INFECTION AND IMMUNITY, July 1974, p. 216-219

Vol. 10, No. 1
Printed in U.S.A.

Streptococcus mutans in a Wild, Sucrose-Eating Rat Population

ALAN L. COYKENDALL, PATRICIA A. SPECHT, AND HARRY H. SAMOL

Veterans Administration Hospital, Dental Research, Newington, Connecticut 06111 and Florida Sugar Cane League Inc., Clewiston, Florida 33440

Received for publication 21 March 1974

Streptococcus mutans, an organism implicated in dental caries and not previously found outside of man and certain laboratory animals, was isolated from the mouths of wild rats which ate sugar cane. The strains isolated fermented mannitol and sorbitol, and failed to grow in 6.5% NaCl or at 45 C. They formed in vitro plaques on nichrome wires when grown in sucrose broth. They also stored intracellular polysaccharide which could be catabolized by washed, resting cells. Deoxyribonucleic acid-deoxyribonucleic acid reassociations revealed two genetic types. One type shared extensive deoxyribonucleic acid base sequences with *S. mutans* strains HS6 and OMZ61, two members of a genetic type found in man and laboratory hamsters. The other type seemed unrelated to any *S. mutans* genetic type previously encountered. It is concluded that the ecological triad of tooth-sucrose-*S. mutans* is not a phenomenon unique to man and experimental animals.

The presence of *Streptococcus mutans* (3, 7, 14) on human teeth has been correlated with decay (caries) of those teeth (8, 16, 21, 26, 27), and the organism has been proven cariogenic for experimental animals on sucrose diets (9-11, 20, 30). The organism's preference for sucrose has been related to its production of glucans from this substrate (15, 19). These glucans have adhesive properties which seem to aid the organism's colonization and survival on tooth surfaces (12). Although *S. mutans* appeared to be indigenous to some experimental animal colonies (9, 10, 19), it has been suggested that they acquired their infection from man (20).

We reasoned that, if a natural, non-human source of *S. mutans* existed, the most likely place where it could be found would be an ecosystem involving a mammal on a high-sucrose diet. A search for such a system led to Florida, where sugar cane is extensively cultivated. Several species of rats inhabit these cane fields, where they feed on the sucrose-rich cane and cause considerable damage (25). We report here the isolation of *S. mutans* from these rats and conclude that the mouth-sucrose-*S. mutans* ecosystem is not unique to man and laboratory animals.

MATERIALS AND METHODS

Sampling procedures. Fourteen rats (thirteen roof rats, *Rattus rattus*, and one cotton rat, *Sigmodon hispidis*) were trapped in a cane field and killed with sodium pentobarbital, their teeth were scraped with a small dental instrument, and their oral soft tissues were swabbed with a cotton-tipped stick. The scraping and the swab were put into separate vials of a transport medium (24). Because experimental animals can transmit *S. mutans* through feces (10, 19), fecal samples were obtained from three animals, and five stools were collected from the field.

All samples were processed within 3 days of collection. Material was dispersed by brief sonic treatment, and a sample was streaked onto plates of Mitis-salivarius agar (M-S) and onto the same agar containing 0.2 U of bacitracin per ml (M-S+B) and 15% extra sucrose (13). Appropriately diluted samples were spread onto additional plates of M-S and M-S+B, as well as lactobacillus agar (LBS; BBL). All plates were incubated at 37 C in an atmosphere of 10% CO_2-90% N_2 for 20 h. Colonies of all types were picked and characterized, but particular attention was paid to colonies resembling those of *S. mutans* (18).

Characterization of isolates. Cultures derived from picked colonies were gram-stained, restreaked on M-S agar, and tested for hemolysis on Trypticase soy agar (BBL) with 10% sheep blood. Fermentations of mannitol, sorbitol, raffinose, and trehalose were tested by inoculating cystine-Trypticase agar (BBL) with one drop of a fresh 18-h culture. Production of ammonia from arginine was determined by the method of Niven et al. (23). Growth in sucrose was tested by using Jordan broth (17) with 5% sucrose. Growth in phenol red broth containing 6.5% NaCl and 1% glucose was used to test salt tolerance. Growth at 45 C was tested by using Jordan broth with 1% glucose.

In vitro plaque formation. The ability of strains to produce adherent "plaques" in vitro was determined by the nichrome wire method of McCabe et al. (22), except that it was not always necessary to reinoculate the fresh broth when the wires were transferred. Plaques were allowed to develop for 3 days (two transfers) and were then transferred again, at which time they were photographed. After 2 more days, without transfer, the tubes were vortexed to judge the tenacity of the plaques.

Production of IPS. Cells grown in 2% glucose broth were assayed for intracellular iodophilic polysaccharide (IPS) by absorption of light at 565 nm in 0.2 I_2-2% KI, as described by Van Houte and Jansen (28). To estimate acid produced from IPS, early stationary-phase cells from 100-ml 2% glucose cultures were washed with ice-cold 50 mM KCl-1 mM phosphate buffer at pH 4.1 and then resuspended in 15 ml of this buffer. The temperature and pH were raised to 37 C and 7.0, respectively. Acid produced by the cells under these conditions was titrated continuously with 0.1 M NaOH by using an automatic titrator (Radiometer, Copenhagen). Cells were allowed to catabolize until exhausted. IPS was determined (as above) on samples taken at the start and end of these experiments. Cells grown in 0.1% glucose served as controls.

DNA base sequence homology. Deoxyribonucleic acid (DNA) was isolated as previously described (4). DNA-DNA hybrids were allowed to form on membrane filters (6) incubated in 3 × SSC (0.45 M NaCl-0.045 M sodium citrate) at 67 or 73 C (5). Labeled DNA was isolated from cells grown with [*methyl*-^{3}H]thymidine (New England Nuclear, Boston) and was sheared by brief sonic treatment.

Bacterial strains used as controls. In many experiments, well-characterized strains of *S. mutans* were used as controls: 10449, FA1, SL1, and HS6 (genetic groups I, II, III, and IV [5; A. L. Coykendall, in press]; serological groups *c*, *b*, *d*, and *a* [1], respectively). A strain of *Streptococcus faecium*, F24, was used as a positive control for the salt tolerance test and for growth at 45 C.

RESULTS

Organisms identical to *S. mutans* were isolated from seven rats. These strains were gram-positive, nonhemolytic cocci in chains, which formed hard, adherent colonies on M-S agar. They fermented mannitol and sorbitol and produced adherent, flocculent growth in 5% sucrose broth. None grew at 45 C or in 6.5% NaCl, or produced ammonia from arginine. Of 19 strains isolated, 7 strains failed to ferment raffinose and 1 strain did not ferment sorbitol. The raffinose-negative strains produced colonies on M-S agar which were darker than the frosty-glass color of the raffinose-positive strains, and lacked the characteristic "puddle" of liquid which usually occurs around *S. mutans* colonies on M-S agar (18). One strain, 5T1, fermented raffinose but produced a colony resembling the raffinose-negative strains. The raffinose- and sorbitol-negative strains and 5T1 did not grow on M-S+B; all others did (Table 1). In Jordan broth (17) with 1% glucose, at an original pH of 7.3, these bacteria produced acid to give the following pH values: raffinose-positive strains, 4.0 to 4.2; raffinose-negative strains, 4.2 to 4.5; and strain 5T1, 5.0.

Bacteria resembling *Streptococcus salivarius* were isolated from 11 animals. These produced large, gelatinous colonies on M-S agar, fermented raffinose, failed to ferment mannitol or sorbitol, and did not hydrolyze arginine. Colonies of gram-positive rods on LBS agar were considered species of *Lactobacillus* and were seen in cultures from 12 rats. None of the 28 streptococcal isolates from the 14 rats produced ammonia from arginine. This implies an absence of *Streptococcus sanguis* which, in humans, is one of the predominant oral streptococci (2). This organism produces glucans from sucrose but is otherwise quite distinct from *S. mutans* and has not been implicated in caries. No colonies resembling *S. mutans* or *S. salivarius* were observed on plates streaked with fecal material.

The plaque-forming ability of 13 strains from the cane-eating rats was compared with 4 well-known *S. mutans* strains (10449, FA1, HS6, and SL1). After 3 days (two transfers) all strains produced abundant plaques (Fig. 1). No adherent plaques were formed in glucose broth. These plaques were of sufficient tenacity to resist significant dislodgement by vortexing.

The storage of IPS is a characteristic of most *S. mutans* strains (M. L. Freedman and A. L. Coykendall, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1974, p. 177, P 196; J. D. de Stoppelaar, Ph.D. thesis, University of Utrecht,

TABLE 1. *Biochemical characteristics of S. mutans strains isolated from cane-rats*[a]

Strain	Mannitol	Sorbitol	Raffinose	M-S+B + sucrose	pH
1S2, 1S6, 1S7, 3T3, 3S1, 10T2, 10S1, 10S2, 10S3	+	+	+	+	4.0–4.2
4S1, 8S1, 8S2, 11S1, 11S3, 11S4	+	+	–	–	4.2–4.5
5T1	+	+	+	–	5.0
11S2	+	–	+	–	4.0

[a] Abbreviations: M-S+B plus sucrose, Mitis-salivarius agar plus 0.2 U of bacitracin per ml and 20% sucrose; +, acid production or growth; –, no acid production or no growth; pH, final pH in 1% glucose broth. Strain designations containing a T were derived from tooth scrapings; S from the swab (which often contacted the teeth).

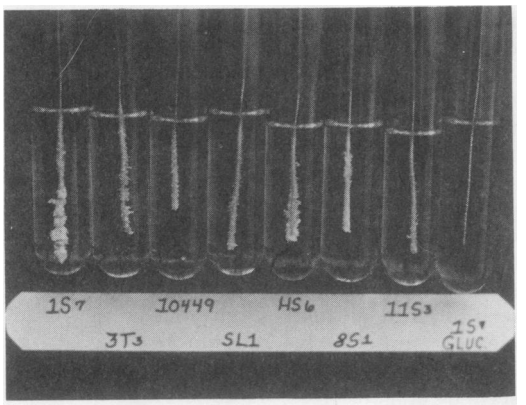

FIG. 1. *In vitro plaque formation by strains of Streptococcus mutans grown in sucrose broth. Thirteen strains (four of which are shown) isolated from cane-eating rats produced adherent masses comparable to the human and laboratory animal strains (10449, HS6, and SL1). These plaques do not form if the cells are given only glucose as the energy source (1S7-GLUC).*

Utrecht, The Netherlands, 1971). Five cane-rat strains were tested for IPS production. All five strains stored IPS and produced acid from it in amounts comparable to other strains of *S. mutans*. Acid production was accompanied by a decrease in 565-nm-absorbing material.

The results of DNA reassociations with 10 cane-rat strains showed that the strains isolated from the rats formed 2 genetic groups (Table 2). Those which did not ferment raffinose (plus strain 5T1) formed a group with considerable common base sequences. The remaining strains shared few base sequences with these raffinose nonfermenters. The reciprocal experiment confirmed the existence of two groups (Table 2). These experiments showed little or no relationship between the cane-rat strains and strain 10449 (genetic group I) or strain SL1 (genetic group III). In subsequent experiments, strain FA1 (genetic group II) also failed to show base sequence similarities with the cane-rat strains (Table 2). However, the representative of the raffinose-fermenting group (strain 1S7) was almost genetically identical to the representative of genetic group IV (strain HS6; Table 2). Hybrid duplexes between 1S7 DNA and HS6 DNA continued to form at the more stringent incubation temperature of 73 C. Under these conditions 1S7 DNA bound 79% to HS6 DNA, 80% to 10S2 DNA, and 72% to OMZ61 DNA. *E. coli* DNA, as a negative control, bound 4%. (Group IV strains are very homologous and include hamster isolates, such as HS6, and human isolates from the United States and Europe, such as OMZ61.) The only difference between the raffinose-fermenters and group IV strains is their growth on M-S+B. We have found that group IV strains fail to grow on this medium. The group of raffinose nonfermenters (and strain 5T1) appeared to be a genetically distinct group not yet found in humans.

DISCUSSION

The isolation, from these rats, of bacteria so similar to human and laboratory animal strains of *S. mutans* leads us to conclude that *S. mutans* is not unique to man and laboratory animals. Unfortunately, this rat population could not provide control animals, i.e., similar rats not eating sucrose. Thus, we cannot unequivocally state that sucrose was essential for the presence of *S. mutans* in these animals. But such a statement would be logical in view of the sucrose-*S. mutans* relationship seen in man and experimental animals (12).

These cane rats probably do not exchange their organisms with humans because they are

TABLE 2. *DNA homologies among 10 cane-rat S. mutans strains, plus strains SL1, 10449, HS6, and FA1*

Strain	Relative binding (%)	
	With 8S1 [^{3}H]DNA	With 1S7 [^{3}H]DNA
Expt 1		
8S1	100 (49.0)[a]	20.8
4S1	87.1	21.8
8S2	86.5	15.9
11S1	75.5	24.0
11S3	84.7	22.6
5T1	84.3	21.2
1S7	27.3	100 (60.1)
3S1	21.1	93.6
10S2	20.8	90.6
11S2	20.2	84.0
10449 (I)[b]	28.5	19.1
SL1 (III)	14.0	41.5
Expt 2		
8S1	100 (77.8)	33.8
11S1	92.3	ND[c]
1S7	33.3	100 (61.2)
10S2	ND	104.0
HS6 (IV)	35.0	88.7
SL1 (III)	32.1	44.9
FA1 (II)	37.2	27.5

[a] Number in parenthesis is the actual percentage of [^{3}H]DNA bound in the homologous reaction compared to total input [^{3}H]DNA.

[b] Roman numerals designate genetic groups.

[c] ND, Not determined.

isolated from most human contact by the vastness of the cane fields, which extend for several kilometers, and by the lattice of canals which drain the area. Nevertheless, the discovery of strains genetically related to human isolates suggests the possibility of animal-man exchange of *S. mutans* under appropriate conditions.

The presence of strains of a distinct genetic type, not yet found in man, implies the independent evolution of an organism phenotypically indistinguishable from *S. mutans*. In fact, the genetic disparity between the two cane-rat groups is no greater than the disparity among the genetic groups of *S. mutans* previously described (5; A. L. Coykendall, J. Gen. Microbiol., in press). What we now call *S. mutans* may actually represent a phenon containing several organisms which have evolved independently in several human and animal populations on a sucrose diet.

ACKNOWLEDGMENT

We appreciate the assistance of Marianne Stolezki in the preparation of the manuscript.

LITERATURE CITED

1. Bratthall, D. 1970. Demonstration of five serological groups of streptococcal strains resembling *Streptococcus mutans*. Odontol. Revy **21**:143-152.
2. Carlsson, J. 1965. Zooglea-forming streptococci, resembling *Streptococcus sanguis*, isolated from dental plaque in man. Odontol. Revy **16**:348-358.
3. Clarke, J. K. 1924. On the bacterial aetiology of dental caries. Brit. J. Exp. Pathol. **5**:141-147.
4. Coykendall, A. L. 1970. Base composition of deoxyribonucleic acid isolated from cariogenic streptococci. Arch. Oral Biol. **15**:365-368.
5. Coykendall, A. L. 1971. Genetic heterogeneity in *Streptococcus mutans*. J. Bacteriol. **106**:192-196.
6. Denhardt, D. T. 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641-646.
7. Edwardsson, S. 1968. Characteristics of caries-inducing human streptococci resembling *Streptococcus mutans*. Arch. Oral Biol. **13**:637-646.
8. Englander, H. R., and H. V. Jordan. 1972. Relation between *Streptococcus mutans* and smooth surface caries in the deciduous dentition. J. Dent. Res. **51**:1505.
9. Fitzgerald, R. J., H. V. Jordan, and H. R. Stanley. 1960. Experimental caries and gingival pathologic changes in the gnotobiotic rat. J. Dent. Res. **39**:923-935.
10. Fitzgerald, R. J., and P. H. Keyes. 1960. Demonstration of the etiologic role of streptococci in experimental caries in the hamster. J. Amer. Dent. Ass. **61**:9-19.
11. Gibbons, R. J., K. S. Berman, D. Knoettner, and B. Kapsimalis. 1966. Dental caries and alveolar bone loss in gnotobiotic rats infected with capsule forming streptococci of human origin. Arch. Oral Biol. **11**:549-560.
12. Gibbons, R. J., and J. Van Houte. 1973. On the formation of dental plaques. J. Periodontol. **44**:347-360.
13. Gold, O. G., H. V. Jordan, and J. Van Houte. 1973. A selective medium for *Streptococcus mutans*. Arch. Oral Biol. **18**:1357-1364.
14. Guggenheim, B. 1968. Streptococci of dental plaques. Caries Res. **2**:147-163.
15. Guggenheim, B., and H. E. Schroeder. 1967. Biochemical and morphological aspects of extracellular polysaccharides produced by cariogenic streptococci. Helv. Odontol. Acta **11**:131-152.
16. Hoerman, K. C., H. J. Keene, I. L. Shklair, and J. A. Burmeister. 1972. The association of *Streptococcus mutans* with early carious lesions in human teeth. J. Amer. Dent. Ass. **85**:1349-1352.
17. Jordan, H. V., R. J. Fitzgerald, and A. E. Bowler. 1960. Inhibition of experimental caries by sodium metabisulfite and its effect on the growth of selected bacteria. J. Dent. Res. **39**:116-123.
18. Jordan, H. V., B. Krasse, and A. Möller. 1968. A method of sampling human dental plaques for certain "caries-inducing" streptococci. Arch. Oral Biol. **13**:919-927.
19. Keyes, P. H. 1960. The infectious and transmissible nature of experimental dental caries. Arch. Oral Biol. **1**:304-320.
20. Krasse, B. 1966. Human streptococci and experimental caries in hamsters. Arch. Oral Biol. **11**:429-436.
21. Krasse, B., H. V. Jordan, I. Svensson, and L. Trell. 1968. The occurrence of certain "caries-inducing" streptococci in human dental plaque material. Arch. Oral Biol. **13**:911-918.
22. McCabe, R. M., P. H. Keyes, and A. Howell, Jr. 1967. An *in vitro* method for assessing the plaque forming ability of oral bacteria. Arch. Oral Biol. **12**:1653-1656.
23. Niven, C. F., K. L. Smiley, and J. M. Sherman. 1942. The hydrolysis of arginine by streptococci. J. Bacteriol. **43**:651-660.
24. Rundell, B. B., L. A. Thomson, W. J. Loesche, and H. M. Stiles. 1973. Evaluation of a new transport medium for the preservation of oral streptococci. Arch. Oral Biol. **18**:871-878.
25. Samol, H. H. 1972. Rat damage and control in the Florida sugar cane industry, p. 503-506. Proc. 14th Cong. Int. Soc. Sugar Cane Technol. Franklin Press Inc., Baton Rouge.
26. Shklair, I. L., H. J. Keene, and L. G. Simonson. 1972. Distribution and frequency of *Streptococcus mutans* in caries active individuals. J. Dent. Res. **51**:882.
27. Stoppelaar, J. D. de, J. Van Houte, and O. Backer Dirks. 1969. Relationship between extracellular polysaccharide-producing streptococci and smooth surface caries in 13-year-old children. Caries Res. **3**:190-199.
28. Van Houte, J., and H. M. Jansen. 1968. The iodophilic polysaccharide synthesized by *Streptococcus salivarius*. Caries Res. **2**:47-56.
29. Wood, J. M. 1967. A dextransucrase from streptococcus FA1. Arch. Oral Biol. **12**:1659-1660.
30. Zinner, D. D., J. M. Jabon, A. D. Aran, and M. S. Saslaw. 1965. Experimental caries induced in animals by streptococci of human origin. Proc. Soc. Exp. Biol. Med. **118**:766-770.