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Characteristics of Yellow-Pigmented Nonfermentative Bacilli (Groups VE-1 and VE-2) Encountered in Clinical Bacteriology

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The morphological and physiological characteristics of 20 strains of motile, gram-negative, yellow-pigmented oxidative bacilli (groups VE-1 and VE-2) isolated in clinical bacteriology are described. Electron micrographs demonstrate the polar multitrichous flagella of group VE-1 and polar monotrichous flagella of group VE-2. Data obtained from guanine plus cytosine ratio studies of 56.8% for VE-1 and 68.9% for VE-2 further distinguish the two groups of bacteria.

In 1972, Weaver et al. (13) applied the designations VE-1 and VE-2 to gram-negative, yellow-pigmented oxidative bacilli resembling pseudomonads. Oxidative microorganisms with similar physiological features were previously designated *Chromobacterium typhiflavum* by Pickett and Pedersen (9). Strains with characteristics attributed to the VE-1 and VE-2 groups have occasionally been recovered from clinical material, but their role as pathogens in man remains questionable (8). Because the strains comprising these groups have features similar to those of other yellow-pigmented pseudomonads and xanthomonads encountered in clinical specimens, morphological, physiological, and guanine plus cytosine (G+C) ratio studies were undertaken to define the characteristics of these bacteria. This paper reports on these studies.

MATERIALS AND METHODS

DNA isolation. Bacteria were grown overnight at 37 C with continuous shaking in 50 ml of brain heart infusion broth (Difco) in 250-ml Erlenmeyer flasks. Cells were collected by centrifugation, resuspended in 0.1 M NaCl-0.1 M sodium ethylenediaminetetraacetate at pH 8.0 (SV), and lysed at 55 C with 1% (wt/vol) sodium dodecyl sulfate. The lysate was cooled to 5 C and deproteinized by shaking with a volume of freshly distilled phenol saturated with SV and adjusted to pH 8.0 with NaOH plus 1 volume of $CHCl_3$-isoamyl alcohol (24 to 1 by volume). The aqueous phase was separated by centrifugation at 5,000 × *g* for 10 min at 5 C, and the nucleic acids were collected by spooling on a glass rod after the addition of 2 volumes of 95% ethanol. The fibers were washed free of phenol in sequential rinses of 70% ethanol, drained, air dried, and redissolved in a minimal amount of 15 mM NaCl containing 1.5 mM Na_3 citrate at pH 7.0.

DNA base composition. Approximately 1 μg of each DNA was mixed with 0.6 μg of reference DNA from subtilis-phage 2 C (P_{CsCl} = 1.742 g/cm³) and centrifuged to equilibrium at 42,040 rpm at 25 C in a Beckman Analytical Centrifuge fitted with an ultraviolet scanner. The buoyant density of the sample and its G+C contents were calculated by the reference method as previously described (6, 10).

Electron microscopy. The microorganisms were washed three times in phosphate buffer (0.1 M, pH

TABLE 1. *Sources of strains*[a]

No.	Source	No.	Source
422	HJD, foot wound	1612[b]	MJP, inhalation therapy equipment
424	HJD, tibia wound	1613[b]	MJP, cervical swab
523	HJD, sputum	1622[b]	RW
572	HJD, leg wound	1623[b]	RW
614	HJD, ear	1715	SR
619	HJD, hip wound	1734	EB
750	HJD, leg wound	1763	EB, urine
1175	EB	1770	EB, eye
1251	DB	1832	RM, throat
1430	HJD, agar plate contaminate	2051	HJD, sputum

[a] HJD, Hospital for Joint Diseases; EB, E. Bottone, Mt. Sinai Hospital, New York; DB, Donna Blazevic, University of Minnesota; MJP, M. J. Pickett, University of California, Los Angeles; SR, S. Rosenthal, Boston City Hospital; RW, R. Weaver, Center for Disease Control, Atlanta; RM, R. Murphy, Nassau County Medical Center, New York.

[b] Designations of reference strains: K217, *Chromobacterium typhiflavum* (1612); K224, *C. typhiflavum* (1613); B5142, VE-2 (1622); B1587A, VE-1 (1623).

7.2) to remove the broth particles. Negative stain was applied by means of mixing a drop of cell suspension in phosphate buffer with a drop of 2% phosphotungstic acid stain (pH 7.2 in phosphate buffer). After 30 s a droplet of the mixture was picked up on 100-mesh, formvar- and carbon-coated copper grids and air dried (7). The examination was performed on a Siemens 101 electron microscope.

Antimicrobial susceptibility was tested and interpreted by the standardized single-agar disk diffusion method of Bauer and associates (1). Other tests and media employed in this study were previously reported (3).

RESULTS AND DISCUSSION

The sources of the 20 clinical isolates, including 4 reference strains, and their characteristics are given in Tables 1 and 2, respectively. Salient features of these gram-negative bacilli include the presence of flagella, either polar monotrichous or polar multitrichous in arrangement, production of an intracellular nondiffusable yellow pigment, lack of indophenol oxidase activity, and utilization of a wide range of organic compounds as a sole source of carbon and energy. When these bacilli were inoculated into OF medium containing glucose and sealed with petrolatum, acid production was not detected. Aggregates of individual cells in chain formation (symplasmata) were observed in 13 strains which produced rough colonial forms. Seven strains producing only smooth colonies lacked these aggregates. The granular, elon-

TABLE 2. *Characteristics of 20 group VE strains*[a]

Test, substrate, or morphology	Group VE-1										Group VE-2									
	422[b]	572	1175	1251	1613	1623	1715	1734	1763	1832	424	523	614	619	750	1430	1612	1622	1770	2051
Rhamnose (1%, OFBM)	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Sucrose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
ONPG	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+	+	+	−	−
Urea	−	−	−	+	−	−	−	−	−	−	+	+	−	−	−	+	−	−	−	−
Nitrite production	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Arginine dihydrolase (DBM)	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Phenylalanine deaminase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−
Esculin hydrolysis	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Lipase	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	+	+	−	−
Gelatinase and caseinase	+	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Growth on SS agar and DC agar	+	+	−	−	+	−	−	+	−	+	−	−	+	−	−	−	−	−	−	−
6.5% NaCl tolerance	+	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	+	+	+	+
pH 5.6 tolerance (SDA)	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+
Growth on cetrimide	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	+	+
Growth at 42 C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	−
Wrinkled colonies, symplasmata	−	+	−	−	−	−	+	−	+	+	+	+	+	+	+	+	−	−	+	+
Polar monotrichous flagellum	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
Polar multitrichous flagella	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Maltose (MBM)[c]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Adipate	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
beta-Alanine	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+	−	−	+	−
L-Arginine	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	−	−	+	−	+
L-Lysine	−	+	+	−	+	−	+	+	+	−	−	+	−	+	−	+	−	+	+	−

[a] All strains gave positive results for the following tests or substrates: acid from glucose, fructose, galactose, mannose, xylose, mannitol (1%, OF basal medium [OFBM]), lactose (10%, purple agar base); growth on MacConkey agar; 2.5% NaCl tolerance; yellow chromogenesis; motile; assimilation of D-glucose, D-fructose, L-arabinose, D-xylose, sucrose, D-trehalose, D-mannitol, *i*-inositol, acetate, pelargonate, malonate, succinate fumarate, D-malate, DL-lactate, citrate, pyruvate, asparagine, DL-aspartate, L-glutamate, DL-serine, betaine, gluconate. All strains gave negative results for the following tests or substrates: acid from lactose (1%, OFBM); gluconate oxidation; indole; hydrogen sulfide; nitrogen gas; indophenol oxidase; lysine, ornithine decarboxylase; amylase (a few strains gave equivocal results); deoxyribonuclease; lecithinase; hemolysis; triphenyl tetrazolium chloride tolerance; pyoverdin; assimilation of suberate, glycine, DL-methionine, DL-valine, acetamide. Symbols: +, positive test; −, negative test; DBM, decarboxylase base Moeller; ONPG, *ortho*-nitrophenyl-*beta*-D-galactopyranoside; SS agar, Salmonella Shigella agar; DC agar, deoxycholate agar; SDA, Sabouraud dextrose agar; MBM, mineral base medium.

[b] Strain number.

[c] For the remaining entries in this column, assimilation was tested for.

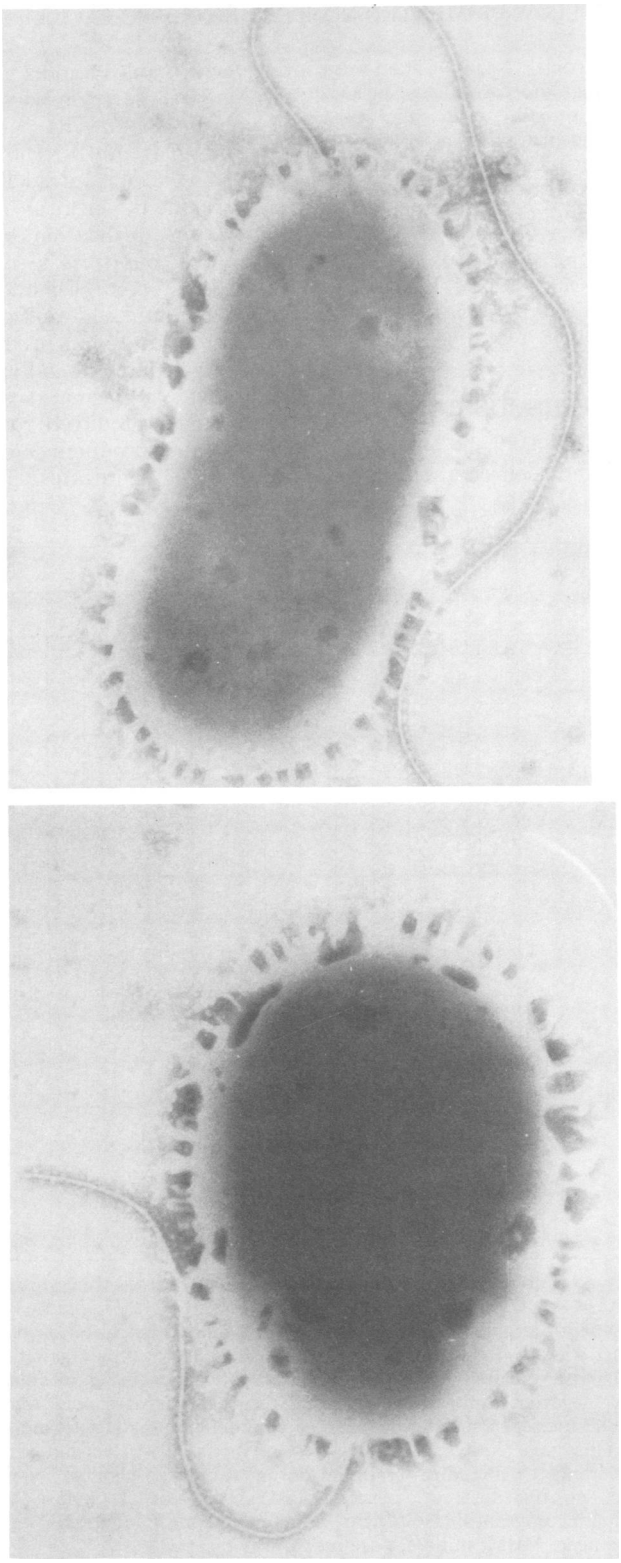

FIG. 1. *Electron micrographs of VE-2 strain 1612 (K217, C. typhiflavum) showing polar monotrichous flagella. ×20,000. Phosphotungstic acid stain.*

FIG. 2. *Electron micrograph of VE-1 strain 1613 (K224, C. typhiflavum) showing polar multitrichous flagella. ×12,000. Phosphotungstic acid stain.*

TABLE 3. *Buoyant density in CsCl and guanine plus cytosine base content of the deoxyribonucleic acids of VE strains*

Group VE-1			Group VE-2		
Strain	P_{CsCl} (g/cm³)	G+C (mol%)	Strain	P_{CsCl} (g/cm³)	G+C (mol%)
1613	1.7155[a]	56.6	424	1.725	66.3
1623	1.716[a]	57.1	750	1.7255[a]	66.8
1715	1.716	56.1	1430	1.726[a]	67.3
1734	1.715	56.1	1612	1.7255[a]	66.8
1763	1,716[a]	57.1	1622	1.725	66.3
1832	1.7155[a]	56.6	1770	1.726[a]	67.3
Grand mean ± SD[b]	1.7157 ± 0.0005	56.8 ± 0.5		1.7256 ± 0.0005	66.9 ± 0.5

[a] Average of two determinations.
[b] SD, Standard deviation.

gated segmented masses were demonstrated after 24 h of incubation by observing a hanging drop preparation of the condensate of an agar slant culture. The distinctive morphology of symplasmata was previously illustrated (4). The tested strains were uniformly susceptible to erythromycin, ampicillin, tetracycline, chloramphenicol, streptomycin, neomycin, kanamycin, polymyxin, gentamicin, and carbenicillin and variably susceptible to nalidixic acid.

Group VE-1 strains, as described by Weaver et al. (13), are polar multitrichous, produce positive tests for arginine dihydrolase and esculin hydrolysis, and display variable results for nitratase activity. Group VE-2 strains are described by these authors as polar monotrichous and negative for these same biochemical features. Ten of the strains examined in the present study, including *Chromobacterium typhiflavum* strain K217 (Fig. 1), were identical to the characteristics attributed to group VE-2 strains. Ten strains, including *C. typhiflavum* strain K224 (Fig. 2), were similar to the features attributed to group VE-1 strains. Tatum et al. (12) believe that the differences between these groups are sufficient to warrant their acceptance as separate species.

There appears to be some confusion, however, as to the use of *Chromobacterium*, since the genus has been defined as containing members which produce a violet pigment, are both oxidative and fermentative with regard to carbohydrate utilization, and demonstrate both polar monotrichous as well as peritrichous flagella (11). In addition, the designation *Bacterium typhiflavum* (2) was first applied to yellow-pigmented fermentative bacilli which were subsequently demonstrated to be members of the genus *Erwinia* (5).

Group VE strains and xanthomonads (group IIK) (12) share common morphological and biochemical features; however, group VE strains are distinguished from xanthomonads since the latter do not attack polyalcohols and are usually indophenol oxidase positive.

As a result of this study, the clinical isolates and strains previously designated *C. typhiflavum* by earlier investigators were found to be similar to strains of the VE group. Data obtained from G+C ratio studies of 56.8% for VE-1 and 66.9% for VE-2 separate the two groups (Table 3). The latter values are close to those reported for *Chromobacterium lividum*, *C. violaceum*, and for many species of *Pseudomonas*, whereas the 56.8% G+C is at the lower limits of the range for *Pseudomonas*. The conclusion can be drawn that the VE-1 and VE-2 groups represent two different genospecies with a low probability of any significant number of identical genes.

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