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Rapid Identification of *Actinomycetaceae* and Related Bacteria

MOGENS KILIAN

Department of Microbiology, Royal Dental College, DK-8000 Aarhus C, Denmark

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Identification of new isolates belonging to the family *Actinomycetaceae* requires extensive numbers of biochemical tests, supplemented with gas-liquid chromatography determination of fermentation end products and, often, analysis of cell wall composition. This paper describes the results of the testing of 162 strains of *Actinomycetaceae* and related taxa for 20 different enzymatic activities including phosphatases, esterases, aminopeptidases, and glycosidases. The results of all tests were read after 4 h of incubation. The results obtained in the study provide significant new information on the biochemical properties of these groups of bacteria. An identification scheme based upon 13 selected tests, which allow the identification of these groups of bacteria within 4 h, is proposed.

The family *Actinomycetaceae* as defined in the eighth edition of *Bergey's Manual of Determinative Bacteriology* (20) comprises five genera: *Actinomyces, Arachnia, Bifidobacterium, Bacterionema,* and *Rothia.* Besides forming part of the normal oral flora of humans and various animals (1), most species of this group of bacteria are well established as human pathogens (1, 5, 21) and have recently attracted considerable attention for their possible implication in periodontal disease and dental caries (21). However, identification of few other groups of bacteria has remained, to the same extent, such a speciality as that of the *Actinomycetaceae.* Although many of the species of *Actinomycetaceae* have been extensively studied, using standard biochemical tests, the applicability of such tests have remained limited because the results are often irreproducible. The identification of new isolates requires a fairly extensive series of biochemical tests, in addition to gas-liquid chromatographic determination of glucose fermentation end products, supported in some cases by analysis of chemical and antigenic composition of cell walls. The performance of biochemical tests on these bacteria requires incubation for 7 to 14 days, and the other procedures can both be time consuming and require specialized methods or equipment.

During recent years, the rapid identification of bacteria has been a growing necessity, and new methods for detection of enzyme activities have been applied to bacteriology. Such methods include the use of chromogenic enzyme substrates which allow the demonstration of a variety of enzymes formed by suspensions of non-growing bacteria (3, 11). Besides being rapid, the interpretation of results obtained by such tests is not complicated by side effects of the multiple reactions that may occur in complex nutrient culture media containing the substrate. A variety of such synthetic substrates are now commercially available and provide a simple way of screening bacteria for enzyme activities. This paper presents the results of the application of the API ZYM system (Societe Analytab Products Inc., La Balme Les Grottes, France; U. S. marketing expected soon) and other rapid tests for individual glycosidases (13) to a collection of representative strains belonging to the family *Actinomycetaceae* and some related taxa. A scheme for the identification of these bacteria within 4 h is proposed.

MATERIALS AND METHODS

Bacterial strains. One hundred and sixty-two strains comprising 121 reference strains and 41 isolates from dental plaque of monkeys (14) and humans (15) were used in the study. The 162 strains included representatives of the genera *Actinomyces, Arachnia, Bacterionema, Bifidobacterium, Rothia, Nocardia, Propionibacterium,* and *Corynebacterium* (Table 1). New isolates were identified with standard biochemical and physiological tests (21) and gas-liquid chromatographic determination of glucose fermentation end products (4).

Cultural conditions. The bacteria were cultured on brain heart agar (Difco Laboratories, Detroit, Mich.) supplemented with yeast extract (Difco), resazurin, vitamin K, hemin, and cysteine-hydrochloride (7). Incubation was performed anaerobically or aerobically with 10% CO_2 according to the optimal growth conditions of the respective taxa. Cultures used for inoculation of the tests were incubated at 37°C until sufficient growth was obtained (2 to 6 days).

API ZYM tests. The API ZYM system, which

TABLE 1. *Strains used in this study*

Species	Strain designation[a]
Actinomyces bovis	NCTC[1] 9429, NCTC 9430, NCTC 9431, ATCC[2] 13683, ATCC 19012, ATCC 19009
A. israelii	NCTC 4860, NCTC 6827, NCTC 6826, NCTC 6830, NCTC 10215, NCTC 10236, ATCC 10049, ATCC 10048, ATCC 12102, ATCC 12103, WVU 307[3], C 65[3], INGB 17[3], A80/77[5], A91/77[5], A332/76[5], A1746/76[5], A309[5], 16.143[7], 16.164[7]
A. naeslundii	NCTC 10301, ATCC 12104, WVU 398a[3], W1096[3], TF 11[3], B 120[4], 2.1[7], 2.27[7], 5.28[7], 6.15[7], 6.23[7], 8.112[7], 9.13[7], 9.180[7], 10.161[7], 15.186[7], 16.179[7], 17.144[7], 5c14[8], Dc8[8]
A. odontolyticus	NCTC 9931, NCTC 9935, ATCC 17982, ATCC 17929, Be 31[3], 3.198[7], 17.2[7]
A. viscosus	
Serotype 1	ATCC 15987, ATCC 15988, WVU 440[3], NY 1[3]
Serotype 2	ATCC 19246, WVU 626[3], WVU 627[4], W 1053[3], WVU 371[3], B25[3], B236[4], RF7[3], H21[3], 6.130[7], 9.14[7], 9.195[7], 9.145[7], 17.8[7], 17.198[7], Ban5[8], Dan68[8], Dan67[8], 5ae94[8]
Arachnia propionica	
Serotype 1	ATCC 15157, WVU 471[9], WVU 1529[9], WVU 449[9], WVU 427[9], WVU 439[9], WVU 1089[9]
Serotype 2	WVU 346[9], WVU 1578[9], WVU 1579[9]
Bacterionema matruchotti	NCTC 10206, NCTC 10592, ATCC 14265, ATCC 14266, J 1337[3], A 1839[3], 9.135[7], 16.23[7], 17.16[7]
Bifidobacterium bifidum	NCTC 10471, NCTC 10472, ATCC 15696
B. adolescentis	ATCC 15703, ATCC 15704, F 167[10]
B. catenulatum	B 669[10], F94[10], F276[10], F320[10]
B. dentium	ATCC 27534, ATCC 27678, B 735[10], B 757[10], B 764[10]
B. ("Actinomyces") eriksonii	ATCC 15423, ATCC 15424
Corynebacterium cervicis	NCTC 10604
C. diphtheriae subsp. *mitis*	NCTC 10349
C. haemolyticum	NCTC 9697, NCTC 9998
C. pyogenes subsp. *hominis*	NCTC 10513
C. ulcerans	NCTC 7907
C. xerosis	NCTC 7238
Nocardia asteriodes	NCTC 630, NCTC 1935, NCTC 4524, NCTC 8595, NCTC 9969, ATCC 10905, ATCC 19247, Mö 5001[6], Mö 5006[6], A 792/77[5], A 410/76[5], A 2201/69[5]
N. brasiliensis	NCTC 10300, ATCC 19019, ATCC 19296, ATCC 19297
N. caviae	NCTC 1934, Mö 5005[6]
N. (Streptomyces) pelletieri	NCTC 3026, NCTC 4162, NCTC 9999, NCTC 10000
Propionibacterium acnes	NCTC 737, ATCC 11827, A 406[5], 1.167[7], 1.171[7], 1.197[7], 2.200[7], 3.124[7], 3.170[7], 4.112b[7], 4.130b[7]
Rothia dentocariosa	NCTC 10207, ATCC 14189, ATCC 14190, ATCC 14191, ATCC 17931, B 28/74[3], B 50/74[3], MG B27[3], 4.19[7], 17.16[7]

[a] Sources of strains: [1] National Collection of Type Cultures, Colindale, London; [2] American Type Culture Collection, Rockville, Md.; [3] G. H. Bowden, MRC Dental Epidemiology Unit, London Hospital, London; [4] E. D. Fillery, MRC Dental Epidemiology Unit, London Hospital, London; [5] J. Bodenhoff, Statens Seruminstitut, Copenhagen, Denmark; [6] K. Holmberg, Statens Bakteriologiska Laboratorium, Stockholm, Sweden; [7] isolated from dental plaque of Tanzanian children (reference 15); [8] isolated from dental plaque of *Macaca irus* monkeys (reference 14); [9] M. A. Gerencser, West Virginia University, Morgantown; [10] V. Scardovi, Instituto di Microbiologica Agraria, Universita di Bologna, Italy.

allows detection of 19 different enzymatic reactions, is a standard device ready for use. The 19 tests performed by this system are: alkaline phosphatase, esterase, esterase lipase, lipase, leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin, chymotrypsin, acid phosphatase, phosphoamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, β-glucosaminidase, α-mannosidase, and α-fucosidase. This battery of tests was applied to all 162 strains to select substrates that could be used to distinguish genera and species. In addition, this screening included the application of the *o*-nitrophenyl-β-D-xylosidase (ONPX) test which detects β-xylosidase activity (13).

Cupules of the API ZYM strip containing the respective substrates were inoculated with 2 drops of a heavy suspension of bacteria in sterile saline (turbidity between MacFarland standards 5 and 6) as recommended by the manufacturers. After incubation aerobically for 4 h at 37°C, the reactions were read after

addition of 1 drop each of API reagents A and B (reagent A: tris(hydroxymethyl)aminomethane, 250 g; HCl, 37%, 110 ml; laurylsulfate, 100 g; distilled water to 1 liter; reagent B: 3.5 g of fast blue BB (Sigma Chemical Co., Saint Louis, Mo.) in 1 liter of 2-methoxyethanol). The intensity of the reactions which developed within 5 min was graded from zero to five with reference to the API ZYM color reaction chart. To determine the reproducibility of the results, a number of strains from each taxon was examined at least twice.

Tests with nitrophenol derivatives. Determination of β-xylosidase activity and the glycosidases included in the API ZYM system was also performed as separate tests by using chromogenic nitrophenol derivatives. The principle of these tests was that applied previously to *Enterobacteriaceae* (13). The compositions of the respective glycosidase test substrates are provided in Table 2. The pH values of the buffers used represent the respective pH optima for the color reactions, determined as previously described (13). A single strain of *Actinomyces viscosus* was used in the pH assays of α- and β-galactosidase, α- and β-glucosidase, and β-xylosidase. For determination of pH optima of the reactions of β-glucuronidase, β-*N*-acetylglucosaminidase, and α-mannosidase, a strain of *Propionibacterium acnes* was used. Finally, a strain of *Actinomyces odontolyticus* was used in the assay for α-fucosidase.

For the standard, routine procedure, one loopful of bacteria (approximately 1 mg of dry weight) was suspended directly in 0.5 ml of the respective enzyme substrates. The development of a yellow color, indicating released nitrophenol, was recorded after incubation for 4 h.

RESULTS

With reference to the API ZYM color chart, the reactions obtained could be divided into negative (grade 0), weak positive (grade 1 to 2), and strong positive reactions (grade 3 to 5). The reactions were consistent on repeated testing, with the exception of minor variations in intensity of the color reaction. These variations never exceeded one grade in the reference color chart.

Twelve of the tests included in the API ZYM system generally yielded strong positive or negative reactions with consistency among strains within the individual taxon. The results obtained with these 12 tests are summarized in Table 3. The remaining seven tests were the tests for alkaline and acid phosphatase, esterase, esterase lipase, lipase, and leucine and cystine aminopeptidases. Only lipase was uniformly absent in all strains. Virtually all of the strains produced esterase, esterase lipase, and leucine aminopeptidase. The reactions obtained in the test for cystine aminopeptidase were generally weak and were not consistent within individual species. Only strains of *Nocardia* and some strains of *Bifidobacterium* produced alkaline phosphatase. In contrast, virtually all strains, except for those of *Arachnia, Rothia,* and *Bifidobacterium,* produced acid phosphatase. However, many of the reactions were weak.

Table 3 also includes the reactions detected in the ONPX test for β-xylosidase. Activity was demonstrated by strains of *Actinomyces, Bifidobacterium,* and *Nocardia,* except for *Nocardia pelletieri.* With strains of *Actinomyces israelii* and *Nocardia* species, this reaction developed almost instantaneously. With other positive strains, a clear reaction developed within 4 h, although the reactions seen with strains of *Actinomyces bovis* and *A. odontolyticus* remained weak.

In combination with information on catalase activity and oxygen requirement, the 12 selected

TABLE 2. *Composition of glycosidase test substrates*

Enzyme	Substrate	Concn (wt/vol)	Buffer	pH	Abbreviation used
α-Glucosidase EC 3.2.1.20	4-Nitrophenyl-α-D-glucopyranoside (Merck)	0.1%	Phosphate (Sørensen) 0.067 M	8.0	PNPG
β-Glucosidase EC 3.2.1.21	4-Nitrophenyl-β-D-glucopyranoside (Merck)	0.1%	Phosphate (Sørensen) 0.067 M	8.0	NPG
α-Galactosidase EC 3.2.1.22	2-Nitrophenyl-α-D-galactopyranoside (Fluka)	0.1%	Phosphate (Sørensen) 0.067 M	8.0	α-Gal
β-Galactosidase EC 3.2.1.23	2-Nitrophenyl-β-D-galactopyranoside (Merck)	0.1%	Phosphate (Sørensen) 0.067 M	8.0	ONPG
α-Mannosidase EC 3.2.1.24	4-Nitrophenyl-α-D-mannopyranoside (Calbiochem)	0.1%	Phosphate (Sørensen) 0.067 M	8.0	PNPM
β-*N*-acetylglucosaminidase EC 3.2.1.30	4-Nitrophenyl-*N*-acetyl-β-D-glucosaminide (Calbiochem)	0.1%	Phosphate (Sørensen) 0.067 M	8.0	GNAC
β/Glucuronidase EC 3.2.1.31	4-Nitrophenyl-β-D-glucopyranosiduronic acid (Merck)	0.1%	Tris(hydromethyl)-aminomethane-hydrochloride 0.05 M	8.5	PGUA
β-Xylosidase EC 3.2.1.37	2-Nitrophenyl-β-D-xylopyranoside (Koch-Light)	0.1%	Phosphate (Sørensen) 0.067 M	8.0	ONPX
α-Fucosidase EC 3.2.1.51	4-Nitrophenyl-α-L-fucopyranoside (Koch-Light)	0.1%	Phosphate (Sørensen) 0.067 M	8.0	PNPF

TABLE 3. *Scheme for rapid identification of Actinomycetaceae and related bacteria*

Species	No. of strains tested	Test reaction[a]														
		Aerobic growth	Catalase	Valine aminopeptidase	Trypsin	Chymotrypsin	Phosphoamidase	α-Galactosidase	β-Galactosidase	β-Glucuronidase	α-Glucosidase	β-glucosidase	β-*N*-acetylglucosaminidase	α-Mannosidase	α-Fucosidase	β-Xylosidase
Actinomyces bovis	6	−	−	+	−	−	(d)	d	+	−	−	+	+	(d)	−	(+)
A. israelii	20	−	−	d	−	−	−	+	+	−	+	+	−	−	−	+
A. odontolyticus	7	(+)	−	d	−	−	−	+	+	−	+	+	−	−	+	(d)
A. naeslundii	20	+	−	d	−	−	−	+	+	−	+	+	−	−	−	+[1]
A. viscosus										−						
Serotype 1	4	+	+	+	−	−	−	+	+	−	+	+	−	−	+	+[1]
Serotype 2	19	+	+	+	−	−	−	+	+		+	+	−	−	−	+[1]
Arachnia propionica																
Serotype 1	7	(+)	−	−	−	(+)	−	+[1]	+[1]	−	+	−	−	−	−	−
Serotype 2	3	(+)	−	−	−	(+)	−	−	−	−	(d)	−	−	−	−	−
Bifidobacterium bifidum	3	−	−	−	−	−	d	+	+	−	+	d	+	−	−	d
B. adolescentis	2	−	−	−	−	−	(+)	+	+	−	+	+	−	+	−	+
B. catenulatum	4	−	−	−	−	−	(+)	+	+	−	+	+	−	(d)	−	+
B. dentium	5	−	−	−	−	−	d	+	+	d	+	+	−	−	−	+
B. eriksonii	2	−	−	−	−	−	d	+	+	+	+	+	−	−	−	+
Bacterionema matruchotti	9	+	+	−	+	−	−	−	−	−	+	+	−	−	−	−
Rothia dentocariosa	10	+	+	+	+	−	−	−	−	−	+	d	−	−	−	−
Propionibacterium acnes	11	−	d	−	−	−	+[1]	−	+	+	−	−	+	+	−	−
Nocardia asteriodes	12	+	+	d	d	−	+[1]	−	−	−	+	+	−	−	−	+
N. brasiliensis	4	+	+	d	d	−	d	−	d	−	−	+	d	−	−	+
N. caviae	2	+	+	d	+	−	+	−	+	−	+	+	−	−	−	+
N. (Streptomyces) pelletieri	4	+	+	+	d	+	−	−	−	−	d	−	d	−	−	−
Corynebacterium species	7	+	+	−	d	−	d	−	−	−	d	−	−	−	−	−

[a] +, More than 90% positive; −, more than 90% negative; d, 11 to 89% positive; (), reactions weak; [1], some reactions may be weak.

tests of the API ZYM system and the ONPX test provide a basis for identification of the species included in the study. As revealed by Table 3, each genus showed a characteristic basic pattern of reactions. The pattern for the *Bifidobacterium* species was very similar to that of the *Actinomyces* species. However, the two groups of bacteria could be distinguished on the valine aminopeptidase reaction. This reaction also separated *Bacterionema matruchotii* and *Rothia dentocariosa,* which were almost identical in all other reactions. Among the *Actinomyces* species, *A. bovis* showed a distinct pattern of reactions which differentiated this species from the other species of *Actinomyces.* The results obtained for strains of *Arachnia propionica* are tabulated as two separate groups comprising serotype 1 and serotype 2 strains, respectively. The former group of strains showed more pronounced biochemical reactivity. The *Corynebacterium* strains representing six species were differentiated from all other strains by almost uniformly negative reactions. One strain of *Bifidobacterium adolescentis* (F167) showed a reactivity pattern different from the two other strains of that species, including the type strain (Table 3) in that strain F167 was positive for β-glucuronidase and β-glucosaminidase and negative for the reactions of α-galactosidase, β-glucosidase, and β-xylosidase.

In addition to the β-xylosidase test (ONPX), all glycosidase tests were also performed as separate tests with the respective chromogenic nitrophenol derivatives (Table 2). Some of these tests have previously been applied to a study of *Enterobacteriaceae* (13). The pH optima determined for test color reactions common to the two studies were identical, except for that of the α-fucosidase test. In this study the optimal color intensity was achieved at pH 8.0 with a strain of *A. odontolyticus,* whereas the optimum with a strain of *Haemophilus parasuis* was determined previously at pH 7.5. The results obtained with these tests were identical with those obtained in the API ZYM system.

DISCUSSION

A common characteristic of all the biochemical tests used in this study is their simplicity. All tests are based on single synthetic substrates in a buffer to which nongrowing bacteria are exposed. Results of such tests are rapid and highly reproducible and are not affected by differences in oxygen requirements of the bacteria being tested. Furthermore, such tests may be applied, with no alterations, to bacteria with various special growth requirements (12, 22). In contrast, standard biochemical tests are often markedly influenced by the composition of the basal medium used and require incubation for several days, particularly with the relatively slow-growing bacteria considered in this paper.

The API ZYM system has been designed for detecting enzyme activities in tissues, tissue cultures, biological fluids, and microorganisms. The system allows demonstration of 19 different enzymatic reactions within 4 h by using small sample quantities. When applied to bacteria, a heavy suspension of bacterial cells harvested from a pure culture on an agar plate or in a fluid medium is used directly as inoculum. The type of growth produced on agar media by the bacteria considered in this study is easily removed, and preparation of a suitable inoculum suspension presents no problems. Since none of the tests depends on enzyme induction, any medium which provides good growth may be used.

Of the 19 enzymatic reactions detectable by the API ZYM system only one, the lipase reaction, was uniformly negative with all 162 strains. Twelve of the remaining tests proved to be of considerable value for differentiating the species studied. The selected 12 tests were those that showed the most clear-cut results with the highest degree of consistency within the individual species. In combination with the ONPX test for β-xylosidase (13) and information on catalase activity and oxygen requirement, this series of tests provides a basis for identifying species of *Actinomycetaceae* and some related species within 4 h (Table 3).

The strains used in this study belonged primarily to the genera *Actinomyces, Arachnia, Bacterionema,* and *Rothia.* Some of the other species were represented by relatively few strains. However, the strains of all taxa included type strains, whenever available, and strains which have been extensively characterized as part of strain collections studied by numerical taxonomic, serological, and genetic principles (6, 8, 9, 10, 19). With the genera *Nocardia, Propionibacterium,* and *Bifidobacterium,* only such species which may, by their ecology, present differential diagnostic problems were included. Further studies may be required to determine the consistency of the enzyme reactions within some of the taxa represented by only a few strains.

In the identification scheme presented in Table 3, differentiation of some species is based upon a single or a few enzyme reactions. Although the present study seems to indicate a high consistency of these reactions, the performance of a few conventional tests may, in cases of doubtful identification, be required for verification of the diagnosis. However, the results obtained after 4 h with the tests listed in Table 3

allow selection of very specific confirmatory tests.

Most of the characteristics upon which the identification scheme is based are new to the classification of bacteria. Thus, the information provided in Table 3 adds significantly to the general characterization of the species studied. Only the β-galactosidase, the α-galactosidase, and the β-glucosidase tests may be partially equated with conventional fermentation tests. The principle of the β-galactosidase test is well known from the *o*-nitrophenyl-β-D-galactopyranoside (ONPG) test, which has been extensively used to detect potential lactose fermenters among *Enterobacteriaceae* (2, 17, 18), *Haemophilus* (12), and various other gram-negative bacteria (16). Because of the different structure of the natural substrate, lactose, and the ONPG molecule, the results obtained by the two tests do not always agree. Thus, the ONPG test usually gives more positive results (16). In this study, a positive β-galactosidase reaction conformed with the known pattern for lactose fermentation by the species examined (21). However, the consistency of results was considerably higher than that reported for these bacteria when tested by the conventional lactose fermentation test (20, 21). Thus, the frequency of lactose fermentation in strains of the *Actinomyces* species is reported to vary from 23 to 93% (21), whereas all 76 actinomyces strains of this study showed β-galactosidase activity. The same pattern is evident when the α-galactosidase test is correlated to its theoretic equivalent, the raffinose fermentation test. Species which are known to ferment raffinose produced positive results in the α-galactosidase test, but with a higher degree of consistency.

A similar parallel may theoretically be drawn between the test for β-glucosidase and the conventional cellobiose fermentation test. However, the results obtained in this study with the former test did not correlate positively with the known ability of these bacteria to ferment cellobiose. Thus, the β-glucosidase test retains a value as an independent test. Both the α- and β-glucosidase tests have previously been applied to enterobacteria (13) and haemophili (12), but, with few exceptions, the two tests yielded weak and uncertain reactions with these bacteria. With the group of bacteria included in this study, all reactions were strong and clear-cut.

All other tests outlined in Table 3 reveal new information on the enzymatic properties of this group of bacteria. The natural substrates for the enzymes demonstrated by these tests are widely distributed in nature (24). The enzyme β-glucuronidase, which has been found to be an exclusive character of *Escherichia coli* and some *Shigella* species among *Enterobacteriaceae* (13), attacks all the natural β-D-glucupyranosidurates present, for example, in many polysaccharides. β-*N*-acetylglucosaminidase has the potential of cleaving the repeating unit of hyaluronic acid and splitting off terminal acetylglucosamine present in some blood group substances. α-Mannosidase attacks α-D-mannoside bonds present, for example, in ovalbumin and ribonuclease B. Natural glycoproteins such as serum proteins, salivary glycoproteins, and glycoproteins of cellular membranes of which fucose is a component are potential substrates for α-fucosidase. β-Xylosidase has the ability to split β-1-4-linked xylose oligosaccharides, from xylobiose to xylohexaose, which are present, for example, in partial hydrolysate of wheat flour. The two serine proteinases, trypsin and chymotrypsin, are usually considered to be animal enzymes. Only trypsin has previously been demonstrated in strains of *Streptomyces* as a component of Pronase (23). Trypsin hydrolyzes peptides, amides, and esters at bonds involving the carboxyl group of L-arginine or L-lysine. Chymotrypsin also cleaves peptides, amides, and esters, but it does so preferentially at carboxyl groups of the hydrophobic amino acids. Finally, valine aminopeptidase attacks bonds at N-terminal valine molecules in peptides.

The results of this study elucidate some of the taxonomic problems of the family *Actinomycetaceae*. *A. bovis* is known to differ from the other *Actinomyces* species in its habitat, its specific type of cell wall peptidoglycan, and its potential biochemical activity (21). The gap between *A. bovis* and the other *Actinomyces* species is further widened by the results of this study, which show that the biochemical activity of *A. bovis* differs considerably from the basic and common reactivity pattern of all the other *Actinomyces* species (Table 3). *Actinomyces naeslundii* and *A. viscosus,* which by many are considered a single species, also produced identical results in the present tests. However, it is noteworthy that all four of the serotype 1 strains of *A. viscosus* (animal isolates), like strains of *A. odontolyticus,* were characterized by α-fucosidase activity, which was absent from all other strains included in the study.

It has previously been indicated that *A. propionica* may be a heterogeneous species. Thus, Johnson and Cummins (10) found that *A. propionica* strain WVU 346, which was so identified on the basis of its morphological and biochemical characteristics, showed no DNA homology or serological cross-reactivity with the type strain. However, no biochemical differences have supported the subdivision of the species. In this study, strain WVU 346, together with two

other serotype 2 strains, was biochemically distinguished from all serotype 1 strains of *A. propionica*. This may indicate that serotype 2 strains warrant recognition as a separate species.

The API ZYM system is commercially available as a complete set containing 19 tests. Although only 12 of the tests seem to be useful for separating species of *Actinomycetaceae* and some related genera, the system represents an invaluable addition to the battery of biochemical tests used in numerical taxonomic studies of this group of organisms and probably others as well. The system possesses many advantages over conventional diagnostic procedures. Besides revealing more rapid results, the system is relatively inexpensive and can be stored for considerably longer periods of time. For specialized purposes, where smaller numbers of tests are required, the glycosidases may be demonstrated with identical results by using tests based upon nitrophenol derivatives (Table 2).

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