JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2002, p. 4375–4381 Vol. 40, No. 11
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.11.4375–4381.2002

Characteristics of Rare or Recently Described *Corynebacterium* Species Recovered from Human Clinical Material in Canada

K. A. Bernard,* C. Munro, D. Wiebe, and E. Ongsansoy

National Microbiology Laboratory, Health Canada, Winnipeg, Manitoba R3E 3R2, Canada

Received 9 May 2002/Returned for modification 22 July 2002/Accepted 19 August 2002

Nineteen new *Corynebacterium* species or taxa described since 1995 have been associated with human disease. We report the characteristics of 72 strains identified as or most closely resembling 14 of these newer, medically relevant *Corynebacterium* species or taxa, as well as describe in brief an isolate of *Corynebacterium bovis*, a rare pathogen for humans. The bacteria studied in this report were nearly all derived from human clinical specimens and were identified by a polyphasic approach. Most were characterized by nearly full 16S rRNA gene sequence analysis. Some isolates were recovered from previously unreported sources and exhibited unusual phenotypes or represented the first isolates found outside Europe. Products of fermentation, with emphasis on the presence or absence of propionic acid, were also studied in order to provide an additional characteristic with which to differentiate among phenotypically similar species.

Nineteen species or taxa consistent with the genus *Corynebacterium* and recovered from human clinical material have been described or validated since 1995 (10, 11). Many of these species were first described from European laboratories and have not as yet been reported from human clinical specimens in other geographic areas. We report here the characteristics observed for 73 strains of *Corynebacterium* which had been studied with a polyphasic approach and could be assigned to one of 15 newer or rarely recovered species or a recently described taxon group.

Many strains exhibited phenotypic features which differed from those presented in the original descriptions of the new species, which were occasionally based on a single strain or small number of strains. Each of the newer or rare species found here, as well as most other medically relevant *Corynebacterium* species, was studied for the detection of propionic acid as a fermentation product. This had been found previously to provide additional chemotaxonomic data with which to discriminate among phenotypically close *Corynebacterium* species (7, 8).

Strains and methods. The strains studied are described in Table 1. Nearly all were referred from Canadian provincial reference centers as isolates in pure culture and were recovered from human clinical material. Identifier numbers are those of the Special Bacteriology Laboratory of the National Microbiology Laboratory (NML), Health Canada, except where otherwise described. Some of the older cultures were originally unidentifiable or poorly identifiable until retrospectively characterized by molecular identification methods. Detailed clinical information on underlying diseases of the patients was generally not available.

Conventional biochemical tests, salt tolerance, Christie-Atkins-Munch-Peterson (CAMP) reaction test, tyrosine hydrolysis, esculin or bile esculin hydrolysis, starch hydrolysis, DNase production, and type and degree of hemolysis on 5% sheep blood agar were done as described previously (2) or by standard methods (19, 20). The *Corynebacterium bovis* strain was the only isolate studied for *O*-nitrophenyl-β-D-galactopyranoside (Sigma-Aldrich Canada Ltd.) production; *C. bovis* has been described as being positive (19). Reduction of both nitrate and nitrite was added to the panel of tests after the description of *C. simulans* (31). Lipophilia was determined by comparing growth after 72 h at 37°C in brain heart infusion broth (Becton Dickinson, Sparks, Md.) with that in brain heart infusion broth with 1% (vol/vol) Tween 80 (Becton Dickinson) (22).

Semiquantitative estimates of growth on plate agar were evaluated as no growth or from 1+ (poor) to 4+ (excellent). Extended (48 to 72 h) incubations were required for some taxa. For each strain, temperature preference was determined by estimating semiquantitatively the amount of growth on 5% sheep blood agar after incubation in air at 25°C, 37°C, or 42°C; its atmospheric preference after incubation in air, 5% CO_2, or anaerobically (Anaerocult anaerobic jar; EM Science, Gibbstown, N.J.) at 37°C; and ability to grow on media lacking blood, such as tryptic soy agar (Becton Dickinson). Products of glucose fermentation for the taxa shown in Table 2 were analyzed here as described previously (1) or as described in other publications. Vibriostatic disk O129 was applied to most of these taxa for at least one strain, and a zone of 11 to 36 mm was considered to show sensitivity while one of 0 to 10 mm was considered to show resistance (34).

API Coryne and API Zym strips were used as described by the manufacturer (Biomérieux, Montréal, Canada). Cellular fatty acid composition extraction and analysis were done after growth on 5% sheep blood agar at 35°C to 37°C in 5% CO_2 for 24 to 72 h, depending on the species' requirements (2). Version 3.9 or version 4.0 of the MIDI method Aerobe (MIDI, Newark, Del.) and library generation system software (MIDI) were used to make entries for an in-house library, LCDC1. Each strain analyzed was compared to LCDC1 and CLIN (MIDI version 3.9 or 4.0) libraries. The methods to extract, amplify, and sequence the 16S rRNA gene, yielding about 1,400 to

* Corresponding author. Mailing address: National Microbiology Laboratory, Health Canada H5040, 1015 Arlington St., Winnipeg, Manitoba R3E 3R2, Canada. Phone: (204) 789-2135. Fax: (204) 789-5009. E-mail: kathy_bernard@hc-sc.gc.ca.

TABLE 1. Newer or rare *Corynebacterium* species in Canada[a]

Taxon (no. of strains if >1)	NML strain no. (GenBank accession no.)	Clinical and geographic source(s)	API Coryne code(s)	Accession no., % identity	Features not found in original descriptions; sensitivity to O129 disk
C. argentoratense	98-0075 (AF537589)	Blood culture, Ontario	2000104	X83955, 99.8	Chymotrypsinase not produced; first report from blood culture; mannose positive; O129 sensitive
C. bovis	99-0033 (AF537590)	Human blood culture, Ontario	0101104	X84444, 99.8	Lipophilic; oxidase positive; API urea positive but conventional urea negative; β-galactosidase negative; fructose slowly fermented; O129 sensitive
C. confusum	99-0145 (AF537591)	Breast abscess, Alberta	3100004	Y15886, 99.6	CAMP positive; tyrosine negative; TBSA detected as cellular fatty acid; O129 resistant
C. coyleae (8)	93-0786, 96-0224, 97-0083, 97-0171 (AF537592) 98-0010, 98-0066, 98-0068, 99-0246	4 blood, 3 urine, 1 abscess; 3 from British Columbia; 3 from Manitoba; 1 each from Ontario and Saskatchewan	2 each of 2100104, 6100304, and 2100304; 1 each of 4100304 and 6100004	X96497,[b] >98.5	Urine as clinical source not previously cited; for O129, 6 of 8 strains ND, remaining 2 strains resistant
C. durum (5)[b]	81-0379, 86-0376, 91-0086, 98-0165, 99-0047 (AF537593)	2 sputum, 2 blood, 1 neck abscess; 2 from Saskatchewan; 1 each from Quebec, Alberta, Ontario	3440125, 2440125 (ND for 1981, 1986, 1991 strains)	Z97069, ≥99.2	99-0047, nitrate negative, lactose positive, β-galactosidase positive; for O129, 3 of 5 strains ND, remaining 2 strains sensitive
C. falsenii	97-0205 (AF537594)	Blood, Ontario	2101104	Y13024, 100	Yellowish pigment observed which did not intensify with time; conventional esculin negative but API esculin positive; both conventional and API urea positive, delayed (29); O129 sensitive
C. glucuronolyticum (11)	79-0685, 90-0199, 93-0676, 93-0709, 93-0735, 94-0190, 94-0425, 96-0032, 96-0275, 98-0013 (AF537595), 98-0098 (AF537596)	4 blood, 2 peritoneal fluid, 1 prostate fluid, 1 disk fluid, 3 unknown; 3 from Ontario, 2 from British Columbia; 1 each from Alberta, Prince Edward Island, and Newfoundland; 3 from Spain	2241325, 6201705, 2241725, 3200505, 2200705, 7240703, 6600705, 6241725, 2201325, 2200725, 3201105	X86688, ≥98.3	Recovery from blood cultures, peritoneal fluid, or disk fluid not previously described for *C. glucuronolyticum* (14) or junior synonym *C. seminale* (6, 23); for O129, 9 of 11 strains ND, remaining 2 strains sensitive
C. imitans (6)	89-0572, 93-0726, 98-0019, 00-0068, 00-0109, 01-0063 (AF537597)	5 blood culture, unknown; 2 from Ontario; 1 each from Alberta, British Columbia, Manitoba, and Seville, Spain	3 of 3100325; 1100325, 2100325, 0100325	Y09044, >99.3	Blood culture isolates not previously described (12); 4 of 6 strains nitrate positive by conventional and API Coryne but 2 negative by both methods; 6 of 6 strains resistant to O129
C. kroppenstedtii (4)	87-0354, 96-0162, 97-0060, 00-0244 (AF537598)	Breast abscess, lung biopsy, sputum, blood culture; 3 from Ontario, 1 from British Columbia	2040104, 2040105, 0101104, 1987 sample ND	Y10077, >99.5	Lipophilic; conventional esculin may be negative, but strains were esculin positive with API Coryne system; TBSA detected; for O129, 2 of 4 strains ND, remaining 2 strains sensitive

Continued on following page

1,500 bp, have been described (1). Establishing the closest known relatives was done by performing database searches of GenBank with the Blast program or of in-house data.

Assignment to specific *Corynebacterium* species was made based on demonstrating phenotypic characteristics consistent with literature values for that taxon group, with differences otherwise being noted in Table 1. Identifications were made based on a strain's having a cellular fatty acid composition and products of fermentation consistent with species of the genus *Corynebacterium* (2, 7, 8, 10) and based on a significantly high degree of identity (>98.0% to 100%) with respect to 1,300 to 1,500 bp of 16S rRNA gene sequence when aligned with sequences derived from validated *Corynebacterium* species (derived from GenBank), with the *Corynebacterium* species assigned being first choice upon sequence comparison. Some strains from the years 1981 to 1998 did not have 16S rRNA

TABLE 1—*Continued*

Taxon (no. of strains if >1)	NML strain no. (GenBank accession no.)	Clinical and geographic source(s)	API Coryne code(s)	Accession no., % identity	Features not found in original descriptions; sensitivity to O129 disk
C. mucifaciens (23)	N85-0494, 87-0360, 87-0436 (AF537599), 88-0085, 88-0977, 92-0106, 93-0528, 94-0282, 95-0019, 95-0186, 95-0290, 95-0369, 96-0217, 97-0088, 97-0096, 97-0097, 97-0158, 97-0167, 97-0216, 98-0057, 99-0203, 00-0064, 01-0118 (AF537600)	10 blood, 3 abscess or wound, 2 peritoneal fluid, 1 each breast cellulitis, knee, hand, nipple discharge, dialysis fluid, paraspinal tissue biopsy, inguinal hernia and sac, unknown; 9 from Ontario, 6 from British Columbia, 4 from Alberta, 1 each from Manitoba, Quebec, New Brunswick, and Saskatchewan	14 of 23 had API Coryne data: 3 of 2000104, 2 each of 2000004 and 6100104, 1 each of 0000004, 2100004, 2100104, 2000105, 6000004, 6100105, and 6100125	Y11200, ≥98.0	All 23 had mucoid or extremely mucoid, yellow or yellowish colonies; all were urease negative; 01-0118 was only slightly mucoid, slightly beta-hemolytic; maltose and sucrose were positive by both API (6100125) and conventional means (see also text); for O129, 15 of 23 strains ND, remaining strains sensitive
Closest to *C. mucifaciens*, smooth white colonies	97-0160 (AF537601)	Blood, Ontario	2101104	Y11200, 98.3[c]	Urea positive; colony smooth, not mucoid or yellow; O129 resistant
C. riegelii (4)	95-0198, 97-0139, 98-0154, 99-0185 (AF537602)	2 adult blood culture, 1 cord blood, 1 urine; 2 from Manitoba, 1 each from Saskatchewan and British Columbia	3 of 2001224; 2101224	Y14651, ≥99.8	First report of recovery from adult or cord blood cultures; for O129, 2 of 4 strains ND, remaining 2 strains sensitive
C. simulans (2)	81-0613[d] (AF537603), 00-0186 (AF537604)	1 bile, 1 blood, Quebec, Alberta	3000125	AJ012837, ≥99.8	Both nitrate and/or nitrite positive, 1981 strain maltose positive; for O129, ND; 00-0180 catalase negative, O129 sensitive
C. sundsvallense (2)	93-0639[e] (AF537605), 99-0121 (AF537606)	Draining sinus, Ontario; blood culture, British Columbia	2001004, 2101125	Y09655, ≥99.6	Close relationships between *C. sundsvallense* and *C. thomssenii*, described further in text; O129 ND for 1993 strain, 99-0121 sensitive
C. thomssenii	97-0130 (AF537607)	Air sample	0101004	AF010474, 99.9	Neither NAG nor PYZ detected (33), sucrose negative, DNase negative; O129 ND
Black-pigmented *Corynebacterium* sp. (2)	91-0032, 92-0360 (AF537608)	Vaginal swab, Ontario; vulval ulcer, Nova Scotia	0000125	AF220220, 99.8	Both had adherent black-pigmented colonies, both O129 sensitive

[a] All identifiers refer to accession numbers for Health Canada's Special Bacteriology section of the NML. ND, not done; NAG, *n*-acetyl-β-glucosaminidase; PYZ, pyrazinamidase; TBSA, tuberculostearic acid. No specimens were received from the Yukon, Northwest, or Nunavut Territory. Where all strains from a single taxon group had been sequenced, the percent identity shown is the lowest value obtained, with others being higher with respect to percent identity to the GenBank accession number for the reference strain of the species.

[b] 81-0379, 86-0376, and 91-0086 were described briefly in reference 21, with the original identification being most like *C. matruchotii*; 16S rRNA gene sequencing data described in reference 21 but not as yet deposited in GenBank.

[c] GenBank sequence contained a large number of ambiguous base pairs, reducing degree of match; 16S rRNA gene sequence for 97-0160 over 1,463 bp with respect to accession no. Y11200, after ambiguous base pairs were edited.

[d] NML 81-0613 was originally identified as "resembling CDC group G-1"; it was characterized in brief by Riegel et al. (22, 27) as strain LCDC 81-0613 and described as a unique taxon with characteristics like those found in the genus *Corynebacterium*.

[e] 93-0639, from a draining sinus, was further described in reference 4.

gene sequence analysis done but were otherwise consistent with the relevant species description. Therefore, some *C. glucuronolyticum* bacteria were identified based on production of β-glucuronidase and other features (14), some CAMP-positive isolates which fermented glucose only slowly plus other features were identified as *C. coyleae* (17), and some *C. mucifaciens* bacteria were identified based on the observance of characteristic yellow, mucoid colonies and other relevant traits (15).

The 16S rRNA gene sequences for 20 of 73 strains reported here have been deposited in GenBank and are further described in Table 1. Identification was based on characteristics described in standard texts (10, 11) as well as for *C. argentoratense* (26), *C. bovis* (13, 19), *C. confusum* (9), *C. coyleae* (17), *C. durum* (21, 24), *C. falsenii* (29), *C. glucuronolyticum* (14), *C. imitans* (12), *C. kroppenstedtii* (3), *C. mucifaciens* (15), *C. riegelii* (16), *C. simulans* (31), *C. sundsvallense* (4), *C. thomssenii* (33), and a black-pigmented *Corynebacterium* taxon group (28).

TABLE 2. *Corynebacterium* strains classified by production of propionic acid[a]

Propionic acid production	Species	Strains	Reference(s)
No	*C. accolens*	NML 98-0005	This study
	C. afermentans	NML 88-0199 = CIP 103499^{TS}	This study
	C. auris	DMMZ 328^{TS}	This study; 8
	C. bovis	NML 99-0033	This study; 7
	C. coyleae	NML 93-0786, 96-0224, 97-0083, 97-0171, 98-0010, 98-0066, 98-0068, 99-0246; DMMZ 214^{TS}	This study; 7
	C. falsenii	NML 97-0205	This study
	C. imitans	NML 98-0019	This study
	C. jeikeium	NML 98-0125, 99-0037	This study; 7, 8
	C. macginleyi	DMMZ 1352^{TS}	This study
	C. minutissimum		7, 8, 32
	C. mucifaciens	NML 96-0217, 97-0088, 97-0096, 97-0097, 97-0158, 97-0167, 97-0202, 97-0216, 98-0057, 99-0203, 00-0064, 01-0118	This study
	C. mucifaciens	97-0160	This study
	C. mycetoides	ATCC 21134	This study
	C. propinquum		8
	C. pseudodiphtheriticum	ATCC 10700	This study; 8
	C. renale		7, 8
	C. riegelii	NML 95-0198, 97-0139, 98-0154, 99-0185	This study
	C. simulans	NML 81-0613, 00-0186	This study
	C. striatum		7, 8, 32
	C. sundsvallense	NML 93-0639, 99-0121	This study
	C. thomssenii	NML 97-0130	This study
	C. urealyticum		This study; 8
	C. xerosis		7, 18, 32
	CDC group G		7, 8
	CDC group F-1	NML 98-0116	This study[b]
	Black-pigmented *Corynebacterium* spp.	NML 91-0032, 92-0360	This study
Yes	*C. amycolatum*	NML 89-0826, 91-0077, 92-0042, 92-0043	This study; 8, 18, 32
	C. argentoratense	NML 98-0075	This study
	C. confusum	NML 00-0145	This study
	C. diphtheriae (all biotypes)		This study; 7, 8, 32
	C. durum		21, 24
	C. glucuronolyticum		8, 14, 32
	C. kroppenstedtii	NML 96-0162, 97-0060	This study
	C. kutscheri		7, 8
	C. matruchotii		24
	C. pseudotuberculosis		7
	C. ulcerans		7, 8
	CDC group F		7

[a] All strains were found to have detectable acetic acid and/or lactic acid and/or succinic acid. Products were detected at the NML with identifiers as shown for taxon or otherwise from the reference cited. NML 88-0199, formerly LCDC 88-0199, was deposited as CIP 103499^{TS}. CIP, Collection of the Institute Pasteur; DMMZ, Department of Medical Microbiology, University of Zurich; ATCC, American Type Culture Collection; TS, type strain. We did not have any representative human or other strains to test for fermentation products from (medically relevant species) *C. lipophiloflavum*, *C. sanguinis*, or *C. singulare*, from (species relevant to animals) *C. auriscanis* (dogs), *C. camporealensis* (sheep), *C. mastitidis* (sheep), *C. phocae* (seals), *C. cystitidis*, or *C. pilosum* (bovines), or from (environmentally derived species) *C. acetoacidophilum*, *C. ammoniagenes*, *C. callunae*, *C. flavescens*, *C. glutamicum*, *C. terpenotabidum*, *C. variabile*, or *C. vitarumen.*

[b] No propionic acid was detected for an isolate identified here as CDC group F-1. In contrast, three strains of CDC group F reported by Estrangin et al. were found to produce propionic acid (7). As previously reviewed CDC group F-2 isolates lacking mycolates and identifiable as *C. amycolatum* should produce propionic acid (13), and so that taxon may have been represented in the Estrangin study.

Of the 15 *Corynebacterium* species or taxa described here, 11 (*C. argentoratense*, *C. confusum*, *C. coyleae*, *C. falsenii*, *C. glucuronolyticum*, *C. imitans*, *C. kroppenstedtii*, *C. mucifaciens*, *C. riegelii*, *C. simulans*, and *C. thomssenii)* have not been previously reported as being recovered outside Europe. The *C. argentoratense* strain (NML 98-0075, GenBank accession no. AF537589) was recovered from a blood culture, not from a throat specimen, lower respiratory tract, or ear (25, 26), and lacked significant chymotrypsinase activity. *C. bovis* identified by contemporary methods appears to be a very rare human pathogen (5, 13). This strain of *C. bovis*, a blood culture isolate (NML 99-0033, GenBank accession no. AF537590), was oxidase positive and did not produce β-galactosidase by two methods (API Coryne and API Zym) but was otherwise consistent with that species (13).

The abscess-derived strain of *C. confusum* (NML 99-0145, GenBank accession no. AF537591) found here contained tuberculostearic acid as a cellular fatty acid and produced propionic acid as a fermentation product (Table 2), but unlike the isolate described previously (9), this isolate was CAMP positive. Glucose was utilized if conventional methods were used, but this isolate was glucose negative with the API Coryne strip,

as found previously (9). Such isolates would have to be further discerned from *C. propinquum* or *C. coyleae* by the CAMP reaction, production of propionic acid, nitrate reduction, and tyrosine hydrolysis.

The originally described strains of *C. coyleae* were derived from sterile body fluids, including blood cultures and pleural fluid (17). We report here the first recoveries of this species from urine and from an abscess. 16S rRNA gene sequence data for one of the urine isolates (NML 97-0171) has been deposited as GenBank accession no. AF537592. Some strains tested with the O129 disk were found to be resistant. Acid was produced from ribose, fructose, and mannose in addition to glucose, as found by Funke et al. (17), which aids in differentiation from other taxa.

Three of the *C. durum* isolates described here were also reported in brief by Rassoulian Barrett et al. (21). *C. durum* is one of the few *Corynebacterium* species which can utilize mannitol and hydrolyze esculin, and these two tests were variable among our isolates. Members of this species have been recovered from respiratory tract specimens (24) or throat cultures of healthy volunteers (30) but not from blood cultures or an abscess, as found here. *C. durum* blood culture isolate NML 99-0047 (GenBank accession no. AF537593) did not reduce nitrate and fermented lactose. Several Canadian strains produced β-galactosidase, the detection of which had been observed previously (30). The *C. falsenii* strain reported here (NML 97-0205, GenBank accession no. AF537594) did not exhibit a yellow pigment which intensified with time but otherwise was highly consistent with the species description (29).

Table 1 test results for 11 strains of *C. glucuronolyticum* were as reported by Funke et al. (14). However, recovery of *C. glucuronolyticum* or its junior synonym *C. seminale* from blood cultures, disk fluid (NML 98-0013, GenBank accession no. AF537595), or peritoneal fluid (NML 98-0098, GenBank accession no. AF537596) had not been reported previously (6, 14, 23). Isolates of *C. imitans* found here were primarily recovered from blood cultures, including NML 01-0063 (GenBank accession no. AF537597), and all isolates were resistant to the O129 disk. Four out of six strains reduced nitrate by either the conventional or rapid strip method, and two of six did not reduce nitrate by either method, as described previously by Funke et al. (12).

C. kroppenstedtii was originally described based on characteristics of one strain from the sputum of a woman with pulmonary disease (3). Strains here were also recovered from respiratory specimens as well as from pus and blood cultures. One strain reduced nitrate (NML 87-0354, from pus), and one blood culture isolate (NML 00-0244, GenBank accession no. AF537598) was weakly reactive by conventional methods for mannitol and lactose but did not hydrolyze esculin. The remaining three strains hydrolyzed esculin, as found by Collins et al. (3).

Twenty-three strains of *C. mucifaciens* are described in Table 1, with 21 of 23 isolates being found to be highly consistent with the description by Funke et al. (15). One blood culture isolate, observed to have typical microbiological and morphological characteristics for this species, was selected for deposit in GenBank (NML 87-0436, GenBank accession no. AF357599). Many of the clinical sources shown in Table 1 had been reported previously by Funke et al. (15), with the exception of recovery from dialysate (NML 95-0019) and peritoneal fluid (NML 95-0189 and 95-0369). Blood culture isolate NML 01-0118 (GenBank accession no. AF537600, with 99.1% identity to *C. mucifaciens* [Gen Bank accession no. Y11200]) was found to have some aberrant characteristics in that it was observed to have an only slightly mucoid colony, exhibited weak beta-hemolysis, and was reactive in both sucrose and maltose with both the rapid strip and conventional methods.

It is interesting that, by phylogenetic analysis of the 16S rRNA gene sequences, *C. coyleae*, *C. afermentans*, and *C. mucifaciens* were most closely related (97.7% to 98.5% identity) with each other (15) but at times are difficult to differentiate even after using a polyphasic approach, for phenotypically aberrant strains. From Table 1, blood culture isolate NML 97-0160 (GenBank accession no. AF357601), by 16S rRNA sequence analysis, was closest to (98.3% identity with) *C. mucifaciens* (GenBank accession number Y11200) (Table 1) but differed from typical strains of that species by lacking pigment, having a smooth, not mucoid colony, hydrolyzing urea, and being resistant to the O129 disk. This and other aberrant strains may actually represent additional taxonomic diversity, requiring further study.

C. riegelii was originally recovered from urine specimens from women with urinary tract infections (16). Here, we report recovery from blood cultures, including NML 99-0185 (GenBank accession no. AF537602), as well as from urine (NML 97-0139).

Two strains of *C. simulans* were found in this review. One of these isolates, NML 81-0613 (GenBank accession no. AF537603), had been recovered from bile and was initially identified as resembling Centers for Disease Control (CDC) group G-1. This strain had been studied along with other lipophilic coryneforms, including CDC group G strains, by Riegel et al. and found to be like members of the *Corynebacterium* genus but could not be definitively assigned to any existing species at that time (22, 27). After molecular studies and demonstration of nitrite reduction, this isolate could be definitively assigned to *C. simulans* but differed from the description by Wattiau et al. by utilizing maltose (31). The second strain of *C. simulans* derived from a blood culture (NML 00-0186, GenBank accession no. AF537604) was aberrant by repeatedly being catalase negative but otherwise consistent with the species description by Wattiau et al.

C. sundsvallense isolates found here include one from a draining sinus, described in brief previously (4) (NML 93-0639, GenBank accession no. AF537605), and a blood culture (NML 99-0121, GenBank accession no. AF537606). *C. sundsvallense* and *C. thomssenii* were both described within a short time period in 1999, based on the description of three strains and one strain, respectively, and share a number of commonalities biochemically (4, 33). *C. sundsvallense* and *C. thomssenii* (GenBank accession no. Y09655 and AF010474, respectively) are closely related by comparative 16S rRNA gene sequence analysis (98.9% identity; data not shown). The *C. thomssenii* isolate found here (NML isolate 97-0130, GenBank accession no. AF537607) was recovered from an air sample of an operating theater being studied due to contamination by a coryneform. The sequence derived from this strain had 99.9% identity with *C. thomssenii* (GenBank accession no. AF010474) and was consistent with that species except that oxidase was weakly

positive and that *N*-acetyl-β-glucosaminidase could not be demonstrated, in spite of several attempts.

Two black-pigmented *Corynebacterium*-like strains similar to the isolate described by Shukla et al. (28) have been characterized here. That isolate is referred to in GenBank as "*Corynebacterium nigricans*," currently a nonvalidated species name. These bacteria were recovered from a vulval ulcer (NML 91-0032) and vaginal specimen (NML 92-0360, GenBank accession no. AF537608), sources similar to that of the Shukla isolate. The strains here were pyrazimamidase and alkaline phosphatase negative with the API Coryne strip. Ribose and glycogen were negative with both the API Coryne strip and conventional methods, differing from the strain reported by Shukla et al. (28).

Products of fermentation were reviewed for each taxon group in Table 2. All taxa produced small or moderate volumes of acetic, lactic, and/or succinic acid. The presence or absence of propionic acid appears to be species dependent, as originally postulated (7), and so observation of that product may be useful for discerning between phenotypically close taxa.

In summary, we demonstrated with a polyphasic approach that rare or new *Corynebacterium* species could be recovered in geographic areas outside Europe. Some isolates were observed to have characteristics or were recovered from clinical sources which had not been described previously, which may prove useful for clinical microbiology laboratories attempting to identify *Corynebacterium* species.

Nucleotide sequence accession number. The following accession numbers (NML isolate designations in parentheses) have been reported to GenBank: AF537589 (98-0075), AF537590 (99-0033), AF537591 (99-0145), AF537592 (97-0171), AF537593 (99-0047), AF537594 (97-0205), AF537595 (98-0013), AF537596 (98-0098), AF537597 (01-0063), AF537598 (00-0244), AF537599 (87-0436), AF537600 (01-0118), AF537601 (97-0160), AF537602 (99-0185), AF537603 (81-0613), AF537604 (00-0186), AF537605 (93-0639), AF537606 (99-0121), AF537607 (97-0130), and AF537608 (92-0360).

ADDENDUM

Two additional *Corynebacterium* species have been described since submission of our manuscript, *Corynebacterium aurimucosum* sp. nov. (32a) and *Corynebacterium appendicis* (32b). It is not known if the Canadian reference center has encountered any strains of these species.

The assistance of Shaun Tyler and the staff of the DNA core facility of the NML is gratefully acknowledged.

REFERENCES

1. **Bernard, K. A., L. Shuttleworth, C. Munro, J. C. Forbes-Faulkner, D. Pitt, J. H. Norton, and A. D. Thomas.** 2002. *Propionibacterium australiense* sp. nov. derived from granulomatous bovine lesions. Anaerobe **8:**41–47.
2. **Bernard, K. A., M. Bellefeuille, and E. P. Ewan.** 1991. Cellular fatty acid composition as an adjunct to the identification of asporogenous, aerobic gram-positive rods. J. Clin. Microbiol. **29:**83–89.
3. **Collins, M. D., E. Falsen, E. Akervall, B. Sjoden, and A. Alvarez.** 1998. *Corynebacterium kroppenstedtii* sp. nov., a novel corynebacterium that does not contain mycolic acids. Int. J. Syst. Bacteriol. **48:**1449–1454.
4. **Collins, M. D., K. A. Bernard, R. A. Hutson, B. Sjoden, A. Nyberg, and E. Falsen.** 1999. *Corynebacterium sundsvallense* sp. nov., from human clinical specimens. Int. J. Syst. Bacteriol. **49:**361–366.
5. **Coyle, M. B., and B. A. Lipsky.** 1990. Coryneform bacteria in infectious diseases: clinical and laboratory aspects. Clin. Microbiol. Rev. **3:**227–246.
6. **Devriese, L. A., P. Riegel, J. Hommez, M. Vaneechoutte, T. De Baere, and F. Haesebrouck.** 2000. Identification of *Corynebacterium glucuronolyticum* strains from the urogenital tract of humans and pigs. J. Clin. Microbiol. **38:**4657–4659.
7. **Estrangin, E., B. Thiers, and Y. Peloux.** 1987. Micromethods and gas chromatography analysis of carboxylic acids produced from the fermentation of glucose in the identification of corynebacteria. Ann. Biol. Clin. (Paris) **45:** 285–289.
8. **Früh, M., A. von Graevenitz, and G. Funke.** 1998. Use of second-line biochemical and susceptibility tests for the differential diagnosis of coryneform bacteria. Clin. Microbiol. Infect. **4:**332–338.
9. **Funke, G., C. R. Osorio, R. Frei, P. Riegel, and M. D. Collins.** 1998. *Corynebacterium confusum* sp. nov., isolated from human clinical specimens. Int. J. Syst. Bacteriol. **48:**1291–1296.
10. **Funke, G., and K. A. Bernard.** 1999. Coryneform gram-positive rods, p. 319–345. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Holden (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
11. **Funke, G., and K. A. Bernard.** Coryneform gram-positive rods. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Holden (ed.), Manual of clinical microbiology, 8th ed., in press. American Society for Microbiology, Washington, D.C.
12. **Funke, G., A. Efstratiou, D. Kuklinska, R. A. Hutson, A. De Zoysa, K. H. Engler, and M. D. Collins.** 1997. *Corynebacterium imitans* sp. nov. isolated from patients with suspected diphtheria. J. Clin. Microbiol. **35:**1978–1983.
13. **Funke, G., A. von Graevenitz, J. E. Clarridge III, and K. A. Bernard.** 1997. Clinical microbiology of coryneform bacteria. Clin. Microbiol. Rev. **10**:125–159.
14. **Funke, G., K. A. Bernard, C. Bucher, G. E. Pfyffer, and M. D. Collins.** 1995. *Corynebacterium glucuronolyticum* sp. nov. isolated from male patients with genitourinary infections. Med. Microbiol. Lett. **4:**204–215.
15. **Funke, G., P. A. Lawson, and M. D. Collins.** 1997. *Corynebacterium mucifaciens* sp. nov., an unusual species from human clinical material. Int. J. Syst. Bacteriol. **47:**952–957.
16. **Funke, G., P. A. Lawson, and M. D. Collins.** 1998. *Corynebacterium riegelii* sp. nov., an unusual species isolated from female patients with urinary tract infections. J. Clin. Microbiol. **36:**624–627.
17. **Funke, G., C. P. Ramos, and M. D. Collins.** 1997. *Corynebacterium coyleae* sp. nov., isolated from human clinical specimens. Int. J. Syst. Bacteriol. **47:**92–96.
18. **Funke, G., P. A. Lawson, K. A. Bernard, and M. D. Collins.** 1996. Most *Corynebacterium xerosis* strains identified in the routine clinical laboratory correspond to *Corynebacterium amycolatum*. J. Clin. Microbiol. **34:**1124–1128.
19. **Hollis, D. G., and R. E. Weaver.** 1981. Gram-positive organisms: a guide to identification. Special Bacteriology Section, Centers for Disease Control, Atlanta, Ga.
20. **MacFaddin, J. F.** 2000. Biochemical tests for identification of medical bacteria. Lippincott, Williams & Wilkins, Philadelphia, Pa.
21. **Rassoulian Barrett, S. L., B. T. Cookson, L. C. Carlson, K. A. Bernard, and M. B. Coyle.** 2001. Diversity within reference strains of *Corynebacterium matruchotii* includes *Corynebacterium durum* and a novel organism. J. Clin. Microbiol. **39:**943–948.
22. **Riegel, P., D. de Briel, G. Prévost, F. Jehl, and H. Monteil.** 1994. Genomic diversity among *Corynebacterium jeikeium* strains and comparison with biochemical characteristics. J. Clin. Microbiol. **32:**1860–1865.
23. **Riegel, P., R. Ruimy, D. de Briel, G. Prévost, F. Jehl, F. Bimet, R. Christen, and H. Monteil.** 1995. *Corynebacterium seminale* sp. nov., a new species associated with genital infections in male patients. J. Clin. Microbiol. **33:** 2244–2249.
24. **Riegel, P., R. Heller, G. Prevost, F. Jehl, and H. Monteil.** 1997. *Corynebacterium durum* sp. nov. from human clinical specimens. Int. J. Syst. Bacteriol. **47:**1107–1111.
25. **Riegel, P., R. Ruimy, R. Christen, and H. Monteil.** 1996. Species identities and antimicrobial susceptibilities of corynebacteria isolated from various clinical sources. Eur. J. Clin. Microbiol. Infect. Dis. **15:**657–662.
26. **Riegel, P., R. Ruimy, D. de Briel, G. Prevost, F. Jehl, F. Bimet, R. Christen, and H. Monteil.** 1995. *Corynebacterium argentoratense* sp. nov., from the human throat. Int. J. Syst. Bacteriol. **45:**533–537.
27. **Riegel, P., R. Ruimy, D. de Briel, G. Prévost, F. Jehl, R. Christen, and H. Monteil.** 1995. Genomic diversity and phylogenetic relationships among lipid-requiring diphtheroids from humans and characterization of *Corynebacterium macginleyi* sp. nov. Int. J. Syst. Bacteriol. **45:**128–133.
28. **Shukla, S. K., D. N. Vevea, D. N. Frank, N. R. Pace, and K. D. Reed.** 2001. Isolation and characterization of a black-pigmented *Corynebacterium* sp. from a woman with spontaneous abortion. J. Clin. Microbiol. **39:**1109–1113.
29. **Sjöden, B., G. Funke, A. Izquierdo, E. Akervall, and M. D. Collins.** 1998. Description of some coryneform bacteria isolated from human clinical specimens as *Corynebacterium falsenii* sp. nov. Int. J. Syst. Bacteriol. **48:**69–74.

30. **von Graevenitz, A., V. Pünter-Streit, P. Riegel, and G. Funke.** 1998. Coryneform bacteria in throat cultures of healthy individuals. J. Clin. Microbiol. **36:**2087–2088.
31. **Wattiau, P., M. Janssens, and G. Wauters.** 2000. *Corynebacterium simulans* sp. nov., a nonlipophilic, fermentative corynebacterium. Int. J. Syst. Evol. Microbiol. **50:**347–353.
32. **Wauters, G., B. van Bosterhaut, M. Janssens, and J. Verhaegen.** 1998. Identification of *Corynebacterium amycolatum* and other nonlipophilic fermentative corynebacteria of human origin. J. Clin. Microbiol. **36:**1430–1432.
32a. **Yassin, A. F., U. Steiner, and W. Ludwig.** 2002. *Corynebacterium aurimucosum* sp. nov. Int. J. Syst. Evol. Microbiol. **52:**1001–1005.
32b. **Yassin, A. F., U. Steiner, and W. Ludwig.** 2002. *Corynebacterium appendicis* sp. nov. Int. J. Syst. Evol. Microbiol. **52:**1165–1169.
33. **Zimmermann, O., C. Spröer, R. M. Kroppenstedt, E. Fuchs, H. G. Köchel, and G. Funke.** 1998. *Corynebacterium thomssenii* sp. nov., a *Corynebacterium* with *N*-acetyl-β-glucosaminidase activity from human clinical specimens. Int. J. Syst. Bacteriol. **48:**489–494.
34. **Zinkernagel, A. S., A. von Graevenitz, and G. Funke.** 1996. Heterogeneity within *Corynebacterium minutissimum* strains is explained by misidentified *Corynebacterium amycolatum* strains. Am. J. Clin. Pathol. **106:**378–383.