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Pseudomonas putrefaciens Isolates from Clinical Specimens

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A total of 109 cultures of *Pseudomonas putrefaciens* isolated from clinical specimens were studied. The cultures were separated into two groups. The majority of the group 1 isolates, comprising 31 cultures, were characterized by (i) growth in plain nutrient broth, but no growth in broth supplemented with NaCl at concentrations of 7% and above, (ii) no growth on Salmonella-Shigella (SS) agar, and (iii) production of acid from the carbohydrates, sucrose, maltose, arabinose, and dextrin. Most group 2 isolates, comprising 78 cultures, were (i) unable to grow in plain nutrient broth, but grew well in broth supplemented with NaCl at a concentration of 7 to 10%, (ii) able to grow on SS agar, and (iii) unable to produce detectable amounts of acid from any of the carbohydrates tested except for variable results with glucose and fructose.

In 1964, King (4) gave a description of 22 cultures of an H_2S-producing gram-negative rod which had been identified from clinical isolates. She provisionally named this nonfermentative, polarly flagellated rod "Ib." In 1970, von Graevenitz and Simon (9) reported the isolation of 13 strains of this bacterium from clinical specimens and 1 strain from a contaminated tissue culture. In 1970, Hugh (3) stated that King's "Ib" microorganism was the same as the bacterium *Pseudomonas putrefaciens*. *P. putrefaciens* was originally isolated from butter and described by Derby and Hammer (1), and was subsequently designated as *P. putrefaciens* by Long and Hammer (6). Hugh (3) listed the characteristics necessary for the identification of *P. putrefaciens* and reviewed the diverse sources of isolation that have been reported for this microorganism.

Levin (5) proposed that there are two species of this organism on the basis of the guanine plus cytosine (GC) content of the deoxyribonucleic acid (DNA) and the ability to grow in 6% NaCl broth. The GC content of the DNA of the isolates which grew in 6% NaCl had a range of 55.9 to 59%, and that of the group unable to grow in 6% NaCl had a range of 47.8 to 50.8%.

This study involved further evaluation of *P. putrefaciens* or the "Ib" cultures of King. Some additional biochemical characteristics of these microorganisms were investigated to determine the likelihood of the existence of two groups or, possibly, two species.

MATERIALS AND METHODS

Bacteria. The bacterial cultures employed were obtained from the stock culture collection maintained by the Special Bacteriology Laboratory (SBL) at the Center for Disease Control. They were identified from clinical isolates submitted for further investigation by this laboratory. A strain of *P. putrefaciens* (RH-1085; ATCC 8073) was generously supplied by R. Hugh. It has been stored in defibrinated rabbit blood at −40 C since 1970, and has not changed from the initial observations recorded in this laboratory on receipt.

Biochemical characteristics. The biochemical tests were those routinely employed in the SBL for the study of submitted cultures. The media and procedures used in performing these tests have been previously described (2, 4). The carbohydrate OF (oxidative-fermentative) media were prepared by using a modification of King's formula (4). The basal OF medium consisted of 0.2% Difco Casitone, 0.003% phenol red, and 0.3% agar. The *p*H of the medium was 7.3. Arginine dihydrolase and lysine and ornithine decarboxylase activities were detected by the method of Moeller (7). The oxidation of potassium gluconate to 2-ketogluconate was determined by the procedure of Moore and Pickett (8). All agar slant media were inoculated with one drop of an 18- to 24-hr Difco heart infusion broth culture.

RESULTS

Some selected metabolic characteristics of the *P. putrefaciens* cultures are presented in Table 1. From the results obtained, the bacteria were separated into two groups. Both groups were catalase- and oxidase-positive, reduced nitrate to nitrite, exhibited proteolysis, and

TABLE 1. *Some characteristics of Pseudomonas putrefaciens*[a]

Test or substrate	Cultures[b]		
	RH-1085	Group 1	Group 2
Catalase	+	+	+
Oxidase	+	+	+
Growth on MacConkey agar	+	+	+
Growth on SS agar	−	− (1)	+
Growth on cetrimide agar	−	−	−
Citrate utilization	−	− (5)[c]	− (25)[c]
Urea hydrolysis	−	− (5)	− (27)
Nitrate reduction	+	+	+
Indole production	−	−	−
Gelatinase	+	+	+
Peptonization of litmus milk	+	+ (10)	+ (10)
Growth on TGY			
25 C	+	+	+
35 C	+	+	+
42 C	−	− (9)	− (22)
2-Ketogluconate	−	−	−
Esculin hydrolysis	−	−	−
Lysine decarboxylase	−	−	−
Arginine dihydrolase	−	−	−
Ornithine decarboxylase	+	+	+

[a] SS, Salmonella-Shigella; TGY, tryptone-glucose-yeast extract.

[b] A total of 31 group 1 cultures and 78 cultures of group 2 were tested. The numbers inside the parentheses indicate the number of strains which deviated from the recorded result.

[c] No color change due to *p*H alteration was observed.

produced ornithine decarboxylase. All of the group 2 cultures grew luxuriantly on Salmonella-Shigella (SS) agar within 48 hr. Of 31 strains of group 1 which were tested, only one grew on SS agar. The named culture of *P. putrefaciens* (RH-1085; ATCC 8073) did not grow on SS agar.

The utilization of various carbohydrates by these bacteria was studied (Table 2). Both groups gave variable results in glucose and fructose OF media. Group 1 strains produced acid from sucrose, maltose, arabinose, and dextrin. Except when grown in glucose and fructose OF media, group 2 cultures did not detectably oxidize any of the substrates tested. Moreover, without the addition of 0.5% NaCl to the basal OF medium, the group 2 cultures grew only in rare instances. The type culture of *P. putrefaciens* exhibited a carbohydrate pattern identical to that of the group 1 bacteria.

The salt tolerance of these bacterial cultures was investigated (Table 3). All group 1 isolates and the type culture of *P. putrefaciens* grew in Difco nutrient broth with no NaCl supplement. However, none of the group 2 cultures grew in this medium. Only 2 of 17 cultures of group 1 grew in nutrient broth containing a NaCl concentration in excess of 5%. In contrast, 24 of 26 group 2 cultures grew in nutrient broth containing 8% NaCl.

DISCUSSION

The results obtained in this study concur with the proposal of Levin (5) that two groups exist within the species *P. putrefaciens*, and that the establishment of an additional species should be considered. The majority of the cultures of group 1 may be characterized as (i) capable of growing in plain nutrient broth or broth supplemented with a maximal NaCl

TABLE 2. *Carbohydrate utilization by Pseudomonas putrefaciens*

Substrate[a]	Acid production[b]		
	RH-1085	Group 1	Group 2
Glucose	+	±	±
Xylose	−	−	−
Mannitol	−	−	−
Lactose	−	−	−
Sucrose	+	+ (1)	−
Maltose	+	+	−
Glycerol	−	−	−
Salicin	−	−	−
L-Arabinose	+	+	−
Adonitol	−	−	−
Dulcitol	−	−	−
Galactose	−	−	−
Fructose	−	±	±
Mannose	−	− (1)	−
Rhamnose	−	−	−
Trehalose	−	−	−
Raffinose	−	−	−
Sorbitol	−	−	−
Inositol	−	−	−
Cellobiose	−	−	−
Inulin	−	−	−
Dextrin	+	+	−
Glycogen	−	−	−
Erythritol	−	−	−
Melibiose	−	−	−
Melezitose	−	−	−
Starch	−	− (1)	−

[a] The filter-sterilized carbohydrates were added aseptically to the OF medium at a concentration of 1%. The carbohydrate OF medium was supplemented with 0.5% sodium chloride for group 2 strains.

[b] Incubation was for 7 days at 35 C. The numbers inside the parentheses indicate the number of strains which deviated from the recorded result. Nineteen cultures of group 1 and 26 cultures of group 2 were tested.

TABLE 3. *Salt tolerance of Pseudomonas putrefaciens*[a]

Culture[b]	NaCl percentage									
	0	0.5	3	4	5	6	7	8	9	10
RH-1085	+	+	+	+	−	−	−	−	−	−
Group 1 (1)	+	+	+	−	−	−	−	−	−	−
Group 1 (8)	+	+	+	+	−	−	−	−	−	−
Group 1 (6)	+	+	+	+	+	−	−	−	−	−
Group 1 (2)	+	+	+	+	+	+	−	−	−	−
Group 2 (1)	−	+	+	+	+	+	−	−	−	−
Group 2 (1)	−	+	+	+	+	+	+	−	−	−
Group 2 (2)	−	+	+	+	+	+	+	+	−	−
Group 2 (5)	−	+	+	+	+	+	+	+	+	−
Group 2 (17)	−	+	+	+	+	+	+	+	+	+

[a] Results indicate the presence (+) or absence (−) of cellular growth.

[b] The broths were inoculated with one drop of a nutrient broth suspension. To prepare the suspension, which had an optical density of 0.01 at a wavelength of 620 nm, growth was removed from a 24-hr slant culture with a straight needle and suspended in the broth. The number inside the parentheses indicates the number of cultures in each category; 26 cultures of group 2 and 17 cultures of group 1 were examined.

concentration of 4 to 5%, (ii) unable to grow on SS agar, and (iii) capable of producing acid from sucrose, maltose, arabinose, and dextrin. In contrast, the majority of the cultures of group 2 were (i) unable to grow in plain nutrient broth, but thrived in broth supplemented with up to 9 to 10% NaCl, (ii) able to grow luxuriantly on SS agar, and (iii) unable to produce detectable amounts of acid from any of the carbohydrate substrates tested, except for variable results with glucose and fructose.

Additional evidence for this separation was presented by Levin's (5) previous report, in which the GC content of the DNA of several cultures used in this study was determined. From his data, those which fit into our group 1, A7310 and A7191, had 49.5 and 50.1% GC, respectively. Those strains which fit into our group 2, B89a, B89b, A7921, A4186, and B23, had 55.9, 57, 57.1, 57.4, and 57.6% GC, respectively.

Therefore, there is evidence for the existence of two groups within the current description of the microorganism *P. putrefaciens*. The type culture studied was categorized as a member of our group 1. Whether or not this division should create an additional species will require further study. DNA-DNA homology studies are a necessity for further clarification of this problem.

Compiled laboratory data indicate no appreciable difference between the two groups in regard to sources of isolation. The sources vary from the mouth and throat to various body wounds. From the salt tolerance differential, one might expect that the group 2 cultures would be isolated from sources located near coastal areas. However, a difference in the geographical distribution of the two groups has not been detected.

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