Pseudomonas vesicularis from Cervical Specimens

L. ANNE OTTO,¹ BEHRAM S. DEBOO,² EVELYN L. CAPERS,³ AND M. J. PICKETT^{4*}

U.S. Food and Drug Administration, Los Angeles, California 90015¹; The Everett Clinic, Everett, Washington 98201²; Bio Science Laboratories, Van Nuys, California 91405³; and Department of Bacteriology, University of California-Los Angeles, Los Angeles, California 90024⁴

Received for publication 4 November 1977

Biochemical characteristics and antibiotic susceptibilities of five strains of *Pseudomonas vesicularis* isolated from cervical cultures are reported. The organisms were recovered from Thayer-Martin medium, which, because of its inhibitory properties, restricts over-growth by most other species. Our findings agree with those of Kaltenbach and associates (J. Clin. Microbiol., 1:339-344, 1975) on the importance of esculin hydrolysis, maltose oxidation, and pigmentation in the identification of *P. vesicularis*. Comparative carbohydrate oxidation studies showed agreement in three of four methods (oxidative attack, buffered single substrate, King oxidative/fermentative medium). No oxidation of carbohydrates was observed in commercial oxidative/fermentative medium. In addition to biochemical characteristics, antibiograms can be useful auxiliary aids in the identification of *P. vesicularis* and in its differentiation from *P. diminuta*, a closely related species.

Pseudomonas vesicularis has rarely been reported in the literature. It was first described under the name *Corynebacterium vesiculare* by Busing et al. (3) and Busing and Freytag (4), who isolated it from a medicinal leech (*Hirsuda medicinalis*). They reported the organism to be nonmotile and gram negative or variable. In 1961, Carrier and McClesky (5) examined a culture of *C. vesiculare* (ATCC 11426) and found it to be gram negative and motile, although the culture otherwise agreed with the original species description. Galarneau and Leifson (6) proposed the species designation *Pseudomonas vesiculare* (Busing et al.) comb. nov. and recommended that ATCC 11426 be designated as the type culture. Extensive biochemical and genetic characterization of the type strain (ATCC 11426) and a strain isolated from a whooping cough plate (no. 230, Lautrop AB 102) was reported by Ballard et al. (1). In 1974, Moss and Kaltenbach reported on GLC (gas-liquid chromatographic) characterization of these two strains (14). A detailed biochemical, cultural, and GLC examination of nine strains of *P. vesiculare* was reported by Kaltenbach et al. (12). The authors did not specify the original sources of their strains, but rather reported only the culture collections from which they were obtained.

The present account reports the biochemical characteristics and antibiotic susceptibilities of two reference strains and five clinical isolates of

P. vesicularis recovered from cervical specimens inoculated on Thayer-Martin medium. The field strains were from women attending an out-patient gynecology clinic. Cultures had been taken to aid in identifying the etiology of cervicitis in these patients. At the time the cultures were obtained, three patients were receiving oral contraceptives and two were fitted with intrauterine devices.

During an 18-month period, 25 similar strains were isolated from cervical cultures, but on subculture at 35°C failed to survive for adequate biochemical characterization. Attention is called to the survival and growth of *P. vesicularis* on Thayer-Martin medium, and additional physiological characteristics and antibiotic susceptibility patterns are presented.

MATERIALS AND METHODS

Clinical details of the strains. A 152: Age unknown; intrauterine device; *Trichomonas vaginalis* on wet mount; moderate growth of *P. vesicularis*. A 250: 17 years old; oral contraceptives; *Candida albicans* also isolated from culture. A 251: 22 years old; oral contraceptives; herpes lesion on cervix; vulvar condylomata; *C. albicans* also isolated from culture. A 252: 20 years old; oral contraceptives; *T. vaginalis* on wet mount. A 253: 20 years old; intrauterine device; profuse vaginal discharge; heavy growth of *P. vesicularis*. A 115: Reference strain Lautrop AB-102. A 117: Reference strain ATCC 11426.

Specimen collection and culture. Cervical specimens were obtained through a vaginal speculum and

inoculated directly onto Thayer-Martin medium obtained as prepared plates from a commercial source. The plates were incubated at 35°C in an atmosphere of 8 to 10% CO₂ and examined daily for 4 days. Colonies of *P. vesicularis* were visible at 3 to 4 days.

Biochemical Tests. The procedures of Pickett and Pedersen (18, 19) were used for conventional tests. Esculin hydrolysis was determined by observing loss of fluorescence at 366 nm after 48 h of incubation at 30°C. The medium was a brucella agar (BBL) slant containing 0.002% esculin.

Carbohydrate acidification was determined by four methods: oxidative attack (OA) (16); buffered single substrate (18, 19); modified King oxidative/fermentative medium (CDC O/F) (20); and commercial OF medium, open tube only (11), prepared from a dry mix product (Difco).

Alkalinization of salts of organic acids, amino acids, amides, and other nitrogen-containing compounds was determined by the OA method.

All systems were inoculated concurrently with cells grown at 30°C for 24 h on brucella agar slants. OA tests were incubated at 30°C; all other tests were incubated at 35°C. Reactions were read at 24 and 48 h.

Antibiotic susceptibility tests. (i) **Minimal inhibitory concentrations.** Antibiotic susceptibilities were determined by the microdilution technique. Preparation of the antibiotic trays using a Canalco Autotiter IV has been previously described (7, 8). Serial dilutions of antibiotics were made in Mueller-Hinton broth. Using a Klett photometer, overnight cultures of the bacteria were standardized against MacFarland turbidity standards and then appropri-

ately diluted in Mueller-Hinton broth to a concentration of approximately 10⁵ to 10⁶ cells per ml. A volume of 50 µl of inoculum was added to the wells using 40-µl disposable droppers (Cooke Laboratory Products). The plates were sealed with self-adhesive transparent plastic and incubated overnight at room temperature. After incubation, the undersides of the wells were examined with the aid of a mirrored viewer for macroscopic evidence of growth. The minimal inhibitory concentration was read as the lowest concentration of antibiotic completely inhibiting growth. Results are reported as the average values of duplicate tests. Control organisms included *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *P. aeruginosa* ATCC 27857.

(ii) **Disk diffusion susceptibilities.** Disk diffusion susceptibility testing was performed by a modification of the method of Bauer et al. (2). Mueller-Hinton plates were inoculated according to the standard procedure, then incubated at room temperature. The plates were examined after 24 h, but 48 h of incubation was required before there was sufficient growth to produce defined zones of inhibition.

RESULTS

Cultural and biochemical characteristics. Cultural and biochemical reactions of all strains are given in Table 1. Isolated colonies appeared only after 48 h of incubation and showed no detectable pigment. Pigments were observed only in (orange) or under (brown) areas of confluent growth and only after 48 h of incubation. Our data essentially agree with those of Kalten-

TABLE 1. *Cultural and biochemical reactions of P. vesicularis*^a

Determination	Strain no.						
	115 ^b	117 ^b	152	250	251	252	253
TSI	N/N	K/N	K/N	K/N	K/N	K/N	K/N
H ₂ S	—	—	—	—	—	—	—
Oxidase	+	+	+	+	+	+	+
Catalase	—	+ _w	—	+	+	+	+
Urease (Christensen)	—	—	—	—	—	—	—
Esculin	+	+	+	+ _w	+ _w	+ _w	+ _w
Insoluble orange pigment	+	+	+	+	+	+	+
Soluble brown pigment	—	—	+	+	+	+	+
Motility	+	+ _w	+ _w	+	+	+	+
NO ₃ ⁻ NO ₂ ⁻	—	—	—	—	—	—	—
NO ₂ ⁻ N ₂	—	—	—	—	—	—	—
NO ₃ ⁻ amines	—	—	—	—	—	—	—
Fluorescence	+ _{yg}	—	—	—	—	—	—
Growth at 42°C	—	—	—	—	—	—	—
Growth on MacConkey agar	—	—	—	+	+	+	+
Citrate (Simmons)	—	—	—	—	—	—	—
Indole	—	—	—	—	—	—	—
Gluconate	—	—	—	—	—	—	—
Lysine decarboxylase	—	—	—	—	—	—	—
Ornithine decarboxylase	—	—	—	—	—	—	—

^a K, Alkaline; N, neutral; +, positive; +_w, weakly positive; —, negative; +_{yg}, yellow-green; TSI, triple sugar iron agar (slant/butt); H₂S, in TSI butt; nitrate was checked with zinc dust. Results were recorded after 48 h of incubation at 30°C.

^b Reference strains.

bach et al. (12). Pigmentation (orange), esculin hydrolysis, and maltose oxidation are important characteristics in the identification of *P. vesicularis*.

The comparative data on carbohydrate oxidation are given in Table 2. Comparable results were observed in three (OA, buffered single substrate, and CDC O/F) of the four assay systems. All strains acidified L-arabinose, cellobiose, galactose, glucose, and maltose. Rhamnose was acidified by field strains only. Xylose was acidified by one reference strain (no. 115). Commercially supplied O/F medium (Difco) was relatively insensitive; only one positive reaction occurred, although there was growth in all tubes.

P. vesicularis was almost completely inert on "salts" substrates (salts of organic acids, amino acids, amides, and other nitrogen-containing compounds). Asparagine was attacked by six of the seven strains (Table 3). Lactate was alkalized only by the field strains. Since these compounds are attacked by many nonfermentative organisms, they are not useful criteria for identification.

Antibiotic susceptibility patterns. The antibiotic susceptibility results are given in Table 4. Because 48 h of incubation was required for

the disk diffusion tests, the results were considered unreliable and therefore are not presented. Interpretation of the minimal inhibitory concentrations was made according to recommendations from the National Committee for Clinical Laboratory Standards (13).

All strains were resistant to colistin, nalidixic acid, and sulfisoxazole. Two strains were susceptible and five strains were of intermediate susceptibility to ampicillin. All strains were of intermediate susceptibility to nitrofurantoin. All were susceptible to gentamicin, kanamycin, cephalothin, tetracycline, and streptomycin.

DISCUSSION

To our knowledge there has been no mention in the literature of sites from which clinical isolates of *P. vesicularis* were recovered. This organism is probably rarely recognized in cultures from the cervix or other specimens because of its inability to compete on conventional culture media with other bacteria. The recovery of *P. vesicularis* on Thayer-Martin medium can be attributed to its resistance to colistin (polymyxin E) and the inhibitory effects of this medium on the growth of many other microbial species.

TABLE 2. Carbohydrate oxidation in various systems by *P. vesicularis*

Carbohydrate	Method ^a							
	OA		BSS		CDC O/F		Difco O/F	
	R (2)	F (5)	R (2)	F (5)	R (2)	F (5)	R (2)	F (5)
Adonitol	—	—	—	—	—	—	—	—
L-Arabinose	+	+	+ ²	+ ²	+	4+	—	—
Cellobiose	+	+	+ ²	+ ²	+	+	—	—
Dulcitol	—	—	—	—	—	—	—	—
Fructose	—	—	—	—	—	—	—	—
D-Galactose	+	+	+	+	+	+	—	1+ _w
D-Glucose	+	+	+	+	+	+ ²	—	—
Glycerol	—	—	—	—	—	—	—	—
m-Inositol	—	—	—	—	—	—	—	—
Lactose	—	—	—	—	—	—	—	—
Maltose	+	+	+	+	+ ²	3+ ²	—	—
D-Mannitol	—	—	—	—	—	—	—	—
D-Mannose	—	—	—	—	—	—	—	—
Melibiose	—	—	—	—	—	—	—	—
Raffinose	—	—	—	—	—	—	—	—
Rhamnose	—	+	—	+	—	4+ ²	—	—
D-Ribose	—	—	—	—	—	—	—	—
Salicin	—	—	—	—	—	—	—	—
Sorbitol	—	—	—	—	—	—	—	—
Sucrose	—	—	—	—	—	—	—	—
Trehalose	—	—	—	—	—	—	—	—
D-Xylose	1+	—	—	—	—	—	—	—

^a BSS, buffered single substrate. R, Reference strains; F, cervical culture isolates. Parentheses indicate number of strains tested. +, All strains tested were positive within 1 day; —, all strains tested remained negative throughout 48 h of incubation; +², all strains became positive after 2 days of incubation; 1+_w, one of the strains tested became weakly positive after 2 days of incubation.

TABLE 3. Substrate alkalization by *P. vesicularis*, OA method

Substrate	Strain no. ^a						
	115 ^b	117 ^b	152	250	251	252	253
Acetamide	—	—	—	—	—	—	—
β -Alanine	—	—	—	—	—	—	—
Allantoin	—	—	—	—	—	—	—
Asparagine	+	—	+	+ _w	+ _w	+ _w ²	+
Benzoate	—	—	—	—	—	—	—
Betaine	—	—	—	—	—	—	—
Butyrate	—	—	+ ²	—	—	+ _w ²	—
Citrate	—	—	—	—	—	—	—
Formate	—	—	—	—	—	—	—
L-Glutamine	—	—	—	—	—	—	—
<i>p</i> -Hydroxybenzoate	—	—	—	—	—	—	—
DL-Lactate	—	—	+ ²	+	+ ²	+ ²	+
Malonate	—	—	—	—	—	—	—
Nicotinamide	—	—	—	—	—	—	—
Nicotinate	—	—	—	—	—	—	—
L-Phenylalanine	—	—	—	—	—	—	—
Propionate	—	+ _w ²	+ _w ²	—	—	—	—
D-Saccharate	—	—	—	—	—	—	—
DL-Serine	—	—	—	—	—	—	—
DL-Tartrate	—	—	—	—	—	—	—

^a Abbreviations and notations as for Tables 1 and 2.^b Reference strains.

TABLE 4. Antibiotic susceptibility

Antibiotic	Susceptibility (MIC [μ g/ml] interpretation ^a) of strain no.:						
	115 ^b	117 ^b	151	250	251	252	253
Ampicillin	3/S	24/I	16/I	8/S	16/I	24/I	16/I
Carbenicillin	8/ ^c	24/	96/	64/	48/	32/	48/
Cephalothin	1.5/S	4/S	8/S	8/	8/S	6/S	4/S
Colistin	16/R	8/R	8/R	8/R	16/R	16/R	16/R
Gentamicin	<0.25/S	<0.25/S	<0.25/S	<0.25/S	<0.25/S	<0.25/S	<0.25/S
Kanamycin	<0.5/S	<0.5/S	<0.5/S	<0.5/S	<0.5/S	<0.5/S	<0.5/S
Nalidixic acid	>32/R	>32/R	>32/R	>32/R	>32/R	>32/R	>32/R
Nitrofurantoin	64/I	64/I	64/I	64/I	64/I	96/I	96/I
Streptomycin	<0.5/S	<0.5/S	1/S	0.5/S	1/S	1/S	2/S
Sulfisoxazole	>512/R	>512/R	>512/R	>512/R	>512/R	>512/R	>512/R
Tetracycline	<0.5/S	<0.5/S	<0.5/S	<0.5/S	<0.5/S	<0.5/S	<0.5/S

^a S, Sensitive; I, intermediate; R, resistant. MIC, Minimum inhibitory concentration.^b Reference strains.^c The National Committee on Clinical Laboratory Standards does not provide interpretative standards of carbenicillin susceptibility for *Pseudomonas* species other than *P. aeruginosa*.

Of the other nonfermentative gram-negative bacilli reported from human sources, *P. diminuta*, *P. pseudomallei*, *P. cepacia*, *Flavobacterium* sp., and CDC's group IIk (*Xanthomonas*) are usually resistant to the polymyxins (9, 15, 17). (Holmes et al. [10] have proposed the name *Pseudomonas paucimobilis* for biotype IIk-1; as yet no Linnean binomial has been proposed for biotype IIk-2. The IIk group is now not considered synonymous with *Xanthomonas*.) The latter four organisms can be readily differentiated from *P. vesicularis* on the basis of carbohydrate oxidation and other biochemical reactions. Differentiation between *P. diminuta* and *P. vesicularis*

is more difficult. Our findings agree with those of Kaltenbach and associates in the importance of pigmentation, esculin hydrolysis, and maltose oxidation in the identification of *P. vesicularis*.

Criteria and systems for the identification of the nonfermentative gram-negative bacilli have not been as universally agreed upon as those used for the identification of *Enterobacteriaceae*. When using the criteria established by a particular investigator, a laboratory must also adhere to that worker's protocol, including media and methodology. We have presented data on the saccharolytic activities of *P. vesicularis*

in four different basic media. Similar reactions were observed in OA, buffered single substrate, and CDC O/F methods, whereas the organisms were almost completely inert on carbohydrates in O/F medium obtained from a commercial source. Differences in composition of the two O/F basal media may account for the differences in the visible indications of carbohydrate oxidation observed in the two media.

LITERATURE CITED

1. Ballard, R. W., M. Doudoroff, R. Y. Stanier, and M. Mandel. 1968. Taxonomy of the aerobic pseudomonads: *P. diminuta* and *P. vesicularis*. *J. Gen. Microbiol.* 53:349-361.
2. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* 45:493-496.
3. Busing, K. J., W. Doll, and K. Freytag. 1953. Die Bakterienflora der medizinischen Blutegel. *Arch. Mikrobiol.* 19:52-86.
4. Busing, K. J., and K. Freytag. 1954. Bakterienflora der Blutegel-Harnblase. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig.* 160:579-582.
5. Carrier, E. B., and C. S. McCleskey. 1961. Motility and flagellation of *Corynebacterium vesiculare* Busing, Doll, and Freytag. *J. Bacteriol.* 82:154-156.
6. Galarneau, T. P., and E. Leifson. 1964. *Pseudomonas vesiculare* (Busing et al.) comb. nov. *Int. Bull. Bacteriol. Nomencl. Taxon.* 14:165-168.
7. Gavan, T. L., and D. A. Butler. 1974. Automated microdilution method for antimicrobial susceptibility testing, p. 63-76. In A. Balows (ed.), *Current techniques for antimicrobial susceptibility testing*. Charles C Thomas, Publisher, Springfield, Ill.
8. Gerlach, E. H. 1974. Microdilution: a comparative study, p. 88-93. In A. Balows (ed.), *Current techniques for antimicrobial susceptibility testing*. Charles C Thomas, Publisher, Springfield, Ill.
9. Gilardi, G. L. 1971. Antimicrobial susceptibility as a diagnostic acid in the identification of nonfermenting gram-negative bacteria. *Appl. Microbiol.* 22:821-823.
10. Holmes, B., R. J. Owen, A. Evans, H. Malnick, and W. R. Willcox. 1977. *Pseudomonas pancimobilis*, a new species isolated from human clinical specimens, the hospital environment, and other sources. *Int. J. Syst. Bacteriol.* 27:133-146.
11. Hugh, R., and G. L. Gilardi. 1974. *Pseudomonas*, p. 250-269. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
12. Kaltenbach, C. M., C. W. Moss, and R. E. Weaver. 1975. Cultural and biochemical characteristics and fatty acid composition of *Pseudomonas diminuta* and *Pseudomonas vesiculare*. *J. Clin. Microbiol.* 1:339-344.
13. Matsen, J. M., and A. L. Barry. 1974. Susceptibility testing: diffusion test procedures, p. 418-427. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
14. Moss, C. W., and C. M. Kaltenbach. 1974. Production of glutaric acid: a useful criterion for differentiating *Pseudomonas diminuta* from *Pseudomonas vesiculare*. *Appl. Microbiol.* 27:437-439.
15. Oberhofer, T. R., J. W. Rowen, and G. F. Cunningham. 1977. Characterization and identification of gram-negative, nonfermentative bacteria. *J. Clin. Microbiol.* 5:208-220.
16. Otto, L. A., and M. J. Pickett. 1976. Rapid method for identification of gram-negative, nonfermentative bacilli. *J. Clin. Microbiol.* 3:566-575.
17. Pedersen, M. M., M. A. Marso, and M. J. Pickett. 1970. Nonfermentative bacilli associated with man. III. Pathogenicity and antibiotic susceptibility. *Am. J. Clin. Pathol.* 54:178-192.
18. Pickett, M. J., and M. M. Pedersen. 1970. Characterization of saccharolytic nonfermentative bacteria associated with man. *Can. J. Microbiol.* 16:351-362.
19. Pickett, M. J., and M. M. Pedersen. 1970. Salient features of nonsaccharolytic and weakly saccharolytic nonfermentative rods. *Can. J. Microbiol.* 16:401-409.
20. Riley, P. S., H. W. Tatum, and R. E. Weaver. 1972. *Pseudomonas putrefaciens* isolates from clinical specimens. *Appl. Microbiol.* 24:798-800.