Replacement of the Type Strain of *Selenomonas sputigena* under Rule 18g

Request for an Opinion

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The type strain of *Selenomonas sputigena* (Flügge) Boskamp 1922, 70, the type species of the genus *Selenomonas*, is strain VPI 10068 (= ATCC 33150). This strain was a reference strain that was arbitrarily designated as the type strain on the 1980 Approved Lists of Bacterial Names before adequate documentation of its characteristics. It is not an original strain of the author of the species; subsequent examination has indicated that it almost certainly is not representative of the species described by Boskamp or by Flügge ($P = <0.006$), nor is it representative of strains that are widely recognized as *S. sputigena*. We propose replacement of strain ATCC 33150 with strain ATCC 35185 as the type strain of *S. sputigena*. The replacement type strain conforms (as determined by deoxyribonucleic acid-deoxyribonucleic acid homology) to the common oral species that Flügge most probably described.

The name "*Spirillum sputigenum*" was used by Flügge (6) to refer to motile bacteria seen microscopically in fluid suspensions of samples from oral cavities. The organisms were described as crescent shaped with a tuft of flagella attached laterally, generally on the concave side of the cell (13). In 1913 von Prowazek proposed the generic name *Selenomonas* for bacteria that he saw in blood smears of African game animals that had been shot and whose blood had become contaminated with gut contents (27). von Prowazek also mentioned the morphological similarity between the organisms that he had seen and "*Spirillum sputigenum*" Flügge and "*Ancyromonas ruminantium*" Certes (*Selenomonas ruminantium*). In 1922 Boskamp (2) formally proposed the combination *Selenomonas sputigena*, but some investigators continued to use the name "*Spirillum sputigena*" (15-17). The taxonomic history of these names has been reviewed by Lessel and Breed (13), by Kingsley and Hoeniger (10), and in Opinion 21 of the International Committee on Bacteriological Nomenclature Judicial Commission, which conserved the generic name *Selenomonas* von Prowazek and designated *Selenomonas sputigena* the type species of the genus (8).

The first description of *Selenomonas sputigena* in *Bergey's Manual of Determinative Bacteriology* was that by Lessel in the 7th ed. (12). The phenotypic characteristics cited were those determined by MacDonald (J. B. MacDonald, Ph.D. thesis, University of Toronto, Toronto, Canada, 1953). Additional characteristics, based on reports of Loesche and Gibbons (14) and Kingsley (V. V. Kingsley, Ph.D. thesis, University of Toronto, Toronto, Canada, 1968), were given by Bryant in *Bergey's Manual of Determinative Bacteriology*, 8th ed. (3). The type strain of the species, strain VPI 10068 (= ATCC 33150), was designated on the Approved Lists of Bacterial Names (26).

In studies of the supragingival and subgingival periodontal flora of people with healthy or diseased gingiva (21-24), more than 160 strains of motile saccharoclastic curved rods with the general characteristics of *Selenomonas sputigena* (3) were isolated from 40 of 102 people, including the person from whom strain VPI 10068^T (T = type strain) originally

was isolated. In this paper we report the results of deoxyribonucleic acid (DNA) homology studies between the oral isolates and the designated type strain of *Selenomonas sputigena*.

MATERIALS AND METHODS

Bacterial strains. Reference DNAs were prepared from strain VPI 10068 (= ATCC 33150 [1]), the present type strain, which was isolated from a tooth scraping coronal to the gingival margin (supragingival sample), and from strain VPI D19B-28 (= ATCC 35185), which was isolated from a subgingival (sulcus) sample from a person with juvenile periodontitis. Other strains used in the DNA homology studies were strains VPI D1C-6, VPI D14A-11, and VPI D48B-10, which were isolated from supragingival samples obtained from areas adjacent to sites affected with severe periodontitis in three other patients; strain VPI D28E-29, which was isolated from a subgingival sample from a site affected with severe periodontitis in a third person; and strain VPI D49B-7, which was isolated from a supragingival sample obtained from an area adjacent to a site with moderate periodontitis in the same individual from whom the type strain was isolated more than 10 years previously. Samples were collected and cultured as described previously (23). Strains VPI D1C-6, VPI D14A-11, and VPI D19B-28 were isolated from anaerobic roll tubes, and strains VPI D28E-29, VPI D48B-10, and VPI D49B-7 were isolated from anaerobic blood agar plates. The type strain was isolated originally on anaerobically incubated brucella agar.

Methods of characterization. Phenotypic characteristics of the bacterial strains were determined in prereduced media by previously described methods (7, 29) and by using RapID ANA panels obtained from Innovative Diagnostics Systems, Decatur, Ga. The panels were inoculated, incubated, and read as recommended by the manufacturer. These panels test for glucosidases, galactosidases, phosphatase, arginine deaminase, tetrazolium reduction, indole production, trehalose fermentation, and aminopeptidases that cleave beta-naphthylamine from various amino acids.

The polyacrylamide gel electrophoresis patterns of soluble cellular proteins were determined as described by Moore et al. (20).

DNA isolation. The strains were grown in a medium that

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TABLE 1. DNA homology results for some strains of *Selenomonas*

Strain	G+C content (mol%)	% Homology with reference DNA from strain:	
		ATCC 35185 (proposed type strain)	ATCC 33150 (present type strain)
ATCC 35185	57	(100)	16
ATCC 33150 ^T	55	22	(100)
VPI D1C-6	57	62	20
VPI D14A-11	58	66	26
VPI D28E-29	58	73	14
VPI D48B-10	59	75	27
VPI D49B-7	59	74	12

was prepared as described previously (5). This medium contained mineral salts, 1% pepticase, 0.5% yeast extract, 1% dehydrated brain heart infusion broth (Difco Laboratories, Detroit, Mich.), 1% glucose, 0.03% cysteine, 0.03% sodium formaldehyde sulfoxalate, 0.01% heme, and 0.05 M potassium phosphate buffer (pH 7.0). At the time of inoculation, 10 ml of 10% sterile NaHCO₃ and 100 ml of inoculum were added to each 1 liter of medium. The inoculated flasks were incubated at 37°C with gentle shaking until maximum growth occurred (about 2 days).

High-molecular-weight DNA was isolated by the method of Marmur (18). Other DNA preparations were isolated by an hydroxyapatite procedure (9). The DNA preparations were stored at -20°C in a solution containing 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.15 M sodium citrate, pH 7.0) and 1 mM HEPES (sodium *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonate) (pH 7.0).

Thermal melting points were used to determine the guanine-plus-cytosine (G+C) contents of the DNA preparations (9, 19). The DNA preparations were fragmented for use in the homology experiments by passing each one three times through a French pressure cell at 16,000 lb/in².

The preparations were denatured by heating in a boiling water bath for 5 min and, after cooling in ice water, were centrifuged at 12,000 × *g* for 15 min. The concentration of each preparation was then adjusted to 0.4 mg/ml, and the preparations were stored at -20°C.

Labeled DNA was prepared by iodination (25). The specific activities of the labeled preparations ranged from 2 × 10⁶ to 4 × 10⁶ cpm/μg.

DNA homology values were determined by using the S1 nuclease procedure (9). The reassociation vials each contained 10 μl of labeled DNA (0.01 to 0.03 μg), 50 μl of unlabeled DNA (20 μg), 25 ml of 5.28 M NaCl in 1 mM HEPES (pH 7.0), and 25 μl of deionized formamide. This resulted in a sodium ion concentration equivalent to that of 6× SSC and a formamide concentration of 23%. The vials were incubated for 24 h at 60°C (25°C below the thermal melting point of native DNA in this buffer system at a C₀ value of 47 mol · s/liter).

RESULTS AND DISCUSSION

All of the strains which we studied were obligately anaerobic, gram-negative, motile, curved rods that fermented carbohydrates to acetic and propionic acids. Flagella were located laterally. The G+C contents of the DNAs of six strains were 57 to 59 mol%. Therefore, these strains were members of the genus *Selenomonas* von Prowazek.

DNA homology results are presented in Table 1. The six

recent isolates tested had little homology with the type strain of *Selenomonas sputigena*, but all had relatively high levels of homology with reference DNA of strain ATCC 35185 (= VPI D19B-28). Therefore, it appears that the type strain is distinct from the more common isolates obtained from human periodontal flora.

Arabinose fermentation by the present type strain (strain ATCC 33150) was the only commonly tested phenotypic difference between this strain and the six other isolates. However, the polyacrylamide gel electrophoresis patterns of the soluble proteins of strain ATCC 33150^T were distinctly different. No other strain in our collection of saccharoclastic selenomonads fermented arabinose or had a polyacrylamide gel electrophoresis pattern like that of strain ATCC 33150^T.

In an attempt to find other isolates like strain ATCC 33150^T, 3 supragingival samples and 21 subgingival samples were cultured from the same person from whom strain ATCC 33150^T was originally isolated more than 10 years previously. None of the 21 resulting isolates of saccharoclastic selenomonads fermented arabinose or had a polyacrylamide gel electrophoresis pattern like that of strain ATCC 33150^T.

Thus, it appears that strain ATCC 33150^T (= VPI 10068^T) represents a rare oral species that, as determined by DNA homology, is genetically distinct from common oral selenomonads and that it is the only strain of that species that we know to be extant. It is extremely unlikely (less than 1 in more than 163 isolates [*P* = <0.006]) that strain ATCC 33150^T represents the bacterium originally described as *Selenomonas sputigena* ("Spirillum sputigenum"). Furthermore, this strain is not representative of the species that has come to be widely recognized by dental microbiologists as *Selenomonas sputigena*.

Although we have detected several electrophoretically and phenotypically distinct selenomonads in the periodontal flora, strains that commonly would be called *Selenomonas sputigena* (similar to strain VPI D19B-28 [= ATCC 35185]) are the first or second most numerous selenomonads in all human populations that we have examined. The strains that would be identified as *Selenomonas sputigena* (not like strain ATCC 33150^T) represented 0.3% of 3,123 isolates taken at random from people with healthy gingivae or with naturally occurring gingivitis, 1.2% of 5,179 isolates associated with moderate chronic periodontitis, 0.73% of 2,876 isolates associated with severe adult periodontitis, 0.34% of 2,335 isolates associated with juvenile periodontitis, 0.59% of 3,035 isolates from adults with experimentally produced gingivitis, and 1.3% of 3,217 isolates from children with experimentally produced gingivitis (21-24).

Because strain ATCC 33150^T almost certainly is not representative of the species as originally described, was not properly documented before its assignment to the status of type strain, is not representative of the species that is widely recognized as *Selenomonas sputigena*, and possibly is the only strain of its genospecies that is extant, we believe that it is not a valid type strain. We propose strain VPI D19B-28 (= ATCC 35185) as the valid type strain of *Selenomonas sputigena*.

We request that the Judicial Commission rule that strain VPI 10068 (= ATCC 33150) is not the valid type strain of *Selenomonas sputigena* according to Rule 18g (28) (formerly Rule 18i [11]), which states that if a type strain has become unsuitable due to changes in its characters or for other reasons, then the matter should be referred to the Judicial Commission, which may take action leading to replacement of the strain. We further request that the Judicial Commis-

sion designate strain VPI D19B-28 (= ATCC 35185) as the type strain of *Selenomonas sputigena*.

We believe that these actions are proper and much preferable to the alternative of creating a new species name to represent a commonly encountered and well-known dental species, an action that would be in direct conflict with Principle 1 (Sections 1, 2, and 3) of the *International Code of Nomenclature of Bacteria* (11).

Emended description of *Selenomonas sputigena*. Strains are obligately anaerobic, nonsporeforming, gram-negative rods. Cells are motile and crescent shaped with tapered ends and occur singly, in short chains, and in clumps. Flagella are lateral and often occur in tufts. Cells of the type strain in peptone-yeast extract-glucose broth cultures are 1.3 to 1.6 by 1.6 to 2.4 μm .

Surface colonies on anaerobic blood agar plates incubated for 2 days are pinpoint to 0.5 mm in diameter, circular, entire, low convex, white, and translucent. Colonies in roll tubes are 1 to 2 mm in diameter, lenticular, and translucent. No growth occurs on the surface of blood agar plates incubated in air enriched to contain 10% carbon dioxide.

Glucose broth cultures are not turbid or are only slightly turbid and have a smooth or slightly stringy sediment; the terminal pH is 4.7 to 5.3. Growth in peptone-yeast extract cultures is 1+ to 2+ (on a negative to 4+ scale), and growth

TABLE 2. Phenotypic characteristics of *Selenomonas sputigena*^a

Characteristic	Strain ATCC 35185 (proposed type strain)	Five other strains	Strain ATCC 33150 (present type strain)	Description by Bryant ^b
Acid from:				
Arabinose	— ^c	—	A	A
Esculin	—	—	—	A
Fructose	A	A	A	NR
Mannitol	—	—	—	A
Raffinose	A	A (80) ^d	A	NR
Salicin	—	— (80)	—	—
Sucrose	A	A	A	NR
Xylose	—	—	—	A
Reduction of:				
Nitrate	—	+ (60)	—	+
Tetrazolium	+	+ (80)	+	NR
Production of:				
Phosphatase	+	+	+	NR
β -Galactosidase	—	— (60)	—	NR
α -Glucosidase	—	— (60)	—	NR
α -Galactosidase	+	+	+	NR
G+C content (mol%)	57 ^e	58 $\pm$ 1 ^c	55 ^c	61 ^f

^a All strains produce acid from glucose, lactose, maltose, and melibiose. No strain ferments amygdalin, cellobiose, erythritol, esculin, glycogen, inositol, mannitol, mannose, melezitose, rhamnose, ribose, sorbitol, starch, trehalose, or xylose. No strain hydrolyzes esculin or starch. No strain produces hydrogen sulfide (in sulfide indole motility medium [BBL Microbiology Systems, Cockeysville, MD.]), catalase, indole, lecithinase, or lipase. No strain digests gelatin, milk, or meat. As tested in RapID ANA panels, no strain produces β -glucosidase, α -fucosidase, or acetyl-glucosaminidase; no strain produces an aminopeptidase for leucylglycine, glycine, proline, phenylalanine, arginine, serine, or pyrrolidine; no strain deaminates arginine; produces indole, or ferments trehalose.

^b See reference 4.

^c +, Positive reaction; —, negative reaction; A, pH below 5.5; NR, not reported.

^d The numbers in parentheses are the percentages of strains giving the reaction indicated.

^e Determined by the thermal melting point method.

^f Determined by the buoyant density method.

TABLE 3. Differential characteristics of species of *Selenomonas*

Characteristic	<i>Selenomonas sputigena</i> ATCC 35185 (proposed type strain)	Strain ATCC 33150 (present type strain)	<i>Selenomonas ruminantium</i> ^a
Acid from:			
Arabinose	— ^b	+	+
Cellobiose	—	—	+
Mannitol	—	—	+
Natural habitat:			
Human mouth	+	Rare	—
Rumen	—	NR	+

^a Characteristics cited by Bryant (4).

^b +, Positive reaction; —, negative reaction; NR, not reported.

with a fermentable carbohydrate is 3+ to 4+. Growth is inhibited by 2% oxgall (20% bile) and is slightly inhibited by 6.5% NaCl. Neither added vitamin K₁ nor hemin is required for growth.

The optimum temperature for growth is 35 to 37°C. Strains grow well at 30°C but poorly, if at all, at 25 and 45°C.

Most strains, including the proposed type strain, produce no detectable gas in peptone-yeast extract-glucose deep agar cultures. A trace amount is observed in some cultures.

The major fermentation acids from 1% peptone–1% yeast extract–1% glucose broth cultures are acetate (1.8 meq/100 ml of culture) and propionate (3.5 meq/100 ml of culture). Little or no hydrogen is detected in headspace gas of glucose broth cultures. Lactate, pyruvate, and fumarate are converted to propionate and acetate. Threonine is not attacked.

The six strains which we studied are susceptible to chloramphenicol (12 $\mu\text{g/ml}$), clindamycin (1.6 $\mu\text{g/ml}$), erythromycin (3 $\mu\text{g/ml}$), penicillin G (2 U/ml), and tetracycline (6 $\mu\text{g/ml}$), as determined by the broth disk methods of Wilkins and Thiel (29).

Other characteristics of the species are given in Table 2. Isolated from tooth scrapings and subgingival plaque.

The G+C content of the DNA is 57 to 59 mol% (as determined by the thermal melting point method).

Proposed type strain: strain ATCC 35185.

Differential characteristics of the described species of *Selenomonas* are shown in Table 3.

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