INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Oct. 1982, p. 410–415 Vol. 32, No. 4
0020-7713/82/040410-06$02.00/0
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Streptococcus gallinarum sp. nov. and *Streptococcus oralis* sp. nov.

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Received 22 February 1982/Accepted 2 September 1982

A numerical taxonomic survey of numerous strains of streptococci showed the presence of two clusters of strains that are distinct from the presently named species of *Streptococcus*. One group, from chicken intestines, is named *S. gallinarum* and the other, from the human mouth, is named *S. oralis*. The type strains are F87/276 (= PB21 = NCTC 11428) and LVG 1 (= PB182 = NCTC 11427), respectively.

As the result of a numerical taxonomic survey of 202 strains of streptococci and closely related organisms (P. D. Bridge, Ph.D. thesis, University of Leicester, 1981), two phenetic groups of strains were observed that were distinct from those representing known species (8) of *Streptococcus*. These groups were referred to as chicken group D and oral group II.

The chicken group D strains were isolated by Barnes et al. (2) while investigating the effects of dietary bacitracin on fecal streptococci in young chicks. These strains showed properties distinct from those of the established enterococci. They were detected only after a long incubation period (8 days) on a selective medium. The oral group II strains were studied by Carlsson (3) in a numerical taxonomy of streptococci isolated from the human oral cavity. Through the kindness of E. M. Barnes and J. Carlsson, strains were made available for study, and the evidence is presented here for considering these as two new species of *Streptococcus*. Certain previously named species of the genus do not appear on the Approved Lists of Bacterial Names (11), and these names are placed in quotation marks in this paper.

MATERIALS AND METHODS

Bacterial strains. The 17 strains of chicken group D and the 13 strains of oral group II are as follows: Chicken group D strains PB18 through PB34 are, respectively, the strains of Barnes et al. (2) numbered F87/273 through F87/279, F87/296, F87/307, F87/311, F87/344, F87/358, F87/361, F87/362, and F87/364 through F87/366. Oral group II strains PB177 through PB190 are, respectively, those of Carlsson (3) numbered KPE2, MVE1, KPE1, LPA1, MPA1, LVG1, OS51, LV51, NS51, OP51, NT61, PT51, and LV81.

Cultural determinants. Cultures were stored at −80°C on glass beads (5). Strains in the laboratory were grown on a basal medium of blood agar base no. 2 (Difco Laboratories) with the addition of 7.5% (vol/vol) horse blood (Difco) or on broth medium consisting of (in grams per liter): proteose peptone (Difco), 15; sodium chloride, 5; yeast extract (Difco), 5; and liver digest (Oxoid Ltd.), 2.5. Acid production from carbohydrates was observed in API 50E galleries (API System Ltd.) inoculated with API media and incubated at 35°C for 48 h. Standard methods were used for the following: Gram stain; hemolysis on horse blood agar; resistance to 60°C for 15 and 60 min; growth at 4, 10, and 45°C; growth at pH 9.6; growth with 3 and 6.5% (wt/vol) NaCl, 0.0004% (wt/vol) crystal violet, and 0.1% sodium azide; reduction of tellurite, tetrazolium, methylene blue in milk, and nitrite; hydrolysis of arginine, hippurate, and esculin; decarboxylation of lysine; final pH in glucose broth; dextran formation; and gelatin liquefaction (details for these, and for other tests used in the numerical taxonomy, are given by P. D. Bridge, Ph.D. thesis).

Growth on thallous acetate-tetrazolium agar was tested as described by Barnes (1) with the exception that Oxoid neutralized bacteriological peptone was used in place of Evans peptone; cultures were incubated at 35°C for 24 h and then at room temperature for 7 days.

DNA was extracted and purified by the method of Garvie (6) with minor adaptations. Melting point determinations were made in standard saline citrate buffer. The denaturation curves were followed with a Beckman model 35 spectrophotometer with a Stanton Redcroft linear temperature variable rate programmer and an Edale thermistor thermometer with a Grant Instruments temperature probe.

The numerical taxonomy of the 202 strains was based on 157 tests with the Gower coefficient and unweighted pair group method with averages (UPGMA) clustering (14). The test for distinctness of clusters was the *W* statistic (12); a computer program was used that utilizes the intercentroid distance and standard deviations along the intercentroid axis (13). The method permits a significance test to determine whether overlap is significantly less than that expected from arbitrary division of a continuous variation, referred to as a rectangular distribution (12).

RESULTS

The strains of chicken group D clustered as a separate group at the 90% similarity level (Fig. 1a). They were most closely linked to "*Streptococcus avium*" (10). They are allied to the classical enterococci *S. faecalis* and *S. faecium* but are not closely related to other species sometimes included among the enterococci, such as *S. bovis* and *S. equinus*. The chicken group D cluster is well separated from "*S. avium*." The disjunction coefficient *W* between the two clusters was 2.75, corresponding to a nominal overlap of less than 0.7%. Because of the small numbers of strains in the "*S. avium*" cluster, it is not possible to prove that this separation is statistically less than a rectangular one (12), for which *W* is close to 1.7 (or a little higher when numbers of strains are small), and the overlap is about 8% (or a little lower for small numbers of strains). The test results, however (Table 1), leave little doubt that the groups are distinct.

The strains of oral group II formed a cluster at the 84% similarity level (Fig. 1b). This was closest to "*Streptococcus milleri*" (7), but the *W* coefficient between the two was 6.24, corresponding to a nominal overlap of 10^{-9} and significantly less than that for a rectangular distribution.

The other species that were close were *S. mitis* and *S. sanguis*. The *W* value between *S. sanguis* and oral group II was 2.62, which indicates overlap of less than 0.9%, but again statistical significance against the rectangular distribution was not achieved because of the small number of strains of *S. sanguis*. The test results (Table 2) show, however, a sufficient number of differences to support their distinctness. The separation of oral group II from *S. mitis* (and from other oral groups such as *S. mutans* and *S. salivarius*), was highly significant.

The results of the physiological tests and DNA base ratios for the new species and those similar to them are given in Tables 1 and 2. The results for the growth of strains of chicken group D on thallous acetate-tetrazolium agar are shown in Table 3.

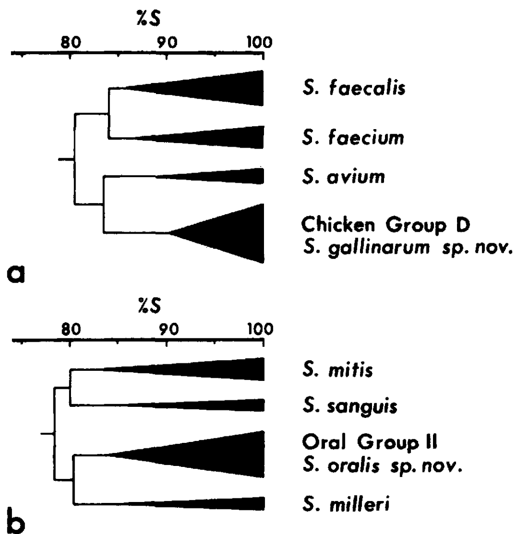

FIG. 1. Dendrograms showing the relations of the new species of *Streptococcus* to similar species, based on the Gower similarity coefficient and unweighted pair group method with averages (UPGMA) clustering. (a) Chicken group D; (b) Oral group II.

DISCUSSION

The distinctness of the two new species clusters (shown by Fig. 1 and the overlap statistics) is as good as that for traditional species of *Streptococcus*. Although uniquely defining characters are few or absent, this is also true for existing species (Tables 1, 2). Neither of them appears to correspond to a previously named species.

The chicken group D strains were noted by Barnes et al. (2) because of their slow growth at room temperature on thallous acetate-tetrazolium agar, on which they produce pink colonies (a property which we confirmed, Table 3). They were of serological group D but did not react with group Q antiserum, unlike "*Streptococcus avium*" (10). Barnes et al. (2) divided their isolates into three groups, A1, A2, and A3, based on arginine hydrolysis and inulin fermentation, but in our study there were no distinct subclusters that reflected these tests. Barnes et al. noted that cells of the strains tended to be more pointed and pleomorphic than those of *S. faecalis* and *S. faecium*.

Twenty-three tests used in this study of the chicken group D strains are comparable to those used by Barnes et al. (2). Table 4 shows a comparison of the results. The results of most tests are in good agreement. Barnes et al. (2) used tube methods for the production of acid from carbohydrates incubated for 7 days at 37°C, with bromcresol purple as pH indicator, which only responds to acidity below about pH 5.8. The results here are from API 50E galleries incubated at 37°C for 48 h, with phenol red as indicator. Although the incubation time was shorter, phenol red detects acidity at about pH 6.8, and it may be that the higher percentage of positive carbohydrate reactions in this study (Table 4) was due to detection of smaller amounts of acidity in the API galleries.

The discrepancy seen in the reduction of methylene blue milk may be accounted for by

TABLE 1. Test results for *S. gallinarum* and similar organisms[a]

Test	% Positive					
	S. faecalis (10 strains)	*S. faecium* (6 strains)	"*S. avium*" (4 strains)	*S. gallinarum* sp. nov. (17 strains)	*S. bovis* (4 strains)	*S. equinus* (4 strains)
Hemolysis[b]	40	83	100	100	50	25
Reduction of tellurite	100	50	50	18	0	0
Reduction of methylene blue	100	67	25	0	0	0
Arginine hydrolysis	100	100	29	0	0	0
Growth at 10°C	100	100	100	100	0	0
Growth at pH 9.6	100	100	100	100	0	0
Survival at 60°C for 15 min	100	100	100	75	75	100
Survival at 60°C for 1 h	100	100	25	0	75	75
Hippurate hydrolysis	90	0	0	12[c]	0	0
Growth with 6.5% NaCl	100	100	100	100	0	0
Growth at 4°C	10	100	0	6	0	0
Acid from glycerol	100	100	100	100	25	0
Acid from *d*-(−)-arabinose	40	0	100	6	0	0
Acid from *l*-(+)-arabinose	30	67	100	100	25	0
Acid from *d*-(+)-xylose	40	0	100	100	0	0
Acid from *l*-(−)-xylose	10	0	100	18	0	0
Acid from adonitol	0	17	100	0	0	0
Acid from methyl xyloside	0	0	0	6	0	0
Acid from *l*-(−)-sorbose	10	0	100	0	0	0
Acid from rhamnose	50	17	100	12	0	50
Acid from dulcitol	0	17	0	6	0	25
Acid from *meso*-inositol	70	0	0	12	0	25
Acid from mannitol	100	33	100	100	50	50
Acid from sorbitol	100	83	100	100	0	0
Acid from methyl-*d*-mannoside	20	0	50	23	0	0
Acid from methyl-*d*-glucoside	20	0	100	100	25	0
Acid from amygdalin	100	100	100	100	75	100
Acid from arbutin	10	100	100	100	75	100
Esculin hydrolysis[d]	100	100	100	100	75	100
Acid from lactose	100	100	100	100	100	0
Acid from *d*-(+)-melibiose	0	50	25	100	100	0
Acid from sucrose	80	67	50	100	100	50
Acid from *d*-(−)-trehalose	100	83	100	100	25	25
Acid from inulin	10	17	25	77	50	25
Acid from *d*-(+)-melezitose	80	0	100	59	0	50
Acid from *d*-(+)-raffinose	0	0	25	100	100	25
Acid from dextrin	90	50	75	100	75	25
Acid from amylose	0	0	0	12	0	25
Acid from starch	100	67	25	100	100	0
Acid from glycogen	10	0	0	6	75	0
Methyl red (API)[d]	80	100	100	94	100	75
DNase (API)[d]	10	0	0	0	0	25
Mucate[d]	10	0	0	12	25	0
Gluconate[d]	70	0	75	100	0	25
Lipase[d]	40	67	100	88	50	100
Tetrathionate reductase[d]	70	100	100	87[c]	100	100
Christensen citrate[d]	50	0	0	0	0	0
Malonate[c]	0	0	0	47	0	0
Mol% G+C[e]	38.4	39.0	39.9	35.6, 39.2	39.3	35.6

[a] All strains were positive for the following: growth at 45°C; esculin hydrolysis; final pH in glucose broth below 4.25; growth with 40% bile; acid from ribose, galactose, glucose, fructose, mannose, *N*-acetyl glucosamine, salicin, cellobiose, and maltose. All strains were negative for the following: gelatin liquefaction; acid from erythritol; attack on pectate (in API); and utilization of acetate (in API).

[b] Positive strains were all alpha-hemolytic.

[c] The reactions of the type strain are the majority reactions except for these tests.

[d] Tests (other than acidification of carbohydrates) performed in API galleries.

[e] Two strains of *S. gallinarum* (PB18, PB21) and one from each of the other species were examined.

TABLE 2. Test results for *S. oralis* and similar organisms

Test	% Positive			
	S. mitis (6 strains)	*S. sanguis* (3 strains)	*S. oralis* sp. nov. (13 strains)	"*S. milleri*" (3 strains)
Hemolysis	83	100	100	33
Nitrite reduction	0	67	92[c]	67
Growth with 3% NaCl	100	100	0	67
Reduction of methylene blue	100	0	8	67
Growth with sodium azide	33	0	54	100
Reduction of tetrazolium	67	0	100	67
Lysine decarboxylase	0	0	85	33
Survival at 60°C for 15 min	83	67	15	33
Growth with 0.0004% crystal violet	0	0	100	33
Production of dextran	17	100	31[c]	0
Acid from glycerol	0	100	54	0
Acid from erythritol	0	67	0	0
Acid from *d*-(−)-arabinose	0	0	8	0
Acid from *l*-(+)-arabinose	0	33	0	0
Acid from ribose	50	67	92	100
Acid from *l*-(−)-sorbose	0	33	0	0
Acid from rhamnose	17	0	8	0
Acid from *meso*-inositol	0	0	31	0
Acid from mannitol	17	0	38	0
Acid from sorbitol	17	0	23	0
Acid from methyl-*d*-mannoside	17	0	15	0
Acid from methyl-*d*-glucoside	50	33	38[c]	0
Acid from *N*-acetyl glucosamine	100	100	92[c]	100
Acid from amygdalin	0	67	15	0
Acid from arbutin	0	33	38	33
Esculin hydrolysis[d]	0	67	15	0
Acid from salicin	67	67	54[c]	67
Acid from *d*-(+)-cellobiose	33	67	31	33
Acid from lactose	67	100	100	100
Acid from *d*-(+)-melibiose	67	0	77[c]	33
Acid from sucrose	83	100	100	100
Acid from *d*-(−)-trehalose	17	100	77	100
Acid from inulin	17	100	31	0
Acid from *d*-(+)-melezitose	50	0	69	0
Acid from *d*-(+)-raffinose	67	0	61[c]	0
Acid from dextrin	100	67	77	0
Acid from starch	50	33	31	0
Acid from glycogen	0	33	23	0
Methyl red[d]	50	33	38	0
DNase[d]	17	0	0	0
Gluconate[d]	17	0	15	0
Lipase[d]	33	0	61[c]	67
Tetrathionate reductase[d]	17	33	92	100
Mol% G+C[e]	38.9	39.3	39.9, 40.0	34.1

[a] All strains were positive for the following: acid from galactose, glucose, fructose, mannose, and maltose. All strains were negative for the following: acid from *d*-(+)-xylose, *l*-(−)-xylose, adonitol, methyl-*d*-xyloside, dulcitol, amylose, and in the mucate, pectate, Christensen citrate, malonate and acetate tests in API.

[b] Positive strains were all alpha-hemolytic.

[c] The reactions of the type strain are the majority reactions except for these tests.

[d] Tests (other than acidification of carbohydrates) performed in API galleries.

[e] Two strains of *S. oralis* (PB179, PB183) and one from each of the other species were examined.

the different incubation times used. The difference in arginine hydrolysis may be due to the isomer used; the original method (9) and that in this study used the *d* isomer of arginine, but Barnes et al. (2) used the *l* isomer.

The difference in hippurate hydrolysis may be due in part to the limited number of strains tested by Barnes et al. (2). The resistance of these strains to 60°C is not high, although different times of testing prevent close comparison.

The reason for the difference in the type of hemolysis is not clear, but in our hands the

TABLE 3. Growth of four strains of *S. gallinarum* on thallous acetate-tetrazolium agar over 9 days

Growth after:	Strain			
	PB18	PB19	PB23	PB24
Day 1 (35°C)	Minimal growth	Minimal growth	Minimal growth	No growth
Days 2 through 4 (20°C)	Small pink colonies	Small pink colonies	Minimal growth	Small pink colonies
Days 5 through 9 (20°C)	Moderate-sized pink colonies	Moderate-sized pink colonies	Small pink colonies	Moderate-sized pink colonies

initial greening (alpha-hemolysis) at 16 h changed to clear zones after 48 h; possibly some constituent in our medium inhibited hemolysis.

The strains of oral group II were found by Carlsson (3) to fall into several of his clusters, principally I and V (other strains of Carlsson's groups II and III clustered in our study close to *S. salivarius*, as expected from his study). Carlsson considered that his group I corresponded to *Streptococcus sanguis* and that group V was similar to but not identical with *S. mitis* (although some were beta-hemolytic or possessed the Lancefield group G, K, or L antigens). Our findings confirm the closeness of oral group II, *S. sanguis*, and *S. mitis*, and in addition indicate that "*S. milleri*" is closely allied (Fig. 1b). However, they also suggest that oral group II is not identical to any of these species. (The type strain of *S. sanguis*, NCTC 7863, and the authentic strains *S. mitis* NCTC 10712 and "*S. milleri*" NCTC 10708 were present in their respective clusters.)

Facklam (4) characterized two biovars of *S. sanguis*. He suggested that strains of *S. sanguis* II (esculin negative, produce acid from raffinose) and certain other dextran-producing, raffinose-positive strains of "*S. mitior*" represented a new taxon. The oral group II strains are mainly esculin negative and raffinose positive and may partly correspond with *S. sanguis* II, although only 31% of strains were found to produce dextran.

The two new species are formally proposed as follows.

Streptococcus gallinarum sp. nov. (gall·in·ar'·um. L. fem. gen. pl. n. *gallinarum*, of hens).

Cells are gram-positive cocci in pairs or short chains, without capsules, and somewhat pleomorphic. They are nonmotile, nonsporing, aerobic and facultatively anaerobic, fermentative, and catalase-negative. Cells react with Lancefield group D antiserum. Grows slowly on thallous acetate-tetrazolium agar at room temperature, producing pink colonies. Hemolytic on horse blood agar, producing greening or complete lysis. *d*-Arginine is not hydrolyzed, and growth occurs at 45°C and pH 9.6; the final pH in glucose broth is below 4.25. Shows growth with 6.5% NaCl. Other test reactions are shown in Table 1. Similar to "*Streptococcus avium*" but differs in being adonitol-negative, *l*-(−)-sorbose negative, raffinose positive, often malonate-positive, and reacts only with group D antiserum. Mole percent G+C, 37.4. Found in intestines of domestic fowls. Type strain: F87/276 (= PB21 = NCTC 11428). The reactions of the type strain are the majority reactions for *S. gallinarum* given in Table 1, with the exceptions indicated by the footnote.

Streptococcus oralis sp. nov. (or·a'·lis. M. L. adj. *oralis*, of the mouth).

Cells are gram-positive cocci in short chains, without capsules. They are nonmotile, nonsporing, aerobic and facultatively anaerobic, fermentative, and catalase negative. Hemolytic on horse blood agar, producing pronounced green-

TABLE 4. Comparison of the results of this study with those of Barnes et al. (2)

Test	% Positive	
	Barnes et al. (37 strains)	This study (17 strains)
Hemolysis (type)	100 (beta)	100 (alpha)
Growth with tellurite	68	18
Arginine hydrolysis	54	0
Methylene blue reduction	100 (10 days)	0 (1 day)
Growth at 10°C	92	100
Growth at 45°C	98	100
Growth at pH 9.6	100	100
Hippurate hydrolysis	100[a]	12
Esculin hydrolysis	100	100
Final pH in glucose broth below 4.3	100	100
Growth with 6.5 NaCl	100	100
Gelatin liquefaction	0	0
Acid from lactose	38	100[b]
Acid from mannitol	54	100[b]
Acid from sorbitol	8	100[b]
Acid from *d*-(+)-arabinose	40	100[b]
Acid from raffinose	98	100[b]
Acid from inulin	54	77[b]
Acid from sucrose	100	100[b]
Acid from starch	0	100[b]

[a] Not all strains tested.
[b] Tests performed in API galleries.

ing (α-hemolytic). Reduces tetrazolium; grows with 0.0004% crystal violet; reduces tetrathionate. Other reactions are as in Table 2. Resembles *Streptococcus sanguis* but is usually esculin negative, raffinose positive, and does not grow with 3% NaCl. Mole percent G+C, 39.9. Found in the human mouth. The type strain is LVG 1 (= PB182 = NCTC 11427). The reactions of the type strain are the majority reactions for *S. oralis* given in Table 2, with the exceptions indicated by the footnote.

ACKNOWLEDGMENTS

This work was supported by a Research Training Award to P.D.B. from the Medical Research Council.

We are grateful to E. M. Barnes and J. Carlsson for the strains examined and to M. J. Sackin of this Department for assistance with computing.

LITERATURE CITED

1. **Barnes, E. M.** 1956. Methods for the isolation of faecal streptococci (Lancefield group D) from bacon factories. J. Appl. Bacteriol. **19**:193–203.
2. **Barnes, E. M., G. C. Mead, C. S. Impey, and B. W. Adams.** 1978. The effect of dietary bacitracin on the incidence of *Streptococcus faecalis* subsp. *liquefaciens* and related streptococci in the intestines of young chicks. Brit. Poultry Sci. **19**:713–723.
3. **Carlsson, J.** 1968. A numerical taxonomic study of human oral streptococci. Odontologisk Revy **19**:137–160.
4. **Facklam, R. R.** 1977. Physiological differentiation of viridans streptococci. J. Clin. Microbiol. **5**:184–201.
5. **Feltham, R. K. A., A. K. Power, P. A. Pell, and P. H. A. Sneath.** 1978. A simple method for storage of bacteria at −76°C. J. Appl. Bacteriol. **44**:313–316.
6. **Garvie, E. I.** 1976. Hybridization between the deoxyribonucleic acids of some strains of heterofermentative lactic acid bacteria. Int. J. Syst. Bacteriol. **26**:116–122.
7. **Guthof, O.** 1956. Ueber pathogene "vergrunende Streptokokken." Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **166**:553–564.
8. **Jones, D.** 1978. Composition and differentiation of the genus *Streptococcus*, p. 1–49. *In* F. A. Skinner and L. B. Quesnel (ed.), *Streptococci*. Academic Press Inc., London.
9. **Niven, C. F., K. L. Smiley, and J. M. Sherman.** 1942. The hydrolysis of arginine by streptococci. J. Bacteriol. **43**:651–660.
10. **Nowlan, S. S., and R. H. Deibel.** 1967. Group Q streptococci. I. Ecology, serology, physiology, and relationship to established enterococci. J. Bacteriol. **94**:291–296.
11. **Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.).** 1980. Approved lists of bacterial names. Int. J. Syst. Bacteriol. **30**:225–420.
12. **Sneath, P. H. A.** 1977. A method for testing the distinctness of clusters: a test of the disjunction of two clusters in Euclidean space as measured by their overlap. J. Math. Geol. **9**:123–143.
13. **Sneath, P. H. A.** 1979. BASIC program for a significance test for two clusters in Euclidean space as measured by their overlap. Computers & Geosciences **5**:143–155.
14. **Sneath, P. H. A., and R. R. Sokal.** 1973. Numerical taxonomy. W. H. Freeman and Co., San Francisco.