JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2001, p. 3955–3961 Vol. 39, No. 11
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.11.3955–3961.2001

Phenotypic Identification of *Actinomyces* and Related Species Isolated from Human Sources

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Received 8 May 2001/Returned for modification 23 July 2001/Accepted 21 August 2001

Recent advancements in chemotaxonomic and molecular biology-based identification methods have clarified the taxonomy of the genus *Actinomyces* and have led to the recognition of several new *Actinomyces* and related species. *Actinomyces*-like gram-positive rods have increasingly been isolated from various clinical specimens. Thus, an easily accessible scheme for reliable differentiation at the species level is needed in clinical and oral microbiology laboratories, where bacterial identification is mainly based on conventional biochemical methods. In the present study we designed a two-step protocol that consists of a flowchart that describes rapid, cost-efficient tests for preliminary identification of *Actinomyces* and closely related species and an updated more comprehensive scheme that also uses fermentation reactions for accurate differentiation of *Actinomyces* and closely related species.

The genus *Actinomyces* consists of a heterogeneous group of gram-positive, mainly facultatively anaerobic or microaerophilic rods with various degrees of branching (22). *Actinomyces* species are frequently found as members of the normal microflora, especially in the mouth; but they are also found to be etiologic agents in infections, such as in classical actinomycosis, human bite wounds and abscesses at different body sites, eye infections, and oral, genital, and urinary tract infections (20, 23). Detection of *Actinomyces* species in clinical specimens is important, as it may affect the prognosis and patient management, but identification by conventional biochemical methods can be difficult.

At present, 15 different *Actinomyces* species are found in humans, with 9 found in the oral cavity. *Actinomyces israelii* is known as the key species responsible for classical actinomycosis (23), but it is often isolated in connection with other oral infections, such as peri-implantitis (N. Sarkonen, E. Könönen, E. Tarkka, P. Laine, M. Könönen, and H. Jousimies-Somer, J. Dent. Res. **79**(special Issue)**:**620, abstr. 3813, 2000). *Actinomyces odontolyticus*, *Actinomyces naeslundii*, and *Actinomyces viscosus* are the primary *Actinomyces* species in infants' mouths (21) as well as in early dental plaque (13, 17). *Actinomyces georgiae*, *Actinomyces gerensceriae*, and *Actinomyces meyeri* have been isolated from gingival crevices of periodontally healthy individuals (3, 10). Two new *Actinomyces* species of oral origin have been described recently: *Actinomyces radicidentis* from infected root canals (4) and *Actinomyces graevenitzii* from respiratory tract secretions (19) and infants' saliva (21). During the past few years, several other new species from nonoral sources have been included in the genus *Actinomyces* (6, 7, 12, 16, 27) and some former *Actinomyces* species have been moved to the closely related genera *Arcanobacterium* and *Actinobaculum* (11, 18). The natural habitats of these species have remained obscure, and their clinical relevance as a part of a polymicrobial infection is not fully established (8, 20). The recent changes in nomenclature among the *Actinomyces* species and closely related genera are presented in Table 1.

The identification and differentiation of the gram-positive rods that belong to the genus *Actinomyces* may pose major problems for clinical and oral microbiology laboratories in terms of labor, time, and cost when conventional biochemical methods are used. Furthermore, currently available commercial identification kits do not include most of newer species in their databases. Sophisticated novel methods such as pyrolysis mass spectrometry, amplified 16S ribosomal DNA restriction analysis (8, 14), and 16S rRNA sequencing will greatly help in the identification of the most problematic *Actinomyces* species. Unfortunately, these methods are still available only in research and reference laboratories. The aim of the present study was to create an easily accessible flowchart that describes rapid, cost-efficient tests for the preliminary identification of *Actinomyces* and closely related species and an updated biochemical scheme for the more definite differentiation of *Actinomyces* species and closely related species in routine clinical and oral microbiology laboratories.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study consisted of 19 reference strains from international culture collections (see Table 2), including 15 *Actinomyces* spp., 3 *Arcanobacterium* spp., and 1 *Actinobaculum* sp., and 70 clinical *Actinomyces* isolates from oral and nonoral sources. The clinical isolates, which originated from infants' saliva ($n = 29$), peri-implantitis samples ($n = 20$), submandibular abscesses ($n = 6$), and nonoral sites ($n = 15$, of which 13 were a kind gift from V. Hall, University Hospital of Wales) in adults, were presumptively assigned as members of the genus *Actinomyces* on the basis of the fact that they were gram-positive branching rods and produced succinic acid as the major end product of glucose metabolism, as determined by gas-liquid chromatography. All strains were revived from frozen (−70°C) stocks, subcultured twice, on

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TABLE 1. Recent taxonomic changes among *Actinomyces* and closely related genera from human sources

Year	Current name	Previous nomenclature or taxonomic position	Source	Reference
1994	*Actinomyces neuii* subsp. *anitratus*	CDC group 1 coryneform	Abscess, blood	7
1994	*Actinomyces neuii* subsp. *neuii*	CDC group 1-like coryneform	Abscess, blood	7
1995	*Actinomyces radingae*	*A. pyogenes*-like (APL1)	Polymicrobial infection	27
1995	*Actinomyces turicensis*	*A. pyogenes*-like (APL10)	Polymicrobial infection	27
1995	*Actinomyces europaeus*	New species	Abscess	6
1997	*Actinomyces graevenitzii*	New species	Respiratory tract	19
2000	*Actinomyces radicidentis*	New species	Oral cavity	4
2000	*Actinomyces urogenitalis*	New species	Urogenital tract	16
2001	*Actinomyces funkei*	New species	Blood	12
1997	*Actinobaculum schalii*	New species	Blood	11
1997	*Arcanobacterium bernardiae*	*Actinomyces bernardiae*	Abscess, blood	18
1997	*Arcanobacterium pyogenes*	*Actinomyces pyogenes*	Polymicrobial infection	18

brucella blood agar, and incubated anaerobically at 37°C for 3 to 4 days before testing.

Morphological and biochemical characteristics. The identification of the isolates was performed by established biochemical methods. Briefly, colony morphology was examined under a dissecting microscope, pigmentation was assessed on brucella and rabbit laked blood agar media after incubation for 5 days, and cell morphology was assessed with Gram-stained preparations. Growth patterns in ambient air, in 5% CO_2, and under anaerobic conditions were recorded after prolonged incubation (5 to 10 days). Production of catalase was tested with 15% H_2O_2, and reduction of nitrate was tested by a disk test (24). *Staphylococcus aureus* ATCC 25923 was used as an indicator strain for the CAMP test (synergistic hemolysis) on brucella blood agar. The enzyme tests described in Fig. 1 and 2 and in Table 2 were performed, and incubation was at 36°C for 4 h in air, according to the manufacturer's instructions, with individual diagnostic tablets (Rosco, Taastrup, Denmark). The tests were for hydrolysis of urea and esculin and production of α-fucosidase, α-glucosidase, β-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside [ONPG]), β-*N*-acetyl-glucosaminidase (β-NAG), α-mannosidase, and arginine dihydrolase (the last two tests were conducted only for *A. israelii* and *A. gerencseriae*), L-arabinose, and β-xylosidase (for differentiation of *Arcanobacterium bernardiae* and *Actinomyces turicensis*, see Fig. 2). To assess the uniformity of reactivity by different test systems, the reference strains were additionally tested in parallel with the API ZYM kit (bioMerieux, Marcy l'Etoile, France) by incubation at 36°C for 4 h and a test based on substrates linked to 4-methylumbelliferyl [4-MU; Sigma, St. Louis, Mo.; 20 μl of substrate in *N*-tris

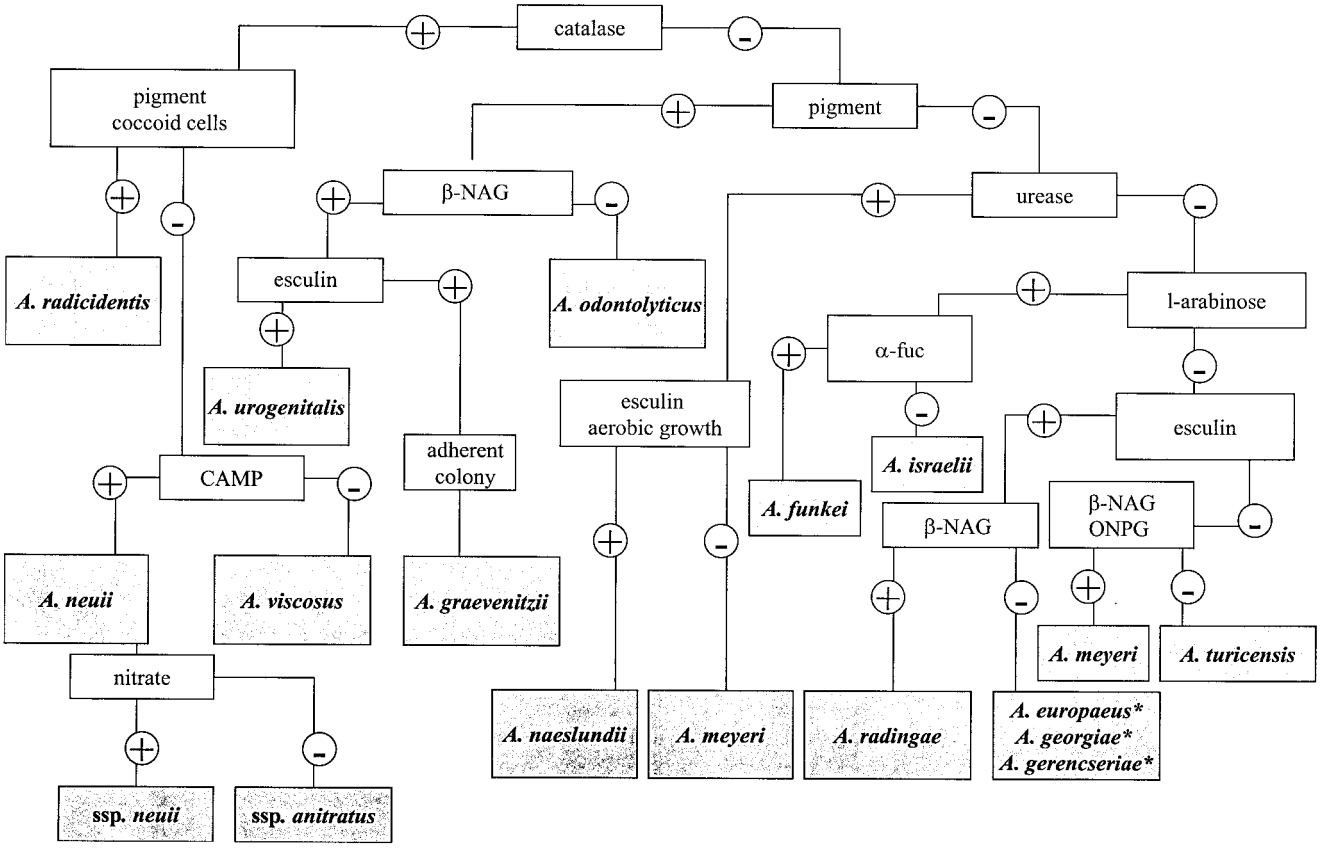

FIG. 1. Flowchart for preliminary identification of *Actinomyces* species. All enzyme reactions were performed with Rosco diagnostic tablets. *, see Table 2; ssp., subsp.; α-fuc, α-fucosidase.

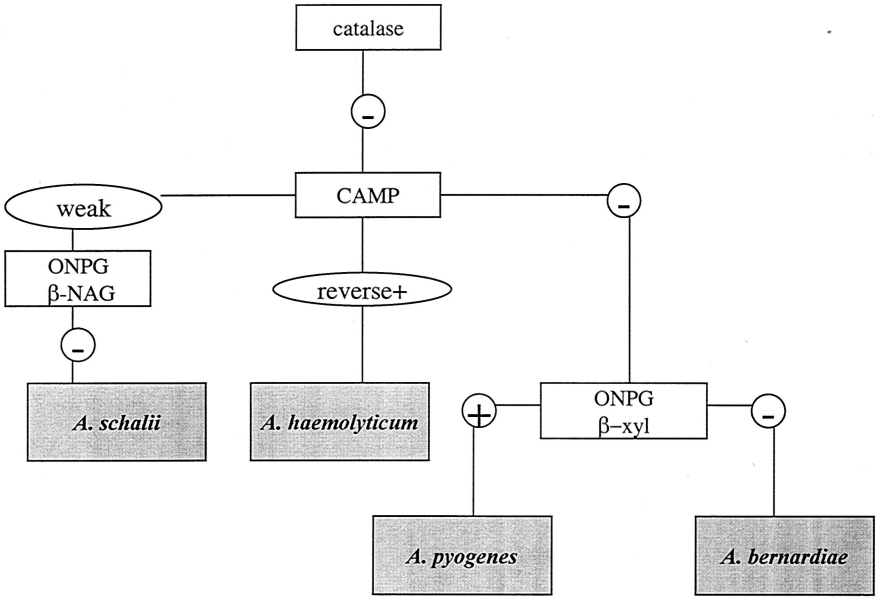

FIG. 2. Flowchart for preliminary identification of *Arcanobacterium* species and *A. schalii*. All enzyme reactions were performed with Rosco diagnostic tablets.

(hydroxymethyl) methyl-2-aminoethanesulfonic acid buffer plus a loopful of bacterial cells from colonies on a blank paper disk (Oxoid, Unipath, Basingstow, England)], with incubation at 36°C for 15 to 30 min (5, 15). Inocula for the testing of enzyme activities were from 3 to 4 days of growth on brucella plates and were adjusted to a cell turbidity equal or greater than a McFarland no. 4 standard in saline for Rosco diagnostic tablets and a McFarland 5 to 6 standard in sterile water for the API-ZYM kit. Tests for the fermentation of arabinose, glucose, maltose, mannitol, raffinose, rhamnose, sucrose, trehalose, and xylose used prereduced, anaerobically sterilized (PRAS) biochemical media incubated at 36°C for a minimum of 5 days (24). If no or scanty growth (<2+) was obtained, 50 μl of 10% Tween 80 was added to 5 ml to promote growth.

RESULTS AND DISCUSSION

The flowchart for the preliminary identification of *Actinomyces* species by a limited number of rapid, cost-effective tests is depicted in Fig. 1. The flowchart for the preliminary identification of *Arcanobacterium* species and *Actinobaculum schalii* is depicted in Fig 2. The more comprehensive identification scheme is presented in Table 2.

The group of gram-positive, non-spore-forming bacilli consisting of several genera can reliably be differentiated from each other only by their metabolic end products. Gas-liquid chromatography should be used for differentiation of these genera. The separation of *Arcanobacterium*, *Actinobaculum*, and *Actinomyces* from other genera can be very difficult without the demonstration of succinic acid as a metabolic end product. In addition to succinic acid, the first two genera produce acetic acid and *Actinomyces* produces considerable amounts of lactic acid. Furthermore, the CAMP test reaction, catalase production nitrate reduction, and the production of β-galactosidase, β-NAG, and β-xylosidase are important tests for discrimination of these three genera from each other (Table 2; Fig. 2). Classically, *Actinomyces* species have been described as branching rods, but many of the recently described species are seldom branching.

In a deviation from the information in the current literature, we noticed that not only *A. odontolyticus* but also three other *Actinomyces* species, namely, *A. graevenitzii*, *A. radicidentis*, and *A. urogenitalis*, produced pigment. All colonies of *A. odontolyticus* showed brown or purple red pigmentation, *A. graevenitzii* showed a dark, almost black, pigmentation, *A. radicidentis* showed brown pigmentation and *Actinomyces urogenitalis* showed a reddish pigmentation on rabbit laked blood agar after incubation for 5 days. However, on brucella agar *A. graevenitzii* colonies were nonpigmented, confirming the original description by Pascual Ramos et al. (19). Colonies of the type strain of *A. radicidentis* were brownish, whereas those of *A. urogenitalis* were pinkish beige on brucella agar (after 5 days) and resembled colonies of *A. odontolyticus* (pinkish, "old rosa"). It is noteworthy that many other *Actinomyces* strains may exhibit some brownish color after prolonged incubation (6 to 11 days) (2); however, this is not usually regarded as real pigment production but, rather, is a result of medium decomposition.

In contrast to smooth and nonadherent colonies of *A. odontolyticus*, *A. radicidentis*, and *A. urogenitalis*, colonies of *A. graevenitzii* were rough and dry and adhered to blood agar, as described previously (19). In addition to deviating colony characteristics, in our study the definite differentiation of *A. odontolyticus*, *A. graevenitzii*, and *A. urogenitalis* was accomplished by testing for production of β-NAG: *A. graevenitzii* and *A. urogenitalis* were positive and *A. odontolyticus* was negative. Furthermore, esculin hydrolysis discriminates *A. graevenitzii* (negative) and *A. urogenitalis* (positive). The strikingly coccoid microscopic morphology of *A. radicidentis* (4) easily separated it from the other three pigment producers. On the other hand, this atypical morphology may lead one to falsely suspect the presence of gram-positive cocci and thus result in failure to identify the species as a member of the *Actinomyces* genus.

Catalase production has previously been considered the key characteristic for *A. viscosus* only. However, two additional

TABLE 2. Identification scheme for *Actinomyces* and closely related species[a]

Species and strain(s)	Pigmentation	Catalase production	Nitrate reduction	CAMP test	Hydrolysis of:		Production of:				Fermentation of:							
					Urea	Esculin	α-Fucosidase	α-Glucosidase	β-NAG	β-Galalactosidase	Arabinose	Maltose	Mannitol	Raffinose	Rhamnose	Sucrose	Xylose	Trehalose
A. europaeus CCUG 32789A^T	−	−	+[b]	−	−	+[b]	−	+	−	+	−	+	−	−	−	−	+[b]	−
A. funkei CCUG 42773^T	−	−	+	+[c]	−	−	+[d]	+	+	+	+[e]	+	−	−	−	+	+	−
A. georgiae																		
ATCC 49285^T	−	−	+	−	−	+	−	+	−	+	−	w	−	−	w	+	+	+
1 clinical strain	−	−	−	−	−	+	−	+	−	+	−	+	−	−	+	+	+	+
A. gerencseriae																		
ATCC 23860^T	−	−	+	−	−	+	−	+	−	+	−	+	+	−[f]	+[f]	+	+[f]	+
12 clinical strains	−	−	v	−	−	+	−	+	−	+	−	+	−	+	−	+	w	v
A. graevenitzii																		
CCUG 27294^T	+[g]	−	−	−	−	−	−	−	+	+	−	+	−	+	−	+	−	−
9 clinical strains	+	−	v	−	−	−	−	v	+	+	−	+	−	−	−	+	w	v
A. israelii																		
ATCC 10049	−	−	+	−	−	+	−	+	−	+	+	+	−	+	+	+	+	+
10 clinical strains	−	−	+	−	−	+	−	+	−	+	+	+	w	+	v	+	+	+
A. meyeri																		
ATCC 35568^T	−	−	−	+[h]	−	−	−	+	+[i]	+[i]	−	+	−	−	−	+	+	−
4 clinical strains	−	−	v	+	−	−	−	+	+	+	+	+	−	−	−	+	+	−
A. naeslundii																		
ATCC 12104^T	−	−	+	−	+	+	−	+	−	+	−	+	−	+	−	+	−	+
8 clinical strains	−	−	v	−	+	v	−	+	−	+	−	+	−	+	−	+	−	+
A. neuii subsp. *neuii* CCUG 32252^T	−	+	+	+	−	−	−	+	−	+	+	+	+	−[j]	w[j]	+	+	−[j]
A. neuii subsp. *anitratus* CCUG 32253^T	−	+	−	+	−	+[k]	−	+	−	+	−	+	+	+	−	+	+	+
A. odontolyticus																		
ATCC 17929^T	+	−	+	−	−	w	+	+[l]	−	+[l]	−	+[m]	−	−	+[m]	+[m]	w	−
10 clinical strains	+	−	+	−	−	v	v	v	−	+	−	+	−	−	+	+	+	−
A. radicidentis CCUG 36733^T	+[n]	+	+	−	−	w	−	+	−	+	−	+	+	+	−	+	−	+
A. radingae																		
CCUG 32394^T	−	−	−	+[o]	−	+	+	+	+	+	+	+	−	−	−	+	+	−
5 clinical strains	−	−	+	+	−	+	+	+	v	+	+	+	−	w	−	+	v	v
A. turicensis																		
CCUG 34269^T	−	−	−	−	−	−	+	+	−	−	−	+	−	−	−	+	+	−[p]
5 clinical strains	−	−	−	−	−	−[+]	v	+	−	−	−	+	−	−	−	+	+	v
A. urogenitalis CCUG 38702^T	+[q]	−	+	−	−	+	−	+	+	+	w	w	−	−[r]	w	+	+	w
A. viscous																		
ATCC 15987^T	−	+	+	−	+	−	−	+	−	+	−	+	−	+	−	+	−	−
6 clinical strains	−	+	+	−	−	−	−	+	−	v	−	+	−	+	−	+	v	−
Arcanobacterium bernardiae CCUG 33419^T	−	−	−	−	−	−	+[s]	+	+[t]	−	w	+	−	−	w	−	−	−
Arcanobacterium haemolyticum ATCC 9345^T	−	−	−	+[Rev]	−	−	+	+	+	+	−	+	−	−	−	+	−	−
Arcanobacterium pyogenes CCUG 13230^T	−	−	−	−	−	−	−	+[u]	−[v]	+	−	+	w	−	−	w	+	+
Actinobaculum schalii CCUG 27420^T	−	−	−	w	−	−	−	+	−	−	+	+	−	−	+	+	+	−

[a] All enzyme reactions in this table are based on results obtained with Rosco diagnostic tablets; fermentation reactions are based on tests with PRAS biochemicals. The other footnotes describe the reactions that are discrepant compared with previously published data. Abbreviations ATCC, American Type Culture Collection, Manassas, Va.; CCUG, Culture Collection, University of Gothenborg, Gothenborg, Sweden; +, positive reaction or result; −, negative reaction or result; w, weak reaction; v, variable reaction; Rev, reverse.
[b] In the original description (6), the type strain is nitrate, esculin, and xylose negative with the API CORYNE kit.
[c] In the original description (12), the strain is not reported to be CAMP test positive.
[d] In the original description (12), the strain is negative with API systems.
[e] In the original description (12), acid is not produced from L-arabinose with API systems.
[f] In the original description (10), the type strain does not ferment rhamnose or xylose and ferments raffinose.
[g] In the original description (19), the strain is nonpigmented.
[h] Wüst et al. (27) reported a negative reaction by the CAMP test.
[i] Schaal (22), in Bergey's manual, reports a negative reaction with the API ZYM system.
[j] In the original description (7), acid is produced from raffinose and trehalose but acid is not produced from L-rhamnose with the API 50CH system.
[k] In the original description (7), the strain is reported to be esculin negative with the API CORYNE kit.
[l] Schaal (22), in Bergey's manual, reports a negative reaction with the API ZYM kit.
[m] Johnson et al. (10) report a negative reaction with PRAS biochemicals.
[n] In the original description (4), the strain is not reported to be a pigment producer.
[o] Vandamme et al. (25) reported a negative CAMP test reaction.
[p] Vandamme et al. (25) reported a positive reaction with a tryptone soy broth plus horse serum (Oxoid) and a variable reaction with the API CORYNE kit.
[q] In the original description (16), the strain was not reported to be a pigment producer.
[r] Nikolaitchouk et al. (16) reported a positive reaction for D-raffinose with the API RAPID ID 32 STREP kit.
[s] In the original description (18), the strain was reported to have a negative reaction with the API ZYM kit.
[t] Funke et al. (6) and Lawson et al. (11) reported negative reactions (the test system was not traceable).
[u] Lawson et al. (11) reported a negative reaction.
[v] Lawson et al. (11) reported a positive reaction.

catalase-producing *Actinomyces* species, *Actinomyces neuii* (two subspecies [7]) and *A. radicidentis* (4), currently exist in the genus. To confirm the separation of these newly described catalase-positive *Actinomyces* species from *A. viscosus*, we recommend testing for pigment production and the CAMP test reaction (Table 2). *A. neuii* can be further differentiated to the subspecies level by the nitrate reaction (7). We also found the type strain of *A. neuii* subsp. *anitratus* to be lipase positive and the type strain of *A. neuii* subsp. *neuii* to be lipase negative, characteristics that may be used for the separation of these two subspecies.

Previous published data on enzyme reactions and fermentation tests can be very difficult to interpret because they are often obtained by using different commercial kits and in-house systems that deviate in their substrate specificities, buffering capacities, and hence, sensitivities. Therefore, to allow direct comparisons, it is of utmost importance to carefully describe in publications the system or method by which the reactions were obtained (see the footnotes to Table 2).

In the present study, to compare different systems for testing, of enzyme activity, the reactivities of α-fucosidase, α-glucosidase, β-galactosidase, and β-NAG were tested for the reference strains in parallel by using individual Rosco tablets, 4-MU-linked substrates as a rapid filter paper spot test, and API ZYM kits. Table 3 presents the reactions obtained by these three test methods. Variation was seen mainly with α-glucosidase reactivities (three negative reactions with 4-MU-linked substrates and two negative reactions and one positive reaction with the API ZYM kit) and β-galactosidase reactivities two negative reactions with the API ZYM kit). In addition to reference strains, the α-fucosidase reactivities of 33 clinical strains of *A. odontolyticus* were tested in parallel by using Rosco tablets and 4-MU-linked substrates. Thirteen (33%) of these *A. odontolyticus* strains were α-fucosidase positive by using Rosco tablets, whereas only one (3%) isolate was positive by the method with 4-MU-linked substrates. The discrepancy may be explained by the substrate avidities or the specificities of the different test systems (1).

The phenotypic differentiation of *A. israelii* and *A. gerencseriae* (previously *A. israelii* serotype II) may pose problems due to a lack of discriminatory tests. Their biochemical reactions are very similar; however, the capability of *A. israelii* to ferment arabinose seems to separate it from *A. gerencseriae* (Table 2). According to the original description by Johnson et al. (10), the majority (89%) of *A. israelii* strains ferment arabinose. In contrast, in a recent study in which species-specific oligonucleotide probes were used for identification of *A. gerencseriae* and *A. israelii*, Jauh-Shun et al. (9) reported that only the reference strain of *A. israelii* fermented arabinose but that none of the clinical strains fermented arabinose. The result may be due to different substrate specificities and buffering conditions in their commercial biochemical test kit (Microbact 24AN system; Pacific Diagnostics) compared to those for PRAS biochemicals. In the present study, the arabinose-fermenting strains were identified as *A. israelii* and arabinose-nonfermenting strains were identified as *A. gerencseriae*. The separation was supported by the finding that all clinical *A. israelii* strains tested were positive for mannitol fermentation and arginine dihydrolase, whereas all strains of arabinose-negative *A. gerencseriae* were negative for these reactions. By using the 4-MU-linked

TABLE 3. Enzyme reactions of three different test methods[a]

Strain	Rosco diagnostic tablets[b]				4-MU-linked substrates[b]				APIZYM kit[c]			
	α-Fucosidase	α-Glucosidase	β-NAG	β-Galactosidase	α-Fucosidase	α-Glucosidase	β-NAG	β-Galactosidase	α-Fucosidase	α-Glucosidase	β-NAG	β-Galactosidase
A. europaeus	−	+	−	+	−	**−**	−	+	**0**	3	0	5
A. funkei	+	+	+	+	+	+	+	+	**0**	4	5	3
A. georgiae	−	+	−	+	−	+	−	+	0	3	0	**0**
A. gerencseriae	−	+	−	+	−	+	−	+	0	5	0	5
A. graevenitzii	−	−	+	+	−	−	+	+	0	**2**	5	5
A. israelii	−	+	−	+	−	+	−	+	0	5	0	5
A. meyeri	−	+	**+**	+	−	+	−	w	0	3	0	3
A. naeslundii	−	+	−	+	−	w	−	w	0	**0**	0	3
A. neuii subsp. *anitratus*	−	+	−	+	−	+	−	+	0	4	0	5
A. neuii subsp. *neuii*	−	+	−	+	−	**−**	−	+	0	5	0	5
A. odontolyticus	+	+	−	+	w	w	−	+	1	1	0	2
A. radicidentis	−	+	−	+	−	+	−	+	0	4	0	5
A. radingae	+	+	+	+	+	+	+	w	5	5	5	5
A. turicensis	+	+	−	−	+	+	−	−	5	1	0	0
A. urogenitalis	−	+	+	+	−	+	+	+	0	5	4	5
A. viscosus	−	+	−	+	−	**−**	−	w	0	1	0	5
A. bernardiae	**+**	+	+	−	−	+	+	−	0	5	5	0
A. haemolyticum	+	+	+	+	w	+	+	+	**0**	3	4	3
A. pyogenes	−	+	−	+	−	+	−	+	0	**0**	0	**0**
A. schalii	−	+	−	−	−	+	−	−	0	5	0	0

[a] The results with major discrepancies are indicated in boldface. β-galactosidase substrates were ONPG for Rosco diagnostic tablets, β-D-galactopyranoside for the 4-MU-linked substrates, and 2-naphthyl-β-D-galactopyranoside for the API ZYM kit.
[b] −, negative reaction; +, positive reaction; w, weak reaction.
[c] Color intensities: 0, negative; 1 to 2, weakly positive; 3 to 5, positive.

fluorogenic substrates, Maiden et al. (15) reported negative α-mannosidase reactivity for *A. israelii* but positive α-mannosidase reactivity for *A. gerencseriae*. This reactivity pattern is listed in the user's guide for Rosco diagnostic tablets as well. Therefore, using Rosco diagnostic tablets, we tested both the type strains and seven clinical isolates representing each species for α-mannosidase reactivity. The type strain and six clinical strains of *A. israelii* were negative, as described previously (15), whereas only the type strain and one clinical strain of *A. gerencseriae* were positive.

In our flowchart (Fig. 1), esculin hydrolysis was used to separate *A. meyeri* and *A. turicensis* (negative) from *Actinomyces radingae* (positive). Furthermore, tests for production of β-NAG glucosaminidase and β-galactosidase were positive for *A. radingae* and negative for *A. turicensis*. Although we found that both type strains were α-fucosidase positive (with Rosco tablets, 4-MU-linked substrates, and the API ZYM kit), our previous experience shows that the production of α-fucosidase is a variable feature of *A. turicensis* among clinical strains. This probably reflects the vast heterogeneity of the former *Actinomyces pyogenes*-like (26) and *A. meyeri*-like (2) organisms that are included in *A. turicensis* (25). The separation of *A. meyeri* from *A. turicensis* is difficult. However, the type strain and four clinical isolates of *A. meyeri* (confirmed by molecular biology-based methods) were positive for both β-galactosidase and β-NAG by the test with Rosco tablets, whereas the type strain and five clinical isolates of *A. turicensis* were negative (see Table 2). Classically, *A. meyeri* has been described as an obligatory anaerobic organism (3). As *A. turicensis* grows both anerobically and aerobically (25), aerotolerance may also be a phenotypic test that can be used to discriminate between these two phenotypically close species. *A. turicensis* may be differentiated from *Arcanobacterium bernardiae* by positivity for xylose fermentation or a rapid β-xylosidase reaction (Fig. 2; Table 2). An unexpected finding was that *Actinomyces funkei*, *A. meyeri*, and *A. radingae* isolates, including the type strains, were positive for the CAMP test reaction.

The rapid enzyme tests that were used in our flowchart (Fig. 1) failed to separate *Actinomyces europaeus*, *A. georgiae*, and *A. gerencseriae* from each other. Instead, the fermentation of raffinose, rhamnose, sucrose, and trehalose could be used for identification (Table 2). According to the original descriptions (6, 10), *A. europaeus* does not ferment any of these carbohydrates, whereas *A. georgiae* ferments rhamnose, sucrose, and trehalose and *A. gerencseriae* ferments raffinose, sucrose, and trehalose. Surprisingly, in our tests, in which we also used the PRAS biochemicals, the type strain of *A. gerencseriae* did not ferment raffinose but was positive for rhamnose fermentation. However, the results for 12 clinical strains tested confirmed the original description of *A. gerencseriae* (10).

Although the identification of these gram-positive rods to the species level possesses major problems, it is important to clarify their roles in both oral and nonoral ecologies and infections. The phenotypic scheme presented here can help to identify the current members of the genera *Actinomyces*, *Arcanobacterium*, and *Actinobaculum* to the species level. In cases of unresolved results with the current scheme for potential actinomycete isolates from invasive sites, such as blood, and from clinically significant infections, the strains should be sent to a reference laboratory for definite confirmation of their identities. Commercial identification kits are widely used in clinical laboratories; however, the lack of data on the novel species interferes with successful precise identification. Therefore, evaluation of the applicability and accuracy of commer-

cial kits for the rapid identification of *Actinomyces* species is in progress in our laboratory with the intent to further facilitate the task of clinical and oral microbiology laboratories.

ACKNOWLEDGMENT

This work was partially funded by the Finnish Dental Society and Research Foundation of Orion Corporation, Espoo, Finland.

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