Achromobacter Species (CDC Group Vd): Morphological and Biochemical Characterization

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Twenty-three isolates of *Achromobacter* species (CDC group Vd) were examined morphologically and biochemically. Gram stains revealed gram-variable bacilli frequently curved or hooked at one pole and often coryneform in shape and arrangement. Electron microscopy revealed the presence of extracellular material in polar accumulations and demonstrated the polar flagella arrangement seen by light microscopy to be lateral. Two colony types were produced; one was minute and watery at 24 h (35°C) progressing to large, mucoid colonies at 48 h, and the other type was shiny, glistening, opaque but nonmucoid. All isolates grew on MacConkey agar and produced catalase, oxidase, and urease. Most grew on salmonella-shigella agar, reduced nitrate to nitrite and gas, hydrolyzed esculin, deaminated phenylalanine (2 to 4 days) and produced H₂S in triple sugar iron agar (4 to 12 days). Oxidation of carbohydrates was weak, delayed, and limited to glucose and xylose. Two isolates also oxidized maltose, mannitol, and sucrose. The ability of miniaturized "nonfermenter" kits to identify *Achromobacter* species was tested. The Minitek (Baltimore Biological Laboratory, Cockeysville, Md.) and N/F (Corning, Roslyn, N.Y.) systems, respectively, identified 21 and 19 of the 23 isolates, whereas the Oxi/Ferm (Roche, Nutley, N.J.) identified 13 and the API 20E (Analytab Products, Plainview, N.Y.) identified only 3.

In the eighth edition of *Bergey's Manual of Determinative Bacteriology*, the various members of the genus *Achromobacter*, although cross-indexed as *Achromobacter*, have been reassigned to several genera, usually *Alcaligenes* but including *Arthrobacter*, *Agrobacterium*, *Acinetobacter*, *Brevibacterium*, *Corynebacterium*, *Lucibacterium*, *Pseudomonas*, and *Vibrio* (2). In fact, some authorities have recommended the rejection of the name *Achromobacter* (7). However, a group of bacteria clinically encountered and designated *Achromobacter xylosoxidans* (16) and *Achromobacter* species (13) remains outside these taxonomic changes and reassignments. The subject of this study, *Achromobacter* species, is referred to as group Vd by the Special Bacteriology Section of the Center for Disease Control (CDC), Atlanta, Ga. (14). *Achromobacter* species isolates possess the general characteristics of the genus *Achromobacter* (8), are gram-negative, oxidase-positive, obligately aerobic, nonfermenting bacilli with peritrichous flagella, and are assigned to one of two biotypes based primarily on ability to oxidize maltose, mannitol, and sucrose.

In this report, 23 strains of *Achromobacter* species have been morphologically and biochemically characterized to provide additional infor-

mation relative to the differentiation of this bacterium from *A. xylosoxidans* and members of similar clinically encountered genera and groups, e.g., *Alcaligenes*, *Bordetella*, *Moraxella*, *Pseudomonas*, and CDC group IVe, IVc-2, and IIk. The biochemical information provided involves both conventional methodology and "miniaturized" identification kits.

MATERIALS AND METHODS

Bacteria. Table 1 lists the 23 strains of *Achromobacter* species studied and indicates for each the body site from which the isolate was obtained, status at time of examination, i.e., reference culture or recent isolate, biotype, and contributor.

Gram stain. The stains, mordant, and decolorizer used were those incorporated into the prepared Gram stain kit (Difco Laboratories, Detroit, Mich.). Application times for reagents were crystal violet, 1 min; Gram iodine solution, 1 min; decolorizer, until solvent ran colorlessly from the slide; and safranin, 10 s (10). Material for Gram staining consisted of growth on 5% sheep blood agar (BA, Baltimore Biological Laboratory [BBL], Cockeysville, Md.) and MacConkey agar with crystal violet (MC, BBL), each incubated at 25 and 35°C for 24 and 48 h. In addition, Trypticase soy broth cultures (TSB, BBL) were Gram stained after 24 h at 35°C.

Agar and broth morphology. Observations of colony formation were made under the following con-

TABLE 1. *Achromobacter species* (CDC group Vd) isolates studied

Isolate no.	Source	Biotype	Contributor	Designation of contributor
104	Blood ^a	2 ^b	R. E. Weaver, CDC, Atlanta, Ga.	D9035
146	Incision ^a	1 ^b		C675
219	Not known ^a	1 ^b		Not known
228	Blood ^a	1 ^b		C5250
229	Blood ^a	2 ^b		D9386
230	Urine ^a	2 ^b		D9053
241	Blood ^a	1 ^b		D2759
252	Throat ^a	2 ^b		D8708
291	Urine ^c	1 ^d		Not known
359	Urine ^c	1 ^d	J. Kittick, Elmhurst Hospital, New York.	Not known
401	Urine ^c	1 ^d	S. B. Wee, Johns Hopkins Hospital, Baltimore, Md.	X468.2
428	Stool ^c	1 ^d	G. L. Gilardi, Hospital for Joint Diseases, New York.	T266
429	Surgical wound ^c	1 ^d		N356.3
779	Urine ^c	2 ^d		Z676.3
781	Oral abscess ^c	1 ^d		O951
782	Urine ^c	1 ^d		V631.3
783	Not known ^c	1 ^d		Not known
784	Ankle wound ^c	1 ^d		3188
785	Hip wound ^c	1 ^d		3194
786	Vaginal ^c	1 ^d		3195
787	Environmental ^c	1 ^d	T. C. J. Cleary, Jackson Memorial Hospital, Miami, Fla.	3200
917	Urine ^c	2 ^d		Not known
925	Not known ^c	1 ^d	L. Garcia, Jackson Memorial Hospital, Miami, Fla.	Not known

^a Stock culture.^b Determined by contributor.^c Fresh isolate.^d Determined by authors.

ditions: BA and MC; 25 and 35°C incubation temperature; 24-, 48-, and 72-h incubation periods. Growth in broth was observed for each strain with TSB incubated at 35°C for 24 and 72 h.

Motility determination. Motility was determined by dispersing a portion of a colony grown on BA (35°C, 24 h) into 2 drops of TSB followed by placing a cover slip over the preparation and observing microscopically (×1,000). Motility tests were also performed via microscopic examination of several drops of a TSB culture incubated overnight at 25°C.

Flagella staining. The presence and pattern of flagella were determined with a modified Fontana silver staining procedure recommended by West et al. (15) and by electron microscopy with phosphotungstic acid staining (6).

Conventional biochemical studies. Catalase was determined by introducing a portion of a colony grown on BA (35°C, 24 h) into a drop of 3% hydrogen peroxide and observing for bubbling (O₂). Oxidase activity was examined both for cultures grown on BA and MC (35°C, 24 h) with cytochrome oxidase strips (General Diagnostics, Warner-Lambert, Morris Plains, N.J.) and N disks (BBL). Phenylalanine deaminase activity was tested daily on phenylalanine agar slants (Difco) incubated at 35°C for 1, 2, 3, 4, and 10 days by

adding 5 drops of 10% (wt/vol) ferric chloride and observing for the formation of a green color, best, and occasionally only, seen by viewing the agar border from the side. Urease testing was done with Christensen urea agar slants (Difco) (35°C, 24 and 48 h). Hydrolysis of esculin was examined on esculin agar slants (Difco) by observing the formation of a blackening of the agar (35°C daily for 10 days) and by loss of fluorescence with a Wood lamp (366-nm wavelength) (4). Utilization of citrate as a sole carbon source was determined by growth on and alkalization of Simmons citrate agar slants (BBL) (35°C daily for 10 days). Oxidation of glucose, xylose, maltose, mannitol, and sucrose was tested with oxidative-fermentative (OF) basal medium containing 1% of the test carbohydrate (BBL) and bromothymol blue indicator. Two glucose tubes were used for each isolate, one with oil overlay. Each was stabbed four times with inoculum from BA (35°C, 24 h), incubated at 35°C, and examined daily for 10 days. Parallel testing of carbohydrates was done with OF basal medium also containing bromothymol blue indicator and 1% carbohydrate in two-part constricted tubes (Corning Medical Microbiology, Roslyn, N.Y.). Oxidation of the test carbohydrate was detected by a perceptible indicator change from green to yellow (9). Alkalinization of

acetamide was examined with the acetamide agar section of the N/F system (Corning) (35°C, daily observation for 10 days). Growth on cetrinide (cetyltrimethylammonium bromide) was tested on cetrinide agar slants (BBL) (35°C, daily observation for 10 days). Growth on salmonella-shigella agar (Difco) was observed daily for 10 days (35°C). Ornithine and lysine decarboxylase and arginine dihydrolase activities were examined by heavily inoculating 5 ml of Moeller decarboxylase broth containing 1% of the test amino acid (BBL), overlaying with 2 ml of oil, incubating at 35°C, and observing daily for 5 days for an indicator change to purple. Deoxyribonuclease production was detected by a clearing of the green indicator color surrounding the growth of the isolate on deoxyribonucleic acid agar containing methyl green (Difco) (35°C, daily for 5 days). Indole formation was tested by xylene extraction of a 1% tryptophane broth culture (tryptone, Difco) (35°C, 48 h and 5 days). The production of beta-galactosidase was tested by adding a tablet of *O*-nitrophenyl- β -D-galactopyranoside (ONPG, Key Scientific Products, Los Angeles, Calif.) to a 1-ml suspension of the test isolate in distilled water and observing for a yellow color (35°C, 8 h and daily for 5 days). Hydrogen sulfide was detected on triple iron agar slants (Difco) (35°C, daily observation for 14 days) as a black color best seen at the junction of the butt and slant. Gelatinase production was examined with the use of gelatin strips (Key Scientific Products) incubated at 25 and 35°C and observed daily for 10 days.

Growth at 42°C. The ability of each isolate to grow at 42°C was tested by lightly inoculating 5 ml of brain heart infusion broth (Difco) previously warmed to 42°C and maintained at 42°C in a temp-block overnight. At the time of interpretation, a brain heart infusion was inoculated with the isolate in the same manner as the 42°C brain heart infusions and the turbidity of the two was compared. Any increase in turbidity of the 42°C brain heart infusion was considered a positive test.

Minitek nonfermenter identification system. The Minitek system for the identification of nonfermenters (BBL) consists of paper disks impregnated with appropriate biochemicals: dextrose, maltose, sucrose, xylose, urea, citrate, nitrate (reduction and denitrification), phenylalanine, ornithine, arginine, lysine, ONPG, and starch. The suspending broth serves as substrate for the indole test. The disks are dispensed into a plastic plate containing wells. Into each well containing a disk, 0.05 ml of organism suspended in broth (Minitek Enteric and Nonfermenter Broth, BBL) is pipetted. After overlaying with oil, disks containing dextrose (a second dextrose disk is not overlaid), urea, ornithine, arginine, and lysine, the plates are incubated in a humidator at 35°C for 48 h (ONPG and urea are read at 24 h). After the addition of appropriate reagents for indole, nitrate reduction and denitrification, phenylalanine deamination, and starch hydrolysis, the observed reactions are interpreted with tables and a code book provided by the manufacturer.

N/F system. The N/F system (Corning) for the identification of nonfermenters consists of two tubes for the purpose of screening for *Pseudomonas aeruginosa*, *P. fluorescens*, and *P. putida* and a plastic "wheel" composed of 12 agar sections to provide in-

formation relative to the identification of nonfermenters other than the fluorescent pseudomonads. One tube (42P) consists of an agar slant used for growth at 42°C and pyocyanin production. The second tube (GNF) consists of two sections: the upper one to demonstrate fluorescein production and the lower one to detect denitrification and fermentation of glucose. Both tubes are incubated overnight, the former at 42°C and the latter at 35°C. The plastic wheel (Uni-N/F-Tek) contains a central agar area for the detection of indole and H₂S and 11 peripheral agar sections for the following tests: carbohydrate control, dextrose, maltose, mannitol, lactose, xylose, ONPG, deoxyribonucleic acid, esculin, urea, and acetamide. The tubes are inoculated with a portion of the isolated colony and each agar section of the wheel is inoculated on the next day with a distilled water suspension of growth from the GNF slant. The wheel is incubated at 35°C for 48 h. Indole is detected by soaking a swab with Kovac reagent, twisting the swab into the growth on the central agar section, and observing the formation of a red color on the swab. Observed reactions are interpreted with a flow chart and code book provided by the manufacturer.

Oxi/Ferm tube identification system. The Oxi/Ferm tube (Roche Diagnostics, Nutley, N.J.) consists of a plastic tube with eight compartments, each containing a different substrate for the following tests: OF glucose fermentation, arginine, denitrification, H₂S, indole, OF glucose and xylose oxidation, urea, and citrate. The use of this system has been previously described (11).

API system. The API enteric system (API 20E, Analytab Products, Plainview, N.Y.) contains 20 microtubes, each with a dehydrated substrate providing for the following tests: ONPG, arginine, lysine, ornithine, tryptophan, citrate, H₂S, indole, Voges-Proskauer, gelatin, oxidation of glucose, mannitol, inositol, sorbitol, rhamnose, melibiose, amygdalin, and arabinose, nitrate reduction, and denitrification. The use of this system has been previously described (12).

Antimicrobial susceptibility testing. Susceptibility testing was performed by the Kirby-Bauer disk diffusion method (1) with the following antimicrobial disks on 150-mm Mueller-Hinton agar plates (BBL): ampicillin (30 μ g), amikacin (10 μ g), carbenicillin (100 μ g), cephalothin (30 μ g), chloramphenicol (30 μ g), nitrofurantoin (300 μ g), penicillin G (10 μ g), tetracycline (30 μ g), tobramycin (10 μ g), and trimethoprim-sulfamethoxazole (1.25, 23.75 μ g) (all BBL). Due to the poor growth of each isolate in TSB after 8 h of incubation (35°C), overnight TSB cultures were used as inocula.

RESULTS

Macroscopic morphology. On BA, two colony types were seen. The more common type, produced by 17 of the 23 isolates after overnight incubation at 35°C, was extremely small, ranging from barely visible to 0.75 mm in diameter; watery, gray, translucent, convex, entire and nonhemolytic in appearance; and butyrous in consistency. Areas of confluent growth were mu-

coid (Fig. 1A). At 48 h, the colonies ranged in size from 0.75 to 4.0 mm, had become opaque and gray-white and were extremely mucoid (Fig. 1B). At this time, a weak beta-hemolysis was apparent in areas of confluent growth. Incubation for an additional 24 to 48 h, whether at 35 or 25°C, resulted in strong beta-hemolysis in confluent areas and a mottled appearance of confluent growth as a result of the more opaque central portions of each colony visible through the large mass of translucent mucoid material forming the colony periphery.

The less common colony type, seen with 6 of the 23 isolates, was pinpoint in size at 24 h with no indication of the watery or mucoid nature seen with the other type, even in areas of confluence, which instead possessed a semiglossy, gray appearance and butyrous consistency. At 48 h, colonies were 0.25 to 1.25 mm in diameter and opaque, glossy, entire and convex in appearance (Fig. 1C). Colonies were either white or white with a gray periphery. Areas of confluent growth were glossy, opaque, and gray or gray-white. Continued incubation caused the appearance of a beta-hemolysis as seen with the mucoid type of colony.

Two strains, 104 and 787, although forming typically mucoid colonies, appeared as mixed cultures due to the presence of some colonies which required 4 days before appearing mucoid. Both the rapid and delayed mucoid colonies produced both variants on subculture.

On MC, mucoid type colonies, after 24 h of incubation at 35°C, were pinpoint in size. After 48 h, colonies ranged in size from 0.5 to 2.25 mm in diameter and were opaque and occasionally mucoid. Continued incubation resulted in colonies with diameters from 3.0 to 5.0 mm, opaque and pink to purple in color. Only six strains produced mucoid areas of growth. Half of the strains produced colonies with a consistency so gummy that attempts to remove them from the agar with a loop was difficult or impossible and resulted in the formation of a sticky mass of growth which remained adherent to the agar surface.

Nonmucoid colonial morphology on MC was similar to that of the mucoid type.

After incubation at 35°C for 8 h, TSB cultures exhibited a barely visible turbidity which, after an additional 16 h, increased to approximately 1.5×10^8 bacteria per ml (one half that of the McFarland number one barium sulfate standard). The growth was uniformly distributed throughout the broth, although two strains demonstrated increased growth near the broth surface after 24 h. A small amount of sediment was produced by most of the isolates and, upon rotation of the TSB, the sediment rose upward

FIG. 1. Mucoid and nonmucoid colony types produced by *Achromobacter* species (CDC group Vd). (A) Strain 104 at 24 h showing minute, watery colonies progressing to (B) large, mucoid colonies at 48 h. Note the mottled appearance of confluent growth. (C) Strain 779 at 48 h showing colonies representative of the nonmucoid type.

in a ropy, spiral pattern. After an additional 48 h, all of the cultures exhibited increased turbidity near the surface and the formation of a slimy pellicle.

Microscopic morphology. Cells of *Achromobacter* species, when examined from BA colonies (35°C, 24 h), appeared as moderate-sized (0.5 to 0.8 by 1.5 to 2.5 μ m) gram-negative bacilli, single and diploid, with some gram-positive forms usually present (Fig. 2). The bacilli were often coryneform in shape, sometimes with a curved axis or with a swollen hook shape at one end.

Cells grown on MC (35°C, 24 h), although exhibiting the same morphology as cells from BA, had a stronger tendency to retain the crystal violet of the Gram stain. The majority of the strains studied exhibited gram-positive forms when stained from MC.

Cells grown in TSB (35°C, 24 h) were similar in shape to those from BA and MC except that fewer gram-positive cells were seen. The "bar-ring" ascribed to cells of *A. xylosoxidans* (13) and some strains of *Achromobacter* species (9) was not observed.

Motility testing. Each of the 23 isolates was motile when seen by microscopic examination of wet preparations from TSB cultures incubated overnight at 25°C. All except one isolate were motile when tested by wet preparations of colonies directly from BA (35°C, 24 h). However, with the latter method, examination of several

high-power fields ($\times 100$) was necessary with five of the isolates before motile cells were seen.

Flagella staining. Examination of smears made from TSB cultures (35°C, 24 h) and stained with a modified Fontana silver staining method (15) revealed what appeared to be predominantly (95%) monopolar flagella cells with an occasional cell possessing a long lateral flagellum. The same pattern of flagellation was seen with TSB cultures incubated at 25°C for 24 h except that many of the cells possessed two to three flagella rather than one.

Electron microscopy. Electron micrographs revealed the accumulation of extracellular material on many of the cells often at one pole (Fig. 3). Apparently these accumulations add irregular contours so that otherwise symmetrical cells, on Gram stain, appear as curved and hooked forms (Fig. 2).

Electron microscopy also demonstrated that most of the apparently monopolar flagellate cells were actually monolateral flagellate cells with the long lateral flagellum running along the surface of the bacillus and eventually trailing freely in the surrounding broth (Fig. 4). Nevertheless, some monopolar forms were seen even with electron microscopy (Fig. 5).

Conventional biochemical testing. Each

FIG. 2. Typical Gram stain appearance of *Achromobacter* species (CDC group Vd). Many of the cells have a coryneform appearance. Curved (solid arrow-head) and hooked-end (arrow) forms are also present.

FIG. 3. Electron micrograph of *Achromobacter* species (CDC group Vd) demonstrating extracellular material surrounding the cell and forming a polar cap. On Gram stain, this material is indistinguishable from the cell and causes the observer to see hooked and swollen-end forms ($\times 22,750$).

FIG. 4. Electron micrograph of *Achromobacter* species (CDC group Vd) showing a cell possessing a monolateral flagellum ($\times 22,750$).

FIG. 5. Electron micrograph of *Achromobacter* species (CDC group Vd) showing a cell possessing a monopolar flagellum ($\times 22,750$).

of the 23 isolates of *Achromobacter* species grew on MC, and all except strain 779 grew on salmonella-shigella agar. All isolates were positive for catalase (strong reaction), oxidase, and urease (Table 2). With one exception, strain 925, all produced phenylalanine deaminase after 2 to 4 days. However, positive reactions for this test were weak and appeared 15 to 30 s after the addition of ferric chloride as a green color at the periphery of the growth on phenylalanine agar. This green color was best seen by viewing the agar from the side. Most isolates (91.2%) denitrified nitrate, hydrolyzed esculin, and produced small amounts of hydrogen sulfide on triple

sugar iron agar visible at the junction of the slant and butt after 4 to 5 days and, occasionally, not until 12 days. A majority of isolates utilized citrate as a sole source of carbon (73.6%). Oxidation of carbohydrates was generally weak and delayed, with positive reactions appearing after 3 days and essentially limited to xylose (78.0%) and glucose (30.8%). Most of the strains tested, including four reference cultures of biotype 2, failed to produce detectable acid within 10 days from maltose, mannitol, or sucrose. Strains 779 and 917 were the only two which oxidized these three carbohydrates, and were also the only strains which produced beta-galactosidase.

TABLE 2. Biochemical characteristics of 23 isolates of *Achromobacter* species (CDC group Vd) determined by conventional testing^a

Isolate	Test of substrate ^b												
	Pa	N ₂	Esc	H ₂ S	SS	Cit	Xy-lose	Gluc	Mn	Malt	Suc	ONPG	42°C
104	2 ^c	2	2	(7)	1	1	(4)	—	—	—	—	—	—
146	2	2	3	(5)	1	—	1	(4)	—	—	—	—	+
219	(2)	2	1	(12)	1	1	1	—	—	—	—	—	—
228	1	2	1	(7)	1	—	3	—	—	—	—	—	(+)
229	2	2	1	(4)	1	2	(4)	—	—	—	—	—	—
230	2	2	2	(7)	1	—	(4)	—	—	—	—	—	—
241	2	2	1	(4)	1	1	—	—	—	—	—	—	—
252	2	2	1	(5)	1	—	—	—	—	—	—	—	—
291	2	—	1	(5)	1	1	3	—	—	—	—	—	—
359	2	2	(2)	(5)	1	—	3	(5)	—	—	—	—	—
401	2	2	2	(5)	1	1	—	—	—	—	—	—	—
428	2	2	2	(5)	1	1	(3)	—	—	—	—	—	—
429	2	2	1	—	1	1	3	—	—	—	—	—	(+)
779	(3)	2	1	—	—	2	3	3	3	3	3	1	—
781	(2)	2	2	(4)	1	1	(3)	—	—	—	—	—	—
782	(4)	2	2	(12)	1	1	(6)	—	—	—	—	—	—
783	3	2	3	(4)	1	1	(4)	—	—	—	—	—	—
784	(3)	2	1	(5)	1	—	(5)	—	—	—	—	—	—
785	2	2	—	(5)	1	1	2	2	—	—	—	—	—
786	(2)	2	—	(5)	(2)	1	3	2	—	—	—	—	—
787	(2)	2	1	(7)	1	1	—	—	—	—	—	—	—
917	(2)	—	1	(4)	(2)	2	2	2	2	2	2	1	—
925	—	2	2	(4)	2	1	—	(2)	—	—	—	—	—
% Positive	95.6	91.2	91.2	91.2	95.6	73.6	78.0	30.8	8.8	8.8	8.8	8.8	13.2

^a All isolates were positive for oxidase, catalase, and urease (779, 785, and 786 required 2 days of incubation) and grew on MacConkey agar. All isolates were negative for arginine, lysine, ornithine, deoxyribonucleic acid, gelatin, indole, acetamide, and anaerobic glucose and failed to grow on cetrimide agar.

^b Abbreviations: Pa, phenylalanine deaminase; N₂, denitrification; Esc, esculin; SS, salmonella-shigella agar; Cit, citrate; Gluc, glucose; Mn, mannitol; Malt, maltose; Suc, sucrose.

^c Numerical value, number of days required for positive test; (), weak reaction; +, positive for test; —, negative for test.

None of the isolates was positive for any of the following tests: arginine, lysine, ornithine, cetrimide, deoxyribonucleic acid, gelatin, indole, acetamide, or fermentation of glucose. Only three isolates grew at 42°C.

Minitex nonfermenter identification system. The Minitex system correctly identified 21 of the 23 isolates as *Achromobacter* species after 48 h (Table 3). The Minitex system correctly demonstrated all positive reactions for urease, phenylalanine deaminase, denitrification, and oxidation of xylose and glucose. In fact, strain 925, phenylalanine deaminase negative with conventional phenylalanine agar, was positive with the Minitex phenylalanine disk. The Minitex glucose and xylose disks appeared to be more sensitive for the detection of acid than the corresponding conventional tests; strains 241, 252, 401, and 787, all negative on xylose (OF media), were positive with the Minitex xylose disk. However, the Minitex maltose and sucrose disks were as unsuccessful as the corresponding conven-

tional tests in demonstrating positive reactions with any of the four reference cultures of biotype 2. Of 17 isolates, 13 were correctly citrate positive. Four false negative citrate reactions were seen with isolates 779, 785, 786, and 917. The Minitex esculin disk, although not used in this system's basic identification setup of 14 disks, was ineffective and failed to identify 17 of the 21 esculin-positive reactions.

Corning N/F system. At present, due to a lack of insufficient data (Corning Technical Information Dept., personal communication), the Corning N/F system does not code for *Achromobacter* species. However, based on positive reactions for urease, denitrification, esculin hydrolysis, and oxidation of glucose and xylose, 19 of the 23 isolates would have been correctly identified (Table 3). The N/F system correctly noted all positive urease reactions and oxidations of glucose. The N/F system demonstrated acid production from oxidation of glucose with all but two of the *Achromobacter* species isolates

TABLE 3. *Identifications established for 23 isolates of Achromobacter species (CDC group Vd) as determined by four commercially available, miniaturized systems*

Achromobacter species isolate	Commercial identification systems			
	Minitek (BBL)	N/F (Corning)	API (Analytab)	Oxi/Ferm (Roche)
104	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	No identification	<i>Achromobacter</i> species
146	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	No identification	<i>Achromobacter</i> species
219	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	<i>Achromobacter</i> species
228	<i>Achromobacter</i> sp.	<i>Flavobacterium</i> IIb	CDC group IVe	<i>Pseudomonas</i> species
229	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	<i>Achromobacter</i> species
230	No identification	<i>Achromobacter</i> sp.	CDC group IVe	<i>Achromobacter</i> species
241	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	No identification	<i>Achromobacter</i> species
252	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	CDC group IVe	<i>Achromobacter</i> species
291	<i>Achromobacter</i> sp.	CDC group IIk-1	No identification	<i>Pseudomonas</i> species
359	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	<i>Alcaligenes faecalis</i>
401	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	No identification	<i>Alcaligenes faecalis</i>
428	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	No identification	<i>Achromobacter</i> species
429	<i>Achromobacter</i> sp.	No identification	No identification	<i>Achromobacter</i> species
779	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	No identification	<i>Achromobacter</i> species
781	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	<i>A. xylosoxidans</i>	<i>Achromobacter</i> species
782	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	<i>A. xylosoxidans</i>	<i>Achromobacter</i> species
783	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	No identification	<i>Achromobacter</i> species
784	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	No identification	<i>Pseudomonas vesiculare</i>
785	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	No identification	<i>Pseudomonas vesiculare</i>
786	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	No identification	<i>Pseudomonas vesiculare</i>
787	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	No identification	<i>Achromobacter</i> species
917	No identification	<i>Achromobacter</i> sp.	No identification	<i>Pseudomonas</i> species
925	<i>Achromobacter</i> sp.	<i>Achromobacter</i> sp.	<i>P. stutzeri</i>	<i>Pseudomonas</i> species
% Correct identification	91.2	82.4	13.2	57.2

(104 and 228), thus surpassing conventional media and the Minitek system in sensitivity for this test. Oxidation of xylose was not demonstrated as well by the N/F system as by the Minitek system and was equivalent to conventional OF xylose medium (19 versus 18 positive reactions). None of the four reference strains of biotype 2 was shown to be positive for the oxidation of maltose or mannitol by the N/F system. The esculin reaction compared favorably with the conventional esculin agar and was correctly positive with 18 of the 20 esculin-positive isolates. However, demonstration of denitrification was falsely negative with isolates 146, 228, and 429, causing their misidentification.

Oxi/Ferm tube. The Oxi/Ferm tube correctly identified 13 of the 23 isolates of *Achromobacter* species (Table 3). Most of the misidentifications were as *Pseudomonas* species and *Pseudomonas vesiculare*. Eighteen of the 23 urease reactions were correctly positive, as were 16 of 21 denitrification reactions. False negative urease reactions occurred with strains 429, 779, 784, 785, and 786. False negative denitrification reactions were obtained with strains 228, 784, 785, 786, and 925. Only four isolates, 219, 784, 785, and 786, were correctly shown to be aerobic

OF glucose positive, as were only two OF xylose reactions (strains 219 and 785). The citrate test was falsely negative with each of the citrate-utilizing isolates.

API system. Three of 23 *Achromobacter* species isolates were correctly identified by the API system (Table 3). Three isolates were misidentified as CDC Group IVe, two were misidentified as *A. xylosoxidans*, one was misidentified as *Pseudomonas stutzeri*, and the remaining 14 were reported as "no identification."

The API glucose microtube which served as the test area for nitrate reduction and denitrification was extremely accurate and, in addition to showing 19 of the *Achromobacter* species isolates positive for these tests, was the only system (including conventional) which demonstrated denitrification with strains 291 and 917. The API OF glucose tests were equivalent to conventional OF glucose media in detecting acid production. Of the 23 urease reactions, 18 were correctly positive, with false negative reactions seen with strains 779, 781, 782, 785, and 925. Fourteen of 17 isolates were correctly citrate positive. False negative citrate reactions occurred with isolates 779, 786, and 917. Positive citrate tests occurred on the API system with

TABLE 4. Antibigrams of 23 isolates of *Achromobacter* species (CDC group Vd) isolates^a

Test isolate	AM ^b	Cp	Cb	C	K	NF	P	Gm	AmK	Te	TMS	Cy	Tb
104	R ^c	R	R	R	S	R	R	S	S	I	S	S	S
146	R	R	R	R	R	R	R	S	S	S	S	S	S
219	R	R	R	R	R	R	R	S	S	S	S	S	S
228	R	R	R	R	R	R	R	S	S	S	S	R	R
229	R	R	R	R	R	R	R	S	S	S	S	S	S
230	R	R	R	R	R	R	R	R	R	S	R	R	R
241	R	R	R	R	R	R	R	S	S	S	S	S	R
252	R	R	S	R	R	R	R	S	S	S	S	S	S
291	R	R	R	R	R	R	R	S	S	S	S	S	S
359	R	R	R	I	R	R	R	R	S	S	S	S	S
428	R	R	R	R	R	R	R	R	S	R	R	S	R
429	R	R	R	R	R	R	R	R	R	S	S	R	R
779	R	S	S	R	R	S	R	R	R	R	R	S	R
781	R	R	R	R	R	R	R	R	S	S	S	S	R
782	R	R	R	R	R	R	R	R	S	R	R	S	R
783	R	R	R	R	R	R	R	R	S	S	R	S	R
784	R	R	R	R	S	R	R	S	R	R	S	S	S
785	R	S	S	R	R	R	R	S	S	S	S	S	S
786	R	S	S	S	R	R	R	S	S	S	S	R	S
787	R	R	R	R	S	R	R	S	R	R	S	S	S
917	R	R	S	R	S	S	R	S	R	S	S	S	R
925	R	R	R	R	R	R	R	R	S	S	S	S	S
401	R	R	R	R	R	R	R	R	R	S	R	S	R
% Sensitive	0	13.2	22.0	4.4	17.6	8.8	0	57.2	70.4	74.8	74.8	83.6	52.8

^a The validity of data obtained from disc-diffusion testing of *Achromobacter* species has not been established.

^b Am, Ampicillin; Cp, cephalothin; Cb, carbenicillin; C, chloramphenicol; K, kanamycin; Nf, nitrofurantoin; P, penicillin; Gm, gentamycin; AmK, amikacin; Te, tetracycline; TMS, trimethoprim-sulfamethoxazole; Cy, colymycin; Tb, tobramycin.

^c R, Resistant; I, intermediate; S, sensitive.

isolates 359 and 784, both of which were negative with conventional Simmon citrate media.

Table 3 summarizes the identifications given for each of the 23 *Achromobacter* species isolates by each of the four miniaturized systems examined.

Antimicrobial susceptibility testing. Table 4 shows the antibiotic susceptibility patterns of the 23 strains when tested by the disk diffusion method. Bearing in mind that the validity of data based on disk diffusion testing with non-fermenters such as *Achromobacter* species has not been established, the following results are presented. Each of the *Achromobacter* species isolates was resistant to penicillin and ampicillin. Most were resistant to chloramphenicol (one sensitive strain), nitrofurantoin (two sensitive), cephalothin (three sensitive), kanamycin (four sensitive), and carbenicillin (five sensitive). The greatest degree of sensitivity was seen with colymycin (19 strains), tetracycline (17 strains), trimethoprim sulfa (17 strains), and amikacin (16 strains). Approximately 50% of the strains were sensitive to gentamicin and tobramycin (Table 4).

Table 5 lists the characteristics that differentiate *Achromobacter* species from similar bacteria.

DISCUSSION

Achromobacter species (CDC group Vd) is one of the most recent additions to the growing list of nonfermenters encountered in clinical specimens. To date, only a few published reports and data sheets are available which provide morphological and biochemical information relative to this bacterium (3, 5, 9, 13, 14). In our study 23 strains of *Achromobacter* species (8 reference strains, 15 recent isolates) were examined morphologically and biochemically in an attempt to provide additional information for use in the identification of this bacillus.

Morphological examination revealed several distinctive features. Microscopically, most of the strains demonstrated a tendency to retain the crystal violet of the Gram stain, with several gram-positive cells visible in each microscopic field. This gram variability was more pronounced when examining growth from MC agar. In addition to the typical rod-shaped bacilli, many apparently curved forms, and cells with a swollen "hook" at one end were seen (Fig. 2). However, electron microscopy revealed the curved forms, in reality, to be dividing cells still joined but at a slight angle to each other and showed the swollen "hook" to be an accumula-

TABLE 5. Characteristics differentiating *Achromobacter* species (CDC group Vd) from similar bacteria^a

Organism	Motility	SS	Urea	N ₂ gas	Pa	Esc	Oxidation	
							Glucose	Xylose
<i>Achromobacter</i> species	+	+	+	+	+	+	d	d
<i>A. xylosoxidans</i>	+	+	—	d	—	—	—	+
<i>Bordetella bronchiseptica</i>	+	+	+	—	—	—	—	—
<i>Alcaligenes denitrificans</i>	+	d	d	+	—	—	—	—
<i>A. faecalis</i>	+	+	—	—	—	—	—	—
<i>A. odorans</i>	+	+	—	—	—	—	—	—
<i>Pseudomonas pickettii</i> (Va-2)	+	—	d	d	d	—	d	+
CDC group Va-1	+	—	d	d	—	—	d	+
CDC group IVe	d	—	+	d	+	—	—	—
CDC group IVc-2	+	—	+	—	—	—	—	—
<i>Moraxella phenylpyruvica</i>	—	—	+	—	+	—	—	—
<i>Flavobacterium odoratum</i>	—	—	+	—	+	—	—	—
CDC group IIIk-2	—	—	+	—	d	+	+	+

^a See footnotes to Table 2 for abbreviations. d, Differing reactions.

tion of extracellular material (Fig. 3).

The most characteristic features of the macroscopic appearance of the *Achromobacter* species isolates were the exceedingly small colonies produced at 24 h (Fig. 1A) and the subsequent rapid increase in colony size after continued incubation due to the elaboration of large amounts of mucoid material (Fig. 1B). In fact, colonies which became mucoid (seen with 17 isolates) were identical in appearance to those of *Klebsiella pneumoniae* but lacked the "stringing" quality of the latter. Due to the smallness of the colonies (average diameter of 0.4 mm, largest diameter of 0.75 mm) after overnight incubation and their watery translucent quality, without the use of a hand lens or other aid growth of *Achromobacter* species on BA and MC agars can easily be overlooked, especially in mixed cultures, e.g. sputum, stool, and urine. An awareness of these morphologically distinguishing characteristics of *Achromobacter* species, i.e., gram-variability, curved and swollen-ended forms, extremely small colonies after overnight incubation, and mucoid nature upon continued incubation, can help direct microbiologists toward the correct identity. However, unaware microbiologists, when confronted with a gram-variable, coryneform bacterium, apparently not growing on MC after overnight incubation, may possibly misidentify an *Achromobacter* species isolate as *Corynebacterium* species. Additionally, the mucoid colonies may direct an identification toward an atypical *Klebsiella* species, especially in the absence of an oxidase determination.

An additional noteworthy morphological feature of *Achromobacter* species is the arrangement of flagella. Although peritrichous, when examined by the modified Fontana silver staining procedure of West et al. (15) most cells

appear monopolar. Only with electron micrographs is the true monolateral arrangement of the flagella readily apparent (Fig. 4). Therefore, the finding of polar flagella when examining preparations with light microscopy does not rule out *Achromobacter* species as an identification.

The biochemical studies revealed a core of reactions useful in the identification of *Achromobacter* species. All isolates grew on MC agar and were positive for urease, oxidase, and catalase enzymes (Table 2). Deamination of phenylalanine (weakly after 2 to 4 days) and growth on salmonella-shigella agar (1 to 2 days) were seen with all strains except one. Denitrification, esculin hydrolysis (1 to 3 days), and H₂S formation (weakly on triple sugar iron agar after 4 to 12 days) occurred with all except two isolates. The finding of deamination of phenylalanine with 22 of 23 isolates was in contrast to the Oberhofer study (9) in which this reaction was positive with only 5 of 16 *Achromobacter* species strains. Possibly, many or most of the 11 negative strains in the latter study would have given positive reactions had the incubation period been extended from the 24-h period used to 3 to 4 days. In our study, only one isolate produced detectable phenylalanine deaminase after overnight incubation, and most strains required 2 to 3 days for the production of weak to strongly positive reactions.

Oxidation of carbohydrates was essentially limited to glucose and xylose. When positive, these reactions were generally weak (barely detectable indicator change) and delayed. Although 18 of 23 isolates, in agreement with other reports (9, 13, 14), oxidized xylose, only 30.8% of the test isolates oxidized glucose within a 10-day incubation period. This was in contrast to the findings of Weaver et al. (14) who found all of 65 isolates glucose positive and to those of Ober-

hofer (9) who reported 81.3% of 16 isolates glucose positive. This discrepancy may be due, in part, to the fact that these two groups used a more sensitive indicator (phenol red) than the bromothymol blue indicator used in our study.

The failure of any of the four reference strains of *Achromobacter* species biotype 2 to oxidize maltose, mannitol, or sucrose was surprising because the oxidation of any or all of these carbohydrates is reportedly characteristic of this biotype (5, 9, 13). Only 2 of the 15 recent clinical isolates in our study oxidized maltose, mannitol, and sucrose. It is possible that the choice of indicator may again be involved. However, Weaver et al. (14) do not separate *Achromobacter* species into biotypes. Furthermore, a recent gas-liquid chromatographic study involving four strains of *Achromobacter* species biotype 1 and three strains of biotype 2 (strains 104, 230, and 252 in our study) failed to detect any differences between the two biotypes (3). The morphological and biochemical analysis of our 23 *Achromobacter* species strains supports the contention and findings of Dees and Moss (3) and Weaver et al. (14) that isolates of *Achromobacter* species form a homogeneous group not readily separated into biotypes.

In view of the availability of several nonfermenter identification kits and the widespread acceptance that they are receiving, at least for use in identifying the more common "nonfermenters" (11, 12; E. R. Bannister, M. E. West, P. A. Buchner, M. M. Alexander, and J. P. Manos, Abstr. Annu. Meet. Am. Soc. Microbiol., 1978, C159, p. 303), the ability of these kits to identify *Achromobacter* species was tested. Both the Minitek (BBL) and the N/F system (Corning) demonstrated the ability to identify *Achromobacter* species, whereas the Oxi/Ferm tube (Roche) was less than satisfactory (57.2% correct identification) and the API 20E (Analytab) was poor (13% correct) (Table 3). The success of Minitek and N/F appears to be the result of the incorporation and sensitivity of substrates for testing urea, phenylalanine, denitrification, glucose, and xylose (Minitek) and urea, esculin, denitrification, glucose, and xylose (N/F). The inability of the API 20E and Oxi/Ferm to identify many or most of the isolates studied resulted from the lack of testing capabilities for phenylalanine and esculin (API, Oxi/Ferm) and xylose (API) and by the frequent insensitivity of some of the key substrates in the Oxi/Ferm tube: urea, denitrification, glucose, and xylose.

The data provided by this study, in combination with other available information (3, 5, 9, 13, 14) are reflected in Table 5. The identification of *Achromobacter* species and its differentiation from similar bacteria are seen to be a relatively

simple task. *Achromobacter* species isolates will typically reflect the following profile: a gram-negative, oxidase-positive, nonfermentative bacillus which grows on MC and salmonella-shigella agars, is motile, oxidizes glucose and xylose weakly or not at all, produces urease and phenylalanine deaminase, metabolizes nitrate to nitrite and nitrogen gas, and hydrolyzes esculin. The presence of some or all of the following morphological features strengthens the identification: minute colonies at 25 h progressing to moderate-sized and extremely mucoid colonies by 48 h; gram-variability; presence in Gram stain of curved and swollen, hooked-end forms; and the presence of monopolar (light microscopic examination) and laterally flagellated cells (electron microscopic examination).

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