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Recognition of *Pseudomonas pickettii* in the Clinical Laboratory: Biochemical Characterization of 62 Strains

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Pseudomonas pickettii strains were studied to determine the characteristics essential for their identification in the clinical microbiology laboratory. Preliminary investigations indicated that these glucose-oxidizing, denitrifying, gram-negative rods were quite similar to an unclassified group of clinical isolates designated VA-2. Gas liquid chromatography of trimethylsilyl derivatives of whole cell hydrolysates of *P. pickettii* and VA-2 strains yielded nearly identical elution profiles. The VA-2 cultures were concluded to be probable strains of *P. pickettii*. A method is presented for differentiating cultures of *P. pickettii* from other similar bacteria encountered in clinical specimens.

Recently Ralston et al. (7) described and proposed a name for a new bacterial species, *Pseudomonas pickettii*, which had been isolated from clinical specimens. Through the use of deoxyribonucleic acid hybridization experiments and nutritional studies, they determined that this organism was distinctly different from a number of *Pseudomonas* species, and also was genetically similar to *P. solanacearum*. Their description of *P. pickettii*, although thorough in many aspects, did not include characteristics which are frequently used by the clinical microbiologist for culture identification.

For this reason we decided to examine strains of *P. pickettii* by using routine diagnostic techniques. Moreover, in preliminary studies, we observed a distinct similarity between *P. pickettii* and a group of unclassified bacteria which had been accumulated by the Special Bacteriology Section (SBS) at the Center for Disease Control. These bacterial cultures, arbitrarily designated VA-2 (10), and the *P. pickettii* strains were investigated morphologically and biochemically to determine the extent of their relatedness.

MATERIALS AND METHODS

Bacteria. The bacterial cultures were obtained from the stock culture collection maintained by the SBS. They were identified from clinical isolates submitted to this laboratory for further investigation (Table 1). The known cultures of *P. pickettii* were kindly supplied by M. J. Pickett. They were K-288 (recently deposited as ATCC 27511), K-214, K-298, K-472, K-487, K-599, K-619, and K-633.

Media and procedures. The biochemical tests were those routinely employed in the SBS for the study of microorganisms submitted for identification. The media preparation and the procedures used in determining biochemical characteristics have been previously described (2, 5, 8). The oxidation of potassium gluconate was determined by the procedure of Moore and Pickett (6). All agar slant media were inoculated with one drop of an 18- to 24-h Difco heart infusion broth culture.

Gas liquid chromatography (GLC). The freeze-dried cells were hydrolyzed by the technique of Farshy and Moss (3). The dried hydrolysate was dissolved in 0.5 ml of TRI-SIL (Pierce Chemical Co.). The vial (Reacti-Vial, Pierce Chemical Co.) containing the sample was flushed with nitrogen, capped, and placed in a 65 C water bath for 15 min. After incubation, the sample was reduced to dryness under nitrogen at 50 C. The dried residue was dissolved in 1 ml of hexane, and the final volume was reduced to 0.1 ml under nitrogen.

The samples were analyzed with a Hewlett-Packard 402-B gas chromatograph equipped with dual flame ionization detectors and 1.2-m long glass columns of 3-mm inner diameter. The detector temperature was 250 C. Helium, with a flow rate of 40 ml/min, was used as the carrier gas. Sample analysis was done on columns consisting of 3% OV-1 on Gas Chrom Q 100-120 mesh (Applied Science Labs, Inc.). The column temperature was held for 4 min at 130 C and then programmed to 230 C at the rate of 3 C per min. Peak areas were determined by the use of a disk integrator. The percentage of each eluted component was calculated from the ratio of the area of its peak to the total area of all peaks in the sample.

RESULTS AND DISCUSSION

The group VA-2 bacteria were thought to be *Pseudomonas* species but their biochemical characteristics did not parallel those of any named species. However, when strains of *P. pickettii* were examined, characteristics indicative of group VA-2 cultures were observed. All of

TABLE 1. *Sources of 54 strains of group VA-2*

Source	No. of isolates
Urine	12
Nasopharynx	11
Abscesses and wounds	10
Blood	7
Cerebrospinal fluid	2
Nursery isolette	2
Water source of artificial kidney machine	2
Unidentified and miscellaneous sources	8

the strains were unevenly staining gram-negative rods (ca. 0.5 by 1.5 to 3 μm) with parallel sides and rounded ends. The polarly flagellated (1 to 2 flagella) cells occurred as singles, in pairs, and infrequently in short chains (3 to 5 cells).

After 24 h of incubation at 35 C on 5% rabbit blood agar plates, the colonies were approximately 1 mm in diameter, circular, convex, and had an entire edge. The cellular growth was glossy, butyrous, and nearly opaque. Lysis was noted under the primary streak of inoculation, but not under isolated colonies, on some of the plates.

Some selected physiological characteristics determined for each of the bacterial cultures are presented in Table 2. Both the *P. pickettii* and VA-2 strains were oxidase and catalase positive, alkalinized citrate, hydrolyzed urea, and grew at each of the temperatures tested. They did not grow in cetrimide agar and produced no detectable proteolytic activity. Nitrate was reduced with the concomitant production of gaseous products. In each instance gas production during denitrification was observed when the semisolid medium of Stanier et al. (9) was used. However, the evolution of gas was not detected in a few samples tested by a second method (5) involving the use of a nitrate broth medium containing a small inverted vial to entrap the evolved gas.

The oxidative production of acid from various carbohydrates by these bacteria was studied (Table 3). Each strain produced acid from glucose, xylose, glycerol, L-arabinose, galactose, fructose, and mannose. No acid production was detected from the sugar alcohols or the carbohydrate polymers.

Examination of the GLC chromatograms of the trimethylsilyl profiles revealed slight variations among the strains (Table 4). The variation was exhibited in terms of quantitative rather than qualitative peak data. The derivatives recorded did not vary considerably either in number of peaks or in relative peak elution time. Twenty-two major peaks (≥1% of total peak area) and a total of 30 to 34 peaks were observed in the trimethylsilyl profiles of each of the bacterial strains. Each of the cultures tested had 10 common peaks, eluting at 7.6, 8.4, 9.6, 12.3, 12.9, 18, 20.2, 22.3, 24.3, and 26.3 min, which accounted for 70 to 77% of the total peak area measured. The peaks recorded at 7.6, 8.4, and 9.6 min represented 36 to 38% of the total peak area. The elution time for each of the peaks recorded did not vary from strain to strain by more than 0.1 min.

After compiling the data obtained in this study, we feel that the VA-2 clinical isolates are strains of *P. pickettii*. We were unable to differentiate between these cultures morphologically, and identical observations were made on substrate oxidation patterns and product elaboration experiments. Moreover, the GLC profiles of whole cell hydrolysates of these bacteria were quite similar. Intraspecies similarities in deriva-

TABLE 2. *Some selected characteristics of Pseudomonas pickettii and VA-2 cultures*

Test or substrate[a]	Result[b]	
	P. pickettii	VA-2
Citrate alkalinization	+	+
Catalase	+	+
Oxidase	+	+
Lysine decarboxylase	−	−
Ornithine decarboxylase	−	−
Arginine dihydrolase	−	−
Growth on:		
MacConkey agar	+	+
SS agar	−	−
Cetrimide agar	−	−
TGY agar at 25 C	+	+
TGY agar at 35 C	+	+
TGY agar at 42 C	+	+(3)[c]
Urea hydrolysis	+	+
Nitrate reduction to gaseous products	+	+
Indole	−	−
Gelatin hydrolysis	−	−
Aesculin hydrolysis	−	−
Peptonization of litmus milk	−	−
Chromogenesis	−	−
2-Keto gluconate	−	−
Lipid hydrolysis	+	+
ONPG	−	−

[a] Abbreviations: SS, *Salmonella-Shigella*; TGY, tryptone-glucose-yeast extract; ONPG, ortho-nitrophenyl-beta-D-galactopyranoside.

[b] Eight cultures of *P. pickettii* and 54 cultures of VA-2 were examined.

[c] The number inside the parentheses indicates the strains which deviated from the recorded result.

TABLE 3. *Carbohydrate oxidation pattern of P. pickettii and VA-2 cultures*

Substrate[a]	Acid production[b]	
	P. pickettii	VA-2
Glucose	+	+
Xylose	+	+
Mannitol	−	−
Lactose	−	−
Sucrose	−	−
Maltose	−	−
Glycerol	+	+
Salicin	−	−
L-Arabinose	+	+
Adonitol	−	−
Dulcitol	−	−
Galactose	+	+
Fructose	+	+
Mannose	+	+
Rhamnose	−	−
Trehalose	−	−
Raffinose	−	−
Sorbitol	−	−
Inositol	−	−
Cellobiose	−	−
Inulin	−	−
Dextrin	−	−
Glycogen	−	−
Erythritol	−	−
Melibiose	−	−
Melezitose	−	−
Starch	−	−

[a] The filter-sterilized carbohydrates were added aseptically to the OF medium to a concentration of 1%. Incubation was for 7 days at 35 C.

[b] Eight cultures of *P. pickettii* and 54 cultures of VA-2 were tested.

TABLE 4. *Relative percentage of peaks obtained from chromatograms of trimethylsilyl derivatives prepared from whole cell hydrolysates*[a]

Retention time (min)	*P. pickettii*		VA-2			
	K-288	K-214	A4657	A3627	A3217	A3742
6.7	1.5	0.8	1.6	0.7	2	1.4
7.2	1	0.8	0.7	1	1	0.8
7.6	4	4	5	3.5	6	7
8.4	25	24	24.5	26.5	23	21.2
9.6	9	9	8.5	7	8	10.1
10.5	T	0.8	T		0.7	T
11.1	0.8	1	0.7	1.5	1	0.8
11.5	0.8	0.7	1	1	3	0.8
12.3	3	4	3.2	3	2	4
12.9	13	13	14	16	10	11
13.3	4	3	3.3	1.5	4	4.2
13.8	3	2.2	3	3	2	2.5
15.0	T		T		T	T
15.7	1.1	0.8	1	2	2	1.3
16.8	1.5	0.9	1	1	0.8	1
17.0	1	0.9	1	0.7	1.6	1.1
18.0	4	3	5	4	4	4.6
19.0	1.5	3	1	2	0.9	1
20.2	4.2	4	5	3.5	6	5.2
21.4	0.8	1	0.7	1.5	1	
22.3	3	5	4	6	5	4
23.6	T	T		0.7	T	T
24.3	3.6	2	4	3	3	3.5
25.5	0.7	T	T	T	T	T
26.3	4	3	4	3	3	4
26.9	1.2	1.5	1	2	1	1.4
27.5	0.9	1	0.7	T		1
28.1	1.8	1	2	1	1.8	1.5
28.7	T	0.7	T	0.8	T	
29.4	T	1	T		0.8	T
30.0	1.8	3	2	1	2.5	2.1
31.7	1.2	1	1	T	0.9	0.9
32.4	T	0.8	T		T	0.7
33.4	1	0.9	0.8	1	1.2	1

[a] Relative percentage reflects the amount of each component in the sample. The symbol "T" refers to a relative percentage of less than 0.6%.

TABLE 5. *Differentiation of Pseudomonas pickettii from other similar microorganisms which are isolated from clinical specimens*[a]

Test	*P. pickettii*	*P. aeruginosa*	*P. fluorescens-P. putida* group	*P. stutzeri*	*P. cepacia*	*P. pseudomallei*	*A. xylosoxidans*	Vd	IIIa
Acid production from:									
Glucose OF open	+	+	+	+	+	+	+	+	+
Glucose OF closed	−	−	−	−	−	−	−	−	−
Mannitol OF open	−	v	v	+	+	+	−	v	−
Arginine dihydrolase	−	+	+	−	−	+	−(+)	−	−(+)
Gas production during nitrate reduction	+	+	−(+)	+	−	+(−)	+	+(−)	−
Growth on SS agar	−	+	+	−(+)	−	−(+)	+	+	+
Flagellar arrangement[b]	1	1	2	1	2	2	P	P	P

[a] Abbreviations: v, variable results in the reaction; SS, *Salmonella-Shigella*. The sign in the parentheses refers to the result obtained in a minority of the strains.

[b] The symbols for flagellar arrangement indicate: 1, 1 to 2 polar flagella; 2, more than 2 polar flagella; and P, peritrichous flagellation.

tive profiles have been shown to be characteristic of some bacterial species (1, 3, 4).

Cultures of *P. pickettii* may be confused with several microorganisms which are frequently encountered in clinical specimens. In particular, several saccharolytic, oxidase-positive species of *Pseudomonas*- and *Achromobacter*-like cultures mimic *P. pickettii* to some extent. However, a comparison of selected characteristics provides a mean of differentiation (Table 5). Growth on *Salmonella-Shigella* agar, carbohydrate oxidation, and arginine dihydrolase production serve to separate *P. pickettii* from the other similar *Pseudomonas* species. The *Achromobacter*-like cultures, *A. xylosoxidans*, IIIa, and Vd, can be identified by their peritrichous flagellation and the ability to grow on *Salmonella-Shigella* agar.

The clinical significance of the *P. pickettii* strains has not been determined. They have been isolated from a diverse number of sources directly related both to human involvement and to environmental origins (Table 1). Presently, no specific pathological condition has been linked to these microorganisms.

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