A Phylogenetic Analysis of Staphylococci, *Peptococcus saccharolyticus* and *Micrococcus mucilaginosus*

By WOLFGANG LUDWIG,¹ KARL-HEINZ SCHLEIFER,¹ GEORGE E. FOX,² ELKE SEEWALDT¹ AND ERKO STACKEBRANDT^{1*}

¹ Lehrstuhl für Mikrobiologie der Technischen Universität München, Arcisstrasse 16, D-8000 München 2, Federal Republic of Germany

² Department of Biophysical Sciences, University of Houston, Houston, Texas 77004, U.S.A.

(Received 13 October 1980; revised 5 January 1981)

The intra- and intergeneric relationships of the genus *Staphylococcus*, and the phylogenetic position of *Peptococcus saccharolyticus* and *Micrococcus mucilaginosus* (*Staphylococcus salivarius*), were investigated by comparative oligonucleotide cataloguing of 16S rRNA. All the staphylococci investigated form a phylogenetically coherent group at the genus level that, in addition, contains the anaerobic species *Peptococcus saccharolyticus*. The genus *Staphylococcus* belongs to the broad *Bacillus*–*Lactobacillus*–*Streptococcus* cluster that is defined by Gram-positive bacteria with a low DNA G+C content.

Micrococcus mucilaginosus is not a genuine member of the genus *Micrococcus*. The binary matching coefficients between the 16S rRNA of *Micrococcus mucilaginosus* and those of representatives of the *Arthrobacter*/*Micrococcus* group and related genera indicate that *Micrococcus mucilaginosus* should be regarded as a member of a new genus.

INTRODUCTION

Fundamental genetic and epigenetic differences exist between *Staphylococcus* and *Micrococcus* as expressed in their DNA G+C content: 31 to 36 mol % for *Staphylococcus*, compared with 68 to 72 mol % for *Micrococcus* (Silvestri & Hill, 1965; Kocur *et al.*, 1971). Differences in peptidoglycan type (Schleifer & Kandler, 1972) and in cytochrome patterns (Faller *et al.*, 1980) as well as differences revealed by numerical taxonomic studies (Feltham, 1979) also indicate the heterogeneity of this group. Despite these distinct differences, taxonomists still include all of these catalase-positive cocci within the family *Micrococcaceae* (Baird-Parker, 1974). Techniques such as DNA–DNA and DNA–rRNA reassociation, and the comparative immunological analysis of homologous proteins, which measure the phylogenetic distances between organisms, have proved useful in unravelling the intrageneric relationships between species of *Staphylococcus* and *Micrococcus* (Schleifer *et al.*, 1979 *a, b*; Meyer & Schleifer, 1978; Kilpper *et al.*, 1980). These studies also strongly support the separation of *Micrococcus* from *Staphylococcus*, but fail to identify specifically the phylogenetic position of these organisms among the bacteria in general.

Recent phylogenetic investigations on members of the family *Micrococcaceae* using comparative oligonucleotide cataloguing of 16S rRNA (Fox *et al.*, 1977; Woese *et al.*, 1976; Fox *et al.*, 1980) led to the conclusion that this taxon should be dissected (Stackebrandt & Woese, 1979; Stackebrandt *et al.*, 1980). All strains of *Micrococcus* investigated were found to be in close phylogenetic relationship to certain coryneforms, especially to *Arthrobacter*, *Cellulomonas*, *Brevibacterium* and *Microbacterium*, whereas *Planococcus* and *Staphylococcus* share a moderate, but distinct relationship to the *Bacillus* cluster (Stackebrandt & Woese, 1979; Fox *et al.*, 1980). In contrast to the large number of

micrococci investigated, only one member of the genus *Staphylococcus*, *S. epidermidis* ATCC 155, was included in these studies. To determine the genealogical relationship of the genus *Staphylococcus* on a broader basis, we have extended these RNA sequencing studies, using an in vitro labelling procedure (Stackebrandt *et al.*, 1981), to several species shown by DNA-DNA reassociation studies to be only rather distantly related (Schleifer *et al.*, 1979 a). *Peptococcus saccharolyticus* was included because this organism was recently described as an anaerobic member of *Staphylococcus* (Kilpper *et al.*, 1980). *Micrococcus mucilaginosus* (*Staphylococcus salivarius*) CCM 2417 was investigated because its DNA G+C content of 59 mol % (Kocur & Bohacek, 1974) does not fall in the usual range for either *Staphylococcus* or *Micrococcus*.

METHODS

Organisms. The following strains were obtained from the Deutsche Sammlung von Mikroorganismen (DSM) and from the American Type Culture Collection (ATCC): *Staphylococcus haemolyticus* DSM 20263; *S. xylosus* DSM 20266; *S. aureus* ATCC 12600; *S. sciuri* subsp. *sciuri* ATCC 29062; *Peptococcus saccharolyticus* ATCC 14953. *Micrococcus mucilaginosus* CCM 2417 was obtained from the Czechoslovak Collection of Microorganisms, Brno, CSSR. All of the strains investigated are type or neotype strains.

Preparation and sequencing of 16S rRNA. *Peptococcus saccharolyticus* was grown anaerobically in a medium described by Kilpper *et al.* (1980). All other strains were grown aerobically in 200 ml of a peptone/yeast extract/glucose broth (0.5:0.25:0.5; %, w/v). To facilitate the rupture of the cells, about 0.5 mg penicillin ml⁻¹ was added to the medium during the early exponential phase of growth. After 1 h incubation cells were harvested by centrifugation and broken by passing through a French press in a buffer containing Macaloid (Baroid Division, National Lead Co., Houston, Texas) (Woese *et al.*, 1976) in the presence of several drops of 15% (w/v) sodium dodecyl sulphate and 100 µg Proteinase K (Merck). Phenol treatment of the crude extract, and isolation and purification of 16S rRNA, followed the methods described by Woese *et al.* (1976) and Stackebrandt *et al.* (1981). Controlled digestion of the RNA by RNAase T₁, dephosphorylation of the 3' end of the oligonucleotides and the enzymic in vitro labelling of the 5' end with [γ -³²P]ATP and polynucleotide kinase were carried out according to Simsek *et al.* (1973) and Silberklang *et al.* (1979), with modifications described by Stackebrandt *et al.* (1981). Partial cleavage of 5'-labelled oligonucleotides by controlled alkali treatment and the determination of the nucleotide sequences by mobility shift analysis followed the description of Stackebrandt *et al.* (1981). Binary matching coefficients (S_{AB} values) were calculated as described by Fox *et al.* (1977). Dendrograms were generated from tabulations of the binary matching coefficients by average linkage (between the merged groups) clustering.

RESULTS

The oligonucleotides of the organisms investigated are listed in Table 1. The phylogenetic positions of various staphylococci and *Micrococcus mucilaginosus* among the Gram-positive bacteria, as derived from the comparative analysis of the 16S rRNA oligonucleotides (Fox *et al.*, 1980), are depicted in Fig. 1. This dendrogram was calculated from the binary matching coefficients, S_{AB} , given in Table 2. All the staphylococci investigated form a genetically highly related cluster which also includes *Peptococcus saccharolyticus*. The *Staphylococcus* group falls into the large *Bacillus* cluster (Fig. 1). The lowest S_{AB} values separating these species are in the range 0.64 to 0.72 (Table 2). These values are comparable to those found within the *Arthrobacter*/*Micrococcus* group (0.69), the cellulomonads (0.72) and the group comprising *Microbacterium lacticum* and the plant pathogenic coryneforms (0.67). In these three cases the status of genus was recently suggested or confirmed (Stackebrandt *et al.*, 1980). The close relationships among the species of *Staphylococcus* seen here also strongly support its status as a defined genus. Figure 2 shows the phylogenetic relatedness of the individual staphylococci and *P. saccharolyticus* within the cluster defined by rRNA similarities. The closest relationships are found between *S. xylosus* and *S. haemolyticus* (S_{AB} 0.85), and between *S. aureus* and *S. epidermidis* ATCC 155 (0.84). These pairs of organisms exhibit an average S_{AB} value of 0.81. *Peptococcus saccharolyticus* falls into the *Staphylococcus* cluster (0.75) in which *S. sciuri* is the most distantly related organism (0.69). *Micrococcus*

Fig. 1. Dendrogram showing the phylogenetic positions of the genus *Staphylococcus* and of *Micrococcus mucilaginosus* within the branch of the phylogenetic tree comprising Gram-positive organisms, derived by average linkage clustering. The *Staphylococcus* cluster contains *S. xylosus* DSM 20266, *S. haemolyticus* DSM 20263, *S. aureus* ATCC 12600, *S. epidermidis* ATCC 155, *S. sciuri* subsp. *sciuri* ATCC 29062 and *Peptococcus saccharolyticus* ATCC 14953. The following genus abbreviations are used: *E.*, *Escherichia*; *B.*, *Bacillus*; *Spl.*, *Sporolactobacillus*; *Sp.*, *Sporosarcina*; *Pl.*, *Planococcus*; *Str.*, *Streptococcus*; *L.*, *Lactobacillus*; *M.*, *Micrococcus*; *A.*, *Arthrobacter*.

Fig. 2. Dendrogram showing the phylogenetic relationships between *Staphylococcus xylosus*, *S. haemolyticus*, *S. aureus*, *S. epidermidis*, *S. sciuri* subsp. *sciuri* and *Peptococcus saccharolyticus*, derived by average linkage clustering.

mucilaginosus (formerly *Staphylococcus salivarius*) CCM 2417 does not fall into the *Arthrobacter*/*Micrococcus* cluster, defined by *Arthrobacter globiformis*, *M. roseus*, *M. luteus* and their relatives (Stackebrandt *et al.*, 1980). S_{AB} values of 16S rRNA between *M. mucilaginosus* and organisms of this cluster range between 0.62 and 0.71. These values, however, are lower than those found between species of the *Arthrobacter*/*Micrococcus* cluster (0.71). The binary coefficients derived by average linkage clustering between *M. mucilaginosus* and representatives of the family I as proposed by Stackebrandt *et al.* (1980), which includes *Cellulomonas*, *Brevibacterium*, *Microbacterium*, *Arthrobacter* and *Micrococcus*, show that *M. mucilaginosus* is more distantly related (S_{AB} values ranging between 0.54 and 0.71). Thus the S_{AB} value for the branching point of *M. mucilaginosus* from the main stem of the coryneform family I is 0.59.

Table 1. *Oligonucleotide catalogues*

The sequences listed were found in the organisms indicated by the numbers: 1, *Staphylococcus aureus* ATCC 12600; 2, *Peptococcus saccharolyticus* ATCC 14953; 3, *S. sciuri* subsp. *sciuri* ATCC 29062; 4, *S. xylosus* DSM 20266; 5, *S. haemolyticus* DSM 20263; 6, *S. epidermidis* ATCC 155; 7, *Micrococcus mucilaginosus* CCM 2417.

Sequence	Organisms	Sequence	Organisms
6-mers			
CCCACG	1-6	AACAAUAG	5
CACAAG	1-7	CCCCUUAG	1-6
AAACCG	1-6	CACUCUCG	5
ACAAAG	1, 3-6	ACCCUCUG	3-5
UCCACG	1-6	CCUAUCAG	7
CUCACG	7	UCAACUCG	7
CAUCAG	7	AUACCCUG	1-7
CACUAG	7	UUCAACAG	2
CUAACG	1-7	CUACAAUG	1-7
ACUAAG	7	ACUACAUG	1, 3-6
UAAACG	1-7	ACUCAUAG	7
AAACUG	1-7	UUCAAAAG	1
AAUACG	1-2, 4-7	AAAACUUG	1-2, 4, 6
UUCCCG	1-7	AUAACAUG	2
CCUUCG	7	CUCCUCUG	2
UCCAUG	7	UUCUAAAG	5
CUCUAG	6	UUCAAUAG	3, 6
UCUCAG	7	AAUUCUG	7
UCUACG	7	UAACUAUG	2, 4
UACCG	7	ACUAUAUG	2
ACUCUG	4, 7	CUUCUCUG	1
AUCCUG	1, 4, 6-7	CUCCUUUG	4-6
UAACUG	1-7	ACUUUCUG	1-2, 4-6
UAAUCG	1-6	AAUUAUUG	1-7
AUACUG	7		
AAAUUG	7	9-mers	
UUUCCG	1-6	CAACCCUCG	7
UCUUCG	1-4, 6	CUCACCAAG	7
UUCUAG	7	CUACACACG	1-6
UCAUUG	1-6	UACACACCG	1-7
UAUUAG	3-6	UAACACCCG	1-6
		AUCAAAACAG	1-5
		CCAUCUACG	2
7-mers		CACUCUACG	1, 4
AACACCG	7	ACUCCUACG	1-7
CACUCCG	1-6	CUUACCAAG	1-2, 4-6
CAACUCG	1-7	CUCAAUCAG	3
UAACACG	1-6	CUAACUACG	1-7
CACAAUG	7	CUAAUACCG	1, 4-5
ACAAAUG	1-6	AUAACUCCG	3
CCCUUCG	4	CCCCUUAUG	1-7
CCCUCUG	3	CACAUCUUG	1, 5-6
CUUCACG	7	AAUCUCCG	1-6
CAUUAAG	1-7	AAUCCAUG	1-6
UAAUACG	1-7	AUAACUUCG	1-2, 4-6
AUAUCAG	7	AUUAAUUCG	7
UAUACAG	7	UUUCUUAAG	3, 5
AAACUUG	3, 5	UUUAAUUCG	1-6
UUCUCAG	1-2, 4-6	UUUUUUAAG	1-2, 4, 6
UUAUCCG	1-6		
CUUAUAG	3	10-mers	
UUAUCAG	6	UCACACCACG	1-6
AAUAUUG	7	AAACUCAAAAG	1-6
UUUAUAG	1-2, 4-5	ACCCUCUAG	2
UUUCCG	7	ACCACUCUAG	3
UUCUAUG	7	CUUAACCCCG	7
		ACAUUCCAG	7
8-mers		CUAAUACCG	2
AACACCAG	1-2, 4-6	ACAACUCUAG	1
ACAAACCG	1-6	ACAACUAUAG	4
CUCAACCG	1-6	AAAACUAUAG	5
CCACACUG	1-7	ACAUCUCUG	2
CUAAACCG	5-6	UCUAAUACCG	7
AACAUACG	4	UAAACCUCUG	7
ACAAAUG	7	UAAAACUCUG	1-6

Sequence	Organisms	Sequence	Organisms
ACAUUACUG	7	13-mers	
AACAAAUUUG	3	CUAAAACUCAAG	7
ACAUCUUUG	1, 3-6	UCAAUUAUCAUG	1-7
UCACUUAUG	1-3, 6	CUAUCACUUAUG	4-5
UAACCAUUUG	4		
UUUCCCUUG	7	14-mers	
UUUCCCUUG	7	CAAACCCCAUAAAG	2
UAACCUUUUG	2	CAAAUCCCAUAAAG	1, 4-6
		CUAUCCCUAAAAG	7
11-mers			
AAUACACAAAG	6	15-mer	
ACAAUACAAAG	1-2, 4-5	UAACCUACCUAUAAG	3-5
CAACCCUUAAG	1-6		
ACCAAUCUCAG	7	16-mer	
UAACUAACCAG	3	AUAACCUACCUAUAAG	1-2, 6
AUAAUACAAAG	3		
CUUAAACAUG	7	17-mer	
CCUAAUACAUG	1-6	AACCUUACCAAAUCUUG	1-5
CCAUCAUUAAG	1-6		
UACCUAAUCAG	1-2, 4-5	18-mer	
UACAAUACAUG	1	AACCUUACCAAAUCUUUG	6
AUAAUUAUUG	5		
AUAAUUAUUG	4	21-mers	
AUAAUUAUUG	1, 3	CAAAUCCCAUAAAUAUACG	3
		UCUCCCCUUCUUUUUAACG	3
12-mers			
UAACACCCAAAG	7	3' end	
AACCUUACCAAG	7	AUCACCUCCUUUCU _{OH}	1-6
UCUCCCCUUCG	6	AUCACCUCCUUUCU _{OH}	7†
CCUAAACUUUUG	7		
UAACCAUUUAUG	5-6	Modified oligonucleotide	
UUUCCCCUUCG	2	UAACAAG	1-7
UAACCUUUUCUG	1, 3		

† For differences in the ... (C)CCUUU ... portion in the 3' end of the 16S rRNA from members of the actinomycetes group and from non-actinomycetes organisms, see Stackebrandt *et al.* (1980).

DISCUSSION

Comparative oligonucleotide cataloguing of 16S rRNA using the in vitro labelling procedure described by Stackebrandt *et al.* (1981) corroborates the high level of relatedness among the staphylococci as previously demonstrated by DNA-DNA reassociation experiments (Meyer & Schleifer, 1978; Kloos & Wolshohl, 1979; Rosenblum & Tyrone, 1979; Schleifer *et al.*, 1979*a*; Goodfellow *et al.*, 1980) and comparative immunological studies (Rupprecht & Schleifer, 1979; Schleifer *et al.*, 1979*a*). A comparison of DNA-DNA homology values and S_{AB} values is given in Table 3. It should be noted that *Staphylococcus epidermidis* ATCC 155 does not fit the description for *S. epidermidis sensu stricto* (Schleifer & Kocur, 1973; Schleifer & Kloos, 1975): it differs in several biochemical characters from a typical *S. epidermidis* and more closely resembles *S. warneri*. However, DNA-DNA reassociation studies established that *S. epidermidis* and *S. warneri*, together with *S. capitis*, form a closely related species group (Schleifer *et al.*, 1979*a*). The closer relationship of *S. epidermidis*/*S. warneri* to *S. haemolyticus*, as determined by DNA homologies, was not detected by the analysis of their RNAs. According to the S_{AB} values, *S. haemolyticus* is more closely related to *S. xylosus* (0.85) than to *S. epidermidis*/*S. warneri* (0.81). These differences may be explained by the highly conserved DNA sequence of the 16S rRNA cistrons, which certainly does not reflect actual changes in the base sequences of the bulk of less-conserved genes. The distinction of coagulase-positive staphylococci (*S. aureus*) from the coagulase-negative species is more of clinical diagnostic value than of taxonomic significance. As previously demonstrated, *S. aureus* does not show separate grouping from the coagulase-negative strains but is phylogenetically closely related to them (Meyer & Schleifer, 1978; Schleifer *et al.*, 1979*a*; Rosenblum & Tyrone, 1979).

Table 2. *Binary comparison of 16S rRNA catalogues*

The number in the upper left of each rectangle is the S_{AB} value for that pair of catalogues; the number in the lower right corner is the number of bases common to that pair of catalogues (for hexamers and larger oligonucleotides). The catalogues of oligonucleotides of 16S rRNA for the reference organisms not listed in Table 1 are from the following sources: Uchida *et al.* (1974) for *Escherichia coli*; Fox *et al.* (1977) for *Bacillus subtilis*, *B. stearothermophilus*, *Sporosarcina urea*, *Sporolactobacillus inulinus*; Stackebrandt & Woese (1979) for *Planococcus citreus*; Stackebrandt *et al.* (1980) for *Micrococcus luteus*, *M. roseus*, *Arthrobacter globiformis*. The catalogues of *Streptococcus lactis* and *Lactobacillus brevis* are from unpublished results of C. R. Woese and E. Stackebrandt.

	<i>S. xylosus</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. sciuri</i>	<i>P. saccharolyticus</i>	<i>M. mucilaginosus</i>
<i>S. xylosus</i>	X						
<i>S. haemolyticus</i>	0.85 554	X					
<i>S. aureus</i>	0.84 542	0.80 513	X				
<i>S. epidermidis</i>	0.80 509	0.81 509	0.84 530	X			
<i>S. sciuri</i>	0.70 440	0.72 450	0.71 444	0.69 426	X		
<i>P. saccharolyticus</i>	0.77 448	0.72 457	0.80 506	0.75 468	0.64 397	X	
<i>B. stearothermophilus</i>	0.48 285	0.47 279	0.49 285	0.49 285	0.46 265	0.47 270	0.34 182
<i>Spl. inulinus</i>	0.43 260	0.42 254	0.44 260	0.44 260	0.40 236	0.43 255	0.26 142
<i>Sp. urea</i>	0.52 320	0.51 314	0.52 320	0.53 320	0.50 300	0.50 305	0.32 180
<i>B. subtilis</i>	0.55 330	0.55 333	0.55 330	0.55 330	0.53 312	0.55 325	0.32 180
<i>Pl. citreus</i>	0.56 347	0.55 341	0.57 347	0.59 354	0.52 312	0.55 332	0.32 180
<i>Str. lactis</i>	0.39 238	0.38 232	0.38 232	0.40 238	0.36 217	0.36 217	0.25 141
<i>L. brevis</i>	0.40 254	0.38 239	0.40 248	0.42 260	0.34 209	0.38 233	0.26 150
<i>M. mucilaginosus</i>	0.30 177	0.28 165	0.29 171	0.31 177	0.28 159	0.29 165	X
<i>M. roseus</i>	0.29 172	0.27 160	0.28 166	0.30 172	0.27 154	0.28 160	0.71 315
<i>M. luteus</i>	0.32 192	0.31 186	0.33 192	0.34 198	0.31 180	0.32 186	0.66 355
<i>A. globiformis</i>	0.31 186	0.29 174	0.30 180	0.31 186	0.29 168	0.29 174	0.65 353
<i>E. coli</i>	0.23 138	0.23 138	0.23 138	0.23 138	0.21 123	0.22 129	0.23 125

Table 3. Comparison of DNA–DNA homology values and S_{AB} values for each pair of staphylococci investigated

DNA–DNA homology (%)† S_{AB} value	<i>S. xylosus</i>	<i>S. haemolyticus</i>	<i>S. aureus</i>	<i>S. epidermidis</i> (<i>S. warneri</i>)*	<i>P. saccharolyticus</i>	<i>S. sciuri</i>
<i>S. xylosus</i>	X	25 ^a 33 ^c 25 ^e	23 ^a 27 ^b	27 ^a 32 ^c	20 ^d	ND
<i>S. haemolyticus</i>	0.85	X	14 ^b 32 ^e	40 ^a 45 ^c 39 ^e	32 ^d	22 ^e
<i>S. aureus</i>	0.84	0.80	X	32 ^a 25 ^b	24 ^d	15 ^b
<i>S. epidermidis</i> (<i>S. warneri</i>)*	0.80	0.81	0.84	X	29 ^d	ND
<i>P. saccharolyticus</i>	0.77	0.72	0.80	0.75	X	15 ^d
<i>S. sciuri</i>	0.70	0.72	0.71	0.69	0.64	X

ND, Not determined.

* See Discussion.

† *a*, Membrane filter technique, 29 °C below T_m (Schleifer *et al.*, 1979*a*, *b*); *b*, hydroxyapatite at 55 °C (Rosenblum & Tyrone, 1979); *c*, membrane filter technique, 25 °C below T_m (Goodfellow *et al.*, 1980); *d*, membrane filter technique, 25 °C below T_m (Kilpper *et al.*, 1980); *e*, hydroxyapatite at 55 °C (Kloos & Wolshohl, 1979).

Peptococcus saccharolyticus, originally described as a member of the genus *Peptococcus* (Foubert & Douglas, 1948), was later removed from this genus when its saccharoclastic properties were detected. In the latest edition of *Bergey's Manual* (Rogosa, 1974), *P. saccharolyticus* is listed as *species incertae sedis*. The first indication of a relationship of this species to *Staphylococcus* was given by the similarity in the peptidoglycan type (Schleifer & Nimmermann, 1973). Later, Kilpper & Schleifer (1978) and Crosa *et al.* (1979) found a significant degree of relationship of *P. saccharolyticus* to coagulase-negative staphylococci, based on DNA–DNA homology studies. More extensive nucleic acid reassociation studies using DNA–DNA and DNA–23S rRNA hybridization techniques established the close genetic relatedness of *P. saccharolyticus* to the staphylococci (Kilpper *et al.*, 1980). This finding is confirmed by our results.

A comparative immunological study between antisera against highly purified catalases of *S. epidermidis*, *S. capitis* and *S. xylosis* and crude extract of *S. sciuri* subsp. *sciuri* ATCC 29062 demonstrated *S. sciuri* to be the most distantly related species within *Staphylococcus* (Schleifer *et al.*, 1979*a*). Although the cell wall composition of *S. sciuri* is typical of staphylococci (Kloos *et al.*, 1976), its cytochrome patterns are different (Faller *et al.*, 1980). Numerical taxonomic studies (Feltham, 1979) placed strains of *S. sciuri* even more distant from staphylococci and micrococci than these latter two groups are from each other. This led to a proposal to place *S. sciuri* and its relatives into a new genus named *Cellobiosococcus*, because its members produce acid aerobically from cellobiose. This ability, however, is not restricted to *S. sciuri*, but was recently found also in strains of *S. xylosus*

(Bucher *et al.*, 1980). The analysis of the 16S rRNA of *S. sciuri* clearly establishes a close phylogenetic relatedness to *Staphylococcus* species. *Staphylococcus sciuri* does, however, represent an early branching among these staphylococci and thus it exhibits significant phenotypic differences.

Within the branch of the prokaryotic phylogenetic tree (Fox *et al.*, 1980) comprising Gram-positive bacteria, the staphylococci are found within the *Bacillus* cluster (Fig. 1). This cluster contains a variety of morphologically different Gram-positive bacteria such as *Sporosarcina urea* (Pechman *et al.*, 1976), *Planococcus citreus* (Stackebrandt & Woese, 1979) and *Thermoactinomyces vulgaris* (Fox *et al.*, 1980). Furthermore, the *Bacillus* cluster includes not only aerobic but also facultative anaerobic or microaerophilic organisms, e.g. *Bacillus polymyxa* and *Peptococcus saccharolyticus*. Further support for the relatedness of staphylococci and bacilli is the fact that the DNA G+C content of *Staphylococcus* (32 to 38 mol %; Schleifer *et al.*, 1979a) lies within the range for *Bacillus* (32 to 53 mol %, except for *B. acidocaldarius* for which a value of 62 mol % is reported; Jones & Sneath, 1970; Gibson & Gordon, 1974) and that purified plasmid DNA coding for drug resistance, and also crude extract of *S. aureus*, can transform *B. subtilis* (Ehrlich, 1977; Feitelson & Lederberg, 1980).

The S_{AB} value for the branching point of the *Staphylococcus* cluster from the main stem of *Bacillus* is about 0.5. The separation of *Staphylococcus* from microaerophilic or aerotolerant Gram-positive bacteria like lactobacilli and *Streptococcus lactis* occurs at an S_{AB} value of about 0.42 (Fox *et al.*, 1980). However, more RNA sequencing studies of different streptococci are necessary to prove the coherency of the genus *Streptococcus*, as well as to find the branching point of this genus within the *Bacillus/Staphylococcus-Streptococcus-Lactobacillus* cluster. Transferability of plasmids between various streptococci and *S. aureus* (Engel *et al.*, 1980) indicates a natural relationship between these bacteria.

The classification of *Micrococcus mucilaginosus* as a member of the genus *Micrococcus* is now seen to be unjustified. Spherical shape and superficial resemblance in simple physiological characteristics as detected in numerical taxonomic studies on micrococci (Feltham, 1979) are not sufficient grounds for classifying an organism as *Micrococcus*. A similar case was recently seen with the radiation-resistant species *M. radiodurans* and *M. radiophilus* (Brooks *et al.*, 1980). The separation of *M. mucilaginosus* from the genuine micrococci is further supported by differences in DNA G+C content of about 10 mol % (59 mol % for *M. mucilaginosus* versus 68 to 72 mol % for *Micrococcus*). According to Sueoka (1961) two strains differing by more than 10 mol % cannot be phylogenetically closely related. A difference of more than 5 mol % has usually been interpreted as implying membership of a different genus (De Ley, 1969). The characterization of its 16S rRNA clearly reveals that *M. mucilaginosus* belongs to a new genus yet to be described. This genus belongs to a larger group of genera (*Arthrobacter*, *Micrococcus*, *Brevibacterium*, *Cellulomonas* and *Microbacterium*) for which the status of a family was recently suggested (Stackebrandt *et al.*, 1980).

Our results demonstrate the advantage of the combined application of nucleic acid reassociation studies and the comparative analysis of 16S rRNA for phylogenetic studies. DNA-DNA homology studies allow investigation of a larger number of organisms within a short period. However, this method is restricted to closely related species, and fails among more remotely related species, as shown in studies on bacilli (Seki *et al.*, 1975), micrococci (Ogasawara-Fujita & Sakaguchi, 1976; Schleifer *et al.*, 1979b) and arthrobacters (Stackebrandt & Fiedler, 1979). DNA-rRNA reassociation studies may be useful for studying less related species since this method permits the elucidation of genetic relationships even at the intergeneric level (Johnson & Francis, 1975; De Smedt & De Ley, 1977). The comparative analysis of 16S rRNA is an excellent tool for studying the relatedness not only of closely related species (Pechman *et al.*, 1976; Stackebrandt *et al.*, 1980) but also of distantly related bacteria (Woese & Fox, 1977). The advantage of investigating the 16S

rRNA from representatives of genetic clusters, defined either as a genus or a family by nucleic acid homology studies, is that the entire cluster can at once be placed into the existing phylogenetic tree of prokaryotes.

We wish to thank Professor H.-J. Gross for the use of part of his laboratory facilities at the Max-Planck Institute for Biochemistry, München, Martinsried. The work reported here was supported by a grant from the Deutsche Forschungsgemeinschaft and by N.A.S.A. Grant NSG 7440 to G.E.F.

REFERENCES

- BAIRD-PARKER, A. C. (1974). *Micrococcaceae*. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 478–490. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- BROOKS, B. W., MURRAY, R. G. E., JOHNSON, J. L., STACKEBRANDT, E., WOESE, C. R. & FOX, G. E. (1980). Red-pigmented micrococci: a basis for taxonomy. *International Journal of Systematic Bacteriology* **30**, 627–646.
- BUCHER, E., BECK, G. & SCHLEIFER, K. H. (1980). Occurrence and distribution of staphylococci and micrococci in soybean oil meal. *Zentralblatt für Bakteriologie (Abteilung I, Originale C)* **1**, 320–329.
- CROSA, J. H., WILLIAMS, B. L., JORGENSEN, J. J. & EVANS, C. A. (1979). Comparative study of deoxyribonucleic acid homology and physiological characteristics of strains of *Peptococcus saccharolyticus*. *International Journal of Systematic Bacteriology* **29**, 328–332.
- DE LEY, J. (1969). Compositional nucleotide distribution and the theoretical prediction of homology in bacterial DNA. *Journal of Theoretical Biology* **22**, 89–116.
- DE SMEDT, J. & DE LEY, J. (1977). Intra- and intergeneric similarities of *Agrobacterium* ribosomal ribonucleic acid cistrons. *International Journal of Systematic Bacteriology* **27**, 222–240.
- EHRLICH, S. D. (1977). Replication and expression of plasmids from *Staphylococcus aureus* in *Bacillus subtilis*. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 1680–1682.
- ENGEL, H. W. B., SOEDIRMAN, N., ROST, J. A., VAN LEEUWEN, W. J. & VAN EMBDEN, J. D. A. (1980). Transferability of macrolide, lincomycin, and streptogramin resistances between group A, B and D streptococci, *Streptococcus pneumoniae*, and *Staphylococcus aureus*. *Journal of Bacteriology* **142**, 407–413.
- FALLER, A. H., GÖTZ, F. & SCHLEIFER, K. H. (1980). Cytochrome-patterns of staphylococci and micrococci and their taxonomic implications. *Zentralblatt für Bakteriologie (Abteilung I, Originale C)* **1**, 26–39.
- FEITELSON, J. S. & LEDERBERG, J. (1980). Crude lysates of *Staphylococcus aureus* can transform *Bacillus subtilis*. *Journal of General Microbiology* **116**, 545–547.
- FELTHAM, R. K. A. (1979). A taxonomic study of the *Micrococcaceae*. *Journal of Applied Bacteriology* **47**, 243–254.
- FOUBERT, E. L. & DOUGLAS, H. G. (1948). Studies on the anaerobic micrococci. *Journal of Bacteriology* **56**, 25–34.
- FOX, G. E., PECHMAN, K. R. & WOESE, C. R. (1977). Comparative cataloging of 16S ribosomal ribonucleic acid: molecular approach to procaryotic systematics. *International Journal of Systematic Bacteriology* **27**, 44–57.
- FOX, G. E., STACKEBRANDT, E., HESPELL, R. B., GIBSON, J., MANILOFF, J., DYER, T. A., WOLFE, R. S., BALCH, W. E., TANNER, R. S., MAGRUM, L. J., ZABLEN, L. B., BLAKEMORE, R., GUPTA, R., BONEN, L., LEWIS, B. J., STAHL, D. A., LUEHRSEN, K. R., CHEN, K. N. & WOESE, C. R. (1980). The phylogeny of prokaryotes. *Science* **209**, 457–463.
- GIBSON, T. & GORDON, R. E. (1974). *Bacillus*. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 529–550. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- GOODFELLOW, M., MORDARSKI, M., TKACZ, A., SZYBA, K. & PULVERER, G. (1980). Polynucleotide sequence divergence among some coagulase-negative staphylococci. *Zentralblatt für Bakteriologie (Abteilung I, Originale A)* **246**, 10–22.
- JOHNSON, J. L. & FRANCIS, B. S. (1975). Taxonomy of the clostridia: ribosomal ribonucleic acid homologies among the species. *Journal of General Microbiology* **88**, 229–244.
- JONES, D. & SNEATH, P. H. A. (1970). Genetic transfer and bacterial taxonomy. *Bacteriological Reviews* **34**, 40–81.
- KILPPER, R. & SCHLEIFER, K. H. (1978). *Peptococcus saccharolyticus*: an anaerobic *Staphylococcus*? In *Abstracts of the XII International Congress of Microbiology*, p. 86, C6.
- KILPPER, R., BUHL, U. & SCHLEIFER, K. H. (1980). Nucleic acid homology studies between *Peptococcus saccharolyticus* and various anaerobic and facultative anaerobic Gram-positive cocci. *FEMS Microbiology Letters* **8**, 205–210.
- KLOOS, W. E. & WOLSHOHL, J. F. (1979). Evidence for deoxyribonucleotide sequence divergence between staphylococci living on human and other primate skin. *Current Microbiology* **3**, 167–172.
- KLOOS, W. E., SCHLEIFER, K. H. & SMITH, R. F. (1976). Characterization of *Staphylococcus sciuri* sp. nov. and its subspecies. *International Journal of Systematic Bacteriology* **26**, 22–37.
- KOCUR, M. & BOHACEK, J. (1974). DNA base composition and the classification of non-pigmented micrococci. *Microbios* **10A**, 31–38.
- KOCUR, M., BERGAN, T. & MORTENSEN, N. (1971). DNA base composition of Gram-positive cocci. *Journal of General Microbiology* **69**, 167–183.
- MEYER, S. A. & SCHLEIFER, K. H. (1978). Deoxyribonucleic acid reassociation in the classification of coagulase-positive staphylococci.

- Archives of Microbiology* **117**, 183–188.
- OGASAWARA-FUJITA, N. & SAKAGUCHI, K. (1976). Classification of micrococci on the basis of deoxyribonucleic acid homology. *Journal of General Microbiology* **94**, 97–106.
- PECHMAN, K. J., LEWIS, B. J. & WOESE, C. R. (1976). Phylogenetic status of *Sporosarcina urea*. *International Journal of Systematic Bacteriology* **26**, 305–310.
- ROGOSA, M. (1974). *Peptococcus*. In *Bergey's Manual of Determinative Bacteriology*, 8th edn., pp. 518–522. Edited by R. E. Buchanan & N. E. Gibbons, Baltimore: Williams & Wilkins.
- ROSENBLUM, E. D. & TYRONE, S. (1979). Deoxyribonucleic acid homologies among staphylococci: coagulase-positive reference strains. *Current Microbiology* **2**, 171–174.
- RUPPRECHT, M. & SCHLEIFER, K. H. (1979). A comparative immunological study of catalases from coagulase-positive staphylococci. *Archives of Microbiology* **120**, 53–56.
- SCHLEIFER, K. H. & KANDLER, O. (1972). The peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriological Reviews* **36**, 407–477.
- SCHLEIFER, K. H. & KLOOS, W. E. (1975). Isolation and characterization of staphylococci from human skin. I: Amended description of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*, and description of three new species: *Staphylococcus cohnii*, *Staphylococcus haemolyticus* and *Staphylococcus xylosus*. *International Journal of Systematic Bacteriology* **25**, 50–61.
- SCHLEIFER, K. H. & KOCUR, M. (1973). Classification of staphylococci based on chemical and biochemical properties. *Archives of Microbiology* **93**, 65–85.
- SCHLEIFER, K. H. & NIMMERMANN, E. (1973). Peptidoglycan types of strains of the genus *Peptococcus*. *Archiv für Mikrobiologie* **93**, 245–258.
- SCHLEIFER, K. H., MEYER, S. A. & RUPPRECHT, M. (1972a). Relatedness among coagulase-negative staphylococci: deoxyribonucleic acid reassociation and comparative immunological studies. *Archives of Microbiology* **122**, 93–101.
- SCHLEIFER, K. H., MEYER, S. A. & HEISE, W. (1979b). Deoxyribonucleic acid hybridization studies among some micrococci. *FEMS Microbiology Letters* **6**, 33–36.
- SEKI, T., OSHIMA, T. & OSHIMA, Y. (1975). Taxonomic study of *Bacillus* by deoxyribonucleic acid-deoxyribonucleic acid hybridization and inter-specific transformation. *International Journal of Systematic Bacteriology* **25**, 258–270.
- SILBERKLANG, M., GILLUM, A. M. & RAJBHANDARY, U. L. (1979). Use of in vitro ³²P labelling in the sequence analysis of nonradioactive tRNAs. *Methods in Enzymology* **59G**, 58–109.
- SILVESTRI, L. G. & HILL, L. R. (1965). Agreement between deoxyribonucleic acid base composition and taxometric classification of Gram-positive cocci. *Journal of Bacteriology* **90**, 136–140.
- SIMSEK, M., ZIEGENMEYER, J., HECKMAN, J. E. & RAJBHANDARY, U. L. (1973). Absence of the sequence -G-T-Ψ-C-G- in several eukaryotic cytoplasmic initiator transfer RNAs. *Proceedings of the National Academy of Sciences of the United States of America* **70**, 1041–1045.
- STACKEBRANDT, E. & FIEDLER, F. (1979). DNA-DNA homology studies among strains of *Arthrobacter* and *Brevibacterium*. *Archives of Microbiology* **120**, 289–295.
- STACKEBRANDT, E. & WOESE, C. R. (1979). A phylogenetic dissection of the family Micrococcaceae. *Current Microbiology* **2**, 317–322.
- STACKEBRANDT, E., LEWIS, B. J. & WOESE, C. R. (1980). The phylogenetic structure of the coryneform group of bacteria. *Zentralblatt für Bakteriologie (Abteilung I, Originale C)* **1**, 137–149.
- STACKEBRANDT, E., LUDWIG, W., SCHLEIFER, K. H. & GROSS, H. J. (1981). Rapid cataloging of ribonuclease T₁ resistant oligonucleotides from ribosomal RNAs for phylogenetic studies. *Journal of Molecular Evolution* **17** (in the Press).
- SUEOKA, N. (1961). Variation and heterogeneity of base composition of deoxyribonucleic acids: a compilation of old and new data. *Journal of Molecular Biology* **3**, 31–40.
- UCHIDA, T., BONEN, L., SCHAUP, H. W., LEWIS, B. J., ZABLEN, L. & WOESE, C. R. (1974). The use of ribonuclease U₂ in RNA sequence determination. *Journal of Molecular Evolution* **3**, 63–77.
- WOESE, C. R. & FOX, G. E. (1977). Phylogenetic structure of the prokaryotic domain. The primary kingdoms. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 5088–5090.
- WOESE, C. R., SOGIN, M., STAHL, D., LEWIS, B. J. & BONEN, L. (1976). A comparison of the 16S ribosomal RNAs from mesophilic and thermophilic bacilli: some modifications in the Sanger method for RNA sequencing. *Journal of Molecular Evolution* **7**, 197–213.