methods recommended by Sneath (1956). Sensitivity to antibiotics was tested by using multodiscs "Oxoid."

RESULTS

Morphology. The cells of all the strains were Gram-positive, spherical, 1.0 to 1.2 μ in diameter and occurred singly, in pairs, in groups of three cells and in tetrads. Regular packets of eight cells as are found in *Sarcina* sp. were not detected nor was there chain formation. None of the strains produced spores. Motile cells occurred in cultures from both liquid and solid media. The motile cells usually possessed one or two flagella (see figures), occasional cells with three or four flagella were detected. The flagella were often irregular but some showed a regular sine curve with a wave length of 3.0 μ and an amplitude of 0.5 to 1.0 μ (Fig. 1). The flagella were approximately 200 Å in diameter and from 5.0 to 8.0 μ in length. The cells of strains CCM 1849, 2069 and 2104 occasionally showed the presence of shorter, stiff flagella.

Cultural characteristics. All strains formed on nutrient agar smooth, circular, and slightly convex colonies producing a yellowish orange, water insoluble pigment. In nutrient broth, uniform turbidity was produced. All strains were aerobic. Good growth was observed within the range of 20–37°C. The strains grew very well in sea water media as well as in media without added salt.

Biochemical characteristics. Biochemical characteristics of the strains studied are given in Table 2.

On the basis of their %GC content in DNA, the strains studied formed two clusters including 4 strains with 39.5–42.2% GC and 3 strains with 48–51% GC in DNA, respectively. In carbohydrate utilization, differences were observed with the use of four different media. The use of MOF medium and sea water medium showed that all strains produced acid from glucose and from some other carbohydrates, whereas in the medium of Evans et al. (1955) and in the Standard medium of the International Subcommittee (1965), none of them produced acid from glucose. In order to compare carbohydrate utilization in planococci, micrococci, and staphylococci, we give the data obtained with the use of the Standard medium and the medium of Evans et al. (1955). As to the other characteristics of the strains studied, differences were observed in gelatin hydrolysis, aesculin hydrolysis, growth in 15% NaCl, and in resistance to heating.

Sensitivity to antibiotics. All strains were sensitive to lysozyme, penicillin, streptomycin, tetracycline, erythromycin, oleandomycin, novobiocin, and chloramphenicol. No one of the strains was sensitive to sulfafurazole.

Figure 1. Planococcus aquivivus CCM 316. A group of cells showing flagella with a regular sine curve; gold palladium shadowed preparation.

Figure 2. Planococcus sp. CCM 2387. A cell bearing two flagella; gold-palladium shadowed preparation.

(Both figures are electron micrographs of cultures grown at 30°C for 24 hours on nutrient agar.)

DISCUSSION

As to the classification of the seven flagellated cocci, we propose placing them in the genus Planococcus Migula 1894. According to the Index Bergeyana (1966) this genus is considered to be valid and includes nine species. None of these species is, however, available in culture collections. We were therefore able to compare our strains only with the original description of the nine species mentioned above. The comparison showed that three of the strains studied (M. aquivivus CCM 316, M. eucinetus CCM 2388 and 2389) which belong to group II (see Table 2) had similar characteristics to those of Planococcus citreus. Therefore we propose to consider the names M. aquivivus Zobell and Upham 1944 and M. eucinetus Leifson 1964 as synonyms of Planococcus citreus Migula 1894. Although the four remaining strains are closely related in their biochemical characters to these strains, in their GC content (40%) they differ from them considerably. For the time being, we have refrained from giving a precise designation of these four strains and label them only as Planococcus sp., as they are being subjected to further studies.

At the same time we propose a revised characterization of the genus Planococcus.

Genus Planococcus Migula 1894, 236

Plan. o. coc'cus Gr. comb. form planos wandering; Gr. noun coccus a grain, berry; Planococcus motile coccus.

Cocci 1–1.2 μ in diameter, occurring singly, in pairs, in groups of three cells and in tetrads. The motile cells usually possess one or two flagella occasionally three and four flagella. Nonsporeforming. Gram-positive.

Chemoorganotrophs: metabolism respiratory, never fermentative.

Catalase positive: benzidine test for porphyrins is positive. Do not produce acid from glucose in standard medium. Do not produce acetoin, coagulase, phosphatase, reduce nitrate and hydrolyze arginine. Hydrolyze gelatine. Produce yellowish-orange water insoluble pigment. Good growth in sea water media as well as in media without added salt. Strict aerobic.

Temperature range: good growth between 20–37°C.

Saprophytic. Distributed in sea water.

The GC content of the DNA is either 39–42 or 48–52 moles percent.

Type species: Planococcus citreus Migula, 1894, 236.

Planococcus citreus Migula 1894, 236.

Micrococcus aquivivus ZoBell and Upham 1944, 239.

Sarcina citrea (Migula) Bergey et al. 1948, 288.

Micrococcus eucinetus Leifson 1964, 41.

From M. L. citreus, lemon-yellow.

Spheres, 1–1.2 μ in diameter, occurring singly, in pairs, in group of three cells and in tetrads. Motile. The motile cells usually possess one or two flagella. Nonsporeforming. Gram-positive.

Agar colonies: Circular, 2–3 mm in diameter, slightly convex, smooth and glistening. Produce water insoluble yellowish-orange pigment.

Table 2. Biochemical characteristics of flagellated cocci.

Strain	CCM No.	% GC in DNA	pH in liquid medium medium (average)		Aesculin hydrolysis	Gelatin hydrolysis	Growth in 15% NaCl	60°C/15 min.	70°C/15 min.	Group
			aerobic	anaerobic						
<u>Micrococcus</u> sp.	1849	39.6*	6.7**	6.7	-	-	+	-	-	I
<u>M. eucinetus</u>	2387	40.0	6.8	6.7	+	+	-	+	+	
<u>Micrococcus</u> sp.	2069	42.2	6.7	6.7	-	-	+	+	-	
<u>Micrococcus</u> sp.	2104	42.2	7.0	6.9	-	+	+	+	+	
<u>M. eucinetus</u>	2388	48.0	7.0	6.6	-	+	+	+	-	II
<u>M. eucinetus</u>	2389	50.3	6.7	6.7	-	+	+	-	-	
<u>M. aquivivus</u>	316	51.2	6.8	6.7	-	+	-	+	-	

All strains studied had positive catalase and benzidine tests, produced a yellowish-orange pigment, grew in a 0-10% concentration of NaCl and at 37°C.

None of the strains studied produced free or bound coagulase, aerobic or anaerobic acid from glucose, mannitol, lactose, maltose, sucrose or fructose, reduced nitrate, produced acetoin or phosphatase, grew on Simmons' citrate agar, hydrolyzed starch, split Tween 80 or arginine, produced hydrogen sulfide, indole, phenylalanine deaminase, haemolysis, urease, or gave a positive oxidase or egg-yolk reaction. None of the strains was resistant to heating to 80°C for 15 min.

Reaction: + = positive; - = negative.

* Data by Boháček et al. (1967, 1968).

** Uninoculated control: pH = 6.8.

Agar slant: Moderate, glistening mucoid growth with orange pigment.
Sea water broth and nutrient broth: Moderate turbidity with sediment.
No pellicle.

Catalase activity is present.

Porphyrin respiratory enzymes are present (method of Deibel and Evans, 1960).

Produce no acid or gas from glucose, lactose, maltose and sucrose in Standard medium (Subcommittee 1965).

Produce acid but no gas from glucose, maltose and mannitol in MOF medium (Leifson 1963). Produce no acid or gas from lactose and sucrose.

Final pH in glucose broth, 6.6—7.0.

Acetylmethylcarbinol not produced.

Methyl red test is negative.

Indole and hydrogen sulfide not produced.

Ammonia not produced from arginine.

Nitrate or nitrite not reduced.

Gelatin is hydrolyzed (method of Clarke 1953).

Starch and aesculin are not hydrolyzed.

Simmons' citrate agar: No growth.

Phenylalanine deaminase, phosphatase, oxidase or urease not produced.

Egg-yolk reaction is negative.

Tween-80 is not split.

Human and rabbit plasma is not coagulated.

Haemolysis is not produced.

Guanine+cytosine content in the DNA: 48—52% (Boháček, Kocur and Martinec 1967, 1968).

Good growth at 20—37°C.

Good growth in sea water media as well as in media without added salt.

Saprophytic.

Habitat: Sea water.

The strain CCM 316 (Micrococcus aquivivus ATCC 14404) is proposed as a neotype.

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