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Characterization of *Actinomyces turicensis* and *Actinomyces radingae* strains from human clinical samples

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Whole-organism protein electrophoresis was used to compare and group unidentified coryneform bacteria resembling *Gardnerella vaginalis* and various *Actinomyces* and *Arcanobacterium* species. The obtained clusters of strains were further characterized by whole-cell fatty acid analysis and a variety of biochemical tests. Species-specific oligonucleotide probes based on 16S rRNA gene sequences were designed. The results demonstrate that the majority of the isolates belonged to *Actinomyces turicensis*; the other strains belonged to *Actinomyces radingae*. The descriptions of both species are emended.

Keywords: *Actinomyces turicensis*, *Actinomyces radingae*, *Arcanobacterium*, protein electrophoresis, *Gardnerella vaginalis*

INTRODUCTION

Catalase-negative, non-spore-forming coryneform bacteria, including species belonging to the genera *Actinomyces*, *Arcanobacterium* and *Gardnerella*, are difficult to classify at the species or even at the genus level (Funke *et al*., 1997), and are often given provisional vernacular names. *Gardnerella vaginalis* is primarily known to cause genitourinary infections in humans (Van Esbroeck *et al*., 1996), while *Actinomyces* and *Arcanobacterium* infections in humans are very diverse and include respiratory tract infections, genitourinary infections, soft tissue infections and bacteraemia. A detailed overview was given recently by Funke *et al*. (1997).

Bacteria resembling *G. vaginalis* and various *Actinomyces* and *Arcanobacterium* species have been isolated as pure cultures or from mixed cultures in a wide range of infections in humans (Brander & Josimies-Somer, 1992; Piot *et al*., 1980; Van Esbroeck *et al*., 1996; Wüst *et al*., 1993, 1995). In the present study, we used whole-cell protein electrophoresis to compare and group representative strains of taxa tentatively named *G. vaginalis*-like (Piot *et al*., 1980), *Actinomyces meyeri*-like (Brander & Josimies-Somer, 1992), *Actinomyces pyogenes*-like (Wüst *et al*., 1993) [*Actinomyces pyogenes* was recently reclassified as *Arcanobacterium pyogenes* (Pascual Ramos *et al*., 1997); however, we will use the original designation for these ten isolates], *Arcanobacterium haemolyticum*-like [own isolates (L.S.)], '*Corynebacterium cervicis*' (Laughton, 1954) (the name '*C. cervicis*' was never validated and is therefore given in quotes), and Centers for Disease Control and Prevention (CDC) group E (Wüst *et al*., 1995). Strains were further characterized by a variety of classical phenotypic tests, whole-cell fatty acid analysis, and a species-specific rDNA-based probe hybridization assay. Five of the *Actinomyces pyogenes*-like bacteria were recently classified as novel *Actinomyces* species: *Actinomyces turicensis* and *Actinomyces radingae* (Wüst *et al*., 1995). Our data allowed us to allocate virtually all of the strains examined to these two species, and to substantiate and emend the descriptions of both species.

METHODS

Bacterial strains. The strains used, their designation as received, and their sources are listed in Table 1. Bacteriological purity was checked by plating and examining living and Gram-stained cells. Cells were grown on Columbia agar (Becton Dickinson) supplemented with 5% horse blood and incubated at 37 °C in a microaerobic

Table 1. Strains studied and their sources

Type strains are indicated by a superscript T. ATCC, American Type Culture Collection, Rockville, MD, USA; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; DSM, DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; IMET, Nationale Kulturensammlung für Mikroorganismen, Zentralinstitut für Mikrobiologie und Experimentele Therapie, Jena, Germany; ITG, Instituut voor Tropische Geneeskunde, Antwerpen, Belgium; LMG, Culture Collection Laboratorium voor Microbiologie, University of Gent, Gent, Belgium; NCFB, National Collection of Food Bacteria, Agricultural and Food Research Council, Institute of Food Research, Aberdeen, UK; NCTC, National Collection of Type Cultures, London, UK.

Strain (name as received)	Other designations	Depositor*	Source/place/year of isolation (if known)*
Actinomyces bovis CCUG 31996^T	NCTC 11535^T	NCTC	Cow lumpy jaw
Actinomyces denticolens CCUG 32758^T	NCTC 11490^T	NCTC	Dairy cattle supragingival plaque (UK)
Actinomyces georgiae CCUG 32935^T	DSM 6843^T	DSM	Human gingivial crevice
Actinomyces gerencseriae CCUG 32936^T	DSM 6844^T	DSM	Human parotid abscess (USA)
Actinomyces hordneovulneris CCUG 32937^T	DSM 20732^T	DSM	Canine ascites fluid
Actinomyces howelii CCUG 32757^T	NCTC 11636^T	NCTC	Dairy cattle dental plaque
Actinomyces hyovaginalis CCUG 35604^T	NCFB 2983^T	NCFB	Porcine vagina (Belgium)
Actinomyces israelii CCUG 18307^T	IMET 10792^T	IMET	Human brain abscess (USA)
Actinomyces meyeri CCUG 21024^T	LMG 16161^T, ATCC 35568^T	ATCC	Human purulent pleurisy
Actinomyces naeslundii CCUG 18310^T	IMET 11091^T	Möller	Human sinus
Actinomyces neuii subsp. *neuii* CCUG 32252^T	GF 97/90^T	Funke	Human infected mammary haematoma (Switzerland)
Actinomyces odontolyticus CCUG 20536^T	NCTC 9935^T, R-750^T	NCTC	Dental caries
Actinomyces odontolyticus APL11 (*Actinomyces pyogenes*-like)	CCUG 32402, LMG 15953	Wüst	Human (Switzerland)
Actinomyces radingae APL1^T (*Actinomyces pyogenes*-like)	CCUG 32394^T, LMG 15960^T	Wüst	Human perineal abscess (Switzerland)
Actinomyces radingae APL3 (*Actinomyces pyogenes*-like)	CCUG 32396, LMG 15955	Wüst	Human pilonidal cyst (Switzerland)
Actinomyces radingae APL12 (*Actinomyces pyogenes*-like)	CCUG 32403, LMG 15992	Wüst	Human pleural empyema (Switzerland)
Actinomyces radingae 9012 (*Arcanobacterium haemolyticum*-like)			Human axillar hydradenitis (The Netherlands)
Actinomyces radingae 9215 (*Arcanobacterium haemolyticum*-like)			Human pilonidal sinus infection (The Netherlands)
Actinomyces radingae JAP320 (*Arcanobacterium haemolyticum*-like)			Human (The Netherlands)
Actinomyces radingae CDC F7338 (CDC group E)		Weaver	Penis
Actinomyces radingae CDC G1068 (CDC group E)	CCUG 34686	Weaver	Human knee fluid (USA)
Actinomyces slackii CCUG 32792^T	NCTC 11923^T	NCTC	Dairy cattle dental plaque
Actinomyces suis CCUG 19206^T	ATCC 33144^T	ATCC	Swine cystitis
Actinomyces turicensis APL2 (*Actinomyces pyogenes*-like)	CCUG 32395, LMG 15954	Wüst	Human subhepatic abscess (Switzerland)
Actinomyces turicensis APL4 (*Actinomyces pyogenes*-like)	CCUG 32397, LMG 15956	Wüst	Human pilonidal cyst (Switzerland)
Actinomyces turicensis APL6 (*Actinomyces pyogenes*-like)	CCUG 32398, LMG 15957	Wüst	Human chronic otitis media (Switzerland)
Actinomyces turicensis APL7 (*Actinomyces pyogenes*-like)	CCUG 32399, LMG 15958	Wüst	Human chronic otitis media (Switzerland)
Actinomyces turicensis APL8 (*Actinomyces pyogenes*-like)	CCUG 32400, LMG 15959	Wüst	Human perineal abscess (Switzerland)
Actinomyces turicensis APL10^T (*Actinomyces pyogenes*-like)	CCUG 32401^T, LMG 15961^T	Wüst	Human perineal abscess (Switzerland)
Actinomyces turicensis FIN 1324 (*Actinomyces meyeri*-like)		Brander	Human (Finland, 1990)
Actinomyces turicensis FIN 880 (*Actinomyces meyeri*-like)		Brander	Human (Finland, 1990)
Actinomyces turicensis 8811 (*Arcanobacterium haemolyticum*-like)			Human perineal abscess (The Netherlands)
Actinomyces turicensis 8819 (*Arcanobacterium haemolyticum*-like)			Human vulvar abscess (The Netherlands)
Actinomyces turicensis 8823 (*Arcanobacterium haemolyticum*-like)			Human perineal abscess (The Netherlands)
Actinomyces turicensis 9202 (*Arcanobacterium haemolyticum*-like)			Human cystitis (The Netherlands)
Actinomyces turicensis 9205 (*Arcanobacterium haemolyticum*-like)			Human perineal abscess (The Netherlands)
Actinomyces turicensis CDC F1959 (CDC group E)	CCUG 18440, LMG 15962	Weaver	Human perineum (USA, 1981)
Actinomyces turicensis CCUG 23484 (CDC group E)	LMG 15963		Human genital wound (Sweden, 1988)
Actinomyces turicensis CCUG 31183B (CDC group E)	LMG 15965		Human blood (Sweden, 1993)
Actinomyces turicensis CDC E9009 (CDC group E)	CCUG 34684	Weaver	Human wound (USA)
Actinomyces turicensis CDC F6390 (CDC group E)		Weaver	Human leg wound (USA)
Actinomyces turicensis CDC F8501 (CDC group E)		Weaver	Human retroperitoneal abscess (USA)
Actinomyces turicensis LMG 14733 (*G. vaginalis*-like)	CCUG 33037, ITG 900	ITG	Penile ulcer (Belgium)
Actinomyces turicensis LMG 14736 (*G. vaginalis*-like)	CCUG 33040, ITG 143	ITG	Human vagina (Belgium)
Actinomyces turicensis LMG 14331 (*G. vaginalis*-like)	CCUG 32390, ITG 137	ITG	Human vagina (Belgium)
Actinomyces turicensis LMG 14330 (*G. vaginalis*-like)	CCUG 32389, ITG 131	ITG	Human vagina (Belgium)
Actinomyces turicensis LMG 14329 (*G. vaginalis*-like)	CCUG 32388, ITG 75	ITG	Human vagina (Belgium)
Actinomyces turicensis LMG 14734 (*G. vaginalis*-like)	CCUG 33038, ITG 886	ITG	Human vagina (Belgium)
Actinomyces turicensis LMG 14738 (*G. vaginalis*-like)	CCUG 33042, ITG 806	ITG	Human vagina (Belgium)
Actinomyces turicensis LMG 14735 (*G. vaginalis*-like)	CCUG 33039, ITG 147	ITG	Human vagina (Belgium)
Actinomyces turicensis LMG 14737 (*G. vaginalis*-like)	CCUG 33041, ITG 284	ITG	Human vagina (Belgium)
Actinomyces turicensis LMG 14332 (*G. vaginalis*-like)	CCUG 32391, ITG 296	ITG	Human vagina (Belgium)
Actinomyces turicensis CCUG 25712 ('*C. cervicis*')	LMG 15964, NCTC 10604, Laughton 9T	NCTC	Human cervix
Actinomyces turicensis CCUG 32765A ('*C. cervicis*')	LMG 15966		Human wound (Sweden, 1994)
Actinomyces viscosus CCUG 14476^T	ATCC 15987^T	ATCC	Hamster periodontal disease
Arcanobacterium bernardiae CCUG 33419^T	GF 750^T	Funke	Human blood (Canada)
Arcanobacterium pyogenes CCUG 13230^T	LMG 16162^T, NCTC 5224^T, ATCC 19411^T	NCTC	Porcine
Arcanobacterium haemolyticum CCUG 33552^T	LMG 16163^T, NCTC 8452^T	NCTC	
G. vaginalis CCUG 3717^T	LMG 7832^T, NCTC 10287^T	NCTC	Human vagina

* Isolated by authors of this paper if not specified.

atmosphere containing approximately 5% O_2, 10% CO_2 and 85% N_2, unless stated otherwise.

PAGE of whole-cell proteins. Cells were grown for 3 d. Preparation of whole-cell protein extracts, PAGE, densitometric analysis, normalization and interpolation of the protein profiles, and numerical analysis were performed as described by Pot *et al.* (1994) using the GelCompar 4.0 software package (Applied Maths). The profiles were recorded and stored on a personal computer. The similarity between all pairs of traces was expressed by the Pearson product moment correlation coefficient converted for convenience to a percentage value.

Cellular fatty acid analysis. After an incubation period of 72 h, a loopful of well-grown cells was harvested; fatty acid methyl esters were extracted, separated and identified as described previously (Vandamme *et al.*, 1992).

Phenotypic characterization. Strains were grown on Columbia agar supplemented with 5% sheep blood and incubated at 37 °C in air enriched with 5% CO_2.

Phenotypic characteristics present in the API CORYNE microtest system (bioMérieux) and the Rapid ANA II system (Innovative Diagnostic Systems) were analysed as recommended by the manufacturer. Readings of the carbohydrate fermentation reactions in the API gallery were done after 24 and 48 h incubation. Carbohydrate fermentation reactions were also performed in macrotubes containing the relevant sugars in tryptone soya broth (Oxoid) enriched with 5% rabbit serum; readings were taken after 7 d.

In addition, catalase activity was tested using a 30% hydrogen peroxide solution. The CAMP reaction was performed on Columbia sheep blood agar with *Staphylococcus aureus* ATCC 25923.

Antimicrobial susceptibility patterns were determined by the radial diffusion assay on Isosensitest agar (Oxoid) supplemented with 5% lysed sheep blood. The plates were inoculated with a 0·1 ml suspension of McFarland no. 0·5 standard and read after 24 h incubation at 37 °C in a 5% CO_2 atmosphere. The breakpoints described by Rosco (Rosco Diagnostica) for staphylococci were used. Sensitivity to tellurite was tested by inoculation on 2·5% Nutrient broth (Oxoid) supplemented with 2% bacteriological agar no. 1 (Oxoid) and 5% sheep erythrocytes and containing 0·03% (w/v) tellurite and 0·001% (w/v) L-cystine.

DNA sequence analysis, probe design and hybridization by reverse line blotting. The 5′ part of the 16S rRNA gene was amplified using broad-host-range primers 16S1F [AGAGTTTGATC(AC)TGG(TC)TCAG] and 16S1RR [CTTTACGCCCA(AG)T(AG)A(AT)TCCG] corresponding to positions 8–27 and 556–575, respectively, in the *Escherichia coli* numbering system (Bergmans *et al.*, 1995). The resulting 600 bp DNA fragment was purified and sequenced directly using fluorescent dye terminators in the cycle sequencing reaction (ABI model 377 sequencer; Perkin-Elmer Cetus). Sequences were compared with the EMBL DNA databases using the FASTA algorithm (Pearson & Lipman, 1988) and aligned with other 16S rRNA gene sequences using the MegAlign module of the DNAStar program.

For identification of the strains, a reverse line blotting assay was developed. Briefly, amino-linked group-specific oligonucleotides were covalently bound in parallel lines to a negatively charged membrane (Biodyne C; Pall Biosupport) using a miniblotter (Immunetics). After binding, the membrane was removed from the miniblotter, turned 90 ° and placed in the miniblotter again. The slots, which were perpendicular to the oligo lines, were filled with heat-denatured, biotin-labelled, PCR products obtained by amplification with a biotinylated variant of primer 16S1F and primer 16S1RR. A 60 min hybridization in 2× SSPE (360 mM NaCl, 20 mM $Na_2HPO_4.H_2O$, 2 mM EDTA, 0·5% SDS) at 50 °C was performed in the miniblotter and the membrane was subsequently incubated for 60 min at 42 °C with 1:4000 horseradish peroxidase-labelled streptavidin (Boehringer Mannheim) in 2× SSPE. Hybridization was visualized by incubation with ECL detection liquid (Amersham) and a 10 min exposure of a hyperfilm (Amersham) to the membrane.

RESULTS

PAGE of whole-cell proteins

Duplicate protein extracts were prepared to check the reproducibility of the growth conditions and the preparation of the extracts. The correlation level between duplicate protein patterns was above 93%.

The dendrogram obtained after numerical analysis of the protein patterns of part of the strains examined is shown in Fig. 1. The type strains of *Actinomyces bovis*, *Actinomyces denticolens*, *Actinomyces georgiae*, *Actinomyces gerencseriae*, *Actinomyces hordneovulneris*, *Actinomyces howelii*, *Actinomyces hyovaginalis*, *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces neuii*, *Actinomyces suis*, *Actinomyces slackii*, *Actinomyces viscosus* and *Arcanobacterium bernardiae* all occupied clearly distinct positions in the dendrogram and were not included in the analysis shown in Fig. 1.

In the numerical analysis shown in Fig. 1, three clusters and four strains with separate positions were differentiated. Cluster I contains 31 strains grouping above a correlation level of 83·2%. This cluster contains all of the *G. vaginalis*-like strains, five *Arcanobacterium haemolyticum*-like strains, the *Actinomyces meyeri*-like strains, six CDC group E strains, the '*C. cervicis*' strains and six *Actinomyces pyogenes*-like strains. Cluster II comprises three *Actinomyces pyogenes*-like strains, three *Arcanobacterium haemolyticum*-like strains and two CDC group E strains grouping above a correlation level of 88·4%. Cluster III comprises the *Actinomyces odontolyticus* type strain (CCUG 20536^T) and one of the *Actinomyces pyogenes*-like strains (LMG 15953) grouping at a correlation level of 81·0%. The type strains of *G. vaginalis*, *Actinomyces meyeri*, *Arcanobacterium pyogenes* and *Arcanobacterium haemolyticum* all occupy separate positions in the dendrogram (Fig. 1) and have clearly distinct whole-cell protein patterns (Fig. 2).

Cellular fatty acid analysis

A selection of 22 cluster I strains, three cluster II strains, strain APL11, and the type strains of *G. vaginalis*, *Actinomyces meyeri*, *Arcanobacterium pyogenes* and *Arcanobacterium haemolyticum*, were included in the cellular fatty acid analysis. When these data were subjected to numerical analysis using unweighted pair group mean linkage of Euclidian dis-

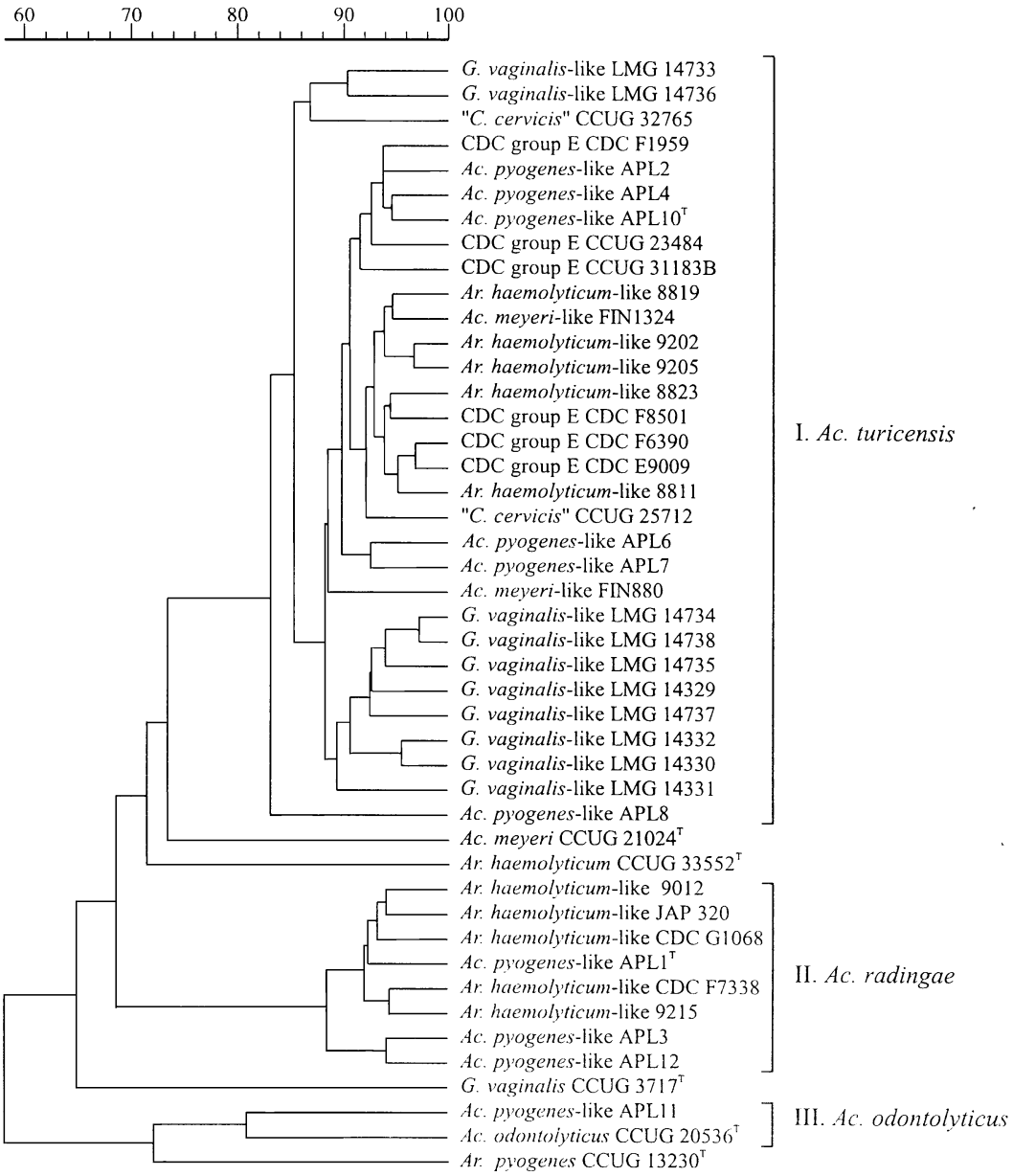

Fig. 1. Dendrogram derived from the unweighted pair group mean linkage of correlation coefficients (expressed for convenience as a percentage value) between the whole-cell protein patterns of some of the strains examined. Roman numerals indicate cluster numbers as discussed in the text. The names used are the designations of the strains as received.

tances between whole-organism fatty acid patterns, all cluster I strains and cluster II strains again formed separate clusters; strain APL11 and the type strains of *G. vaginalis*, *Actinomyces meyeri*, *Arcanobacterium pyogenes* and *Arcanobacterium haemolyticum*, occupied separate positions (data not shown).

The mean fatty acid ester components of cluster I and II strains and of the other strains examined are shown in Table 2. All strains are characterized by high percentages (about 20–40%) of 16:0 and 18:1ω9*c*, and varying levels of 10:0, 12:0, 14:0, 16:1ω9*c*, 18:0, and the summed features 4 and 6 (summed feature 4 comprises 16:1ω7*c*, 16:1ω7*t*, 15:0 iso 2-OH, or any combination of these fatty acids; summed feature 6 comprises 18:2ω6,9*c* or 18:0 anteiso or both). Quantitative differences in the percentages of 16:0 and 18:0 allowed differentiation between cluster I and cluster II strains; both qualitative and quantitative differences were present in the other strains or taxa examined (Table 2).

Phenotypic characterization

Results of the phenotypic analyses are listed below and are based on a selection of 12 cluster I strains and all eight cluster II strains. The 12 *Actinomyces turicensis*

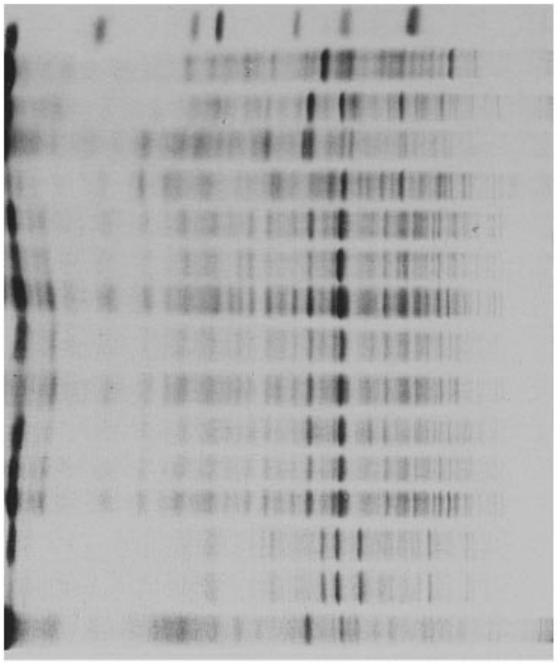

Fig. 2. Electrophoretic protein profiles of a selection of the strains studied. The molecular mass markers (in Da) given in the top lane are (from left to right): lysozyme, 14500; trypsin inhibitor, 20100; trypsinogen, 24000; carbonic anhydrase, 29000; glyceraldehyde-3-phosphate dehydrogenase, 36000; egg albumin, 45000; and bovine albumin, 66000. The names used are the designations of the strains as received.

Table 2. Mean fatty acid composition of the strains examined

Those fatty acids for which the mean amount for all taxa was less than 1 % are not included. Therefore, the percentages may not add up to 100 %. T, Trace amount (less than 1 %); ND, not detected.

Taxon	Fatty acid composition (%)									
	10:0	12:0	14:0	16:0	16:1ω9*c*	18:0	18:1ω9*c*	20:1ω9*c*	Summed feature 4*	Summed feature 6†
Cluster I (22 strains)	6·6±2·4	2·6±0·6	1·8±0·3	20·8±1·3	T	12·9±1·1	32·3±2·3	ND	2·8±0·3	19·1±1·6
Cluster II (3 strains)	10·7±2·0	2·9±0·4	1·5±0·1	16·6±0·5	T	9·2±0·3	33·4±0·5	ND	3·0±0·4	20·0±0·3
Actinomyces meyeri CCUG 21024T	2·0	4·7	4·5	29·0	ND	8·5	30·9	ND	5·0	15·4
Actinomyces odontolyticus APL11	4·4	1·8	4·6	24·4	T	16·6	37·4	1·2	1·1	6·6
Arcanobacterium pyogenes CCUG 13230T	1·1	1·4	12·9	40·4	7·3	3·8	28·3	ND	T	1·2
Arcanobacterium haemolyticum CCUG 33552T	2·5	1·0	T	17·0	T	14·5	26·9	ND	2·3	32.9
G. vaginalis CCUG 3717T	4·2	2·4	10·2	34·5	T	10·5	25·3	ND	1·7	10·5

* Summed feature 4 comprises 16:1ω7*c*, 16:1ω7*t*, 15:0 iso 2-OH, or any combination of these fatty acids.

† Summed feature 6 comprises 18:2ω6,9*c* or 18:0 anteiso or both.

strains were chosen at random; we had no objective criterion to select particular isolates as all seemed equally typical for the entire species. The twelve isolates comprised strains received as *Actinomyces meyeri*-like, *Actinomyces pyogenes*-like and *Arcanobacterium haemolyticum*-like.

Determination of the 16S rRNA gene sequence and hybridization assay

To investigate genetic relationships between the various isolates used in the study, the 16S rRNA gene of 13 cluster I strains and three cluster II strains was amplified by PCR and subjected to comparative sequencing. The sequences consisted of approximately 600 nucleotides and comparative sequence searches of the EMBL database revealed that these sequences were virtually identical to deposited sequences for *Actinomyces turicensis* (cluster I strains) and *Actinomyces radingae* (cluster II strains) (Wüst *et al.*, 1995) (similarity levels were always above 97 % with a maximum value of 98·9 %). Based on the sequences derived from our own sequencing experiments and those present in the EMBL database, specific oligonucleotide DNA probes were designed for *Actinomyces turicensis* (CAACAAAGTTGGAGCATCA-

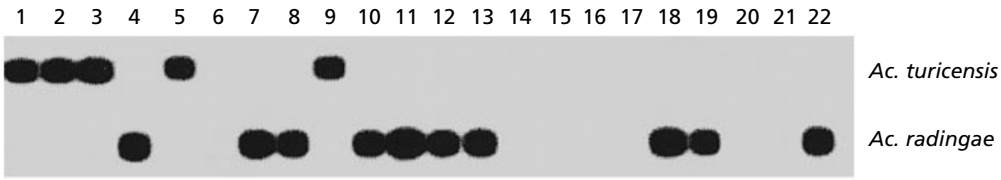

Fig. 3. Reverse line hybridization assay of a representative selection of cluster I and cluster II strains and of strains belonging to other *Actinomyces* species. The oligonucleotides used were the *Actinomyces turicensis*-specific (upper row) and the *Actinomyces radingae*-specific (lower row) probes derived from the 16S rRNA sequences. Lanes 1–3, 5 and 9 represent cluster I strains; lanes 4, 7, 8, 10–13, 18, 19 and 22 represent cluster II strains (including two duplicates); lanes 6 and 14 represent *Arcanobacterium pyogenes* strains; lanes 16 and 17 represent *Actinomyces odontolyticus* strains; and lanes 15, 20 and 21 represent unidentified coryneform bacteria.

TCG; corresponding to positions 59–38 in the *E. coli* numbering system) and for *Actinomyces radingae* (AGAAACCACAAAGGCCCCT; corresponding to positions 211–193). A random selection of 20 cluster I strains and eight strains from cluster II were included in the reverse line blot assay. All cluster I strains hybridized with the *Actinomyces turicensis*-specific probe, whereas all cluster II strains hybridized with the *Actinomyces radingae* oligo probe. Cross-hybridizations or hybridizations with strains representing other *Actinomyces* or *Arcanobacterium* species, including *Arcanobacterium haemolyticum*, *Actinomyces meyeri*, *Actinomyces pyogenes* and *Actinomyces odontolyticus* were not observed. An example of a hybridization assay is shown in Fig. 3.

DISCUSSION

Identification of strains examined

Catalase-negative coryneform bacteria are often difficult to classify (Funke *et al*., 1997). Researchers from Belgium, Finland, Sweden, Switzerland, The Netherlands, the UK and the USA have all described phenotypically similar bacteria but gave their organisms different provisional names. In the present study, we compared biochemical, chemotaxonomic and genotypic characteristics of several of these taxa.

It has been extensively documented for a variety of Gram-negative and Gram-positive bacteria that a high similarity in whole-cell protein content correlates with a high percentage of DNA–DNA hybridization (Costas, 1992). The virtually identical whole-cell protein patterns of the cluster I strains (Figs 1 and 2) strongly suggest that these isolates, which comprise *G. vaginalis*-like strains, *Arcanobacterium haemolyticum*-like strains, *Actinomyces meyeri*-like strains, *Actinomyces pyogenes*-like strains, CDC group E strains and '*C. cervicis*' strains, all constitute a single species, which is clearly different from *G. vaginalis*, *Actinomyces meyeri*, *Arcanobacterium haemolyticum* and *Arcanobacterium pyogenes* (as represented by their respective type strains) (Figs 1 and 2) and from the other *Actinomyces* and *Arcanobacterium* reference species (data not shown). This is further substantiated by the results of several other analyses. All cluster I strains examined have very similar fatty acid components (Table 2) and biochemical characteristics. In addition, analysis of partial 16S rRNA gene sequences of 13 cluster I strains revealed no differences, while this region was successfully used to design a specific identification assay. Finally, analysis of the entire 16S rRNA gene revealed a 98·4% similarity between one of the *G. vaginalis*-like strains (LMG 14331) and one of the *Actinomyces pyogenes*-like strains (APL10^T) (Van Esbroeck *et al*., 1996). It has been established that organisms sharing more than 97% of their 16S rRNA sequence may or may not belong to a single species and that whenever similarity values above 97% are recorded, species level identification must be performed by other techniques (Fox *et al*., 1992; Stackebrandt & Goebel, 1994). We believe that the combined evidence of biochemical tests, whole-cell protein (Figs 1 and 2) and fatty acid (Table 2) analyses, and 16S rRNA gene sequence analyses indicates that all cluster I strains belong to the same species. The name *Actinomyces turicensis* was proposed (Wüst *et al*., 1995) for two of the *Actinomyces pyogenes*-like bacteria [hybridization group IV of Wüst *et al*. (1993)], with strain APL10^T as the type strain; we therefore consider all cluster I strains as *Actinomyces turicensis*. Likewise, the name *Actinomyces radingae* was proposed for three *Actinomyces pyogenes*-like strains [hybridization group I of Wüst *et al*. (1993)] with APL1^T as the type strain (Wüst *et al*., 1995). We conclude that the remaining cluster II strains also belong to *Actinomyces radingae*.

These data are partly at variance with the conclusions of Wüst *et al*. (1995). Within their collection of *Actinomyces pyogenes*-like bacteria, Wüst *et al*. (1995) differentiated five genotypic sub-groups using a slot hybridization method and assigned strains APL1^T, APL3 and APL7 to hybridization group I; APL2, APL4 and APL8 to hybridization group II; APL6 to hybridization group III; APL10^T and APL12 to hybridization group IV; and APL11 to hybridization group V. Strain APL11 was identified as *Actinomyces odontolyticus* (Wüst *et al*., 1993, 1995), which was confirmed by whole-cell protein analysis (Fig. 1). In our analyses, strains APL2, APL4, APL6, APL7, APL8 and APL10^T all belong to the same taxon (and

were phenotypically indistinguishable), which was identified as *Actinomyces turicensis*; strains APL1^T, APL3 and APL12 were identified as *Actinomyces radingae*. However, as mentioned above, strain APL12 was originally classified as *Actinomyces turicensis*, while strain APL7 was originally classified as *Actinomyces radingae* (Wüst *et al*., 1993, 1995). From our data, it is obvious that both species can easily be identified by whole-cell protein and fatty acid analysis, using the 16S rRNA probe, and by at least eight clear-cut differential phenotypic tests (see below). Data on biochemical characteristics and fatty acid components reported by Wüst *et al*. (1993) did not provide differential features between the two species. Furthermore, if our identification results for strains APL7 and APL12 are used to evaluate the data of Wüst *et al*. (1993), both species are easily differentiated by fatty acid analysis and multiple phenotypic tests confirming our data. J. Wüst and colleagues kindly provided new subcultures of these isolates and repeated the analyses in their laboratories. Their repeat analyses confirmed our identifications of strain APL7 as *Actinomyces turicensis* and APL12 as *Actinomyces radingae* (Funke *et al*., 1997). Both species can therefore be differentiated by the following tests: aesculin hydrolysis (present in *Actinomyces radingae*), acid production from lactose and salicin (present in *Actinomyces radingae*), and the presence of leucine arylamidase activity in *Actinomyces turicensis*, and α- and β-galactosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase activities in *Actinomyces radingae*. In addition, pyrazinamidase, β-disaccharidase and α-L-arabinosidase activities are present in the majority of the *Actinomyces radingae* strains (in five to seven of the eight strains tested), while these features are absent in *Actinomyces turicensis* strains. Emended descriptions of *Actinomyces turicensis* and *Actinomyces radingae* are given below.

Emended description of *Actinomyces turicensis* Wüst, Stubbs, Weiss, Funke and Collins 1995

Cells are straight and slightly curved Gram-variable rods with some pseudobranching. They are 1·3–4·0 μm long and about 0·6 μm in diameter. A few club-shaped cells may be present. After 48 h incubation on sheep blood agar in a 5% CO_2 atmosphere at 37 °C, all strains appear as small, grey, convex circular colonies, with a glistening surface, opaque, with a butyrous consistency and an entire edge. Growth is slightly better under CO_2 enrichment or anaerobiosis compared to aerobiosis, and the small haemolytic zone is more visible. Growth is variable at 43 °C, slow at 30 °C, and absent at 25 °C. Catalase activity is not detected and indole is not produced. Aesculin, urea and gelatin are not hydrolysed. Nitrate is not reduced to nitrite. Enhancement or inhibition of haemolysis are not detectable in the CAMP assay. Acid is produced from glucose, ribose, xylose, saccharose, starch, trehalose and maltose (acid production is variable with the latter two substrates in the API microtest system but is constant in macrotubes with horse serum added), but not from mannitol, lactose, glycogen or salicin. β-Disaccharidase, α-L-arabinosidase, β-glucosidase, pyrazinamidase, pyrrolidonyl arylamidase, alkaline phosphatase, β-glucuronidase, α- and β-galactosidase and *N*-acetyl-β-glucosaminidase are not detectable. α-Glucosidase, and glycine, leucine, leucylglycine, proline, phenylalanine, arginine and serine arylamidases are always present.

Additional phenotypic characteristics have been described by Van Esbroeck *et al*. (1996) on *Actinomyces turicensis* strains previously classified as *G. vaginalis*-like. Neither strain is sensitive to mupirocin and all are inhibited by tellurite. Susceptibility to antibiotics is high: penicillin, oxacillin, erythromycin, cefazolin, amoxicillin/clavulanic acid, cotrimoxazole, phosphomycin and doxycycline are all active. The DNA G+C ratio is 57·5 mol% (Wüst *et al*., 1995), and the type strain is CCUG 32401^T. *Actinomyces turicensis* strains have been isolated from various infections in humans.

Emended description of *Actinomyces radingae* Wüst, Stubbs, Weiss, Funke and Collins 1995

Cells appear as Gram-positive coccoid rods, 0·65 μm in diameter and 1 μm long. Pseudobranching is rare. After 48 h incubation on sheep blood agar in a 5% CO_2 atmosphere at 37 °C, all strains appear as small, grey, convex circular colonies, with a glistening surface, opaque, with a butyrous consistency and an entire edge. A haemolytic zone appears slowly and starts mostly with α-haemolysis. Growth is slightly better under CO_2 enrichment or anaerobiosis compared to aerobiosis. Growth is variable at 43 °C, slow at 30 °C and absent at 25 °C. Growth in broth is promoted by the addition of 5% rabbit or horse serum. Catalase activity is not detected and indole is not produced. Aesculin is hydrolysed. No hydrolysis of gelatin (except for one strain which gave a weak reaction) or urea is observed. Nitrate is not reduced to nitrite. Enhancement or inhibition of haemolysis is not detectable in the CAMP assay. Acid is produced from glucose, ribose, xylose, maltose, lactose (for the latter, acid production is observed in only 1 out of 8 in the API gallery but 8 out of 8 in macrotubes with 5% horse serum), salicin and saccharose, but not from mannitol or glycogen. *N*-Acetyl-β-glucosaminidase, α- and β-glucosidase, α- and β-galactosidase, and leucylglycine, glycine, proline, phenylalanine, arginine and serine arylamidases are always present. Leucine arylamidase, pyrrolidonyl arylamidase, alkaline phosphatase and β-glucuronidase are always absent. β-Disaccharidase, α-L-arabinosidase and pyrazinamidase are variably present (in five to seven of the eight strains tested). Neither strain is sensitive to mupirocin and all are inhibited by tellurite. All strains are susceptible to penicillin, oxacillin, erythromycin, cefazolin, amoxicillin/clavulanic acid, cotrimoxazole,

phosphomycin and doxycycline. The DNA G+C ratio is 60 mol% (Wüst *et al*., 1995), and the type strain is CCUG 32394^T. *Actinomyces radingae* strains have been isolated from various infections in humans.

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