Molecular Taxonomic Studies of *Actinomyces*-Like Bacteria Isolated from Purulent Lesions in Pigs and Description of *Actinomyces hyovaginalis* sp. nov.

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The 16S rRNA gene sequence of some *Actinomyces*-like bacteria isolated from purulent lesions in pigs was determined. A comparative analysis of the rRNA sequence data revealed that the bacteria are members of the genus *Actinomyces*, but are phylogenetically distinct from *Actinomyces suis*. On the basis of our findings and the results of previous phenotypic studies it is formally proposed that the bacteria from pigs should be designated a new species, *Actinomyces hyovaginalis*.

During the course of a study of *Actinomyces*-like bacteria isolated from purulent lesions in pigs, two groups of organisms of uncertain taxonomic position were isolated (6). One of these groups, containing organisms isolated exclusively from purulent vaginal discharge and aborted fetuses, phenotypically resembled "*Actinomyces suis*", which was originally described by Franke (5). "*A. suis*" (Franke) was listed as a species incertae sedis in *Bergey's Manual of Systematic Bacteriology* (12) as no type strain exists for this species. Recently, Ludwig et al. (9) proposed that the pig pathogen *Eubacterium suis* (14) should be transferred to the genus *Actinomyces* as *Actinomyces suis* comb. nov. Phenotypically, this organism differs markedly from the description of "*A. suis*" published by Franke and from the unknown porcine isolates (6). In this study we determined the 16S rRNA gene sequence of the unknown *Actinomyces*-like pig bacteria (6) in order to determine the precise phylogenetic position of these organisms.

MATERIALS AND METHODS

Cultivation. Strains were grown in Todd-Hewitt broth (Oxoid) supplemented with 5% sterile horse serum at 37°C. Cultures were harvested at the late exponential phase by centrifugation.

Sequence determination of rRNA genes. Chromosomal DNA was isolated from wet packed cells as described by Pitcher et al. (10). The 16S rRNA gene was amplified by using a polymerase chain reaction and universal primers pA and pH* (1). Approximately 2 µg of chromosomal DNA was amplified in a total volume of 100 µl containing 2 U of *Taq* polymerase (Amersham). The reaction involved 36 cycles consisting of denaturation at 92°C for 2 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 1.5 min. DNA was extracted with chloroform and purified by using a GeneClean II kit (Bio 101) according to the manufacturer's instructions. A qualitative analysis of the DNA fragments was performed by agarose gel electrophoresis. Sequencing of the amplified product was performed by using α-³⁵S-dATP and a Sequenase version 2.0 sequencing kit (United States Biochemical Corp.) as described by Hutson et al. (8). Reaction products were separated on 55-cm

wedge-shaped (0.2 to 0.6 mm) 6% acrylamide–7 M urea gels at 55°C by using an LKB Macrophor 2010 sequencing unit operated at 50 W per gel.

Analysis of sequence data. The sequences generated were aligned with other *Actinomyces* sequences (9, 13), and similarity values were determined by using the Beckman Microgenie program (11). Evolutionary distance values (K_{nuc}) were calculated (7), and an unrooted phylogenetic tree was constructed by using the distance matrix method (3, 4).

Nucleotide sequence accession number. The nucleotide sequence of the 16S rRNA of strain BM 1192/5^T (T = type strain) has been deposited in the GenBank (EMBL) data base under accession number X69616.

RESULTS AND DISCUSSION

The 16S rRNA gene of porcine isolate BM 1192/5^T was amplified in vitro, and its nucleotide sequence was determined directly (8). The derived 16S rRNA primary structure is shown in Fig. 1. The sequence consisted of 1,496 nucleotides (ranging from position 30 to position 1542; *Escherichia coli* numbering [2]). The sequence of a short fragment (ca. position 50 to position 450, which includes variable regions V1 and V2) of the 16S rRNA of a second porcine isolate (strain BM753/6) was also determined. This sequence was found to be identical to the sequence of strain BM 1192/5^T, confirming the genetic homogeneity of the group II porcine isolates of Hommez et al. (6). The sequence of strain BM 1192/5^T was compared with a data set containing more than 50 16S rRNA sequences of high-G+C-content gram-positive bacteria. The highest levels of 16S rRNA sequence similarity were with members of the genus *Actinomyces*. The similarity values for a ca. 1,332-nucleotide region (ranging from position 107 to position 1464 [*Escherichia coli* numbering system]) of the strain BM 1192/5^T sequence and the sequences of previously described *Actinomyces* species are shown in Table 1. Approximately 100 nucleotides at the 5' end of the rRNA were not included in these similarity calculations because of incomplete sequence data for some reference *Actinomyces* species. The closest relative of the porcine isolate was *Actinomyces odontolyticus*, which exhibited a 16S rRNA similarity value of ca. 95%. Considerably lower similarity values were observed with other *Actinomyces* species (ca. 89 to 91%). A phylogenetic

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CGAACGCTGGCGCGTCTTAACACATGCAAGTCGAACGCACGGCCTTGGGGTTTTCTTGGGGTGGTGAGTGGCGAACGGGTGAGTAACACGTGAGTA
 ACCTNCCCTCTTCTTTGGGATAACGGTCGGAACCGGNTGCTAATACTGGATATTCACACATTGTCGCATGGTGGTGTGTGGAAAGGTTTTCTGGTGGG
 GGATGGGCTCGCGGCTATCAGCTTGTGGTGGGGTATGGCTTACCAAGGCTTTGACGGGTAACGGGCTGAGAGGGTGACCGGTACATTGGGACTGA
 GATACGGCCAGACTCTACGGGAGGAGCAGTGGGGAATTTTGCACAATGGACGGAAGTCTGATGCAGCGACGGCGGTGAGGGATGGAGGCCTTCGGG
 TTGTGAACCTCTTTCGCTCATGGTCAAGCCGACGCTTGGGTGTGGTGGGGTAGTGGGTAAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCCGGTA
 ATACGTAGGGCGCGANCGTTGTCCGGAATTATTGGGCGTAAAGGGCTTGTAGGCGGTTGGTTGCGTCTGCCGTGAAAATCCTCTGGCTTAACCTGGGGCG
 TGGGTGGGTACGGCCTGGCTTGTAGTGGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGG
 CGGGTCTCTGGCCGTTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCTGGTAGTCCATNCTGTAACGTTGGGCACTAGGT
 GTGGGGGCCACCGTGGTTTCTGCGCCGTAGCTAACGCTTTAAGTNCCTGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGG
 CCCGCAAGCGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCACCCAGAGCCTCCAGAGATGGGGTGCATT
 TGGTTGGGGTGTGCAAGTGGTGCATGGTTGTCTGTCAGCTCGTGTCTGAGATGTTGGGTAAGTCCCGCAACGAGCGCAACCCCTTGCCTATGTTGCCAG
 CACGTTGTGGTGGGACTCGTGGGGGACTGCCGGGTAACTCGGAGGAAGTGGGGATGACGTCAAATCATATGCCCTTATGCTAGGGCTTCACGC
 ATGCTACAATGGCTGGTACAGAGGGTTGCGATGCTGTGAGGCGGAGCGAATCCCTTAAAGCTGGTCTCAGTTCCGATCGAAGTCTGCAACTCGACTTCGT
 GAAGGTGGAGTCGCTAGTAATCGCAGATCAGCAACGCTCGGGTGAATACGTTCTCGGGCCTGTACACACCGCCGTCACGTACGAAAGTTGGTAACAC
 CCGAAGNCATGGCTAACCGTTTTCCGGGNGAGTGGTCGAGGTGGGATTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGG

FIG. 1. Nucleotide sequence of derived 16S rRNA of *A. hyovaginalis*.

tree depicting the interspecific relationships of the porcine isolate and other *Actinomyces* species is shown in Fig. 2.

A comparative 16S rRNA sequence analysis clearly showed that the unknown bacterium is a member of the genus *Actinomyces*. The sequence analysis results demonstrated that the porcine bacterium and *Actinomyces pyogenes*, which also occurs in pigs, are genealogically only remotely related. The two groups differ in a large number of phenotypic characteristics (6). It was also evident from this analysis that the unknown *Actinomyces* taxon is genotypically distinct from the porcine pathogen designated *A. suis* (formerly *Eubacterium suis*) by Ludwig et al. (9). The unknown porcine isolates differ from *A. suis* as described by Ludwig et al. (9) in producing acid from L-arabinose, salicin, sucrose, D-xylose, D-mannose, and D-fructose. The unknown bacterium also differs from *A. suis* in hydrolyzing esculin, reducing nitrate, and not having urease activity. In view of the phenotypic and phylogenetic distinctiveness of the porcine bacterium, a new species, *Actinomyces hyovaginalis*, is proposed for this organism.

Description of *Actinomyces hyovaginalis* sp. nov. *Actinomyces hyovaginalis* (hy.o.va.gi.na'lis. Gr. n. *hys*, pig; L. n. *vagina*, sheath, vagina; N. L. masc. adj. *hyovaginalis*, pertaining to the vagina of a pig). The following morphological and physiological description is based on a study of 14 strains (6).

The cells are predominantly diptheroidal, and the rods are arranged in clusters or V or Y forms; coccoid elements may

occur. Colonies are flat with outrunning edges, a trait which is particularly pronounced after anaerobic incubation or incubation under CO₂ on horse blood agar. Nonhemolytic. Catalase negative. Acid is produced from adonitol, L- and D-arabinose, N-acetylglucosamine, L-arabitol, galactose, glucose, gluconate, fructose, D-mannose, maltose, D-lyxose, salicin, sucrose, turanose, and D-xylose. Some strains produce acid from cellobiose, inositol, lactose, and β-methyl-xyloside. Acid is not produced from D-arabitol, dulcitol, α-methyl-D-glucoside, β-gentiobiose, inulin, melezitose, melibiose, raffinose, rhamnose, sorbitol, D-tagatose, trehalose, L-xylose, and xylitol. Alkaline phosphatase, α-galacto-

FIG. 2. Unrooted tree showing the phylogenetic interrelationships of *A. hyovaginalis* and some other *Actinomyces* species. The tree is based on a comparison of ca. 1,332 nucleotides.TABLE 1. Levels of 16S rRNA sequence similarity between *A. hyovaginalis*, some other *Actinomyces* species, and *Bifidobacterium bifidum*

Species	% 16S rRNA sequence similarity with ^a :						
	<i>A. hyovaginalis</i>	<i>A. bovis</i>	<i>A. naeslundii</i>	<i>A. odontolyticus</i>	<i>A. pyogenes</i>	<i>A. suis</i>	<i>A. viscosus</i>
<i>A. bovis</i>	90.7						
<i>A. naeslundii</i>	89.7	94.1					
<i>A. odontolyticus</i>	94.9	91.6	90.4				
<i>A. pyogenes</i>	88.9	88.1	87.1	88.7			
<i>A. suis</i>	89.0	87.5	86.9	88.1	91.0		
<i>A. viscosus</i>	90.4	94.5	96.8	91.5	88.1	87.8	
<i>Bifidobacterium bifidum</i>	85.1	85.4	84.4	85.6	84.5	83.6	85.1

^a Similarity values are based on a comparison of ca. 1,332 nucleotides.

sidase, β -galactosidase, and leucine arylamidase positive. Pyrrolidonylarylamidase, pyrazinamidase, and β -glucuronidase negative. Esculin, hippurate, and Tween 80 are hydrolyzed. Arginine, gelatin, and urea are not hydrolyzed. Nitrate is reduced to nitrite. The cell wall murein type is type A5 β (Orn-Lys-Glu). The DNA base composition is 63 mol% G+C (as determined by the thermal denaturation method). The type strain is strain NCFB 2983 (= BM 1192/5). The habitat is the porcine genital tract.

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