INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Oct. 1988, p. 436–439 Vol. 38, No. 4
0020-7713/88/040436-04$02.00/0

Staphylococcus delphini sp. nov., a Coagulase-Positive Species Isolated from Dolphins

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A new coagulase-positive species of the genus *Staphylococcus*, *Staphylococcus delphini*, is described on the basis of a study of two strains isolated from purulent skin lesions of dolphins. The new species is established and differentiated from the other coagulase-positive *Staphylococcus* species primarily on the basis of its deoxyribonucleic acid-deoxyribonucleic acid hybridization relationships, its cell wall composition, its bacteriolytic activity pattern, its penicillin-binding protein profile, its biochemical reactions, and the relatively high guanine-plus-cytosine content of its deoxyribonucleic acid. The type strain is strain Heidy (= DSM 20771).

Natural staphylococcal populations are mainly associated with the skin of warm-blooded animals. The wideness of the host range may vary considerably, depending on the individual *Staphylococcus* species, and the residency status of certain species on definite mammalian or avian hosts has been clearly documented (13, 14). A total of 26 species are currently recognized in the genus *Staphylococcus*, including (i) the 19 species listed in *Bergey's Manual of Systematic Bacteriology* (14), (ii) the 2 species *Staphylococcus lentus* and *Staphylococcus chromogenes*, which were recently elevated to the rank of separate species from subspecies originally ascribed to *Staphylococcus sciuri* and *Staphylococcus hyicus*, respectively (10, 19), and (iii) the 5 newly described species *Staphylococcus arlettae*, *Staphylococcus equorum*, *Staphylococcus kloosii* (21), *Staphylococcus lugdunensis*, and *Staphylococcus schleiferi* (8).

The ability to clot plasma is not very common among *Staphylococcus* species; only two coagulase-positive species and one coagulase-variable species are currently recognized. In fact, besides being the most conventional distinguishing feature of *Staphylococcus aureus*, coagulase production is a general property of *Staphylococcus intermedius* (9) and may be encountered in some strains (generally less than one-half of the strains) of *S. hyicus* (5).

In this paper we describe two coagulase-positive staphylococci which were isolated from dolphins and occupy a unique taxonomic position. It is worth noting that such a particular order of marine mammals as cetaceans (of which dolphins are members) have not previously been described as hosting any *Staphylococcus* strains or species. The two strains were isolated in 1975 on two occasions about 3 months apart, from purulent material taken from two dolphins living in an aquarium. In both cases the animals were suffering from multiple suppurating skin lesions and recovered quickly after antibiotic treatment. These dolphin strains are allocated on the basis of phenetic and genomic data to a new species, *Staphylococcus delphini*.

MATERIALS AND METHODS

Bacterial strains. The two dolphins strains were designated strains Heidy^T (T = type strain) and Nono after the names given to the two aquarium dolphins from which the strains were isolated.

The type strains of 10 *Staphylococcus* species, *Lactococcus lactis*, and *Enterococcus faecalis* were used for deoxyribonucleic acid (DNA)-DNA homology studies.

Methods. Morphological and physiological characteristics were determined essentially as described by Kloos and Schleifer (14). The results of carbohydrate reactions, hemolysis tests, and tests for the production of coagulase, phosphatase, urease, deoxyribonuclease, and acetoin were determined as described previously (22). In the coagulase tests, however, pig, bovine, and human plasmas were used in addition to rabbit plasma, and clot formation examination was prolonged up to 48 h. The other biochemical characteristics were determined by conventional methods (6, 8).

Susceptibility to novobiocin, susceptibility to lysostaphin, and susceptibility to lysozyme (all purchased from Sigma Chemical Co., St. Louis, Mo.) were expressed as the minimal inhibitory concentrations of the antimicrobial agents, which were determined by the agar dilution method (25), using Mueller-Hinton agar as the test medium. Levels of susceptibility to a variety of antibiotics were determined by a standard agar diffusion technique (1), using commercial disks (BBL Microbiology Systems, Cockeysville, Md.). β-Lactamase production was assayed by a tube method, using a 500-μg/ml solution of nitrocefin (Oxoid Ltd., Basingstoke, United Kingdom) (16).

The bacteriolytic activity pattern was determined by using the same assay system, substrates, test media, and criteria as used previously for staphylococci of both human (17, 24) and animal (22, 25) origin.

The penicillin-binding protein (PBP) profile was determined as described previously (3).

For cell wall preparation and determination the methods described by Schleifer and Kandler (20) and Schleifer (18) were used. The composition of cell wall teichoic acids was analyzed as previously described (7).

DNA base composition was determined by thermal denaturation with a model 2600 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) (4). DNA from *Escherichia coli* K-12 was used as a standard (12). DNA-DNA hybridization experiments were carried out by using the filter method under optimal conditions (25°C below the melting temperature of the DNA), as described previously (11).

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TABLE 1. DNA-DNA hybridization studies between *S. delphini* Heidy^T and various other staphylococci[a]

Source of filter-bound DNA	% Homology with labeled DNA in solution from *S. delphini* Heidy^T
S. delphini Heidy^T	100
S. delphini Nono	99
S. intermedius CCM 5739^T	35
S. aureus ATCC 12600^T	8
S. chromogenes NCTC 10530^T	8
S. sciuri DSM 20345^T	6
S. caseolyticus ATCC 13548^T	4
S. simulans DSM 20322^T	7
S. saprophyticus DSM 20229^T	5
S. capitis DSM 20326^T	7
S. haemolyticus DSM 20263^T	4
S. auricularis DSM 20609^T	6
L. lactis DSM 20481^T	4
E. faecalis DSM 20478^T	4

[a] The values were determined under optimal hybridization conditions (25°C below the melting temperature of the DNA).

To determine the plasmid profile, plasmid DNA was isolated by a modification of the procedure described by Birnboim and Doly (2), using lysostaphin (30 μg/ml) to lyse the cells. Samples were electrophoresed in 0.8% agarose gels in 0.089 M tris(hydroxymethyl)aminomethane borate–0.002 M ethylenediaminetetraacetate in a horizontal gel electrophoresis system for 6 h at 70 V at room temperature.

The strains were sent to the reference laboratory at the National Institute of Health, Rome, Italy, for bacteriophage typing studies.

RESULTS AND DISCUSSION

The assignment of the two dolphin strains to the genus *Staphylococcus* was indicated by their being gram-positive, catalase-positive cocci occurring predominantly in clusters, by the guanine-plus-cytosine (G+C) content of their DNA, by their peptidoglycan type, and by the presence of teichoic acid in their cell walls. The two strains are almost identical and differ only slightly in their rate of coagulase reaction and acid production from mannitol and in their susceptibility to lysostaphin.

The two dolphin strains have many biochemical characteristics in common with the other coagulase-positive species. Their failure to produce fibrinolysin, their β-hemolysin activity, and their growth as positive type E on crystal violet agar (V. Hajek, personal communication) indicated a close relationship to *S. intermedius* (9). However, in addition to some differences in carbohydrate reactions (acid produced from maltose but not from trehalose), the two dolphin strains differ strikingly from *S. intermedius* and the other coagulase-positive staphylococci in their lytic activity pattern (22, 25). In particular, the optimal NaCl concentration (2.0 to 3.0%) for bacteriolytic activity in the dolphin strains is markedly higher than the optimal concentration in *S. aureus* and *S. intermedius* (0.5 to 1.0%) or *S. hyicus* (0.25 to 1.0%). Moreover, the two-band PBP profile of the dolphin staphylococci is unique compared with the four-band profile of *S. aureus*, the three-band profile of *S. intermedius*, or the one-band profile of *S. hyicus* (3). Finally, determinations of the cell wall composition and the G+C content of DNA, as well as the results of parallel DNA-DNA hybridization studies, definitively confirmed the unique taxonomic position of the two dolphin staphylococci. The G+C content of the DNA (about 39 mol% in both strains) lies in the upper range for staphylococcal DNAs and is considerably higher than the G+C contents of *S. aureus* (32 to 36 mol%), *S. intermedius* (31 to 36%), or *S. hyicus* (33 to 34 mol%) (14). The two strains are highly related to each other on the basis of DNA-DNA hybridization studies. In contrast, they are not closely related to any of the various staphylococcal type strains belonging to different species groups which we studied (Table 1). *S. intermedius* appears to be the closest relative, but the observed hybridization value is far below the degree of DNA homology consistent with a relationship at the species level.

Therefore, the staphylococci isolated from dolphins are not closely related to any of the previously described species of the genus *Staphylococcus* and are proposed as belonging to a new species, *Staphylococcus delphini*. A description of this new species is given below.

***Staphylococcus delphini* sp. nov.** *Staphylococcus delphini* (del.phi'ni. L. gen. n. *delphini*, of a dolphin). Cocci, 0.8 to 1.0 μm in diameter, arranged predominantly in clusters but occasionally occurring in pairs or singly. Gram positive. Nonsporeforming. Nonmotile. Colonies on nutrient agar are circular, entire, 5 to 7 mm in diameter, slightly convex, smooth, glistening, butyrous, and opaque with a tendency to become slightly translucent after continued incubation. Pigment not produced. Facultative anaerobes; growth is best under aerobic conditions. Fairly good growth at 45°C or in the presence of 15% NaCl. Anaerobic growth in semisolid thioglycolate medium is evident after 24 to 48 h.

Rabbit, pig, bovine, and human plasmas are coagulated; clot formation is slightly faster with strain Heidy^T than with strain Nono (1 h versus 4 h with rabbit and pig plasmas, 4 h

TABLE 2. Characteristics useful in distinguishing between *S. delphini* and related *Staphylococcus* species[a]

Species	Coagulase	Heat-stable deoxyribonuclease	Acid produced aerobically from:		Acid produced anaerobically from mannitol	Optimal NaCl concn (%) for lytic activity	Mol wt of PBPs (×10^3)				G+C content of DNA (mol%)
			Maltose	Trehalose			PBP-1	PBP-2	PBP-3	PBP-4	
S. delphini	+[b]	−	+	−	−	2.0–3.0	85	79			39
S. aureus	+	+	+	+	+	0.5–1.0	79	77	74	44	32-36
S. intermedius	+	+	−	+	−	0.5–1.0	85	82	79		31-36
S. hyicus	v	+	−	+	−	0.25–1.0	79				33-34
S. chromogenes	−	−	v	+	−	0–0.5	84	82	79		33-34
S. simulans	−	(v)	−	v	+	0.25–1.0	83	80	79		34-38

[a] See Table 3 for differences in bacteriolytic activity patterns. With the exception of the *S. delphini* data, the data are from references 3, 5, 9, 10, 14, and 23.

[b] +, 90% or more of the strains are positive; −, 90% or more of the strains are negative; v, 11 to 89% of the strains are positive. Parentheses indicate a weak or delayed reaction.

TABLE 3. Bacteriolytic activity patterns of *S. delphini* and related *Staphylococcus* species[a]

Species	Bacteriolytic activity on the following test media:							
	TP1	TP2	TP2P	T0	T1	T3	B15TP1	a61TP2
S. delphini	±	++	±	−	±	++	−	+
S. aureus	+++	+	−	±	++	−	+	−
S. intermedius	++	+	+	+	++	−	−	−
S. hyicus	++	+	−	+	++	±	−	−
S. chromogenes	++	±	−	++	+	−	−	−
S. simulans	++	+	+	+	++	−	−	−

[a] Test media and symbols for lytic activity have been described by Varaldo et al. (24). With the exception of the *S. delphini* data, the data are from references 22 through 25.

versus 24 h with bovine plasma, and 24 h versus 48 h with human plasma). The strains produce catalase, phosphatase, urease, caseinase, and arginine dihydrolase. Deoxyribonuclease activity is weak and not resistant to heating. Acetoin, gelatinase, clumping factor, and egg yolk factor are not produced. Hemolysis is demonstrated on bovine, sheep, and human blood agar. Nitrates are reduced.

Acid is produced aerobically from glucose, fructose, mannose, maltose, lactose, and sucrose, and moderately from mannitol (acid is produced from mannitol more slowly by strain Heidy^T than by strain Nono). No acid is produced from xylose, arabinose, trehalose, or xylitol. Glucose, but not mannitol, is utilized anaerobically.

The strains are susceptible to novobiocin and lysostaphin and resistant to lysozyme. As determined by agar dilution tests, the lysostaphin minimal inhibitory concentrations are 3.12 μg/ml for strain Heidy^T and 6.25 μg/ml for strain Nono; for both strains, the minimal inhibitory concentration of novobiocin is 0.2 μg/ml, and that of lysozyme is >1 mg/ml). The strains show no β-lactamase activity and are susceptible to the most common antistaphylococcal antibiotics; as determined by disk diffusion tests, both strains are susceptible to penicillin G, oxacillin, cephalothin, cefamandole, cefoxitin, cefuroxime, cefotaxime, vancomycin, teicoplanin, rifampin, cotrimoxazole, chloramphenicol, erythromycin, lincomycin, tetracycline, gentamicin, netilmicin, and ofloxacin.

The bacteriolytic activity pattern strongly resembles that of human staphylococci of lyogroup V (i.e., *Staphylococcus epidermidis*) (23), with an optimal NaCl concentration for lytic activity of 2 to 3%.

The PBP profile is characterized by the occurrence of two bands having molecular weights of 85,000 and 79,000 (3).

Nontypable when the basic sets of phages are used. Both strains show a plasmid profile characterized by one small plasmid (about 2.2 kilobases).

The cell wall peptidoglycan type is Lys-Gly$_{5-6}$. The cell wall teichoic acid consists of glycerol and *N*-acetylglucosamine.

The G+C content of the DNA is about 39 mol%.

The strains appear to be potentially pathogenic for dolphins.

Strain Heidy (= DSM 20771) is the type strain of *S. delphini*.

S. delphini can be differentiated from all other known staphylococci primarily on the basis of coagulase, phosphatase, and heat-labile deoxyribonuclease production, carbohydrate reaction pattern, bacteriolytic activity pattern, PBP profile, and a rather high G+C content. The major features useful in distinguishing between *S. delphini* and the most closely phenotypically related *Staphylococcus* species are summarized in Table 2. The bacteriolytic activity patterns of the same species are compared in Table 3.

ACKNOWLEDGMENTS

We are indebted to V. Hajek for his early examination of the dolphin isolates and comments on them and to G. Orefici for the phage typing studies.

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