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Chemical and Cultural Characterization of CDC Group WO-1, a Weakly Oxidative Gram-Negative Group of Organisms Isolated from Clinical Sources

D. G. HOLLIS,[1]* R. E. WEAVER,[1] C. W. MOSS,[2] M. I. DANESHVAR,[2] AND P. L. WALLACE[2]†

Special Bacteriology Reference Laboratory[1] and Analytical Chemistry Laboratory,[2] Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

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Ninety-six strains of weakly oxidative gram-negative rods isolated primarily from clinical specimens form a distinct group that has been designated Centers for Disease Control (CDC) group WO-1 (WO stands for weak oxidizer). The phenotypic characteristics of CDC group WO-1 were most similar to those of *Comamonas acidovorans*, *Pseudomonas mallei*, and CDC pink coccoid group III. The WO-1 group can be differentiated from *C. acidovorans* by the oxidation of glucose (often weak and sometimes delayed), motility by means of one or two polar flagella, and, when positive, the complete reduction of nitrate and nitrite. Motility and usually the failure to produce arginine dihydrolase distinguish this group from *P. mallei*. The WO-1 strains differ from the pink coccoid group III by the absence of pink growth pigment, the lack of predominantly coccoid cellular morphology, and usually the inability to produce acid from xylose. The cellular fatty acid compositions of 29 group WO-1 strains were characterized by large amounts of $C_{16:0}$ and $C_{16:1w7c}$; smaller amounts of $C_{18:1w7c}$, $C_{14:0}$, $C_{12:0}$, and 3-OH-$C_{10:0}$; and trace to small amounts of $C_{15:1w6}$ and $C_{17:0}$ acids. The fatty acid profile of WO-1, compared with the profiles of other bacteria we have tested previously, was most similar to the profiles of two phenotypically different organisms, *Comamonas terrigena* (a nonoxidative, multipolar gram-negative rod) and *Chromobacterium violaceum* (a fermentative gram-negative rod). Ubiquinone-8 was the major quinone in the five WO-1 strains examined. Eighty-five percent of the WO-1 strains were isolated from human specimens. Thirty-three percent were from blood, and 10% were from cerebrospinal fluid.

The Special Bacteriology Reference Laboratory of the Centers for Disease Control (CDC) receives bacterial isolates for identification and classification from reference and clinical laboratories throughout the United States and some foreign countries. Since 1964 this laboratory has received 96 strains of unidentified oxidative gram-negative rods which are phenotypically similar to *Comamonas* (formerly *Pseudomonas*) *acidovorans* (1, 3, 7, 8), *Pseudomonas mallei* (1), and CDC pink coccoid group III (9) but appear to be distinct. These 96 strains were grouped together and were designated CDC group WO-1 (WO stands for weak oxidizer). Strains of this group were isolated from a variety of clinical sources, such as blood, urine, cerebrospinal fluid (CSF), lung, and wound, and some environmental sources.

In this study, the cultural and biochemical properties of 96 CDC group WO-1 strains were compiled from data obtained when these cultures were received and examined in the laboratory. A number of these strains were examined for cellular fatty acids and isoprenoid quinones. In this report, the phenotypic (including chemical) characteristics of group WO-1 are compared with those of *C. acidovorans*, *P. mallei*, and CDC pink coccoid group III because of the similarity of their phenotypic characteristics (1, 7, 8, 9) and with those of *Comamonas terrigena* and *Chromobacterium violaceum* because of their similar fatty acid compositions. With these data, clinical laboratories can recognize and distinguish group WO-1 organisms from bacteria with similar characteristics.

* Corresponding author.
† Present address: Department of Biology, Georgia Institute of Technology, Atlanta, GA 30332.

MATERIALS AND METHODS

Strains. The laboratory records of 96 strains of CDC group WO-1 bacteria were reviewed for their cultural characteristics, which had been determined at CDC (Table 1), and their clinical sources (Table 2). The clinical and demographic information that accompanied the blood isolates was summarized (Table 3). Twenty-nine of the strains were subcultured from stocks frozen at −50 to −70°C and analyzed for their cellular fatty acid content. Five of the 29 strains were also examined for their isoprenoid quinones. Eighteen strains of *C. acidovorans*, including the type strain RYS14 (ATCC 15668); the type strain of *C. terrigena* (ATCC 8461); 8 strains of *P. mallei*, including the type strain 3873 (ATCC 23344); and 13 *C. violaceum* strains, including NCTC 9694, were also examined in this study.

Biochemical tests. The biochemical characteristics of each group WO-1 strain and the type strain of *C. terrigena* were determined by previously described methods (1) at the time each culture was received for examination.

Gas-liquid chromatography analysis of fatty acids and isoprenoid quinone analysis. Cells grown for 24 to 48 h on heart infusion agar supplemented with 5% rabbit blood were processed and analyzed for cellular fatty acids as described previously (6, 9). Isoprenoid quinones were extracted from 100 mg of lyophilized whole cells according to the procedure of Minnikin et al. (4) and analyzed by reverse-phase high-performance liquid chromatography (2, 6).

RESULTS AND DISCUSSION

The cultural and biochemical characteristics of the WO-1 bacteria are shown in Table 1. The WO-1 strains were

TABLE 1. Characteristics of CDC group WO-1 (96 strains)

Test performed	Result[a]
Morphology	Gram-negative, coccoid to medium, straight rods
Action on blood	11 gr, 9 LG, 8 lav, 6 ly, 5 gr + br, 3 br, 1 β
Oxidase	98
Growth on MacConkey agar	19 (32)[b]
Catalase	87[c]
Acid from (OF base):	
D-Glucose	78 (98)[c]
D-Xylose	4 (5)[c]
D-Mannitol	86 (100)[c]
Nitrate reduction to nitrite	97[d]
Gas from nitrate	20[c]
Simmons citrate	18 (21)
Urea, Christensen's	15 (58)
Litmus milk	33 k,2 IR, 1 pep
Motility	37 (85),[e] one or two polar flagella
Soluble pigment	35 YA, 9 OG, 5 br
Growth at:	
25°C	91
35°C	100
42°C	30
Arginine dihydrolase	6[f]
Nutrient broth	
0% NaCl	97
6% NaCl	9 (10)

[a] Expressed as percent posiative at 48 h (7 days). Symbols and abbreviations: gr, green; LG, lavender-green; lav, lavender; ly, lysis; gr + br, green and brown; br, brown; β, beta-like; k, alkaline; IR, indicator reduction; pep, peptonization; YA, yellow or amber; OG, olive green. All strains were negative in the following tests: acid production from lactose, sucrose, and maltose; growth on salmonella-shigella and cetrimide agars; indole; acidification of triple sugar iron agar; H_2S (triple sugar iron butt); gelatin and esculin hydrolysis; and lysine and ornithine decarboxylases.

[b] Growth was often light.

[c] Reaction was often weak.

[d] 45% completely reduced nitrite without gas formation.

[e] Motility often detected only by wet preparation or flagella stain.

[f] 32 strains tested.

TABLE 2. Sources of 96 CDC group WO-1 cultures

Source	No. of isolates
Blood	27
Urine	10
CSF	8
Lung or lung associated	6[a]
Wound	3
Peritoneal fluid	3
Fluid: joint, hip, or tumor	3
Eye	2
Leg ulcer	2
Lymph node	2
Cervix, vagina	2
Miscellaneous	14[b]
Nonhuman	14[c]

[a] Lung, lung tissue, lung biopsy, lung washing, lung abscess, or pleural fluid.

[b] One each: gall bladder, throat, bone—foot, toe amputation, amputation stump, knee, finger, muscle, bone marrow, infant shunt, colon tumor exudate, abdominal cavity, breast milk, and blood bag.

[c] Two each: environmental, enzyme production, water, and dialysis fluid. One each: water cooler, water scrub, neonatal humidifier, tap water in ambulance, whirlpool, and dialysate.

TABLE 3. CDC group WO-1 blood isolate data (27 strains)

WO-1 strain	Diagnosis or diagnosis and associated condition	Sex[a]/ age (yr)	Geographic source, year received
A5485		M	Washington, 1966
C5924		F	Puerto Rico, 1973
C6309		F/1	Canada, 1973
C7975		F/30	Arizona, 1973
C9925	Cerebrovascular accident, postsurgical pulmonary emboli, hypertension, bacteremia	F/74	Indiana, 1974
D1040[b]	Cystocele, (?) after surgery	F	Tennessee, 1974
D4134[c]		M	Connecticut, 1975
D4135[c]		M	Connecticut, 1975
D7079	(?) Septicemia	M/64	Arizona, 1976
E2039[d]		F/infant	Maryland, 1978
E2056		M/64	Canada, 1978
E2195[b,e]	Fever of unknown origin	M/infant	North Carolina, 1978
E2710	Septicemia	F/55	Tennessee, 1978
E3318		M/15	Arizona, 1978
E3784	Pneumonia, acute pharyngitis	F/18	Michigan, 1978
E3903	Creutzfeld-Jakob disease (no other organism)	M/63	Hawaii, 1978
F3949	Pneumonia	M	Wisconsin, 1982
F4040[b]	Factor VIII deficiency with inhibitor (postpartum), Hickman catheter placement septicemia, recurrent bacteremia	F/28	Indiana, 1983
F4199[b]	Pelvic abscess following appendectomy, sepsis	F/9	Ohio, 1983
F4455[b,f]	Bacteremia, relapse myelomonoblastic leukemia	M/71	Hawaii, 1983
F6708	Seizure disorder, sepsis, UTI, prostate hypertrophy, cerebrosvascular disease	M/86	New Mexico, 1985
F8376	Pneumonia, surgical patient (on respirator)	F/76	Tennessee, 1986
F8416		F/neonate	Ohio, 1986
F8575[d]	AIDS, (?) bacteremia	M/29	Maryland, 1986
F9884	Fever, abdominal discomfort	F/20	Washington, 1987
G1660[g]	Sepsis	M	Texas, 1988
G1984[b]	Septicemia	F/72	Oregon, 1988

[a] F, female; M, male.

[b] Isolated one time.

[c] Patients shared a hospital room for a period of time.

[d] Isolated two times.

[e] Patient deceased.

[f] *Acinetobacter* sp. also isosolated.

[g] "*Corynebacterium aquaticum*" also isolated.

gram-negative, coccoid to medium, straight rods. They oxidized mannitol and glucose (98%), often weakly and sometimes delayed (3 to 7 days), and 97% reduced nitrate (45% completely reduced nitrate and nitrite without gas

formation, 20% produced some gas, and 33% only reduced nitrate to nitrite). The majority of the strains were motile with one or two polar flagella; however, motility was usually delayed in motility medium or was detected only by wet preparation. They were usually oxidase and catalase positive. Different strains gave different results for urease and growth on MacConkey agar. Indole was not produced, and esculin and gelatin were not hydrolyzed. Some strains produced soluble pigment (yellow, tan, amber, olive green, or brown).

The cellular fatty acid composition of the 29 group WO-1 strains tested was distinct from that observed in the 18 *C. acidovorans* strains examined in this study (Table 4) and also from that found in an earlier study (5). Although the overall fatty acid profiles were similar, all CDC group WO-1 strains contained palmitoleic ($C_{16:1w7c}$) as the major acid, whereas palmitic ($C_{16:0}$) was the major component in all *C. acidovorans* strains. In addition, each strain of *C. acidovorans* contained small (3 to 8%) to moderate (9 to 15%) amounts of a 17-carbon cyclopropane acid ($C_{17:0cyc}$), which was absent in all WO-1 strains except F6662 and F8376 (GLC group B [Table 4]). All WO-1 strains, including strains F6662 and F8376, contained trace to 1% amounts of $C_{15:1w6}$ and $C_{17:0}$ acids, which were absent in *C. acidovorans*. Thus, CDC group WO-1 strains were distinguished from *C. acidovorans* by the presence of $C_{16:1w7c}$ as the major acid, by the presence of small amounts of $C_{15:1w6}$ and $C_{17:0}$ acids, and by the absence of $C_{17:0cyc}$ (27 of 29 strains). The isoprenoid quinone content of *C. acidovorans* was identical to that of CDC group WO-1. The type strain of *C. acidovorans* (ATCC 15668) contained ubiquinone-8 (Q-8) as its major quinone with small amounts of Q-7 and Q-9. Essentially identical quinone profiles were observed for five group WO-1 strains (A700, A2437, F6662, F8376, and G1660) with Q-8 as the major component.

The cellular fatty acid composition of the type strain of *C. terrigena* was essentially the same as that of WO-1 except for the presence of 2% 2-OH-$C_{16:0}$ (Table 4). Tamaoka et al. (8) detected trace amounts of this acid in all four strains that they tested. The overall fatty acid profile of *C. violaceum* was most similar to that of WO-1 (Table 4) but contained small amounts (1 to 2%) of 2- and 3-hydroxydodecanoic acid (2-OH-$C_{12:0}$, 3-OH-$C_{12:0}$), octadecadienoic ($C_{18:2}$), and oleic ($C_{18:1w9c}$) acids, which were absent in WO-1. The biochemically similar CDC pink coccoid group III organisms were clearly differentiated from group WO-1 and all other organisms listed in Table 4 by the presence of large amounts of a 19-carbon cyclopropane acid ($C_{19:0cyc}$), a 2-hydroxy-19-carbon cyclopropane acid (2-OH-$C_{19:0cyc}$), larger amounts of $C_{18:1w7c}$, and only small amounts of $C_{16:1w7c}$. *P. mallei* strains differed from WO-1 strains and all other organisms listed in Table 4 by the presence of 3-OH-$C_{14:0}$ (5%) and 2-OH-$C_{16:1}$ (2%).

Shown in Table 5 are some key biochemical tests useful for distinguishing group WO-1 strains from the five organisms listed in Table 4. The WO-1 strains were compared with *C. acidovorans* because both were nonfastidious, oxidase- and nitrate-positive, motile gram-negative rods that sometimes oxidized only mannitol, often weakly, in 1 to 2 days. WO-1 strains, however, usually oxidized glucose, although often weakly and sometimes delayed, were motile by means of only one or two polar flagella, and often completely reduced nitrate and nitrite. In contrast, *C. acidovorans* did not oxidize glucose, had polar tufts of three or more flagella, and did not reduce nitrite (1, 7, 8). The phenotypic characteristics of WO-1 were also similar to those of *P. mallei*,

TABLE 4. Cellular fatty acid composition of various organisms[a]

Organism (no. of strains)	% Fatty acids[b]																		
	3-OH-$C_{10:0}$	$C_{12:0}$	2-OH-$C_{12:0}$	3-OH-$C_{12:0}$	$C_{14:0}$	$C_{15:1w6}$	$C_{15:0}$	$C_{16:1w7c}$	$C_{16:0}$	$C_{17:0cyc}$	$C_{17:0}$	2-OH-$C_{16:0}$	3-OH-$C_{16:0}$	$C_{18:2}$	$C_{18:1w9c}$	$C_{18:1w7c}$	$C_{18:0}$	$C_{19:0cyc}$	2-OH-$C_{18:1}$ 2-OH-$C_{19:0cyc}$
CDC group WO-1																			
GLC group A (27)	4	7	—	—	2	1	1	43	27	—	Tr	—	—	—	—	11	—	—	—
GLC group B (2)	4	5	—	—	5	1	1	40	29	2	Tr	—	—	—	—	11	—	—	—
C. acidovorans (18)	3	2	—	—	1	—	Tr	29	43	9	—	—	—	—	—	11	—	—	—
C. terrigena (1)	5	4	—	—	Tr	—	Tr	40	27	—	Tr	2	—	—	—	21	—	—	—
C. violaceum (13)	3	5	1	2	3	Tr	2	35	28	—	—	—	—	2	2	13	Tr	—	—
CDC pink coccoid group III (90)	—	—	—	—	—	—	—	1	18	—	—	—	1	—	—	43	2	19	3
P. mallei (8)[c]	—	—	—	—	5	—	Tr	7	24	8	Tr	4	4	Tr	1	27	1	8	1

[a] Data published previously for CDC pink coccoid group III (9).

[b] Number before the colon is the number of carbon atoms, and the number after the colon is the number of double bonds. 2-OH indicates a hydroxyl group at the 2-carbon; 3-OH indicates a hydroxyl group at the 3-carbon; w, double bond position from hydrocarbon end of chain; c, cis isomer. Values are percentages of total fatty acids and are arithmetic means; tr, 0.7 to 0.9%; —, not detected.

[c] Also contains 5% 3-OH-$C_{14:0}$ and 2% 2-OH-$C_{16:1}$.

TABLE 5. Results of some key biochemical tests for various organisms[a]

Test	CDC group WO-1 (n = 96)	C. acidovorans (n = 64)	C. terrigena[b] (n = 1)	CDC pink coccoid III (n = 79)	P. mallei (n = 8)	C. violeceum (n = 37)
Carbohydrate base	OF	OF	OF	OF	OF	F
Acid from:						
D-Glucose	78 (98)[c]	0	−	39 (89)[c]	100	100
D-Xylose	4 (5)[c]	0	−	46 (74)[c]	12w (72)	0
D-Mannitol	86 (100)[c]	100	−	53 (100)[c]	62w (76)[c]	0
Lactose	0	0	−	0	12w (74)	0
Sucrose	0	0	−	0	0	20 (6)
Maltose	0	0	−	0	0 (75)	0 (3)
Oxidase	98	100	+	51	25	67
Growth on MacConkey	19 (32)[c]	100	(+w)	60 (82)	88w	97, 3w
Urea	15 (43)	0 (3)	+	59 (98)	12	5 (14)
Nitrate reduction	97[d]	98	+	23	100	97
Gas from nitrate	20	0	−	0	0	0
Indole	0	0[e]	−	0	0	21
Pigment						
Insoluble	0	0	−	Pink[f]	0	91 violet
Soluble	35 yel-amber, 9 olive green, 5 br	47 yel-tan[g]	Slight yel[g]	0	0	0
Arginine dihydrolase	6	0	−	10 (20)	100	100
Motility	37 (48)[h]	89 (100)	+	11[h]	0	100
Flagella	1-2 polar	>2 polar, tuft	>2 polar, tuft	1-2 polar	0	1 polar, 1-4 subpolar or lateral

[a] Data were previously published (1, 9) with the exception of data for CDC group WO-1 and *C. terrigena*. Results are given as percent positive at 48 h (7 days). Symbols and abbreviations: OF, oxidation-fermentation; F, fermentation; w, weak; yel, yellow; br, brown; −, negative reaction; +, positive reaction within 48 h.

[b] ATCC 8461, type strain.

[c] Reaction often weak.

[d] 45% completely reduced nitrate and nitrite without gas formation.

[e] Some strains caused a vivid yellow reaction.

[f] Sometimes very pale pink. Also usually mucoid to runny growth.

[g] Some strains of *C. acidovorans* produced a weak fluorescent pigment; *C. terrigena* produced a trace of fluorescent pigment.

[h] Motility often detected only by wet preparation or flagella.

particularly when motility of WO-1 was not detected early; however, *P. mallei* is consistently nonmotile, may oxidize a wide range of carbohydrates, and produces arginine dihydrolase (1). The pink coccoid group III differed from WO-1 strains by the presence of a pale pink growth pigment, mucoid to runny growth, cellular morphology that contained predominately coccoid forms usually occurring in chains, and usually acid production from xylose. Of the other species, *C. violaceum* is a fermenter, and *C. terrigena* is a nonfermenter and a nonoxidizer. Each of these species have three or more key biochemical differences from those of the group WO-1.

On the basis of the biochemical characteristics and the cellular fatty acid composition, group WO-1 is distinct from other organisms we have studied. It is likely that this group represents yet another species (or more than one species) belonging in the *Comamonas* genus (3, 8); however, genetic studies will be required to determine the taxonomic status of this group. A reference strain, A700 (ATCC 49825), has been deposited with the American Type Culture Collection.

CDC group WO-1 strains were isolated from human and nonhuman specimens (Table 2). Of the 82 clinical isolates, 33% (27 strains) were from blood and 10% (8 strains) were from CSF. Diagnoses or diagnoses and associated conditions were given for 17 of the 27 blood isolates (Table 3). The most frequent diagnosis (five patients) was sepsis or septicemia. Three patients had pneumonia. From two of the patients, a second organism was isolated. Information about the number of times the organism was isolated was received for eight patients. From six patients, WO-1 was isolated only one time; from two patients, the organism was isolated twice. The blood isolates were received from 14 states, Puerto Rico, and Canada.

Diagnoses or diagnoses and limited clinical information were given for four of the eight CSF isolates. Two patients had meningitis. From one of them, a 67-year-old male, no other bacteria were isolated. Fungal and viral culture results were not available. The second patient with meningitis, a 14-month-old female, had *Haemophilus influenzae* isolated from the CSF 12 days before the isolation of WO-1. The WO-1 was isolated from a subculture but not on chocolate and blood agar plates directly inoculated with the specimen. The other two diagnoses were cerebellar hemorrhage and bilateral otitis media. A fifth patient was a baby who developed fever, irritability, and bleeding gums after an exchange transfusion.

Although the foregoing information indicates WO-1 is associated with illness in humans, additional information is needed on patients, from whom these organisms are isolated, to clarify the clinical significance of this organism.

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