

Characterization of *Slackia exigua* Isolated from Human Wound Infections, Including Abscesses of Intestinal Origin[∇]

Keun-Sung Kim,^{1,5,7} Marie-Claire Rowlinson,⁵ Robert Bennion,³ Chengxu Liu,⁵ David Talan,⁴ Paula Summanen,⁵ and Sydney M. Finegold^{1,2,6*}

Department of Medicine¹ and Department of Microbiology, Immunology, and Molecular Genetics,² University of California at Los Angeles School of Medicine, Los Angeles, California; Surgical Service³ and Emergency Medicine Service,⁴ Olive View-UCLA Hospital, Los Angeles, California; Research Service⁵ and Infectious Diseases Section,⁶ Veterans Affairs Medical Center West Los Angeles, Los Angeles, California; and Department of Food Science and Technology, Chung-Ang University, Ansung, South Korea⁷

Received 15 August 2009/Returned for modification 9 October 2009/Accepted 14 January 2010

Eleven clinical strains isolated from infected wound specimens were subjected to polyphasic taxonomic analysis. Sequence analysis of the 16S rRNA gene showed that all 11 strains were phylogenetically related to *Slackia exigua*. Additionally, conventional and biochemical tests of 6 of the 11 strains were performed as supplementary methods to obtain phenotypic identification by comparison with the phenotypes of the relevant type strains. *S. exigua* has been considered an oral bacterial species in the family *Coriobacteriaceae*. This organism is fastidious and grows poorly, so it may easily be overlooked. The 16S rRNA gene sequences and the biochemical characteristics of four of the *S. exigua* strains isolated for this study from various infections indicative of an intestinal source were almost identical to those of the validated *S. exigua* type strain from an oral source and two of the *S. exigua* strains from oral sources evaluated in this study. Thus, we show for the first time that *S. exigua* species can be isolated from extraoral infections as well as from oral infections. The profiles of susceptibility to selected antimicrobials of this species were also investigated for the first time.

In recent years, there have been reports that *Slackia exigua* is a poorly growing pathogen in periodontitis and periapical infections (1, 5, 8, 20, 23, 27). This bacterium was originally classified as *Eubacterium exiguum* in 1996 (20) and was reclassified as *S. exigua* in 1999 (27). *S. exigua* is a Gram-positive, non-spore-forming, nonmotile, and strictly anaerobic bacillus that has been considered an oral asaccharolytic bacterial species in the family *Coriobacteriaceae*. The species has proven to be difficult to culture and is unreactive in conventional biochemical tests (27).

S. exigua has frequently been isolated from periradicular lesions (8, 13, 22), in addition to other oral sites (9, 18, 28). In particular, *S. exigua* has been found in infected necrotic pulps and periradicular lesions (8, 22). These facts suggest that this species may play a pathogenic role in oral infectious diseases, including pulpal infections that spread to the periradicular tissues. In addition, *S. exigua* has been reported to be associated with clinical indicators of periodontal disease (1). However, *S. exigua* was isolated from five infections clearly of intestinal origin during our investigation of the microbiology of human wound infections and abscesses (6). Therefore, the current study was carried out to see if there are any significant differences in the characteristics of clinical isolates of oral and intestinal origin.

In this report, we provide a profile of the role of the bacterium *S. exigua* in various wound infections. We have isolated *S. exigua* strains not only from oral infections but also from var-

ious extraoral wound infections and abscesses. The *S. exigua* isolates were characterized genotypically and phenotypically. To our knowledge, this is the first report of the isolation and extensive characterization of *S. exigua* from human infections other than oral infections. It is also the first time that its antimicrobial susceptibility has been studied.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Four hundred surgical wound or closed abscess specimens were collected by needle and syringe at the Olive View-University of California Medical Center between 2005 and 2007 and were transported to the Wadsworth Anaerobic Bacteriology Laboratory in Port-A-Cul anaerobic transport medium (BBL), usually on the day of collection and occasionally on the day after collection.

To obtain quantitative cultures of bacteria, each specimen was plated and grown on brucella blood agar (Anaerobe Systems) or CDC blood agar (Becton Dickinson) at 37°C under anaerobic conditions (N₂-CO₂-H₂, 90:5:5). Counts of the different colonies were made, and representative colonies were subcultured and subjected to standard procedures for bacterial identification, including Gram staining, catalase and oxidase reactions, and 16S rRNA gene sequencing, as described in the *Wadsworth-KTL Anaerobic Bacteriology Manual* (12) and elsewhere (7). In all, 1,456 isolates were obtained. Among these, there were 11 *S. exigua* isolates identified by 16S rRNA gene sequencing (Table 1). All strains were kept in 10% skim milk at -70°C until further use.

The type strain of *Slackia exigua* (ATCC 700122^T) was obtained from the American Type Culture Collection (ATCC), Manassas, VA. *Bacteroides fragilis* (ATCC 25285), *Bacteroides thetaiotaomicron* (ATCC 29741), and *Eubacterium lentum* (ATCC 43055) were included as control strains in each test run of antimicrobial susceptibility.

16S rRNA gene sequencing analysis. Genomic DNA was extracted and purified from bacterial cells in the mid-logarithmic growth phase by using a QIAamp DNA minikit (Qiagen, Inc., Chatsworth, CA). The 16S rRNA gene fragments were amplified by standard methods. Two subregions of the 16S rRNA gene were amplified by using two pairs of primers, as described previously (2, 25, 26). The first part of the 16S rRNA gene was defined as an approximately 800-bp region between primers 8UA and 907B. The second part of the 16S rRNA gene, defined as approximately 700-bp sequences between primers 774A and 1485B,

* Corresponding author. Mailing address: Infectious Diseases Section (111 F), VA Medical Center West Los Angeles, 11301 Wilshire Blvd., Los Angeles, CA 90073. Phone: (310) 268-3678. Fax: (310) 268-3594. E-mail: sidfinegol@aol.com.

[∇] Published ahead of print on 27 January 2010.

TABLE 1. Culture results for 11 wound samples involving *Slackia exigua* species

Specimen no.	Clinical source(s)	No. of CFU of <i>Slackia exigua</i> isolates/ml	Accompanying isolates (no. of CFU/ml)
06-1724	Abdominal wound abscess	10 ⁹	<i>Peptostreptococcus micros</i> (10 ⁹), <i>Solobacterium moorei</i> (10 ⁸), <i>Bacteroides fragilis</i> (10 ⁷), <i>Eikenella corrodens</i> (10 ⁵), <i>Enterococcus avium</i> (10 ⁵), uncultured bacterium (human fecal flora) (10 ⁵), <i>Escherichia coli</i> (10 ⁴), <i>Bilophila wadsworthia</i> (10 ³)
07-1812	Abscess under chin (not dental related)	10 ⁵	<i>Campylobacter rectus</i> (10 ⁵), <i>Campylobacter showae</i> (10 ⁵), <i>Staphylococcus pasteurii</i> / <i>Staphylococcus warneri</i> (10 ⁵), <i>Tannerella forsythia</i> (10 ⁵)
07-1852	Breast abscess	10 ⁸	<i>Anaerococcus vaginalis</i> (10 ⁹), <i>Atopobium rimae</i> (10 ⁹), <i>Bacteroides ureolyticus</i> (10 ⁸), <i>Eubacterium timidum</i> (10 ⁸), <i>Fingoldia magna</i> (10 ⁸), <i>Flexistipes species</i> / <i>Synergistes species</i> (10 ⁸), <i>Peptoniphilus lacrimalis</i> (10 ⁸), <i>Peptostreptococcus stomatis</i> (10 ⁸), <i>Streptococcus anginosus</i> (10 ⁷)
07-1892	Buttock abscess	10 ⁷	<i>Prevotella disiens</i> (10 ⁸), <i>Actinomyces radingae</i> (10 ⁶)
07-1945	Finger paronychia abscess (nail biter)	10 ⁵	<i>Enterobacter cloacae</i> / <i>Enterobacter aerogenes</i> (10 ⁷), <i>Fingoldia magna</i> (10 ⁵), <i>Gemella palaticanis</i> (10 ⁵), <i>Peptostreptococcus anaerobius</i> (10 ⁵), <i>Prevotella species</i> (10 ⁵), <i>Veillonella species</i> (10 ⁵), <i>Dialister microaerophilus</i> (10 ⁴), <i>Kluyvera ascorbata</i> (10 ⁴), <i>Prevotella buccalis</i> (10 ³)
07-1951	Infection, jaw (not dental related)	10 ⁵	<i>Parvimonas micra</i> (10 ⁶), <i>Dialister invisus</i> (10 ⁵), <i>Dialister pneumosintes</i> (10 ⁵), <i>Olsenella uli</i> (10 ⁵)
07-1974	Intra-abdominal abscess, perforated appendix	10 ⁸	<i>Bacteroides thetaiotaomicron</i> (10 ⁸), <i>Dialister pneumosintes</i> (10 ⁸), <i>Fusobacterium nucleatum</i> (10 ⁸), <i>Parvimonas micra</i> (10 ⁸), <i>Bacteroides fragilis</i> (10 ⁷), <i>Bacteroides species</i> (10 ⁷)/ <i>Bacteroides ovatus</i> / <i>Bacteroides acidofaciens</i> , <i>Campylobacter gracilis</i> (10 ⁷), <i>Escherichia coli</i> (10 ⁵), <i>Candida species</i> (ND ^a)
07-2033	Peritonsillar abscess	10 ⁷	<i>Prevotella intermedia</i> (10 ⁹), <i>Peptostreptococcus stomatis</i> (10 ⁸), <i>Prevotella baroniae</i> (10 ⁸), <i>Prevotella buccae</i> (10 ⁸), <i>Atopobium rimae</i> (10 ⁷), <i>Dialister pneumosintes</i> (10 ⁷), <i>Fusobacterium nucleatum</i> (10 ⁷), <i>Lactobacillus catenaformis</i> (10 ⁷), <i>Peptostreptococcus micros</i> (10 ⁷), <i>Prevotella oral clone</i> / <i>Prevotella melaninogenica</i> (10 ⁷), <i>Streptococcus intermedius</i> / <i>Streptococcus constellatus</i> / <i>Streptococcus anginosus</i> (10 ⁶), <i>Streptococcus pneumoniae</i> / <i>Streptococcus mitis</i> (10 ⁴)
07-2037	Buttock abscess	10 ⁹	<i>Dialister pneumosintes</i> (10 ⁹), <i>Peptostreptococcus anaerobius</i> (10 ⁹), <i>Porphyromonas somerae</i> (10 ⁹), <i>Bacteroides caccae</i> (10 ⁸), <i>Bacteroides coagulans</i> (10 ⁸), <i>Bacteroides ureolyticus</i> (10 ⁸), <i>Prevotella bergensis</i> (10 ⁸), <i>Peptoniphilus lacrimalis</i> (10 ⁷), <i>Prevotella disiens</i> (10 ⁷), <i>Actinomyces europaeus</i> (10 ⁶), <i>Staphylococcus aureus</i> (10 ⁵)
07-2068	Pilonidal abscess	10 ⁷	<i>Fusobacterium gonidiaformans</i> (10 ⁸), <i>Parvimonas micra</i> (10 ⁸), <i>Solobacterium moorei</i> (10 ⁸), <i>Atopobium minutum</i> (10 ⁷), <i>Bacteroides vulgatus</i> (10 ⁷), <i>Peptostreptococcus stomatis</i> (10 ⁷), <i>Prevotella intermedia</i> (10 ⁷), <i>Tannerella forsythia</i> (10 ⁷), <i>Actinomyces turicensis</i> (10 ⁶), <i>Porphyromonas asaccharolytica</i> (ND)
07-2133	Dental abscess	10 ⁵	<i>Neisseria flava</i> / <i>Neisseria sicca</i> / <i>Neisseria mucosa</i> (10 ⁶), <i>Eubacterium timidum</i> (10 ⁵), <i>Neisseria perflava</i> / <i>Neisseria mucosa</i> (10 ⁵), <i>Neisseria subflava</i> (10 ⁵), <i>Dialister pneumosintes</i> (10 ⁴), <i>Porphyromonas gingivalis</i> (10 ⁴)

^a ND, not determined.

was sequenced to obtain the complete 16S rRNA gene sequence. The PCR products were excised from a 1% agarose gel after electrophoresis and purified with a QIAquick gel extraction kit (Qiagen). The purified PCR products were sequenced directly with BigDye Terminator cycle sequencing kits (Biotech Di-

agnostics, Tustin, CA) on an ABI 3100 Avant genetic system (Applied Biosystems, Foster City, CA). The sequencing data were analyzed by comparison of the consensus sequences with GenBank sequences by using Ribosomal Database Project (RDP-II) and Basic Local Alignment Search Tool (BLAST) software.

Sequence similarities (in percent) with respect to the other sequences were determined. Strains were considered to be accurately identified when the sequence similarities of the clinical strains with the sequences of the GenBank entries were equal to or greater than 99%, provided that no other GenBank sequences (representing different species) had greater than 98% sequence similarity to the clinical strains.

Biochemical characterization. Eleven isolates were recovered from the human wound specimens. Six of the 11 strains were characterized biochemically by using a combination of conventional tests, as described previously in the Wadsworth and VPI anaerobic bacteriology manuals (10, 12). The API Rapid ID 32A and API ZYM systems (bioMérieux, Hazelwood, MO) were inoculated and incubated, and the results were interpreted according to the manufacturer's instructions. All biochemical tests were performed in duplicate.

The API Rapid ID 32A system consists of 29 tests for preformed enzymes, and the API ZYM system allows the semiquantitative and rapid determination of 19 enzymatic reactions.

Antimicrobial susceptibility assay. MICs were determined by the Etest method (AB Biodisk, Solna, Sweden), according to the manufacturer's recommendations. The method was previously shown to produce accurate results for the susceptibility testing of anaerobic bacteria (3, 4, 21). Brucella agar supplemented with laked sheep blood, hemin, and vitamin K₁ was used, as recommended by the manufacturer. The following antimicrobial agents were tested: ampicillin, benzylpenicillin, amoxicillin-clavulanic acid, ertapenem, cefoxitin, ceftriaxone, vancomycin, clindamycin, erythromycin, metronidazole, and trimethoprim-sulfamethoxazole. Because these clinical isolates are slow growers, reading of the results was performed after 72 h of incubation. Interpretation was according to the manufacturer's recommendations.

The isolates were categorized, by using interpretative criteria, as susceptible and resistant if the MICs for each antimicrobial were as follows: ampicillin, ≤ 0.5 and > 2 mg/liter, respectively; benzylpenicillin, ≤ 0.5 and > 2 mg/liter, respectively; amoxicillin-clavulanic acid, ≤ 4 and > 16 mg/liter, respectively; ertapenem, ≤ 4 and > 16 mg/liter, respectively; cefoxitin, ≤ 16 and > 64 mg/liter, respectively; ceftriaxone, ≤ 16 and > 64 mg/liter, respectively; and clindamycin, ≤ 2 and > 8 mg/liter, respectively. The breakpoints for vancomycin, erythromycin, and sulfamethoxazole-trimethoprim against strict anaerobes have not been determined. For those antimicrobials, therefore, the breakpoints recommended by the manufacturer against aerobes were used in this study. The production of β -lactamase was tested for with nitrocefin discs (cefinaise; BD Diagnostics, Erembodegem, Belgium).

Nucleotide sequence accession numbers. The nucleotide sequences of the 16S rRNA gene fragments described in this paper have been deposited in the GenBank database under accession numbers GU395300 and GU395299 for two isolates, one of oral origin (specimen number 07-1951) and one of intestinal origin (specimen number 07-2037), respectively.

RESULTS

Bacterial strains. During our comprehensive microbiological evaluation of 400 skin and soft tissue infections and closed abscesses, 11 strains of a Gram-positive-staining, strictly anaerobic, nonmotile, non-spore-forming, rod-shaped organism were isolated from 11 different human clinical sources: 5 from intestine-related cases and 6 from oral cavity-related cases (Table 1). The six oral-source strains were isolated from an abscess under the chin (not dental related), a breast abscess, a finger paronychia abscess (a nail biter), a jaw infection (not dental related), a peritonsillar abscess, and a dental abscess. The five strains related to a bowel source were from an abdominal wound abscess, two buttock abscesses, an intra-abdominal abscess secondary to a perforated appendix, and a pilonidal abscess. The identities of the 11 strains were determined by 16S rRNA gene sequencing analysis; and those of 6 strains from specimens 06-1724, 07-1812, 07-1951, 07-1974, 07-2037, and 07-2068 were further confirmed by biochemical characterization. The other five strains could not be characterized biochemically due to their fastidious nature and poor growth.

Identification of clinical isolates by 16S rRNA gene sequencing analysis. A total of 12 strains, including 1 reference strain (*S. exigua* ATCC 700122^T) and the 11 strains isolated from various clinical sources as described above, were subjected to comparative 16S rRNA gene sequencing analysis. The 16S rRNA gene sequence of the reference strain matched that deposited in GenBank with 100% sequence similarity. Similarly, for all of the clinical strains tested, the 16S rRNA gene sequencing identified *S. exigua* species as the first choice with $\geq 99\%$ sequence similarity to the reference strain sequence (differences at 4 to 7 bp of the 1,450 bp sequenced). In addition, all of the clinical strains tested were closely related to each other genetically, as demonstrated by the 16S rRNA gene sequence similarities ($\geq 99\%$; differences of 1 to 5 bp of the 1,450 bp sequenced). On the other hand, the maximum identities of all 11 clinical isolates tested were 94 to 95% when they were aligned to type strain *S. heliotrinireducens* DSM 20476, 91 to 93% when they were aligned to type strain *S. faecicanis* CCUG 48399, and 90 to 92% when they were aligned to type strain *S. isoflavoniconvertens* DSM 22006. Therefore, all of the 11 strains isolated from various clinical sources as described above were assigned to the species *S. exigua*.

Biochemical characterization. Cells of the six isolates originating from various human clinical sources were obligately anaerobic, non-spore-forming, nonmotile, Gram-positive rods. The colonies that grew on brucella blood agar or CDC blood agar plates after 72 h of incubation at 37°C under anaerobic conditions (N₂-CO₂-H₂, 90:5:5) were circular, convex, and translucent and were less than 1 mm in diameter even after prolonged incubation (5 to 7 days) under anaerobic conditions. The optimum temperature for growth was 37°C. No hemolysis was observed. The six strains were biochemically characterized by using the API Rapid ID 32A and API ZYM systems (Tables 2 and 3). Positive reactions were obtained for arginine dihydrolase, alanine arylamidase, arginine arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, phenylalanine arylamidase, proline arylamidase, serine arylamidase, tyrosine arylamidase, cystine arylamidase, valine arylamidase, esterase (C₄), esterase lipase (C₈), acid phosphatase, and naphthol-AS-BI-phosphohydrolase. Negative results were obtained for urease, alkaline phosphatase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, leucyl glycine arylamidase, pyroglutamic acid arylamidase, α -arabinosidase, α -fucosidase, α - and β -galactosidases, β -galactosidase-6-phosphate, α - and β -glucosidases, β -glucuronidase, β -N-acetylglucosaminidase, α -mannosidase, lipase (C₁₄), trypsin, and α -chymotrypsin. Mannose and raffinose were not fermented. Indole was not produced. Nitrate was not reduced.

Antimicrobial susceptibility assay. The results of *in vitro* testing of the susceptibilities of six clinical isolates to 11 commonly used antimicrobial agents by the Etest method are shown in Table 4. All six strains were susceptible to ampicillin (MICs, 0.094 to 0.19 mg/liter), benzylpenicillin (MICs, 0.064 to 0.125 mg/liter), amoxicillin-clavulanic acid (MICs, 0.094 to 0.25 mg/liter), ertapenem (MICs, 0.016 to 2.0 mg/liter), cefoxitin (MICs, 0.5 to 3.0 mg/liter), ceftriaxone (MICs, 0.002 to 0.25 mg/liter), vancomycin (MICs, 0.032 to 0.50 mg/liter), clindamycin (MICs, 0.016 to 0.023 mg/liter), erythromycin (MICs, 0.016 to 0.023 mg/liter), and metronidazole (MICs, 0.064 to 0.38 mg/liter). However, all six strains were resistant to sulfa-

TABLE 2. Biochemical characteristics of the six *Slackia exigua* isolates from human wound infections, as determined by conventional tests and with the API Rapid ID 32A system

Characteristic	Result for ^a :				
	Clinical isolates from this study	<i>S. exigua</i> ATCC 700122	<i>S. heliotrinireducens</i> DSM 20476	<i>S. faecicanis</i> CCUG 48399	<i>S. isoflavoniconvertens</i> DSM 22006
Cell shape	Rods	Rods	Cocci	Rods	Rods
Gram stain	+	+	+	+	+
Arginine dihydrolase reaction	+	+	+	+	+
Amino acid arylamidase reactions					
Alanine	+	+	+	-	-
Arginine	+	+	v	-	-
Glycine	+	+	+	-	-
Histidine	+	+	+	-	-
Leucine	+	+	+	-	-
Leucyl glycine	-	-	v	-	-
Phenylalanine	+	+	+	-	-
Proline	+	+	+	-	-
Serine	+	+	+	-	-
Tyrosine	+	+	+	-	-
Nitrate reduction	-	-	v	+	-

^a Data for the reference species were obtained from Wade et al. (27) for *S. heliotrinireducens*, Lawson et al. (15) for *S. faecicanis*, and Matthies et al. (17) for *S. isoflavoniconvertens*. +, positive; -, negative; v, variable. In the API Rapid ID 32A system, all strains are negative for urease, alkaline phosphatase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, pyroglutamic acid arylamidase, nine carbohydrate-degradation enzymes (α-arabinosidase, α-fucosidase, α- and β-galactosidases, β-galactosidase-6-phosphate, α- and β-glucosidase, β-glucuronidase, and β-N-acetyl-glucosaminidase), two carbohydrate fermentation enzymes (mannose and raffinose), and indole production.

methoxazole-trimethoprim (MICs, >32 mg/liter). They were β-lactamase negative. The results with the control strains were within the predefined quality control (QC) ranges.

DISCUSSION

All 11 isolates from various human infections gave scanty growth on brucella blood agar and CDC blood agar plates, as well as in peptone-yeast extract-glucose broth. Assignment of the isolates to the species *S. exigua* was based on a significantly high degree of identity (≥99%) to 16S rRNA gene sequences derived from validated *S. exigua* species (GenBank). The phenotypic characteristics of the six isolates were studied by the use of biochemical tests and determination of their suscepti-

bilities to various antimicrobials. The typical *S. exigua* isolates from oral cavity-related lesions in previous studies are inert in most biochemical tests.

The 16S rRNA gene sequences of the 11 *S. exigua* isolates tested in this study (6 isolates from oral sources and 5 isolates from intestinal sources) were determined and aligned with the 16S rRNA gene sequence of the *S. exigua* type strain from oral lesions obtained in other previous studies. The biochemical characteristics of six *S. exigua* isolates in this study (two from oral sources and four from intestinal sources) were determined and compared with those of the isolates from oral lesions described in other reports (20, 27). All things considered, the 16S rRNA genes sequences and the biochemical characteristics of the *S. exigua* strains isolated during this study are almost

TABLE 3. Enzymatic profiles of the six *Slackia exigua* isolates from human wound infections, as determined with the API ZYM system

Enzyme	Result for ^a :				
	Clinical isolates from this study	<i>S. exigua</i> ATCC 700122	<i>S. heliotrinireducens</i> DSM 20476	<i>S. faecicanis</i> CCUG 48399	<i>S. isoflavoniconvertens</i> DSM 22006
Esterase (C ₄)	2-3	3	+	+	+
Esterase lipase (C ₈)	1-2	1	w	w	-
Lipase (C ₁₄)	0	0			-
Leucine arylamidase	5	5	+	-	-
Valine arylamidase	5	5	+	-	-
Cystine arylamidase	1	1	-	-	-
Acid phosphatase	1-2	2	-	+	+
Naphthol-AS-BI-phosphohydrolase	1	1	-	w	+
α-Fucosidase	0	0			-

^a Data for the reference species were obtained from Wade et al. (27) for *S. heliotrinireducens*, Lawson et al. (15) for *S. faecicanis*, and Matthies et al. (17) for *S. isoflavoniconvertens*. The numerical values, from 1 (weak) to 5 (strong), for the clinical isolates from this study and *S. exigua* ATCC 700122 indicate color intensities that correspond to enzymatic reactivity. Zero indicates no enzymatic reactivity. +, positive; w, weakly positive, -, negative. In the API ZYM system, all strains are negative for alkaline phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, and N-acetyl-β-glucosaminidase.

TABLE 4. Antimicrobial susceptibility profiles of the six *Slackia exigua* strains isolated from human wound infections

Antimicrobial(s)	MIC range (mg/liter)	Susceptibility rate (%) ^a		
		S	I	R
Ampicillin	0.094–0.19	100	0	0
Benzylpenicillin	0.064–0.125	100	0	0
Amoxicillin-clavulanic acid	0.094–0.25	100	0	0
Ertapenem	0.016–2.0	100	0	0
Cefoxitin	0.5–3.0	100	0	0
Ceftriaxone	0.002–0.25	100	0	0
Vancomycin ^b	0.032–0.50	100	0	0
Clindamycin	0.016–0.023	100	0	0
Erythromycin ^b	0.016–0.023	100	0	0
Metronidazole	0.064–0.38	100	0	0
Sulfamethoxazole-trimethoprim ^b	>32	0	0	100

^a S, susceptible; I, intermediate; R, resistant.

^b The breakpoints recommended by the manufacturer for aerobes were used.

identical to those of the validated *S. exigua* type strain from an oral source.

In 1999, Wade et al. (27) reported a 94.5% level of 16S rRNA gene sequence similarity between *E. exiguum* from human oral lesions and *Peptostreptococcus heliotrinireducens* from sheep rumen and a 60 to 64 mol% G+C content for the DNA base composition of the two species. On the basis of these sequence similarity data, the new genus *Slackia* was created for these two bacterial species, and *E. exiguum* and *Peptostreptococcus heliotrinireducens* were reclassified as *S. exigua* and *S. heliotrinireducens*, respectively. Since then, on the basis of phenotypic and phylogenetic analyses, four more species have been reported in the genus *Slackia*: *S. faecicanis* (15), *S. isoflavoniconvertens* (17), *S. equolifaciens* (11), and *S. piriformis* (19). *S. exigua* was isolated from human oral lesions (20); *S. heliotrinireducens* was isolated from sheep rumen (14); *S. faecicanis* was isolated from dog feces (15), and *S. isoflavoniconvertens*, *S. equolifaciens*, and *S. piriformis* were isolated from human feces (11, 17, 19).

S. exigua can be identified phenotypically and can be differentiated from the other three species of the genus *Slackia* by using certain enzymatic and morphological characteristics. *S. exigua* and *S. heliotrinireducens* have alanine, glycine, histidine, leucine, phenylalanine, proline, serine, tyrosine, and valine arylamidases; but *S. faecicanis* and *S. isoflavoniconvertens* do not. The last two species can be distinguished by the fact that *S. faecicanis* is nitrate positive. *S. exigua* can be further differentiated from *S. heliotrinireducens* by using the following three enzymatic characteristics and one morphological characteristic. *S. exigua* has cystine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase; but *S. heliotrinireducens* does not. *S. exigua* has a rod shape, and *S. heliotrinireducens* has a coccal shape. If the test for arginine arylamidase is negative, that would rule out *S. exigua* (the result for *S. heliotrinireducens* is variable with this enzyme). Table 2 gives the key characteristics used for the differentiation of *Slackia exigua* from other *Slackia* species.

S. exigua was susceptible to all antimicrobials tested except sulfamethoxazole-trimethoprim. Many bacteria are obligate folic acid synthesizers (16, 24). Although further verification

should be obtained, our results indicate that the six *S. exigua* isolates may not be obligate folic acid synthesizers since sulfamethoxazole-trimethoprim is a folic acid antagonist. Further studies in which a greater number of strains of the species are tested are required.

Since 1996, when Poco et al. (20) originally identified *S. exigua* as an oral asaccharolytic species, all of the species have been isolated only from human oral lesions and have been characterized by using biochemical tests and 16S rRNA gene sequence analyses. In our study of clinical wound infections (6), 11 of 400 samples from both intestinal and oral sources yielded *S. exigua* isolates (Table 1). For all 11 of these samples, *S. exigua* isolates were accompanied by other aerobic and/or anaerobic bacteria. Most *Slackia* isolates were present at high counts (Table 1). Concerning these infections, the role of *Slackia exigua* in the infectious process is uncertain. Previous research on *S. exigua* has focused on oral infections. The present study indicates that research on *S. exigua* should extend to infections from intestinal sources.

ACKNOWLEDGMENTS

This work has been supported in part by DOD grant W81XWH-0510134 and VA Merit Review funds.

REFERENCES

- Booth, V., J. Downes, J. Van den Berg, and W. G. Wade. 2004. Gram-positive anaerobic bacilli in human periodontal disease. *J. Periodontol. Res.* **39**:213–220.
- Brosius, J., M. L. Palmer, P. J. Kennedy, and H. F. Noller. 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* **75**:4801–4805.
- Citron, D. M., and D. W. Hecht. 2007. Susceptibility test methods: anaerobic bacteria, p. 1214–1222. In P. R. Murray, E. J. Baron, J. H. Tenover, and M. A. Tenover (ed.), *Manual of clinical microbiology*, 9th ed. ASM Press, Washington, DC.
- Citron, D. M., M. I. Ostovari, A. Karlsson, and E. J. C. Goldstein. 1991. Evaluation of the E test for susceptibility testing of anaerobic bacteria. *J. Clin. Microbiol.* **29**:2197–2203.
- Downes, J., M. A. Munson, D. A. Spratt, E. Könönen, E. Tarkka, H. Jousimies-Somer, and W. G. Wade. 2001. Characterisation of *Eubacterium*-like strains isolated from oral infections. *J. Med. Microbiol.* **50**:947–951.
- Finogold, S. M., D. Talan, S. Hiyama, M.-C. Rowlinson, P. Summanen, D. Molitoris, C.-X. Liu, N. Ulger, J. Wooton, Y. Song, and R. Bennion. 2008. Analysis of 400 surgical and traumatic wound infections and closed abscesses by molecular techniques for development of a real-time PCR procedure, abstr. C-110, p. 065/D. Abstr. 108th Gen. Meet. Am. Soc. Microbiol. American Society for Microbiology, Washington, DC.
- Finogold, S. M., M. Vaisanen, M. Rautio, E. Eerola, P. Summanen, D. Molitoris, Y. L. Song, C. Liu, and H. J. Somer. 2004. *Porphyromonas uenonis* sp. nov., a pathogen for humans distinct from *P. asaccharolytica* and *P. endodontalis*. *J. Clin. Microbiol.* **42**:5298–5301.
- Hashimura, T., M. Sato, and E. Hoshino. 2001. Detection of *Slackia exigua*, *Mogibacterium timidum* and *Eubacterium sapenum* from pulpal and periradicular samples using the polymerase chain reaction (PCR) method. *Int. Endod. J.* **34**:463–470.
- Hill, G. B., O. M. Ayers, and A. P. Kohan. 1987. Characteristics and sites of infection of *Eubacterium nodatum*, *Eubacterium timidum*, *Eubacterium brachy*, and other asaccharolytic eubacteria. *J. Clin. Microbiol.* **25**:1540–1545.
- Holdeman, L. V., E. P. Cato, and W. E. C. Moore. 1977. Anaerobe laboratory manual. Virginia Polytechnic Institute and State University, Blacksburg, VA.
- Jin, J. S., M. Kitahara, M. Sakamoto, M. Hattori, and Y. Benno. 4 September 2009, posting date. *Slackia equolifaciens* sp. nov., a human intestinal bacterium capable of producing equol. *Int. J. Syst. Evol. Microbiol.* doi: 10.1099/ijs.0.016774-0.
- Jousimies-Somer, H., P. Summanen, D. M. Citron, E. J. Baron, H. M. Wexler, and S. M. Finogold. 2002. Wadsworth-KTL anaerobic bacteriology manual, 6th ed. Star Publishing, Belmont, CA.
- Kiryu, T., E. Hoshino, and M. Iwaku. 1994. Bacteria invading periapical cementum. *J. Endod.* **20**:169–172.
- Lanigan, G. W. 1976. *Peptococcus heliotrinireducens*, sp. nov., a cytochrome-producing anaerobe which metabolizes pyrrolizidine alkaloids. *J. Gen. Microbiol.* **94**:1–10.

15. Lawson, P. A., H. L. Greetham, G. R. Gibson, C. Giffard, E. Falsen, and M. D. Collins. 2005. *Slackia faecicanis* sp. nov., isolated from canine faeces. *Int. J. Syst. Evol. Microbiol.* **55**:1243–1246.
16. Libecco, J. A., and K. R. Powell. 2004. Trimethoprim/sulfamethoxazole: clinical update. *Pediatr. Rev.* **25**:375–380.
17. Matthies, A., M. Blaut, and A. Braune. 2009. Isolation of a human intestinal bacterium capable of daidzein and genistein conversion. *Appl. Environ. Microbiol.* **75**:1740–1744.
18. Moore, W. E. C., L. V. Holdemen, E. P. Cato, R. M. Smibert, J. A. Burmeister, and R. R. Ranney. 1983. Bacteriology of moderate (chronic) periodontitis in mature adult humans. *Infect. Immun.* **42**:510–515.
19. Nagai, F., Y. Watanabe, and M. Morotomi. 18 December 2009, posting date. *Slackia piriformis* sp. nov. and *Collinsella tanakaei* sp. nov., novel members of the family *Coriobacteriaceae*, isolated from human faeces. *Int. J. Syst. Evol. Microbiol.* doi:10.1099/ijs. 0.017533-0.
20. Poco, S. E., F. Nakazawa, T. Ikeda, M. Sato, T. Sato, and E. Hoshino. 1996. *Eubacterium exiguum* sp. nov., isolated from human oral lesions. *Int. J. Syst. Bacteriol.* **46**:1120–1124.
21. Rosenblatt, J. E., and D. R. Gustafson. 1995. Evaluation of the Etest for the susceptibility testing of anaerobic bacteria. *Diagn. Microbiol. Infect. Dis.* **22**:279–284.
22. Sato, T., E. Hoshino, H. Uematsu, and T. Noda. 1993. Predominant obligate anaerobes in necrotic pulps of human deciduous teeth. *Microb. Ecol. Health Dis.* **6**:269–275.
23. Sato, T., M. Sato, J. Matsuyama, S. Kalfas, G. Sundqvist, and E. Hoshino. 1998. Restriction fragment-length polymorphism analysis of 16S rDNA from oral asaccharolytic *Eubacterium* species amplified by polymerase chain reaction. *Oral Microbiol. Immunol.* **13**:23–29.
24. Smith, C. L., and K. R. Powell. 2000. Review of the sulfonamides and trimethoprim. *Pediatr. Rev.* **21**:368–371.
25. Song, Y. L., E. Könönen, M. Rautio, C. Liu, A. Bryk, E. Eerola, and S. M. Finegold. 2006. *Alistipes onderdonkii* sp. nov. and *Alistipes shahii* sp. nov., of human origin. *Int. J. Syst. Evol. Microbiol.* **56**:1985–1990.
26. Summanen, P. H., M. C. Rowlinson, J. Wooton, and S. M. Finegold. 2009. Evaluation of genotypic and phenotypic methods for differentiation of the members of the anginosus group streptococci. *Eur. J. Clin. Microbiol. Infect. Dis.* **28**:1123–1128.
27. Wade, W. G., J. Downes, D. Dymock, S. J. Hiom, A. J. Weightman, F. E. Dewhirst, B. J. Paster, N. Tzellas, and B. Coleman. 1999. The family *Coriobacteriaceae*: reclassification of *Eubacterium exiguum* (Poco et al. 1996) and *Peptostreptococcus heliotrinireducens* (Lanigan 1976) as *Slackia exigua* gen. nov., comb. nov. and *Slackia heliotrinireducens* gen. nov., comb. nov., and *Eubacterium lentum* (Prévot 1938) as *Eggerthella lenta* gen. nov., comb. nov. *Int. J. Syst. Bacteriol.* **49**:595–600.
28. Wade, W. G., M. A. Lewis, S. L. Cheeseman, E. G. Absi, and P. A. Bishop. 1994. An unclassified *Eubacterium* taxon in acute dento-alveolar abscess. *J. Med. Microbiol.* **40**:115–117.