Phylogenetic Analysis of the Genus *Actinomyces* Based on 16S rRNA Gene Sequences: Description of *Arcanobacterium phocae* sp. nov., *Arcanobacterium bernardiae* comb. nov., and *Arcanobacterium pyogenes* comb. nov.

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A systematic phylogenetic analysis of the genus *Actinomyces* was performed. The 16S rRNA gene sequences of 13 *Actinomyces* species, an unnamed *Actinomyces* strain (ATCC 49338), and an *Actinomyces*-like isolate from sea mammals were determined. Comparative sequence analysis with closely related taxa revealed phylogenetic diversity and internal structure within the genus *Actinomyces*. In addition, some members of other genera (viz., the genera *Arcanobacterium*, *Mobiluncus*, and *Rothia*) were shown to be phylogenetically intermixed with the *Actinomyces* species. It was evident from both distance and tree topology considerations that the genus *Actinomyces* is in urgent need of taxonomic revision and requires subdivision into several genera. Based on the results of the present study it is proposed that *Actinomyces bernardiae* and *Actinomyces pyogenes* be assigned to the genus *Arcanobacterium* as *Arcanobacterium bernardiae* comb. nov. and *Arcanobacterium pyogenes* comb. nov., respectively. In addition, a new species, *Arcanobacterium phocae*, is proposed for *Actinomyces*-like bacteria isolated from seals.

The genus *Actinomyces* was created by Harz in 1877 for *Actinomyces bovis*, the causative agent of bovine actinomycosis. Originally, the description of the genus was solely based upon the morphology of actinomycete elements in the granules produced by *Actinomyces bovis* in bovine infections, as it was some years before the organism was successfully cultured (19, 20). Subsequently, cultural, physiological, and other characteristics were incorporated into the definition of the genus, and many new species were assigned to the genus. Today, the genus *Actinomyces* contains 18 species which are gram-positive, non-acid-fast, nonmotile, non-spore-forming, filamentous or diphtheroidal, rod-shaped organisms with high DNA G+C contents. They require increased CO₂ for optimal growth and have a fermentative type of metabolism (20). The taxonomy of the genus *Actinomyces* is generally, if not universally, acknowledged as being unsatisfactory. Traditional phenotypic tests used for delineating species often lack discrimination and/or are variable for many species. Consequently, reliable identification of many species and recognition of new taxa are often very problematic. Furthermore, the genus *Actinomyces* as currently defined embraces such a phenotypically broad range of species that it is now thought that some organisms may be members of subgenera or even independent genera (19, 20). For example, the genus *Arcanobacterium*, which was described by Collins et al. (4) to accommodate the species *Corynebacterium haemolyticum*, closely resembles some *Actinomyces* species (e.g., *Actinomyces pyogenes*). Currently, clear phenotypic distinction between the genera *Actinomyces* and *Arcanobacterium* is not possible.

Knowledge of the phylogenetic structure within the genus *Actinomyces* has improved in recent years because of the advent of 16S rRNA sequencing (9, 10, 22). Nevertheless, infor-

mation is still somewhat fragmentary because many species have not been sequenced yet. Furthermore, some sequences are based on reverse transcriptase analysis results, which are prone to considerable sequencing errors and ambiguities. Despite these limitations, 16S rRNA analyses have already facilitated the recognition of several new *Actinomyces* species (viz., *Actinomyces bernardiae*, *Actinomyces hyovaginalis*, *Actinomyces neuui*, *Actinomyces radingae*, *Actinomyces turicensis* [2, 9, 10, 23]) and are particularly valuable when phenotypic approaches are of limited suitability. Because of the incomplete picture of the phylogenetic structure within the genus *Actinomyces* and the enormous value of discriminatory ribosomal DNA sequences for recognition and identification of new species, we compared the 16S rRNA gene sequences of all currently described *Actinomyces* species and in this paper report the results of a detailed phylogenetic analysis. In addition we used the rRNA sequence data to clarify the taxonomic position of some *Actinomyces*-like organisms isolated from sea mammals. As a result of these studies, a new species, *Arcanobacterium phocae*, is described, and it is proposed that *Actinomyces bernardiae* and *Actinomyces pyogenes* should be reclassified as *Arcanobacterium bernardiae* comb. nov. and *Arcanobacterium pyogenes* comb. nov., respectively.

MATERIALS AND METHODS

Cultures and cultivation. The origins of the six strains from seals and their clinical sources are shown in Table 1. All animals came from coastal waters around Scotland. Isolation was made on Columbia agar (Difco, East Molesey, United Kingdom) supplemented with 5% citrated sheep blood incubated at 37°C in an atmosphere containing 10% CO₂ for 24 h.

For 16S rRNA sequence analysis, reference *Actinomyces* strains were cultivated on yeast-glucose-phosphate broth (11) supplemented with 1% lactose and 5% sterile horse serum in an anaerobic atmosphere containing 10% CO₂, 5% H₂, and 85% N₂. The six isolates from seals (M2122/93/7, M847/93/1, M2464/93/8, M2546/94/1, M1590/94/3^T [T = type strain], and M186/93/3) were cultivated on nutrient agar (Oxoid, Basingstoke, United Kingdom) supplemented with 5% sterile sheep blood at 37°C under anaerobic conditions (GasPak system; BBL Microbiology Systems, Cockeysville, Md.).

Biochemical tests. The six isolates from seals and reference strains of *Arcanobacterium haemolyticum* (CIP103370^T, CIP81-16) were biochemically char-

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TABLE 1. Sources of *Arcanobacterium phocae* sp. nov.

Strain ^a	Host animal	Tissue source(s)
M186/93/3	<i>Halichoerus grypus</i> (grey seal)	Lung, spleen, intestine, mesenteric and submaxillary lymph nodes
M847/93/1	<i>Phoca vitulina</i> (common seal)	Lung
M1590/94/3 ^T (= DSM 10002 ^T)	<i>Phoca vitulina</i> (common seal)	Lung
M2122/93/7	<i>Halichoerus grypus</i> (grey seal)	Mouth lesion
M2464/93/8 (= DSM 10003)	<i>Halichoerus grypus</i> (grey seal)	Nasal swabs
M2546/94/1 (= DSM 10004)	<i>Phoca vitulina</i> (common seal)	Peritoneal fluid

^a M, SAC Veterinary Services Inverness, Inverness, United Kingdom; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.

acterized by using the API ZYM (enzymatic profiling), API 50CH (carbohydrate fermentation), and API CORYNE (identification of coryneform bacteria) systems according to the instructions provided by the manufacturer (API bio-Mérieux, Basingstoke, United Kingdom).

Determination of 16S rRNA gene sequences. Bacterial DNA was isolated by a standard miniprep method, as described by Ausubel et al. (1). A large fragment of the 16S rRNA gene was amplified by the PCR by using universal primers pA (5'-AGAGTTTGATCTGGCTCAG; positions 8 to 27 [*Escherichia coli* numbering]) and pH* (5'-AAGGAGGTGATCCAGCCGCA; positions 1541 to 1522) as previously described (14). The PCR products were purified by using a Prep-A-Gene kit (Bio-Rad, Hercules, Calif.) according to the manufacturer's instructions and were sequenced by using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Inc., Foster City, Calif.) and a model 373A automatic sequencer (Applied Biosystems, Inc.). The following sequencing primers were used: 5'-ACTGCTGCCTCCCGTAGGAG (positions 358 to 339), 5'-CTCCTACGGGAGGCAGCAGT (positions 339 to 358), 5'-TTACCGCGGCTGCTGGCACGT (positions 536 to 516), 5'-CGTGCCAGCAGCCGCGGT AAT (positions 517 to 537), 5'-GTTGCGCTCGTTGCGGGACT (positions 1109 to 1090), 5'-AGTCCCGCAACGAGCGCAAC (positions 1090 to 1109), and 5'-CGGTGTGTACAAGGCC (positions 1398 to 1382).

Analysis of sequence data. The 16S rRNA gene sequences which we determined and previously published sequences of other high-G+C-content actinomycetes (obtained from the EMBL Data Library) were aligned by using the program PILEUP (6), and the alignment was corrected manually. Distance matrices were produced by using the DNADIST program of the PHYLIP package (7). Unrooted phylogenetic trees were constructed with the algorithm of Fitch and Margoliash (8) and by using the neighbor-joining method of Saitou and Nei (18). The statistical significance of the groups obtained was assessed by bootstrapping (500 replicates) by using the programs SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE (7).

Nucleotide sequence accession numbers. The 16S rRNA gene sequences determined in this study have been deposited in the EMBL Data Library under the accession numbers shown in Table 2.

RESULTS

The almost complete 16S rRNA sequences of 13 validated *Actinomyces* species, an unnamed *Actinomyces* strain (ATCC 49338), and an *Actinomyces*-like isolate from a seal (M2546/94/1) were determined. These new sequences were compared with other rRNA sequences available from the EMBL and GenBank data libraries. The 16S rRNA sequences of four of the *Actinomyces* species examined in this study had been determined previously by reverse transcription (RT). Although all of the RT-determined sequences contained approximately 2 to 3% ambiguities, with the exception of *Actinomyces israelii*, they generally showed good agreement (ca. 1% sequence difference) with the newly determined sequences. The previously published RT-determined 16S rRNA sequence of *Actinomyces israelii* DSM 43020 (22), however, displayed >13% sequence difference with the sequence determined for *Actinomyces israelii* CIP 103259^T in the present study. To minimize possible errors in phylogenetic analysis due to sequencing ambiguities, the RT-determined sequences were not used in tree construction. The sequences determined in this study were aligned with the 16S rRNA sequences of other *Actinomyces* species and closely related organisms. In the comparative phylogenetic analysis approximately 100 bases were omitted from the 5' end of the rRNA because of alignment uncertainties in hypervari-

able region V1. Derived distance matrices were used to elucidate the phylogenetic relationships of members of the genus *Actinomyces* and their close relatives. A phylogenetic tree constructed by using the neighbor-joining method, including the confidence values of branching, is shown in Fig. 1. To ascertain whether the inclusion of highly variable moieties of the rRNA affected the inferred phylogenies, the analyses were repeated but variable regions V2, V3, V5, and V6 were excluded from the multiple alignment and tree construction (Fig. 2). The two sets of analyses produced essentially similar trees, and all significant relationships (bootstrap values, ≥90%) shown in Fig. 1 were confirmed by using sequences with reduced variable regions.

The phylogenetic placement of six *Actinomyces*-like organisms (*Arcanobacterium phocae*) from sea mammals was also investigated. The six isolates examined were similar to each other; they were gram-positive, short, diphtheroid organisms which displayed strong β-hemolysis on sheep blood. The biochemical properties of these six strains are summarized in Table 3. In order to investigate the genotypic relatedness of the

TABLE 2. *Actinomyces* and *Arcanobacterium* strains analyzed and their 16S rRNA sequence accession numbers

Species or subspecies	Strain ^a	EMBL accession no. (reference)
<i>Actinomyces bernardiae</i>	DSM 9152 ^T	X79224 (9)
<i>Actinomyces bovis</i>	NCTC 11535 ^T	X81061 (this study)
<i>Actinomyces denticolens</i>	NCTC 11490 ^T	X80412 (this study)
<i>Actinomyces georgiae</i>	DSM 6843 ^T	X80413 (this study)
<i>Actinomyces gerencseriae</i>	DSM 6844 ^T	X80414 (this study)
<i>Actinomyces hordeovulneris</i>	CIP 103149 ^T	X82448 (this study)
<i>Actinomyces howellii</i>	NCTC 11636 ^T	X80411 (this study)
<i>Actinomyces hyovaginalis</i>	NCFB 2983 ^T	X69616 (2)
<i>Actinomyces humiferus</i>	ATCC 25174 ^T	X82449 (this study)
<i>Actinomyces israelii</i>	CIP 103259 ^T	X82450 (this study)
<i>Actinomyces meyeri</i>	CIP 103148 ^T	X82451 (this study)
<i>Actinomyces naeslundii</i>	NCTC 10301 ^T	X81062 (this study)
<i>Actinomyces neuii</i> subsp. <i>anitratus</i>	DSM 8577 ^T	X71862 (9)
<i>Actinomyces neuii</i> subsp. <i>neuii</i>	DSM 8576 ^T	X71861 (9)
<i>Actinomyces odontolyticus</i>	NCTC 9935 ^T	X80504 (22a)
<i>Actinomyces pyogenes</i>	NCTC 5224 ^T	X79225 (this study)
<i>Actinomyces radingae</i>	DSM 9169 ^T	X78719 (23)
<i>Actinomyces slackii</i>	NCTC 11923 ^T	X82452 (this study)
<i>Actinomyces</i> sp.	ATCC 49338 ^T	X81063 (this study)
<i>Actinomyces suis</i>	DSM 20639 ^T	S83623 (16)
<i>Actinomyces turicensis</i>	DSM 9168 ^T	X78720 (23)
<i>Actinomyces viscosus</i>	NCTC 10951 ^T	X82453 (this study)
<i>Arcanobacterium haemolyticum</i>	NCTC 9697	X73952 (22a)
<i>Arcanobacterium phocae</i>	DSM 10004	X97049 (this study)

^a ATCC, American Type Culture Collection, Rockville, Md.; CIP, Collection of Institut Pasteur, Paris, France; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; NCFB, National Collection of Food Bacteria, Reading, United Kingdom.

FIG. 1. Unrooted tree showing the phylogenetic relationships of members of the genus *Actinomyces* and some related species. The tree, constructed by using the neighbor-joining method, was based on a comparison of approximately 1,320 nucleotides. Bootstrap values, expressed as percentages of 500 replications, are given at branch points.

six isolates, partial 16S rRNA gene sequencing was performed. The sequence of a short stretch of approximately 800 bases, including variable regions V1 to V4, which are of discriminatory value for species recognition, was determined. The 16S rRNAs of all of the isolates were found to be identical in these regions, which demonstrated that there was a close genealogical affinity among the strains. Because of the close phenotypic and genotypic resemblance of these isolates, the complete 16S rRNA sequence of a single strain (M2546/94/1) was determined and subjected to phylogenetic analysis. It was evident from both tree topology and distance considerations that this isolate has a close phylogenetic affinity with *Arcanobacterium haemolyticum* (Fig. 1). Bootstrap analysis data (bootstrap value, 100%) confirmed that this association was highly significant.

DISCUSSION

Species currently classified in the genus *Actinomyces* (Harz) display a broad range of morphological, biochemical, and physiological characteristics (19). Consistent with this considerable phenotypic heterogeneity, our comparative gene sequencing clearly showed that the genus *Actinomyces* is phylogenetically very diverse. Among the 18 currently validated species, 16S rRNA sequence divergence values of up to ca. 15% were observed (Table 4). Both distance calculations and treeing analyses revealed considerable phylogenetic diversity within this

so-called genus, with some species forming distinct groups while others formed individual lines. Some members of other genera (viz., the genera *Arcanobacterium*, *Mobiluncus*, and *Rothia*) were also found to be phylogenetically intermixed with *Actinomyces* species.

Actinomyces bovis, the type species of the genus, formed a distinct subgroup with *Actinomyces denticolens*, *Actinomyces gerencseriae*, *Actinomyces howellii*, *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces slackii*, *Actinomyces viscosus*, and *Actinomyces* sp. strain ATCC 49338. This subgroup displayed divergence values of <7% and was statistically highly significant (it was recovered in 100% of the bootstrapped trees) (Fig. 1 and 2). Although *Actinomyces bovis* did not exhibit a particularly close affinity with any of the other species, within this subgroup *Actinomyces israelii* and *Actinomyces gerencseriae* did exhibit a highly significant association, as did *Actinomyces naeslundii*, *Actinomyces viscosus*, and *Actinomyces* sp. strain ATCC 49338. The close affinity between *Actinomyces naeslundii* and *Actinomyces viscosus* shown in Fig. 1 and 2 was not in accord with the branching pattern reported by Stackebrandt and Charfreitag (22), in which *Actinomyces naeslundii* clustered with *Actinomyces israelii*. In the latter study (22), *Actinomyces naeslundii* was on a very short branch. Such a lack of ultrametricity was not observed in the present treeing anal-

FIG. 2. Unrooted tree constructed by the neighbor-joining method, showing the phylogenetic relationships of members of the genus *Actinomyces* and some related species. The tree is based on a comparison of ca. 1,100 nucleotides, excluding variable regions V1, V2, V3, V5, and V6. Bootstrap values, expressed as percentages of 500 replications, are given at branch points.

TABLE 3. Biochemical characteristics of *Arcanobacterium phocae* sp. nov.

Characteristic	No. of strains positive/ no. of strains tested
β -Hemolysis in sheep blood	6/6
Catalase	5/6
Nitrate reduction	0/6
Hydrolysis of:	
Esculin	0/6
Gelatin	0/6
Urea	0/6
Fermentation (after 120 h) of:	
Adonitol	0/6
Amygdalin	0/6
D-Arabinose	0/6
L-Arabinose	0/6
D-Arabitol	0/6
L-Arabitol	0/6
Arbutin	0/6
Cellobiose	0/6
Dulcitol	0/6
Erythritol	0/6
Fructose	6/6
D-Fucose	0/6
L-Fucose	0/6
Galactose	6/6
Gentiobiose	0/6
Glucuronate	1/6
2-Keto-gluconate	0/6
5-Keto-gluconate	6/6
N-Acetylglucosamine	6/6
Glucose	6/6
α -Methyl-D-glucoside	0/6
Glycerol	6/6
Glycogen	6/6
Inositol	5/6
Inulin	0/6
Lactose	6/6
D-Lyxose	0/6
Maltose	6/6
Mannitol	2/6
Mannose	4/6
α -Methyl-mannoside	0/6
Melezitose	4/6
Melibiose	0/6
D-Raffinose	0/6
Rhamnose	0/6
Ribose	6/6
Saccharose	6/6
Salicin	0/6
Sorbitol	0/6
Sorbose	0/6
Starch	6/6
D-Tagatose	2/6
Trehalose	5/6
D-Turanose	6/6
Xylitol	0/6
D-Xylose	1/6
L-Xylose	0/6
β -Methyl-D-xyloside	0/6

Continued

TABLE 3—Continued

Characteristic	No. of strains positive/ no. of strains tested
Enzyme activities (API CORYNE test)	
Pyrrolidonyl arylamidase	0/6
Pyrrazinamidase	6/6
Enzyme activities (API ZYM test) ^a	
Cystine arylamidase	4w,2m/6
Leucine arylamidase	6m/6
Valine arylamidase	0/6
Chymotrypsin	0/6
Esterase (C4)	2w,4m/6
α -Fucosidase	0/6
α -Galactosidase	1w,5m/6
β -Galactosidase	6w/6
N-Acetyl- β -glucosaminidase	0/6
α -Glucosidase	6s/6
β -Glucosidase	0/6
β -Glucuronidase	0/6
Esterase-lipase (C8)	2w,4m/6
Lipase (C14)	0/6
α -Mannosidase	0/6
Acid phosphatase	6s/6
Alkaline phosphatase	4w,2m/6
Naphthol-AS-BI-phosphohydrolase	0/6
Trypsin	1w,2m/6

^a w, hydrolyzed substrate, approximately 5 nmol; m, hydrolyzed substrate, approximately 20 nmol; s, hydrolyzed substrate, >40 nmol.

myces sp. strain ATCC 49338, which was originally designated *Actinomyces naeslundii* serotype IV (13), displays >2% 16S rRNA sequence divergence with *Actinomyces naeslundii* NCTC 10301^T. The rRNA data, together with data from earlier genomic DNA-DNA reassociation studies (15), indicate that strain ATCC 49338 represents a new *Actinomyces* species.

A second highly significant subcluster was formed by six species (viz., *Actinomyces georgiae*, *Actinomyces hyovaginalis*, *Actinomyces meyeri*, *Actinomyces odontolyticus*, *Actinomyces radin-gae*, and *Actinomyces turicensis*). Members of this subcluster displayed divergence values of <6.5%, and the group was supported by a bootstrap value of 100%. *Actinomyces hordeovulneris* formed a distinct line which was loosely associated with this subcluster. However, the latter association was not statistically significant. A third and highly significant subgroup (bootstrap value, 98%) was formed by *Actinomyces bernardiae*, *Actinomyces pyogenes*, the *Actinomyces*-like isolate from seals (*Arcanobacterium phocae*), and *Arcanobacterium haemolyticum*. Within this subgroup *Actinomyces bernardiae* was shown to be closely and specifically related to *Actinomyces pyogenes*, which is consistent with an earlier report (10). The unknown isolate from seals showed a similarly close genotypic affinity with *Arcanobacterium haemolyticum*. An observed sequence divergence value of 2.7%, however, demonstrated that the organism from seals represents a species that is genealogically separate from *Arcanobacterium haemolyticum*. *Actinomyces suis* (formerly *Eubacterium suis*) formed a distinct lineage which was peripherally associated with the *Arcanobacterium haemolyticum* subgroup (bootstrap value, 86%). *Actinomyces suis* displays approximately 8 to 11% sequence divergence with the *Arcanobacterium* cluster and clearly represents a separate genus.

The remaining *Actinomyces* species (viz., *Actinomyces hordeovulneris*, *Actinomyces humiferus*, and *Actinomyces neuii*) formed relatively long individual lines and showed no specific affinity with each other or any other described *Actinomyces* species. *Actinomyces neuii* appears to display a loose associa-

ysis. The very close phylogenetic affinity between *Actinomyces naeslundii* and *Actinomyces viscosus* observed in the present study is consistent with the results of phenotypic (12, 21) and DNA-DNA reassociation (5) studies. The close phylogenetic association of *Actinomyces* sp. strain ATCC 49338, *Actinomyces naeslundii*, and *Actinomyces viscosus* is consistent with the close phenotypic resemblance of these organisms (15). *Actino-*

TABLE 4. Levels of 16S rRNA sequence similarity for members of the genus *Actinomyces* and some related taxa

Taxon	% Sequence similarity																										
	<i>Actinomyces bernardiae</i>	<i>Actinomyces bovis</i>	<i>Actinomyces denticolens</i>	<i>Actinomyces georgiae</i>	<i>Actinomyces gerencseriae</i>	<i>Actinomyces hordeovulneris</i>	<i>Actinomyces howellii</i>	<i>Actinomyces humiferus</i>	<i>Actinomyces hyovaginalis</i>	<i>Actinomyces israelii</i>	<i>Actinomyces meyeri</i>	<i>Actinomyces naeslundii</i>	<i>Actinomyces neuii</i> subsp. <i>anitratus</i>	<i>Actinomyces neuii</i> subsp. <i>neuii</i>	<i>Actinomyces odontolyticus</i>	<i>Actinomyces pyogenes</i>	<i>Actinomyces radingae</i>	<i>Actinomyces slackii</i>	<i>Actinomyces</i> sp. strain ATCC 49338	<i>Actinomyces suis</i>	<i>Actinomyces turicensis</i>	<i>Actinomyces viscosus</i>	<i>Arcanobacterium haemolyticum</i>	<i>Arcanobacterium phocae</i>	<i>Mobiluncus curtisii</i>	<i>Rothia dentocariosa</i>	
<i>Actinomyces bernardiae</i>	100																										
<i>Actinomyces bovis</i>	88.5	100																									
<i>Actinomyces denticolens</i>	87.9	94.3	100																								
<i>Actinomyces georgiae</i>	87.9	91.3	91.5	100																							
<i>Actinomyces gerencseriae</i>	87.7	93.4	93.9	91.8	100																						
<i>Actinomyces hordeovulneris</i>	90.5	91.2	90.2	91.7	89.6	100																					
<i>Actinomyces howellii</i>	88.4	95.5	94.5	91.5	95.7	90.2	100																				
<i>Actinomyces humiferus</i>	89.2	88.2	87.4	87.3	87.2	90.2	88.5	100																			
<i>Actinomyces hyovaginalis</i>	88.8	90.1	90.6	95.8	90.8	91.7	90.5	86.9	100																		
<i>Actinomyces israelii</i>	85.9	93.0	93.2	91.0	96.0	88.2	94.5	86.9	89.9	100																	
<i>Actinomyces meyeri</i>	88.6	91.1	90.6	97.3	91.7	92.6	91.0	86.9	96.0	89.7	100																
<i>Actinomyces naeslundii</i>	89.0	95.2	95.3	91.2	95.2	90.7	96.0	88.1	90.3	93.2	91.3	100															
<i>Actinomyces neuii</i> subsp. <i>anitratus</i>	88.9	88.7	87.6	88.5	87.7	88.9	88.1	85.1	89.8	87.1	89.0	88.5	100														
<i>Actinomyces neuii</i> subsp. <i>neuii</i>	89.2	88.8	87.6	87.9	87.4	88.8	87.9	85.3	89.6	86.6	89.0	88.5	99.8	100													
<i>Actinomyces odontolyticus</i>	89.4	90.8	90.2	96.5	91.0	91.9	90.7	87.7	95.8	89.5	97.6	91.0	89.4	89.2	100												
<i>Actinomyces pyogenes</i>	98.3	88.6	88.4	88.4	88.2	90.2	89.0	89.4	89.4	87.3	89.0	89.4	88.9	89.1	89.8	100											
<i>Actinomyces radingae</i>	88.7	89.6	90.0	93.7	89.7	90.6	89.2	86.2	94.6	88.7	94.7	89.7	89.5	88.5	93.9	88.9	100										
<i>Actinomyces slackii</i>	90.6	97.1	96.1	92.9	95.2	92.4	96.6	91.1	92.2	93.9	92.8	97.3	90.0	90.2	92.8	90.8	91.9	100									
<i>Actinomyces</i> sp. strain ATCC 49338	88.5	94.8	95.5	91.1	94.9	90.4	95.4	88.1	90.2	93.0	91.5	97.9	88.3	88.3	90.4	89.0	89.6	97.2	100								
<i>Actinomyces suis</i>	92.1	87.6	88.0	87.9	87.0	88.3	87.3	87.0	88.5	86.7	88.0	87.8	87.8	87.9	88.8	92.0	88.3	89.7	87.6	100							
<i>Actinomyces turicensis</i>	88.8	90.0	89.6	95.8	90.1	92.0	89.9	87.4	96.6	88.9	96.7	90.1	89.3	89.2	96.8	89.5	93.4	92.2	89.5	88.1	100						
<i>Actinomyces viscosus</i>	89.1	95.3	96.0	91.5	94.6	90.7	95.9	88.2	90.6	93.8	91.3	98.8	88.3	88.5	91.1	89.3	89.6	97.2	97.7	87.7	90.1	100					
<i>Arcanobacterium haemolyticum</i>	94.0	88.7	87.7	88.0	87.6	90.2	89.2	88.4	88.5	86.6	88.8	88.7	88.8	88.6	89.4	94.4	87.9	90.2	88.0	89.5	89.1	88.7	100				
<i>Arcanobacterium phocae</i>	95.2	89.4	88.7	89.4	88.1	90.9	89.5	89.5	89.8	87.1	89.7	89.7	89.8	89.8	91.3	95.5	88.6	90.8	89.0	91.3	90.4	90.0	97.3	100			
<i>Mobiluncus curtisii</i>	90.1	90.4	89.2	92.2	89.4	91.9	90.1	89.4	91.5	89.2	92.2	90.5	90.7	90.8	92.2	90.2	90.8	91.9	90.0	89.7	92.1	90.9	90.5	91.0	100		
<i>Rothia dentocariosa</i>	89.7	87.6	87.3	87.1	87.1	89.0	87.8	91.7	87.0	86.0	87.3	88.5	86.2	86.3	89.4	90.3	86.0	91.3	87.9	87.0	87.5	88.1	88.9	90.0	88.1	100	

TABLE 5. Characteristics for differentiation of species of the genus *Arcanobacterium*

Species	Fermentation of:					
	Adonitol	D-Arabitol	Cellobiose	D-Turanose	Gentiobiose	Xylitol
<i>Arcanobacterium bernardiae</i>	+	+	ND	ND	ND	+
<i>Arcanobacterium haemolyticum</i>	V	—	—	—	+	—
<i>Arcanobacterium phocae</i>	—	—	—	+	—	—
<i>Arcanobacterium pyogenes</i>	V	+	V	V	—	+

+, positive; V, variable; —, negative; ND, no data.

tion with *Mobiluncus curtisii*. However, a 16S rRNA sequence divergence value of >9%, together with low bootstrap values, suggests that *Actinomyces neuui* and *M. curtisii* are not members of the same genus. By contrast, the closest phylogenetic relative of *Actinomyces humiferus* is *Rothia dentocariosa*. It is pertinent to note that *Actinomyces humiferus* differs from typical members of the genus *Actinomyces* in several respects (e.g., sensitivity to lysozyme), and its affiliation with the genus has been questioned (19). Although the association between *Actinomyces humiferus* and *R. dentocariosa* was evident in 100% of the bootstrap trees and therefore is highly significant, a sequence divergence value of 8% indicates that the relationship is probably at the suprageneric level.

The results of the comparative 16S rRNA sequence analysis demonstrate that the genus *Actinomyces* as currently defined (19) comprises a phylogenetically diverse group of organisms. The levels of 16S rRNA sequence divergence observed between described species range from approximately 2 to 15%, which is clearly inconsistent with the divergence found in a single genus. As outlined above, several phylogenetically significant subgroups and individual lines are apparent within the genus *Actinomyces*. Furthermore, it is evident from the 16S rRNA data that three validated non-*Actinomyces* genera (viz., the genera *Arcanobacterium*, *Mobiluncus*, and *Rothia*) are phylogenetically intermixed with *Actinomyces* species. There is little doubt that the genus *Actinomyces* as constituted in *Bergey's Manual of Systematic Bacteriology* (19) requires subdivision into several genera. Two of the three major subgroups, as exemplified by *Actinomyces bovis* and *Actinomyces odontolyticus*, clearly represent the nuclei of two different genera, and the third subgroup corresponds to the genus *Arcanobacterium* (4). However, it seems highly probable that the other lineages currently represented by single species (viz., *Actinomyces hordeovulneris*, *Actinomyces humiferus*, *Actinomyces neuui*, and *Actinomyces suis*) also represent separate genera. Thus, based on currently defined species, the genus *Actinomyces* probably embraces no fewer than seven genera. Presumably, this number can be expected to increase as new species conforming to the current definition of the genus are described in the future. Although it would be premature to propose a major taxonomic revision of the genus *Actinomyces*, it is nevertheless apparent in light of current phylogenetic evidence that *Actinomyces bernardiae* (10) and *Actinomyces pyogenes* (17) are misclassified and are members of the genus *Arcanobacterium* (3, 4). Furthermore, the previously undescribed bacterium from seals clearly merits classification as a new species of the latter genus. We therefore formally propose that *Actinomyces bernardiae* and *Actinomyces pyogenes* be assigned to the genus *Arcanobacterium* as *Arcanobacterium bernardiae* comb. nov. and *Arcanobacterium pyogenes* comb. nov., respectively. In addition, a new species, *Arcanobacterium phocae*, is proposed for the isolates from seals. Full descriptions of the new combinations and the new species are given below, and the differential characteristics of *Arcanobacterium* species are shown in Table 5.

Description of *Arcanobacterium bernardiae* comb. nov. The following description is taken from the study of Funke et al. (10). Cells are gram-positive rods, with coccobacilli predominating. Cells are sometimes arranged in clusters, but primary branching is not observed. Cells are nonmotile and do not form spores. Colonies are circular, smooth, and slightly convex with a glassy appearance. Colony diameters range from 0.2 to 0.5 mm after 48 h of incubation in the presence of 5% CO₂ on sheep blood agar. Facultatively anaerobic. Catalase is not produced. Acid is produced from glycerol, ribitol, adonitol, D-glucose, D-fructose, maltose, starch, xylitol, D-arabitol, and 5-keto-gluconate. Most strains ferment erythritol, ribose, glycogen, and L-arabitol. Acid is not produced from xylose, mannitol, α-methyl-D-mannoside, amygdalin, arbutin, sucrose, and 2-keto-gluconate. Leucine arylamidase and α-glucosidase positive. Alkaline phosphatase, cystine arylamidase, chymotrypsin, trypsin, acid phosphatase, α-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, and α-fucosidase negative. Esculin and urea are not hydrolyzed. Nitrate is not reduced. Palmitic and stearic acids are the main straight-chain cellular fatty acids, while oleic acid is the predominant unsaturated fatty acid. The interpeptide bridge of the peptidoglycan is L-alanine-L-lysine-D-glutamic acid (type A5α). The DNA base composition is 63 to 66 mol% G+C. Strains are isolated from various clinical sources, with blood and abscesses predominating. The habitat of the human-derived strains is not known. The type strain is DSM 9152.

Description of *Arcanobacterium pyogenes* comb. nov. The following description is taken from the study of Reddy et al. (17). Gram-positive, nonmotile, non-spore-forming coccobacilli and short rods that occur singly, in pairs (V, T, and palisade formations), or in clusters. Short diphtheroid forms with clubs are also seen. Streptococcal forms in small clumps and short crooked chains are occasionally observed. Cells vary in shape and size (0.2 to 0.9 by 0.3 to 2.5 μm) in different media. Cells from 24-h-old broth cultures are gram positive, but cells from older cultures may be gram variable. The cell wall ultrastructure is typical of gram-positive bacteria. The cell walls are 29 to 30 nm thick and have a characteristic double-track appearance. Pinpoint, β-hemolytic colonies occur on sheep blood agar after 24 h of incubation. The zones of hemolysis are typically two to three times the diameter of the colony. After 48 to 72 h, the colonies (diameter, 0.5 to 1.5 mm) are convex, circular, opaque, white, and soft with entire edges. Colonies develop faster and are bigger (diameter, 1.5 to 3.0 mm) on SFM agar plates. Good growth occurs under aerobic and strictly anaerobic conditions. Metabolism is strictly fermentative. Acid but not gas is produced from glucose, fructose, galactose, lactose, cellobiose, trehalose, maltose, melezitose, mannose, glycogen, dextrin, xylose, and starch. The fermentation of adonitol, arabinose, erythritol, glycerol, sucrose, mannitol, and sorbitol varies with the strain. No acid is produced from amygdalin, esculin, melibiose, raffinose, rhamnose, or salicin. Alanine, arginine, aspartate, glycine, and threonine are not fermented.

Most strains are catalase negative, although one strain has been reported to be catalase positive. Acid clotting and digestion of clots in litmus milk and liquefaction of gelatin are characteristic of all strains. Nitrates are not reduced, and indole is not produced. The optimum temperature is 37°C; the temperature range is 20 to 40°C. Lactic acid is the primary metabolic product in brain heart infusion or tryptose broth with no added HCO_3^- ; acetate is a minor product. Glucose is fermented in the presence of $\text{CO}_2\text{-HCO}_3^-$, and this reaction yields succinate, acetate, formate, and lactate as major products. For each 1 mol of $\text{CO}_2\text{-HCO}_3^-$ fixed, 1 mol of succinate, 1 mol of acetate, and 1 mol of formate are produced. In identical media without added bicarbonate or hemin, lactate is the major product, and smaller amounts of acetate, succinate, and formate are produced. $\text{CO}_2\text{-HCO}_3^-$ is required for growth. Hemin is highly stimulatory or required for growth. Peptides are required for growth even in the presence of a complete complement of 20 amino acids and $(\text{NH}_4)_2\text{SO}_4$. Inositol can replace the peptide requirement for growth. Riboflavin and nicotinic acid are required for optimal growth. Adenine and uracil are required for optimal growth of some strains. Characteristic cell wall sugar components are rhamnose and glucose. The peptidoglycan is type A5 α (L-Lys-L-Ala-L-Lys-D-Glu). No mycolic acids are present. Culture filtrates are fatal to mice and rabbits after intravenous injection. The soluble hemolysin produced is active against human, guinea pig, sheep, horse, and rabbit erythrocytes. Both toxic and hemolytic activities of crude cell extracts are neutralized by antitoxin. The DNA base composition is 56 to 58 mol% G+C. This organism is frequently isolated from a wide variety of pyogenic disease conditions in many species of domestic animals and in humans. Presumably, *Arcanobacterium pyogenes* occurs as a commensal organism on the mucous surfaces of warm-blooded animals. The type strain is ATCC 19411 (= NCTC 5224).

Description of *Arcanobacterium phocae* sp. nov. *Arcanobacterium phocae* (phoc'ae. N.L. gen. n. *phocae*, of *Phoca*, because the organism has been isolated from seals [the genus *Phoca*]). The following description of morphological and physiological characteristics is based on the results of studies of six strains. Gram-positive, nonmotile, non-spore-forming, non-acid-fast coccobacilli and short rods that occur singly, in pairs, or in clusters; primary branching is not observed. Short diphtheroid forms may occur. Colonies on blood agar (incubated for 24 h) are white, tiny, circular, low convex and surrounded by a zone of β -hemolysis which may be two to three times the diameter of the colony. Facultatively anaerobic. Growth is not enhanced by increased concentrations of CO_2 (5 to 10%). The catalase reaction is variable. Metabolism is strictly fermentative. Acid but not gas is produced from glucose. Acid is produced from glycerol, ribose, galactose, glucose, fructose, *N*-acetylglucosamine, maltose, lactose, saccharose, starch, glycogen, D-turanose, and 5-keto-gluconate. Most strains ferment mannose, inositol, trehalose, and melezitose, and a few strains ferment mannitol, D-xylose, D-tagatose, and gluconate. Acid is not produced from erythritol, cellobiose, D-arabinose, L-arabinose, L-xylose, adonitol, β -methyl-D-xyloside, sorbose, rhamnose, dulcitol, sorbitol, α -methyl-mannoside, α -methyl-D-glucoside, amygdalin, arbutin, salicin, melibiose, inulin, D-raffinose, xylitol, gentiobiose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, and 2-keto-gluconate. Nitrate reduction is negative. Gelatin, esculin, and urea are not hydrolyzed. Pyrazinamidase, acid phosphatase, alkaline phosphatase, α -galactosidase, β -galactosidase, α -glucosidase, esterase (C4), esterase-lipase (C8), cystine arylamidase, and leucine arylamidase are produced. Production of trypsin is variable. Pyrrolidonyl arylamidase,

lipase (C14), valine arylamidase, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase, and α -fucosidase are not produced (Table 3). *Arcanobacterium phocae* has been isolated from various tissues and fluids of common seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*), for which its pathological significance is unclear, but it has been recovered in mixed cultures from pneumonic and septicemic seals. Type strain M1590/94/3 has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, as strain DSM 10002. The type strain possesses the characteristics of the species except that it is catalase positive and produces trypsin. M1590/94/3^T ferments inositol, mannitol, mannose, melezitose, trehalose, and D-xylose but does not ferment D-tagatose and gluconate.

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