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主 論 文

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1）Determination of 16S rRNA Sequences of *Streptococcus mitis* and *Streptococcus gordonii* and Phylogenetic Relationships among Members of the Genus *Streptococcus* 1冊
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Determination of 16S rRNA Sequences of *Streptococcus mitis* and *Streptococcus gordonii* and Phylogenetic Relationships among Members of the Genus *Streptococcus*

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We determined the 16S rRNA sequences of the type strains of *Streptococcus mitis* and *Streptococcus gordonii* and calculated the phylogenetic distances between those organisms and other members of the genus *Streptococcus*. The viridans group streptococci were separated into five phylogenetic groups; we named these groups the anginosus group, the mitis group, the salivarius group, the bovis group, and the mutans group. *S. mitis* and *S. gordonii* clustered in the mitis group together with *Streptococcus pneumoniae*, *Streptococcus oralis*, *Streptococcus sanguis*, and *Streptococcus parasanguis* at levels of sequence homology of more than 96%. Within this group, *S. mitis*, *S. oralis*, and *S. pneumoniae* exhibited more than 99% sequence homology with each other, although the DNA-DNA similarity values for their total chromosome DNAs were less than 60%.

In the past 10 years, several new members have been added to the viridans streptococcus group (3, 11, 18–20). *Streptococcus oralis*, *Streptococcus parasanguis*, and *Streptococcus gordonii* are the major new species that have been described. These organisms are often isolated from human clinical specimens and thus are clinically important species. However, workers at clinical laboratories have found that it is difficult to identify viridans group streptococci because of the lack of decisive phenotypic characteristics.

Some of the confusion concerning identification of these bacteria came from the type strain of *Streptococcus mitis*, strain NCTC 3165. This type strain had traits different from the traits described for the species. Therefore, Coykendall et al. (5) proposed that *S. mitis* NCTC 3165 should be rejected as the type strain, and in Opinion 66 (10) strain NCTC 12261 was designated the neotype strain of *S. mitis*. The rejected type strain is now identified as an *S. gordonii* strain, and we have confirmed this identification by DNA-DNA hybridization (unpublished data). In a previous study, we developed quantitative microplate DNA-DNA hybridization methods to identify streptococci (6, 7) and found that many strains which were identified as *S. mitis*, *S. oralis*, and *Streptococcus sanguis* by phenotypic methods were misidentified. Another problem arose when we applied quantitative DNA-DNA hybridization methods to the identification of viridans group streptococci. Many strains identified biochemically as *S. mitis* were difficult to differentiate from *S. oralis* and *Streptococcus pneumoniae* even by the hybridization method because clinical strains

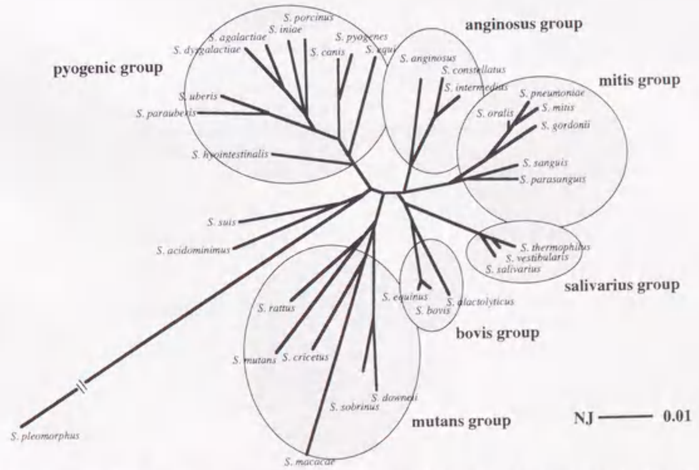

FIG. 1. Phylogenetic relationships among 34 *Streptococcus* species. Distances were calculated by the neighbor-joining (NJ) method. *S. pleomorphus* was located far from other species, so its distance is indicated with an ellipsis; its true distance from the junction was 0.16944.

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TABLE 1. Levels of 16S rRNA sequence homology among *S. mitis*, *S. gordonii*, and other *Streptococcus* species

Group	Species	% Homology with:	
		S. mitis	*S. gordonii*
Mitis	*S. mitis*	100.00	
	S. gordonii	97.63	100.00
	S. pneumoniae	99.01	97.24
	S. oralis	99.39	98.16
	S. sanguis	96.71	97.01
	S. parasanguis	96.78	96.25
Anginosus	*S. anginosus*	94.41	94.64
	S. constellatus	95.86	95.48
	S. intermedius	95.56	96.17
Salivarius	*S. salivarius*	95.10	95.86
	S. thermophilus	94.49	95.41
	S. vestibularis	94.78	95.70
Bovis	*S. bovis*	94.49	95.10
	S. equinus	94.72	95.18
	S. dactolyticus	95.02	95.25
Mutans	*S. mutans*	93.42	94.64
	S. rattus	93.57	94.10
	S. cricetus	92.73	93.87
	S. downei	93.41	93.64
	S. sobrinus	93.80	94.18
	S. macacae	90.72	91.49
Pyogenic	*S. pyogenes*	94.49	94.87
	S. agalactiae	94.79	94.03
	S. canis	94.26	94.87
	S. dysgalactiae	94.86	94.10
	S. equi	93.57	93.95
	S. iniae	95.02	94.18
	S. porcinus	94.63	93.64
	S. uberis	93.65	94.26
	S. parauberis	93.79	93.41
	S. hyointestinalis	94.56	94.87
None[a]	*S. acidominimus*	94.33	94.72
	S. suis	94.26	94.64
	S. pleomorphus	82.46	82.30

[a] No group name is proposed for these three species.

strongly hybridized to both *S. mitis* and *S. oralis* and sometimes to *S. pneumoniae*. To solve this problem, we decided to determine the 16S rRNA sequence of the type strain of the viridans streptococcus group in order to identify members of this group by sequencing. Fortunately, Collins and other workers have published 16S rRNA sequences of most of the members of the genus *Streptococcus* (2, 17, 18, 21). However, the sequences of two type strains, *S. mitis* NCTC 12261 and *S. gordonii* NCTC 7865, have not been determined previously, and thus we were not able to identify viridans group streptococci on the basis of sequence data.

Type strains NCTC 12261 and NCTC 7865 were purchased directly from the National Collection of Type Cultures, and their 16S rRNA genes were amplified as described previously (8, 13). The sequences were determined by using the dye primer method and an ABI automatic sequencer (model 373A; Applied Biosystems, Foster City, Calif.). The sequence of each 16S rRNA from position 8 to position 1392 (*Escherichia coli* numbering) was determined. The sequences of the other members of the genus *Streptococcus* used for alignment and for calculating levels of homology were obtained from the GenBank and EMBL databases. The ODEN program set of the DNA Data Bank of Japan was used to align the sequences, and phylogenetic distances were calculated by using the neighbor-joining method (15).

A phylogenetic tree for 34 species of the genus *Streptococcus* is shown in Fig. 1, and the levels of homology for *S. mitis*, *S. gordonii*, and other species are shown in Table 1.

S. mitis and *S. gordonii* formed one cluster together with *S. pneumoniae*, *S. oralis*, *S. sanguis*, and *S. parasanguis*; (we named this group the mitis group). Within this group, *S. mitis*, *S. oralis*, and *S. pneumoniae* exhibited more than 99% sequence homology with each other. Thus, these three species are closely related. Recently, Stackebrandt and Goebel (16) found that 16S rRNA sequence analysis can be used to determine the phylogenetic relationships of prokaryotic species when the levels of sequence homology are less than 97% and that DNA-DNA hybridization experiments are necessary to confirm the taxonomic positions when homology values are greater than 97%. The DNA-DNA similarity values for all of the species belonging to the mitis group are shown in Table 2. All of the members of the mitis group exhibited less than 60% DNA similarity with each other; thus, our data clearly demonstrated that all of these species are distinct taxa. *S. oralis* and *S. mitis* exhibited less than 55% DNA similarity with each other as determined by quantitative DNA-DNA hybridization, even though they exhibited 99.39% sequence homology. While *S. gordonii* and *S. sanguis* exhibited almost the same level of DNA similarity, they exhibited only 97.01% sequence homology. *S. gordonii* was described by Kilian et al. (11) as a new species that was distinct from *S. sanguis*. These authors also reported that *S. gordonii* was more closely related to *S. sanguis* than to *S. oralis* as determined by DNA similarity data. Our hybridization data showed almost the same results. However, our sequence data showed that *S. gordonii* is more closely related to *S. oralis*, *S. mitis*, and *S. pneumoniae* than to *S. sanguis* and *S. parasanguis*. In this case, whether the data came from 16S rRNA sequence homology studies or from DNA-DNA hybridization similarity studies made a difference.

TABLE 2. Levels of DNA-DNA hybridization between strains belonging to the mitis group

Strain	% DNA-DNA hybridization with[a]:					
	S. mitis NCTC 10234	*S. gordonii* ATCC 10558	*S. oralis* NCTC 11427	*S. sanguis* ATCC 10556	*S. pneumoniae* NCTC 7465	*S. parasanguis* ATCC 15912
S. mitis NCTC 10234	100					
S. gordonii ATCC 10558	9–31	100				
S. oralis NCTC 11427	44–55 (30–38)	13–38 (20–40)	100			
S. sanguis ATCC 10556	16–24 (28)	34–57 (40–60)	23–30 (20–40)	100		
S. pneumoniae NCTC 7465	30–46	0–7	10–19	1–11	100	
S. parasanguis ATCC 15912	22–52	11–24	14–36	20–37	14–37	100

[a] Data from a previous study (11). The data in parentheses are data from reference 11.

We divided the genus *Streptococcus* into six major clusters (the pyogenic group, the anginosus group, the mitis group, the salivarius group, the bovis group, and the mutans group), which included 31 species (Fig. 1). *Streptococcus suis* and *Streptococcus acidominimus* were not related to either the viridans group or the pyogenic group.

Four strictly anaerobic streptococcal species were described in *Bergey's manual of Systematic Bacteriology* (9). Three of these species, *Streptococcus morbillorum*, *Streptococcus parvulus*, and *Streptococcus hansenii*, have been transferred to the genera *Gemella* (12), *Atopobium* (4), and *Ruminococcus* (8), respectively. Thus, *Streptococcus pleomorphus* is now the only anaerobic member of the genus. Ludwig et al. (14) reported that *S. pleomorphus* was not closely related to streptococci but was more closely related to certain clostridia. In our study we found that *S. pleomorphus* exhibited less than 85% sequence homology with *S. mitis* or *S. gordonii* (Table 1) or any other member of the genus *Streptococcus* (data not shown). Our neighbor-joining data (Fig. 1) also revealed that *S. pleomorphus* was not related to any member of the genus *Streptococcus*. Our data supported the observation of Ludwig et al., and we concluded that *S. pleomorphus* should be removed from the genus *Streptococcus*.

Nucleotide sequence accession number. The 16S rRNA sequences of *S. mitis* and *S. gordonii* have been deposited in the DNA Data Bank of Japan under accession numbers D38482 and D38483, respectively.

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Transfer of *Streptococcus adjacens* and *Streptococcus defectivus* to *Abiotrophia* gen. nov. as *Abiotrophia adiacens* comb. nov. and *Abiotrophia defectiva* comb. nov., Respectively

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We performed this study to determine the 16S rRNA sequences of the type strains of *Streptococcus adjacens* and *Streptococcus defectivus* and to calculate the phylogenetic distances between these two nutritionally variant streptococci (NVS) and other members of the genus *Streptococcus*. *S. adjacens* and *S. defectivus* belonged to one cluster, but this cluster was not closely related to other streptococcal species. A comparative analysis of the sequences of these organisms and other low-G+C-content gram-positive bacteria revealed that the two NVS species formed a distinct cluster and were only remotely related to the *Aerococcus* and *Carnobacterium* clusters. The highest level of homology (93.7%) was found between *S. adjacens* and *Carnobacterium divergens*. *Carnobacterium* species have *meso*-diaminopimelic acid in their cell walls, but *S. adjacens* and *S. defectivus* have L-lysine as the diamino acid at position 3 in their peptidoglycan tetrapeptides. On the basis of our findings and the results of previous phenotypic studies, we propose that the NVS species should be placed in a new genus, the genus *Abiotrophia*, as *Abiotrophia adiacens* comb. nov. and *Abiotrophia defectiva* comb. nov.

In 1961, Frenkel and Hirsch (13) described a new type of gram-positive bacteria that exhibited satellitism around colonies of other bacteria, and they identified this new type of bacteria as streptococci. Since then, many names have been given to these bacteria. These names include satelliting streptococci (20), thiol-requiring streptococci (6), vitamin B_6- or pyridoxal-dependent streptococci (4, 21), symbiotic streptococci (14), and nutritionally variant streptococci (NVS) (10, 11, 16); the latter has been the most popular name. Many researchers have investigated the growth (4, 21), morphology (2), biochemical traits (3, 10), fermentation products (5), and cell wall components (24) of these organisms. On the basis of the resulting data it has been suggested that these taxa are closely related to the genus *Streptococcus* and belong to *Streptococcus mitis* or "*Streptococcus mitior*" (4, 15). In 1989, Bouvet et al. (1) performed a DNA-DNA hybridization study with NVS strains and *Streptococcus* species and validly named the NVS *Streptococcus adjacens* and *Streptococcus defectivus*. This is the only study in which the genetic relationships of these organisms were clarified. However, the data of Bouvet et al. revealed that *Streptococcus adjacens* and *Streptococcus defectivus* exhibited levels of similarity of less than 10% with all other members of the genus *Streptococcus* and exhibited levels of similarity of less than 6% with *Streptococcus mitis*, the most closely related species as determined by a phenotypic study. The genetic relationship between the NVS species and the other members of the genus *Streptococcus* remained uncertain.

During our study to determine phylogenetic relationships among members of the genus *Streptococcus* by using 16S rRNA sequences, we noticed that the two NVS type strains were not related to any species belonging to the genus *Streptococcus*. We compared the NVS 16S rRNA sequences with the sequences of other species which have low G+C contents, determined the phylogenetic positions of the NVS, and decided to create a new genus for the two NVS species.

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MATERIALS AND METHODS

Bacterial strains. The strains which we used are the type strains of *Streptococcus adjacens* (GIFU 12706 [= ATCC 49175]) and *Streptococcus defectivus* (GIFU 12707 [= ATCC 49176]). These strains were grown on Columbia blood agar base (Difco) supplemented with 5% defibrinated sheep blood (CBA) and 0.01% L-cysteine (Sigma Chemical Co., St. Louis, Mo.) at 37°C under aerobic conditions. To confirm the purity and the nutritional requirements, the following three tests were performed: (i) a satellite test on CBA with *Staphylococcus epidermidis* GIFU9123^T (T = type strain), (ii) a growth test in Todd-Hewitt broth (THB) (Difco Laboratories, Detroit, Mich.) containing or lacking 0.001% pyridoxal hydrochloride (E. Merck, Darmstadt, Germany) or 0.01% L-cysteine, and (iii) a biochemical test in which we used API-Strep preparations (BioMerieux, Marcy l'Etoile, France).

Analysis of diaminopimelic acid. The diaminopimelic acid was analyzed by the thin-layer chromatography method (19). Briefly, 50 mg (wet weight) of cells was harvested from THB containing pyridoxal hydrochloride, and the cells were hydrolyzed with 1 ml of 6 N HCl at 100°C for 18 h. The hydrolysates were filtered and dried with a rotary evaporator. Each sample was applied to a cellulose thin-layer chromatography plate (catalog no. 5552; Merck). Methanol–water–6 N HCl–pyridine (40:13:2:5, vol/vol/vol/vol) was used as the developing solution. After the plate was developed, the spots were visualized by spraying it with a 0.2% ninhydrin solution. DL-Diaminopimelic acid (Sigma) and cell wall hydrolysate from *Carnobacterium funditum* IFO 15549 (12) were used as the standard and reference preparations, respectively.

16S rRNA gene sequence determination and analysis. The 16S rRNA genes were amplified by using the PCR method described previously (17). The sequences were determined by using the dye primer method and an automatic sequencer (model 373A; Applied Biosystems, Inc., Foster City, Calif.). The 16S rRNA sequence from position 8 to position 1392 (*Escherichia coli* numbering) was determined for each organism.

The sequences of the other members of the genus *Streptococcus* and low-G+C-content gram-positive bacteria used for alignment and for calculating levels of homology were obtained from the GenBank and EMBL databases. The CLUSTAL W software originally described by Thompson et al. (23) was used to align the sequences, and phylogenetic distances were calculated by using the neighbor-joining method. An unrooted phylogenetic tree was drawn by using tree tool software.

Nucleotide sequence accession numbers. The nucleotide sequences of the 16S rRNAs of *Abiotrophia adiacens* (*Streptococcus adjacens*) and *Abiotrophia defectiva* (*Streptococcus defectivus*) have been deposited in the DNA Data Bank of Japan under accession numbers D50540 and D50541, respectively.

RESULTS AND DISCUSSION

The type strains of *Streptococcus adjacens* and *Streptococcus defectivus* grew only around colonies of *Staphylococcus epidermidis* on CBA plates and in THB supplemented with 0.001%

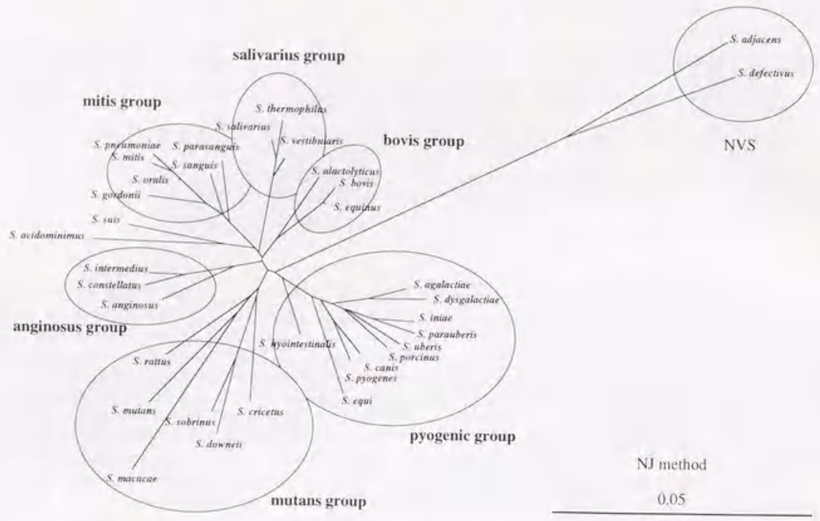

FIG. 1. Phylogenetic relationships between the two NVS species and members of the genus *Streptococcus*. Distances were calculated by the neighbor-joining (NJ) method.

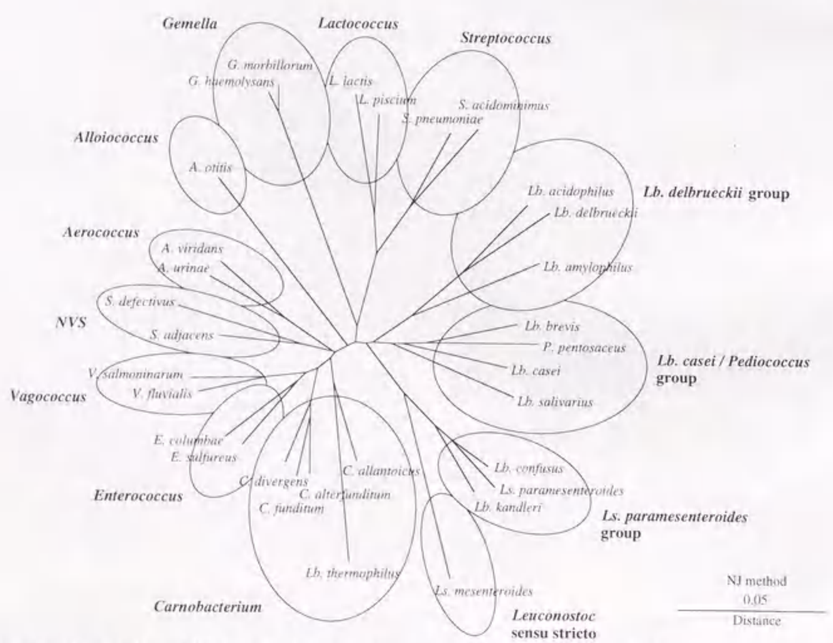

FIG. 2. Phylogenetic positions of the two NVS species and some selected low-G+C-content gram-positive bacteria. Distances were calculated by the neighbor-joining (NJ) method. *Lb.*, *Lactobacillus*; *Ls.*, *Leuconostoc*.

TABLE 1. Levels of 16S rRNA sequence homology among *Streptococcus adjacens*, *Streptococcus defectivus*, and some low-G+C-content gram-positive bacteria

Genus or group	Species	% Homology with:	
		Streptococcus adjacens	*Streptococcus defectivus*
NVS	*Streptococcus adjacens*	100.0	
	Streptococcus defectivus	93.0	100.0
Aerococcus	*Aerococcus urinae*	91.7	90.6
	Aerococcus viridans	91.0	89.5
Alloiococcus	*Alloiococcus otitis*	88.8	86.8
Carnobacterium	*Carnobacterium allantoicus*	93.3	90.4
	Carnobacterium alterfunditum	93.1	90.1
	Carnobacterium divergens	93.7	90.4
	Carnobacterium funditum	93.1	90.1
	Lactobacillus thermophilus	88.4	86.8
Enterococcus	*Enterococcus columbae*	91.3	89.4
	Enterococcus sulfureus	92.1	89.4
Gemella	*Gemella haemolysans*	86.1	86.5
	Gemella morbillorum	85.8	86.6
Lactococcus	*Lactococcus lactis*	86.2	86.4
	Lactococcus piscium	86.8	87.4
Lactobacillus casei-Pediococcus group	*Lactobacillus brevis*	89.6	87.7
	Lactobacillus casei	91.3	89.0
	Lactobacillus salivarius	89.6	88.7
	Pediococcus pentosaceus	89.7	87.8
Lactobacillus delbrueckii group	*Lactobacillus acidophilus*	87.8	88.0
	Lactobacillus amylophilus	87.7	88.0
	Lactobacillus delbrueckii	87.0	86.7
Leuconostoc paramesenteroides group	*Lactobacillus confusus*	88.9	87.4
	Lactobacillus kandleri	88.8	86.7
	Leuconostoc paramesenteroides	88.3	87.1
Leuconostoc sensu stricto	*Leuconostoc mesenteroides*	86.5	86.3
Streptococcus	*Streptococcus acidominimus*	85.5	85.6
	Streptococcus pneumoniae	86.7	86.5
Vagococcus	*Vagococcus fluvialis*	91.8	88.6
	Vagococcus salmoninarum	92.1	88.9

pyridoxal hydrochloride and THB supplemented with 0.01% L-cysteine. Both type strains exhibited positive reactions in pyrrolidonyl arylamidase (PYR), β-glucuronidase, and leucine aminopeptidase tests, and the *Streptococcus defectivus* type strain also exhibited positive reactions in α-galactosidase, β-galactosidase, trehalose fermentation, and lactose fermentation tests and weakly positive reactions in starch fermentation and acetoin production tests. All of these data are consistent with the previously described characteristics of *Streptococcus adjacens* and *Streptococcus defectivus* (1, 3).

The 16S rRNA gene sequences of the two NVS type strains were investigated to determine the relationships of these organisms to other members of the genus *Streptococcus* and to other low-G+C-content gram-positive bacteria. Data for 33 species of the genus *Streptococcus* were prepared, and the sequences were aligned with the sequences of the two NVS type strains.

A phylogenetic tree containing the two NVS strains and other members of the genus *Streptococcus* is shown in Fig. 1. In a previous study, we found that the members of the genus *Streptococcus* belonged to six major clusters and two loosely related species (*Streptococcus suis* and *Streptococcus acidominimus*) (17). The two NVS type strains belonged to one cluster, but this cluster was not closely related to the other six clusters, *Streptococcus suis* and *Streptococcus acidominimus*. The most closely related species was *Streptococcus hyointestinalis*, but the levels of homology between this organism and *Streptococcus adjacens* and *Streptococcus defectivus* were only 88.3 and 89.2%, respectively. These data demonstrated that the two NVS type strains were not closely related to any species belonging to the genus *Streptococcus* and should be removed from this genus.

To investigate the phylogenetic position of the NVS among the low-G+C-content gram-positive bacteria, we prepared another data set, which contained data for representative species of 49 genera (including mycoplasmas), and calculated the phylogenetic position of the NVS. Our results showed that the NVS cluster was near the *Aerococcus-Vagococcus-Enterococ-*

TABLE 2. G+C contents and peptidoglycan structures of selected genera

Genus	G+C content (mol%) (method)[a]	Peptidoglycan structure		
		Position 3	Crossbridge	Type(s)[b]
Abiotrophia (NVS)	37–47 (T_m)	Lys	Ala_{1-2}	A3α
Aerococcus	35–40 (T_m)	Lys	None (direct)	A1α
Alloiococcus	44–45 (T_m)	ND[c]	ND	ND
Carnobacterium	35–37 (T_m)	*meso*-DAP[d]	None (direct)	A1γ
Enterococcus	37–45 (T_m)	Lys	Asp, Ala_{2-3}	A4α, A3α
Gemella	32–35 (Bd)	Lys	Ala_{1-3}	A3α
Lactobacillus	32–53 (T_m, Bd)	Lys	Asp[e]	A4α[e]
Lactococcus	34–43 (T_m)	Lys	Asp, Ala-Gly-Ala[e]	A4α, A3α
Leuconostoc	38–44 (T_m, Bd)	Lys	Ser-Ala_2, Ala[e]	A3α
Pediococcus	34–42 (T_m)	Lys	Ala-Asp, Ala	A4α, A3α
Streptococcus	34–46 (T_m, Bd)	Lys	Ala_{1-3}, Ala-Ser, none (direct)[e]	A3α, A1α
Vagococcus	33–37 (T_m)	Lys	Asp	A4α

[a] T_m, thermal denaturation method; Bd, buoyant density method.
[b] Peptide types described by Schleifer and Kandler (22).
[c] ND, no data available.
[d] *meso*-DAP, *meso*-diaminopimelic acid.
[e] Predominant data for the genus.

rus-Carnobacterium cluster (data not shown). We then prepared a third data set, which contained data for 29 species belonging to 11 genera, including the genera *Streptococcus*, *Aerococcus*, and *Carnobacterium*, and other lactic acid and related bacteria to determine the exact phylogenetic position of the two NVS species. The resulting phylogenetic tree is shown in Fig. 2, and the levels of homology of *Streptococcus adjacens* and *Streptococcus defectivus* with other species are shown in Table 1.

On our phylogenetic tree, each established genus was well separated, and its members formed a distinct cluster. The members of the genera *Lactobacillus*, *Pediococcus*, and *Leuconostoc* were divided into four groups (the *Lactobacillus delbrueckii* group, the *Lactobacillus casei-Pediococcus* group, the *Leuconostoc paramesenteroides* group, and the genus *Leuconostoc* sensu stricto), in accordance with the results of previous studies (7, 8). *Streptococcus adjacens* and *Streptococcus defectivus* clearly belonged to one cluster, which was not closely related to the genus *Streptococcus* cluster and was loosely related to the *Aerococcus* cluster.

The level of homology between *Streptococcus adjacens* and *Streptococcus defectivus* sequences was 93.0%. The highest levels of sequence homology to *Streptococcus adjacens* or *Streptococcus defectivus* were exhibited by *Carnobacterium divergens* (93.7%) and *Aerococcus urinae* (90.6%).

Streptococcus adjacens and *Streptococcus defectivus* lack diaminopimelic acid in their cell walls, while *C. fundilium* cell walls contain *meso*-diaminopimelic acid. The cell walls of NVS strains were analyzed by van de Rijn (24), who found that the mean ratio of muramic acid to glucosamine to glutamic acid to lysine to alanine was 1.0:1.3 to 2.1:1.0:1.0:3.3 to 3.7. These data indicate that L-lysine may be the diamino acid at position 3 and that alanine or alanine-alanine may be the peptide cross bridge, and the presumptive peptide type is type A3α (Table 2). On the other hand, *Carnobacterium* species contain *meso*-diaminopimelic acid and have a type A1γ direct cross-linkage (9), whereas in *Aerococcus* species L-lysine is the diamino acid at position 3 and the direct cross-linkage type is type A1α (22). The two NVS strains could be clearly distinguished from the genera *Carnobacterium* and *Aerococcus* on the basis of these data.

We investigated the relationships between the two NVS type strains and other members of the genus *Streptococcus* and the low-G+C-content gram-positive bacteria. The 16S rRNA sequence data clearly demonstrated that the two NVS type strains were phylogenetically distinct from the genus *Streptococcus*.

TABLE 3. Phenotypic characteristics that differentiate the genus *Abiotrophia* from other catalase-negative, gram-positive cocci

Genus	Satellitism	Susceptibility to vancomycin (30-μg disk)[a]	Gas production from glucose	PYR activity	Leucine aminopeptidase activity	Growth in broth containing 6.5% NaCl	Growth at:		Motility	Hemolysis on blood agar (5% sheep blood)
							10°C	45°C		
Abiotrophia	+[b]	S	−	+	+	−	−	−	−	Alpha
Aerococcus	−	S	−	+	−	+	−	+	−	Alpha
Enterococcus	−	S	−	+	+	+	+	+	v	Alpha, beta, none
Gemella	−	S	−	+	v	−	−	−	−	Alpha, none
Lactococcus	−	S	−	+	+	v	+[c]	−[c]	−	Alpha, none
Leuconostoc	−	R	+	−	−	v	+	v	−	Alpha, none
Pediococcus	−	R	−	−	+	v	−	+	−	Alpha
Streptococcus	−	S	−	−[d]	+[e]	−[e]	−	v	−	Alpha, beta, none
Vagococcus	−	S	−	+	+	+	+	−	+	Alpha, none

[a] S, susceptible; R, resistant.
[b] +, more than 90% of the strains are positive; −, less than 10% of the strains are positive; v, variable.
[c] Some strains grow very slowly at 45°C.
[d] *Streptococcus pyogenes* strains exhibit PYR activity.
[e] Some beta-hemolytic streptococci grow in broth containing 6.5% NaCl.

On the basis of previous data, including data on biochemical traits, fermentation products, and cell wall components, it has been demonstrated that the most characteristic traits of the NVS strains which distinguish these organisms from members of the genus *Streptococcus* are nutrient requirements, satellitism, and PYR test results. Furthermore, the NVS strains but not all other *Streptococcus* species require vitamin B_6 or cysteine for growth. Only one exception to this was described by Bouvet et al. (2), but these authors used a special medium in their study. No NVS strains can grow properly on common commercial media for streptococci (e.g., blood agar based on Trypticase soy medium, CBA, and THB). The NVS strains exhibit satellitism on blood agar around other bacteria (e.g., *Staphylococcus epidermidis*). Some *Haemophilus* species also exhibit satellitism around staphylococci on blood agar. It is known that *Haemophilus* satellitism requires X factor (hemin) and V factor (NAD) and that growing staphylococci supply V factor (18). In the case of the NVS strains, X factor and V factor did not affect growth (10). The factor which is required by the NVS has yet to be determined, but sulfydryl compounds seem to be good candidates for compounds that are required for NVS strain growth (10, 13). This characteristic is also useful for distinguishing NVS strains from members of the genus *Streptococcus*. The NVS strains exhibited a positive reaction in the PYR test; on the other hand, almost all *Streptococcus* species except *Streptococcus pyogenes* had negative reactions in this test (11). *Streptococcus pyogenes* can be easily differentiated from the NVS strains by its beta-hemolysis on blood agar and by many different biochemical characteristics.

The 16S rRNA sequence data obtained for other low-G+C-content gram-positive bacteria also clearly showed that the NVS species were independent and only remotely related to the genera *Aerococcus* and *Carnobacterium*, from which they differ in cell wall peptidoglycan structure.

In view of the data presented above, we propose that *Streptococcus adjacens* and *Streptococcus defectivus* should be classified in a new genus, the genus *Abiotrophia*, as *Abiotrophia adiacens* comb. nov. and *Abiotrophia defectiva* comb. nov., respectively.

Description of *Abiotrophia* gen. nov. *Abiotrophia* (Abi. o. tro'phi. a, G. prefix *a-*, negative (un-); G. n. *bios*, life; G. n. *trophe*, nutrition; M. L. n. *Abiotrophia*, life nutrition deficiency). The description below is based on data from this study and previously described studies (1, 3, 5, 10, 24).

Cells are nonsporulating, nonmotile, and gram positive. Cells are mainly cocci, but pleomorphic ovoid cells, coccobacilli, and rod-shaped cells may occur in THB or CBA supplemented with pyridoxal hydrochloride or L-cysteine. Facultative anaerobes. Catalase and oxidase negative. Lactic acid is the compound that is predominantly produced during glucose fermentation. Does not produce gas from glucose. Growth does not occur at 10 and 45°C or in the presence of 6.5% NaCl. Nutritionally fastidious. No or slight growth occurs in THB or on CBA. Sulfydryl compounds (0.01% L-cysteine is usually used) or vitamin B_6 (0.001% pyridoxal hydrochloride is usually used) is required for growth. Grows as satellite colonies adjacent to *Staphylococcus epidermidis* on blood agar. Alpha-hemolytic on sheep blood agar supplemented with 0.01% L-cysteine or 0.001% pyridoxal hydrochloride. PYR positive. Resistant to optochin and susceptible to vancomycin. L-Lysine is the diamino acid at position 3, and alanine or alanine-alanine is the peptide cross bridge, so the presumptive peptide type is type A3α. The G+C content of the DNA is 36.6 to 46.6 mol%.

The type species of the genus *Abiotrophia* is *Abiotrophia defectiva*.

Tests that are useful for distinguishing the genus *Abiotrophia* from other catalase-negative, gram-positive cocci are shown in Table 3.

Descriptions of *Abiotrophia adiacens* comb. nov. and *Abiotrophia defectiva* comb. nov. The descriptions of *A. adiacens* and *A. defectiva* are the same as the descriptions given for *Streptococcus adjacens* and *Streptococcus defectivus*, respectively (1).

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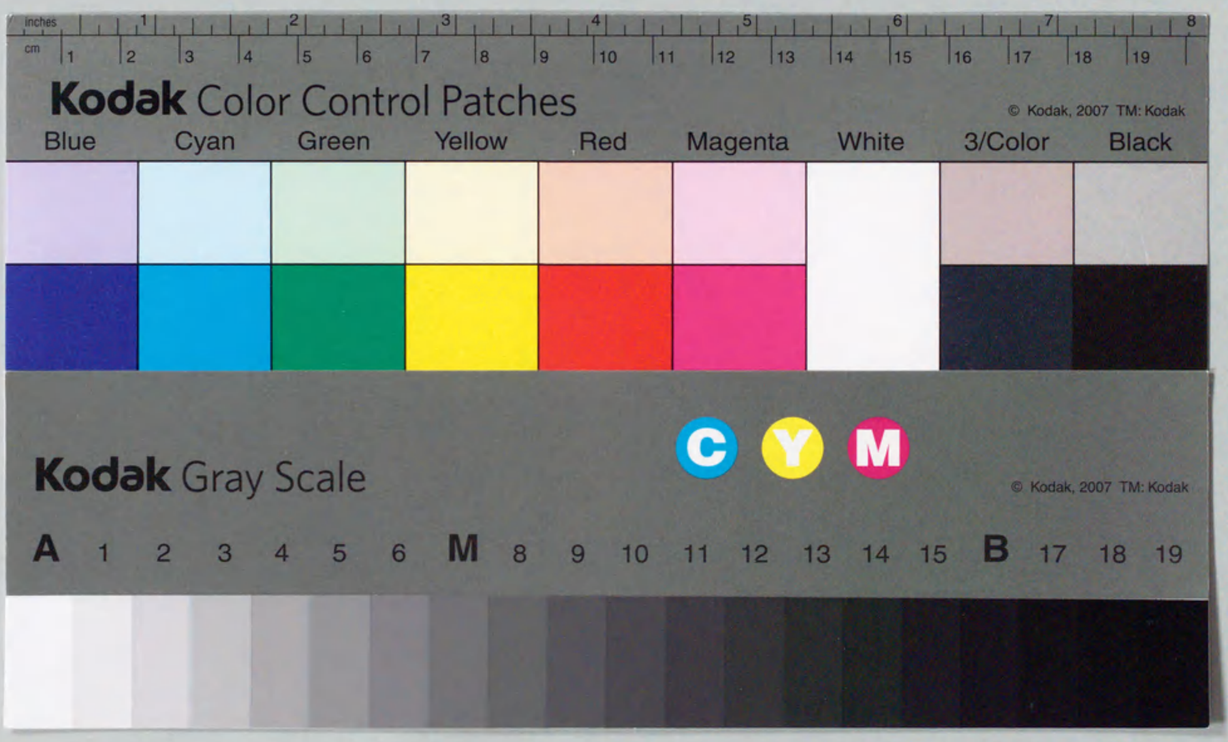
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