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Unidentified Streptococci from Plants

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The unidentified streptococci from plants are catalase negative, gram positive, usually lanceolate cells occurring in pairs and short chains. More than one-half of the 505 strains investigated conform generally to the description of *Streptococcus faecium*, but many deviate in one or more of Sherman's characteristics, growth on bile-esculin agar, and acidification of milk. A few strains superficially resemble *Streptococcus lactis*. The strains not termed *S. faecium*-like are heterogeneous in adherence to Sherman's characteristics, growth and pigmentation on tellurite and tetrazolium agars, and the heme peroxidase reaction; 35 fermentation patterns were established from the fermentations of arabinose, raffinose, melezitose, melibiose, mannitol, and sorbitol. The diversity in properties indicates that the cultural reactions do not permit definitive characterization or speciation. It is suggested that the *S. faecium*-like strains have provided the ancestral pool from which the species as now described has become adapted to life within the animal host.

The unidentified streptococci of plants and vegetables form a heterogeneous group which is second numerically and in frequency of distribution only to *Leuconostoc mesenteroides* (20). Some have been described as being *Streptococcus faecium*-like (21, 22) and sharing characteristics with *S. faecalis* (6). Splittstoesser et al. (29, 30) and White and White (33) isolated atypical streptococci from frozen vegetables. Atypical streptococci bearing the group D antigen have been isolated from feces of pigs (8, 9), feces and tonsils of pigs (13), canned hams (2), and humans (18). A characteristic feature of several studies is the highly variant pattern in the fermentations of selected carbohydrates (2, 9), which appears to be shared by members of *S. bovis* (16, 17, 25) and *S. lactis* (31).

The streptococci reported in this paper were isolated from plants and vegetables (20). They are usually lanceolate, but sometimes spherical, catalase-negative, homofermentative bacteria occurring in pairs and in short chains. They were studied and are reported as two groups based upon segregation at the time of isolation. One group is termed *S. faecium*-like, and the second group is other streptococci.

MATERIALS AND METHODS

Maintenance and propagation of cultures. All cultures have been maintained since isolation in 3% tryptic(ase) soy-16% glycerol-84% water broth at −18 C. Upon recall from storage, they were cultivated routinely in the basal medium of Mallmann and Seligmann (15), which was modified to exclude the sodium azide and to include 1 ml of 1.6% alcoholic solution of bromocresol purple (MJ medium) per liter. Inoculations of media were made from young broth cultures upon solid media with loops or needles and into liquid media as single drops from pipettes. Colonial characteristics and pigmentation on 2,3,5-triphenyltetrazolium chloride agar (1) were obtained by streaking for isolated colonies. The most reliable reactions on heme peroxidase medium (34) were obtained by making short, heavy streaks upon the medium with a loop.

Culture media. Most of the culture media used in this study have been described elsewhere (19-22). Bile-esculin and thallium acetate with added 2,3,5-triphenyltetrazolium chloride (3) agars were used. Heat resistance was determined by introducing 0.5 ml of overnight MJ broth culture into 6 ml of MJ medium, prewarming in a water bath at 45 C for 3 min, and then immersing the tubes in a water bath at 60 C for 31 min. Phenol red-meat extract (PR) broth was used as the basal medium to determine capabilities in the fermentations of sugars and the initiation of growth in salt and alkaline broths. Aerogenesis in glucose-4% malate broth was determined by sealing inoculated tubes with 1.5% plain agar overlaid with 5 ml of water (34). The pH was determined in PR-0.5% glucose broth at 48-h incubation.

RESULTS

Properties of all strains. Most (95%) of the strains examined produce both a strongly acidic reaction in MJ and PR-glucose broths and also a marked sediment and dense turbidity to within the uppermost 2 to 3 cm of liquid media. Very few strains have a pH outside the range 4.1 to 4.5 in PR-glucose broth. More than 95% of all strains ferment cellobiose, galactose, maltose, salicin, and trehalose. Nearly 70% produce a

sedimented, compact blue button in ethyl violet-azide broth. The remainder, except for 12 strains which fail to grow in the broth, produce a grainy or flocculent white sediment, or the growth remains suspended with no further change upon prolonged incubation.

Properties of S. faecium-like strains. The 130 strains of this group adhere generally to Sherman's criteria (28) for the enterococci (Table 1). Only two strains do not hydrolyze esculin, and three strains do not tolerate bile salts. The percentage of strains initiating growth at pH 9.6 compares favorably with Facklam's observation (7); however, his medium was adjusted before sterilization, whereas here the medium was adjusted with 20% K_3PO_4 after sterilization and maintained in tightly closed screw-capped tubes.

Many strains differ in one or more traits from the accepted descriptions of *S. faecium* (6, 7, 23) through the failure to deaminate arginine and reduce methylene blue in milk and hemolysis. The percentage of plant strains decarboxylating tyrosine, a variable property among strains of *S. faecium* (5), is low in comparison with the 57% of decarboxylating strains of *S. faecium* of animal origin (6). Many strains use citrate and produce acetyl-methyl-carbinol. These traits are shared with *S. cremoris* (32) and *S. faecalis*.

All but two strains ferment galactose in PR broth, 80% ferment lactose, sucrose, mannitol, and xylose, slightly more than one-half ferment arabinose and melibiose, slightly less than one-half ferment inulin, melezitose, sorbitol, and rhamnose, 39% ferment raffinose, and 22% ferment glycerol anaerobically. The fermentations of arabinose, melezitose, melibiose, lactose, and sorbitol by some strains were either weak or delayed, and with indicators other than phenol red the reactions of some strains may have been termed negative (2).

About one-half of the strains impart either a transient (1 to 2 days) or permanent reduction to litmus milk, 19% produce a reduced, acidic reaction with or without curd formation, and the remainder are inert (Table 2). The strains exhibiting a transient reduction do not ferment lactose, whereas those exhibiting a permanent reduction ferment lactose slowly, with questionable or no change in the color of the litmus. The reduced, acidic reaction with or without curd formation is produced by 87% of the strains in litmus milk supplemented with 0.5% glucose.

Other streptococci. The 375 strains of this group form 16 of 32 possible patterns according to the initiation of growth at 10 and 45 C, in salt and in alkaline broths, and the deamination of arginine (Table 3). Nearly one-half the strains, scattered among 13 types, grow on bile-esculin agar. Some strains hydrolyze esculin slowly to produce a delayed, light brown discoloration on day 3 or 4 of incubation. Among the bile-esculin negative strains, 67% hydrolyze esculin and do not grow on glucose medium containing bile salts.

The fermentations of arabinose, raffinose, melezitose, melibiose, mannitol, and sorbitol yield 35 fermentation patterns. The numbers of patterns associated with the 16 types are included in Table 3. The six sugars were fermented by 22% of the strains; 12 strains fermented none of these sugars; and the remainder ferment two to five sugars. Raibaud et al. (25) report that among strains of streptococci isolated from porcine feces sorbitol and mannitol were always fermented together, as were melibiose and raffinose. This relationship is not observed among the plant strains. The percentage of strains fermenting each sugar, and other properties ascertained for the streptococci, are recorded in Table 4.

The numbers of strains producing a reduced, acidic reaction or a reduced, acidic curd in litmus milk are distributed among 9 of the 16 types (Table 3). Among the negative strains (67%;

TABLE 1. *Reactions of 130 strains of streptococci resembling S. faecium*

Property tested	Positive (%)
Growth	
bile-esculin agar	98
at 10 C	97
at 45 C	78
in 6.5% NaCl broth	94
in broth (pH 9.6)	82
Deamination of arginine	27
Survival to heating (60 C, 30 min)	94
Reduction, methylene blue in milk	31
Decarboxylation of tyrosine	14
Greening hemolysis	29
Acetyl-methyl-carbinol produced	22
Citrate fermented	27
Gas in malate broth	83
Sodium hippurate hydrolyzed	5

TABLE 2. *Reactions in litmus and litmus-glucose milks by 130 strains resembling S. faecium*

Reaction	Litmus milk (%)	Litmus-glucose (%)
None	32	10
Reduction only	49	3
Reduced, acidic	11	16
Reduced, acidic curd	8	71

TABLE 3. *Cultural characteristics of unspeciated streptococci*

Type	No. of strains	Growth initiated					Deamination of arginine	Litmus milk	No. of fermentation patterns
		BE[a] agar	10 C	45 C	6.5% NaCl	pH 9.6			
A	98	84	+	+	+	+	+	70	13
B	33	15	+	+	+	+	−	9	9
C	75	37	+	−	+	+	−	12	20
D	37	12	+	−	+	−	−	6	11
E	17	2	+	−	−	+	+	11	7
F	14	0	+	−	−	−	+	0	10
G	29	20	+	−	−	+	−	7	9
H	29	4	+	−	−	−	−	1	12
I	4	1	−	+	−	−	−	0	3
J	8	1	−	−	−	−	−	2	6
K	6	4	−	−	−	−	+	0	4
L	5	2	−	−	−	+	+	0	3
M	5	2	+	−	+	−	+	4	4
N	9	1	+	+	−	+	+	0	4
O	4	0	−	+	+	−	−	0	2
P	2	0	+	+	+	−	−	0	2
Percentage		49	93	40	67	71	41	33	

[a] BE, Bile-esculin.

TABLE 4. *Properties of unidentified streptococci*

Property tested[a]	Positive (%)
Fermentation of	
Arabinose	65
Raffinose	62
Melezitose	39
Melibiose	72
Mannitol	78
Sorbitol	76
Lactose	58
Acid or acid curd in litmus	33
MBM reduced	54
Growth on KT agar	53
Reduction of TTC	
Pink	19
S. faecalis-like	9
Tiny, intensely red	9
Heme peroxidase	53
Survival (60 C, 30 min)	66
Growth on m-TAC agar	74
Gas in glucose-malate	99
Decarboxylation of tyrosine	21

[a] MBM, Methylene blue in milk; TTC, 2,3,5-triphenyltetrazolium chloride; m-TAC, thallium acetate with added TTC.

Table 4), 88% produce the reactions in litmus milk containing 0.5% glucose. Reduction in 0.1% methylene blue in milk is independent of the ability to produce reactions in litmus milk.

Some strains produce *S. faecalis*-like and some produce tiny, intensely red, colonies on 2,3,5-triphenyltetrazolium chloride and thallium acetate with added 2,3,5-triphenyltetrazolium chloride agars. Similar variation has been reported among strains of *S. lactis* (4). The thallium acetate (3) and citrate (14), upon which 74% of the strains grow, presumably restrict the growth of *S. lactis*. Growth of the strains on agar containing 0.04% potassium tellurite ranges from vigorous, with intensely black colonies, to that which is barely visible and hazy gray, with no point of demarcation.

Strains among all types of the plant streptococci exhibit the heme peroxidase reaction and survive heating to 60 C for 30 min. Among enterococci, survival upon heating is a borderline property, with only a few cells surviving in an inoculum of millions (27).

DISCUSSION

The plant streptococci share in common with the enterococci and the group N streptococci their morphology, the fermentation of cellobiose, maltose, salicin, trehalose, and sucrose, their strongly fermentative ability, and growth in ethyl violet-azide broth. Divided according to Sherman's criteria, some resemble *S. faecium* and some remotely resemble *S. lactis*. Although the guanine plus cytosine content of strains selected from among the second group of streptococci is in the same range as that of the streptococci, the deoxyribonucleic acid/deoxyribonucleic acid homologies indicate only a distant relationship to *S. faecalis*, *S. faecium*, and *S. lactis* (26). The diversity in properties does not permit a definitive speciation of the many strains.

Bile tolerance, essential to survival of streptococci adapted to residence in the human and animal intestinal tracts, is useful in the recognition of strains of clinical (7, 12) and fecal (24) origins. The property is common among the plant streptococci, some of which have little cultural resemblance to the enterococci. Although not peculiar to any cultural group, the property is found most frequently among those strains which closely resemble *S. faecium*.

Thus, the plant streptococci may have served as the ancestral pool from which strains adapted to more restrictive environments, as suggested by Stark and Sherman (31) and Hirsch (11). Alternatively, bile tolerance among streptococci isolated from plants may reflect a continual shedding of streptococci from animal sources, and it may involve the distribution of bile tolerance to various strains by the several mechanisms of exchange of genetic material.

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REPRINT REQUESTS

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LITERATURE CITED

1. Barnes, E. M. 1956. Tetrazolium reduction as a means of differentiating *Streptococcus faecalis* from *Streptococcus faecium*. J. Gen. Microbiol. **14**:57-68.
2. Barnes, E. M., and M. Ingram. 1955. The identity and origin of faecal streptococci in canned hams. Ann. Inst. Pasteur Lille **7**:115-119.
3. Bulich, A. A., and P. A. Hartman. 1969. Evaluation of thallium acetate-citrate medium for isolation of enterococci. Appl. Microbiol. **18**:944-945.
4. Cavett, J. J., G. J. Dring, and A. W. Knight. 1965. Bacterial spoilage of thawed frozen peas. J. Appl. Bacteriol. **28**:241-251.
5. Deibel, R. H. 1964. The group D streptococci. Bacteriol. Rev. **28**:330-366.
6. Deibel, R. H., D. E. Lake, and C. F. Niven, Jr. 1963. Physiology of the enterococci as related to their taxonomy. J. Bacteriol. **86**:1275-1282.
7. Facklam, R. R. 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. Appl. Microbial. **23**:1131-1139.
8. Fewins, B. G., L. G. M. Newland, and C. A. E. Briggs. 1957. The normal intestinal flora of the pig. II. Qualitative studies of lactobacilli and streptococci. J. Appl. Bacteriol. **20**:234-242.
9. Fuller, R., L. M. Newland, C. A. E. Briggs, R. Braude, and R. Mitchell. 1960. The normal intestinal flora of the pig. IV. The effect of dietary supplements of penicillin, chlortetracycline or copper sulfate on the faecal flora. J. Appl. Bacteriol. **23**:195-205.
10. Gledhill, W. C., and L. E. Casida. 1969. Predominant catalase-negative soil bacteria. Appl. Microbiol. **17**:208-213.
11. Hirsch, A. 1952. The evolution of the lactic streptococci. J. Dairy Res. **18**:290-293.
12. Isenberg, H. D., D. Goldberg, and J. Sampson. 1970. Laboratory studies with a selective enterococcus medium. Appl. Microbiol. **20**:433-436.
13. Koehler, J., and H. Mochmann. 1958. Streptokokken der Tonsillen und Antistreptolysintiter bei Schlachtschweinen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Abt. 1 Orig. **173**:58-65.
14. McDonald, I. J. 1957. Effect of acetate, citrate, and divalent metal ions on utilization of sodium caseinate by lactic streptococci. Can. J. Microbiol. **3**:411-417.
15. Mallmann, W. L., and E. B. Seligmann, Jr. 1950. A comparative study of media for the detection of streptococci in water and sewage. Am. J. Public Health **40**:286-289.
16. Medrek, T. F., and E. M. Barnes. 1962. The distribution of Group D streptococci in cattle and sheep. J. Appl. Bacteriol. **25**:159-168.
17. Mieth, H. 1960. Untersuchungen ueber das Vorkommen von Enterokokken bei Tieren und Menschen. I. Ihr Vorkommen im Darm von gesunden Schlachtschweinen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Abt. 1 Orig. **179**:456-482.
18. Mieth, H. 1961. Untersuchungen ueber das Vorkommen von Enterokokken bei Tieren und Menschen. II. Ihr Vorkommen in Stuhlproben von gesunden Menschen. Zentralkl. Bakteriol. Parasitenkd. Infektionskr. Abt. 1 Orig. **183**:68-89.
19. Mundt, J. O. 1973. Litmus milk reaction as a distinguishing feature between *Streptococcus faecalis* of human and nonhuman origins. J. Milk Food Technol. **36**:364-367.
20. Mundt, J. O., W. F. Graham, and I. E. McCarty. 1967. Spherical lactic acid-producing bacteria of southern grown raw and processed vegetables. Appl. Microbiol. **15**:1303-1308.
21. Mundt, J. O., and A H. Johnson. 1959. Physiological properties of Group D streptococci isolated from plants. Fcod Res. **24**:218-223.
22. Mundt, J. O., A. H. Johnson, and R. Khatchikian. 1958. Incidence and nature of enterococci on plant materials. Food Res. **23**:186-193.
23. Orla-Jensen, S. 1919. The lactic acid bacteria. Andr. Fred. Host and Son, Copenhagen.
24. Pavlova, M. T., F. T. Brezenski, and W. Litsky. 1972. Evaluation of various media for isolation, enumeration, and identification of fecal streptococci from natural sources. Health Lab. Sci. **9**:289-298.
25. Raibaud, P., M. Gaulet, J. V. Galpin, and J. Mocquot. 1961. Studies on the bacteria flora of the alimentary tract of pigs. II. Streptococci: selective enumeration and differentiation of the dominant group. J. Appl. Bacteriol. **24**:285-306.
26. Roop, D. R., J. O. Mundt, and W. S. Riggsby. 1974. Deoxyribonucleic acid hybridization studies among some strains of group D and group N streptococci. Int. J. Syst. Bacteriol. **24**:330-337.
27. Shannon, E. L., G. W. Reinbold, and W. S. Clark. 1970. Heat resistance of enterococci. J. Milk Food Technol. **33**:192-196.
28. Sherman, J. M. 1937. The streptococci. Bacteriol. Rev. **1**:1-97.
29. Splittstoesser, D. F., and I. Gadjo. 1966. The groups of microorganisms composing the "total" count population in frozen vegetables. J. Food Sci. **31**:234-239.
30. Splittstoesser, D. F., R. Wright, and G. J. Hucker. 1961. Studies on media for enumerating enterococci in frozen vegetables. Appl. Microbiol. **9**:303-308.
31. Stark, P., and J. M. Sherman. 1935. Concerning the habitat of *Streptococcus lactis*. J. Bacteriol. **30**:639-646.
32. Swartling, P. F. 1951. Biochemical and serological properties of some citric acid fermenting streptococci from

milk and milk products. J. Dairy Res. **18**:256–267.
33. White, A., and H. R. White. 1962. Some aspects of the microbiology of frozen peas. J. Appl. Bacteriol. **25**:62–71.
34. Whittenbury, R. 1964. Hydrogen peroxide formation and catalase activity in the lactic acid bacteria. J. Gen. Microbiol. **35**:13–26.
35. Whittenbury, R. 1965. The differentiation of *Streptococcus faecalis* and *S. faecium*. J. Gen. Microbiol. **38**:279–287.