COMPARATIVE STUDIES OF THE GENUS *MALLEOMYCES* AND SELECTED *PSEUDOMONAS* SPECIES

I. Morphological and Cultural Characteristics

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Infections caused by *Pseudomonas aeruginosa* have assumed a new importance since the advent of antibiotic therapy. Stanley (1947) noted the increasing number of secondary pseudomonad infections following disturbance of the normal bacterial flora as a result of such therapy. Pigmented strains of *P. aeruginosa* are easily recognized, but the frequency with which achromogenic strains of this genus are encountered presents a problem in diagnostic identification of *Pseudomonas* species, particularly with respect to differentiation from *Malleomyces pseudomallei*.

Descriptions of the biological affinities of *P. aeruginosa* and *M. pseudomallei* have been reported by Legroux and Genevray (1933), Legroux and Blanc (1943), and Pons (1927). Based on morphological studies demonstrating polar flagellation in both *Pseudomonas* species and *M. pseudomallei*, Brindle and Cowan (1951) and Lajudie *et al.* (1953) suggested that *M. pseudomallei* should be incorporated taxonomically into the family *Pseudomonadaceae*.

The purpose of this paper is to note the differences in cultural characteristics of these species and to present electron photomicrographs to further substantiate the cephalotrichous nature of both *M. pseudomallei* and *Pseudomonas* species. Because of their close relationships, *Malleomyces mallei* and other pseudomonads have been included in this study.

MATERIALS AND METHODS

Cultures used. Data, including source and date of isolation of 18 cultures of *Malleomyces* and 28 cultures of *Pseudomonas* are given in tables 1 and 2, respectively. All but 4 cultures were maintained in this laboratory from one to three years on nutrient agar (BBL 138), pH 6.8, and glycerol (3 per cent). They were transferred at 6-week intervals and stored at room temperature.

Morphological studies. Electron photomicrographs were prepared from broth cultures fixed by osmic acid or formalin. The pseudo agar replication method, described by Houwink and Van Iterson (1950) as method 2b, was used to transfer the cells to stainless steel specimen grids. Specimens were shadowed with either chromium or a gold manganin alloy. Observations were made with a RCA electron microscope Model EMU 2C equipped with a 30 μ objective aperture and an extended range lens. The photomicrographs were taken at an original magnification of 4,940 × or 6,440 ×.

Oxidation of carbohydrates. Production of acid in a medium containing carbon compounds was used to determine oxidation of carbohydrates. The basal medium consisted of $(NH_4)_2HPO_4$, 0.2 per cent; $Mg\ SO_4 \cdot 7\ H_2O$, 0.02 per cent; NaCl, 0.02 per cent; K_2HPO_4, 0.02 per cent; and $CaCl_2$, 0.01 per cent. Bromthymol blue was added—5.0 ml of a 0.4 per cent solution per L of medium. The basal medium was dispensed in 2.5 ml amounts in screw cap tubes and autoclaved 15 min at 121 C. The carbohydrates and alcohols were sterilized by Seitz filtration in 10 or 5 per cent aqueous solution and 0.25 ml added aseptically to each tube of basal medium. The inoculum was prepared by adding one loop of a 24-hr trypticase soy agar culture to a tube of basal medium. The inoculum consisted of 2 drops of this suspension (approximately 0.1 ml) to each tube of carbohydrate medium. The carbohydrates employed were: arabinose, xylose, glucose, levulose, galactose, lactose, sucrose, maltose, trehalose, raffinose, inulin, glycerol, mannitol, dulcitol, and salicin.

Proteolysis. Nutrient gelatin (Difco B11), litmus milk (Difco B107), 33 per cent horse serum slants, and Locke's egg medium were employed to detect proteolysis.

Hemolysis. Sheep blood and rabbit blood, 10

TABLE 1

Malleomyces cultures studied

Species	Original Designation	Designation in this Laboratory	Isolation		
			Source	Place	Year
M. mallei	China 5*	MM-A	Equine	Kweiyang	1942
M. mallei	3873*	MM-B	Human	Burma	1944
M. mallei	China 4*	MM-C	Equine	Kweiyang	1942
M. pseudomallei	Pf. mallei‡	MP-D	Equine	Philippines	1947
M. pseudomallei	Horse 49§	MP-E	Equine	Malaya	Unknown
M. pseudomallei	294¶	MP-F	Unknown	Unknown	Unknown
M. pseudomallei	295¶	MP-G	Unknown	Unknown	Unknown
M. pseudomallei	C-3*	MP-H	Unknown	Unknown	Unknown
M. pseudomallei	1691†**	MP-J	Human	Kuala Lumpur	1921
M. pseudomallei	4845†**	MP-K	Monkey	Singapore	1935
M. pseudomallei	4846†**	MP-L	Monkey	Singapore	1935
M. pseudomallei	6700†**	MP-M	Human	England‖	1942
M. pseudomallei	7431†**	MP-N	Unknown	Unknown	Unknown
M. pseudomallei	8707†**	MP-O	Unknown	Unknown	Unknown
M. pseudomallei	8708†**	MP-P	Unknown	Unknown	Unknown
M. pseudomallei	7383†**	MP-Q	Human	Burma	1945–1946
M. pseudomallei	56-B-1*	MP-R	Human	Colorado‖	1946
M. pseudomallei	286*	MP-S	Human	Louisiana‖	1953

* Cultures from collection of Army Medical Service Graduate School, Washington, D. C.

† Received through courtesy of Dr. Martha K. Ward, Communicable Disease Center, Chamblee, Georgia.

‡ Received from Bureau of Animal Industry, Republic of Philippines.

§ Received from Veterinary Service Laboratory, Ipoh, Malaya.

¶ Old stock cultures from the Calcutta School of Tropical Medicine.

‖ These patients had evidence of infection acquired while living in the Far East.

** British National Collection of Type Cultures.

per cent each in tryptone agar plates were used for detection of hemolysis.

Nitrate utilization. The methods described in the *Manual of Methods for Pure Culture Study of Bacteria* (1947) for detection of nitrite and residual nitrates in nitrate broth cultures were used to determine utilization of nitrate by these species.

Hydrolysis of urea. Slants of Christensen's agar and urease test medium (BBL 165) were used to determine presence of urease.

Indole production. Two per cent tryptone broth cultures were tested at 24 and 48 hr by Kovac's method for indole.

Utilization of inorganic nitrogen, and organic acid salts as a source of carbon. Simmon's citrate agar (Difco B91) and Koser's medium were used in this part of the study.

Oxidation of potassium gluconate. A modification of Haynes' (1951) method was used to determine oxidation of potassium gluconate. Cultures were grown in Erlenmeyer flasks containing 100 ml Haynes potassium gluconate medium. After 3 and 7 days' incubation at 37 C, 1.0 ml of the culture was transferred to a tube containing 10 ml Benedict's copper sulphate solution. After 10 min in a boiling water bath the tube was rapidly cooled and examined for copper precipitate.

Pigment production. A chemically-defined medium of Burton, Campbell, and Eagles (1948), and two slants of Gessard's peptone glycerol agar, one at pH 6.8 and the other at pH 7.6, were used to determine pigment production. Georgia and Poe's medium (1931) was used to detect fluorescin. Cultures in the latter medium were incubated at 37 C for 24 hr after which they were removed to a dark cupboard at room temperature. The cultures in Burton's and Gessard's media were incubated at 37 C for one week or until color developed. The Burton's cultures were tested for three pigments, pyocyanin, pyorubin, and fluorescin, as described by Meader *et al.* (1925). The culture was first examined for fluorescin. It was then extracted with chloroform after rendering slightly alkaline with dilute

TABLE 2

Pseudomonas cultures studied

Species	Original Designation	Designation in this Laboratory	Isolation Source	Received Year
P. aeruginosa	135†§	PA-17	Human	1952
P. aeruginosa	157†	PA-18	Human urine	1952
P. aeruginosa	187†	PA-19	Human urine	1952
P. aeruginosa	196†	PA-20	Human urine	1952
P. aeruginosa	197†	PA-21	Unknown	1952
P. aeruginosa	226†	PA-22	Human urine	1952
P. aeruginosa	250†	PA-23	Unknown	1952
P. aeruginosa	458†	PA-24	Human urine	1952
P. aeruginosa	482†	PA-25	Unknown	1952
P. aeruginosa	528†	PA-26	Human urine	1952
P. aeruginosa	616†	PA-27	Unknown	1952
P. aeruginosa	632†	PA-28	Human urine	1952
P. aeruginosa	793†	PA-29	Unknown	1952
P. aeruginosa	833†	PA-30	Human blood	1952
P. aeruginosa	870†	PA-31	Human urine	1952
P. aeruginosa	7700‡	PA-32	Well water	1951
P. aeruginosa	7701‡	PA-33	Well water	1951
P. aeruginosa	8709‡	PA-34	Unknown	1951
P. aeruginosa	9027‡	PA-35	Human ear	1951
P. aeruginosa	7153*	PA-36	Dog urine	1953
P. fluorescens	910†	PF-1	Spinal fluid	1952
P. ovalis	401†	PO-1	Kidney	1952
P. ovalis	403†	PO-2	Kidney	1952
P. ovalis	868†	PO-3	Sheep brain	1952
P. ovalis	959†	PO-4	Bronchial aspiration	1952
P. stutzeri	11607‡	PS-1	Unknown	1953
P. stutzeri	2555*	PS-2	Baby formula	1951
P. stutzeri	1394*	PS-3	Human	1953

* Cultures from collection of Army Medical Service Graduate School, Washington, D. C.

† Received through the courtesy of Dr. Martha K. Ward, Communicable Disease Center, Chamblee, Georgia.

‡ American Type Culture Collection.

§ Subculture of organism reported by Garry and Koch 1951 to be *Malleomyces pseudomallei*.

NaOH. The extract containing blue pyocyanin was treated with approximately 2 ml 0.1 N HCl to produce red acid pyocyanin. The red water soluble pigment pyorubin was unaffected by change of pH.

The effect of incubation temperatures on growth. Glycerol agar slants were used to determine the effect of different temperatures on growth. Slants previously maintained at the test temperature conditions were inoculated with 0.05 ml of an 18-hr broth culture and incubated at 10 C in the refrigerator, at 21 C in a thermostatically controlled cubicle, and at 42 C in a water bath. Observations were made daily for a week or longer.

Sodium chloride concentration. The effect of sodium chloride was determined by the use of plates containing veal infusion agar (Difco B343) with varying concentrations of NaCl from 2 to 6 per cent.

Acid cultivation. Slants of Sabouraud's dextrose agar were used to determine the ability of the organisms to grow at pH 5.6.

Bile salts. SS agar (Difco B74) plates and desoxycholate agar (Difco B273) plates were prepared according to the *Difco Manual*, Ninth Edition. Brilliant green bile plates were prepared by adding 2 per cent agar to brilliant green bile 2 per cent broth (Difco B7) and sterilizing in the usual manner before pouring plates.

Cationic detergents. Cetyltrimethyl ammonium bromide (CTAB), has been used in culture media experimentally since Williams *et al.* (1944) reported that coliform organisms and *P. aeruginosa* were not inhibited by this agent. Lowberry (1951) compared various concentrations of this detergent in culture media and concluded that a concentration of 0.1 per cent was most favorable for the isolation of *P. aeruginosa*. This concentration of CTAB in trypticase soy agar was employed in this study. A stock 5 per cent aqueous solution was prepared and sterilized by Seitz filtration; 2.0 ml of this solution was added to 100 ml of melted agar just before pouring plates. Precipitation was prevented by cooling the agar to 45 C before adding the solution of CTAB.

Sodium azide. Sodium azide agar was prepared by using glucose, 0.1 g; tryptose, 2.0 g; NaCl, 0.5 g; $NaNO_3$, 0.006 g; agar, 2.0 g; H_2O, 100 ml and adjusting to pH 6.8 before autoclaving.

EXPERIMENTAL RESULTS

Morphological observations. All strains were gram negative rods and all were motile, except

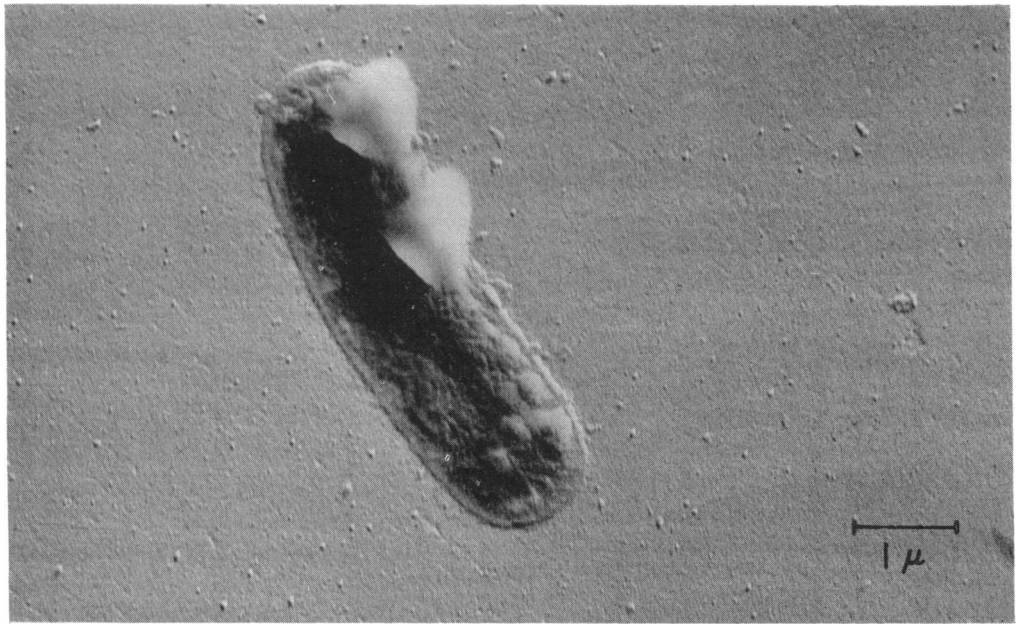

Figure 1. Malleomyces mallei strain MM-A formalin fixed. Magnification 27,400 ×

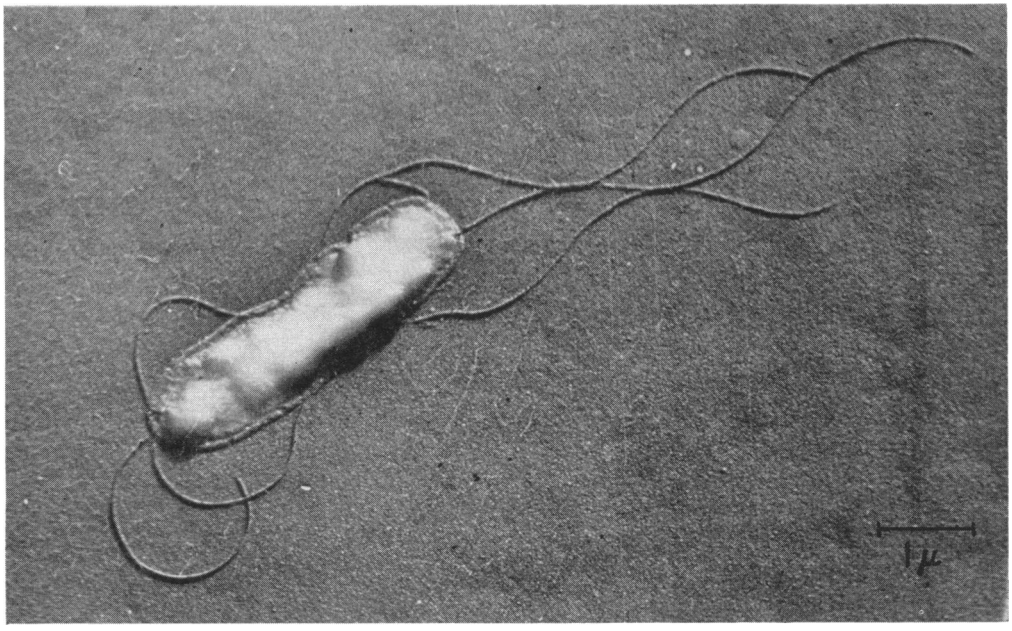

Figure 2. Malleomyces pseudomallei strain MP-M, osmic acid fixation, showing attachment of one flagellum within cytoplasm. Magnification 21,000 ×

three cultures of *M. mallei*. Studies of *M. mallei* using the electron microscope showed no evidence of flagellation. *M. pseudomallei* as well as pseudomonads showed varying numbers of flagella attached at one or both poles of the cell. From the limited number of flagellated cells examined, some of the *M. pseudomallei* strains appear to be monotrichate, some lophotrichate. A point of attachment of a flagellum may be seen in the photograph of *M. pseudomallei* strain MP-M (figure 2). Another cell showing what may be the origin of the flagellum is seen in figure 3. In one of the cultures in the collection, strain MP-J, several polar flagella appear to be in the process

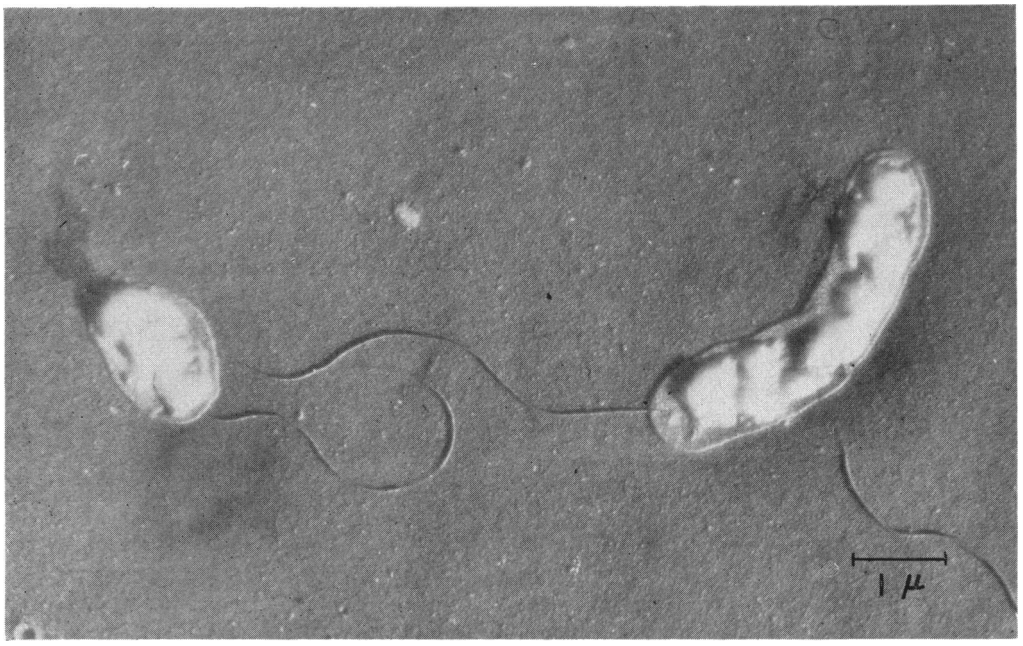

Figure 3. Malleomyces pseudomallei strain MP-D, osmic acid fixation. Magnification 21,500 ×

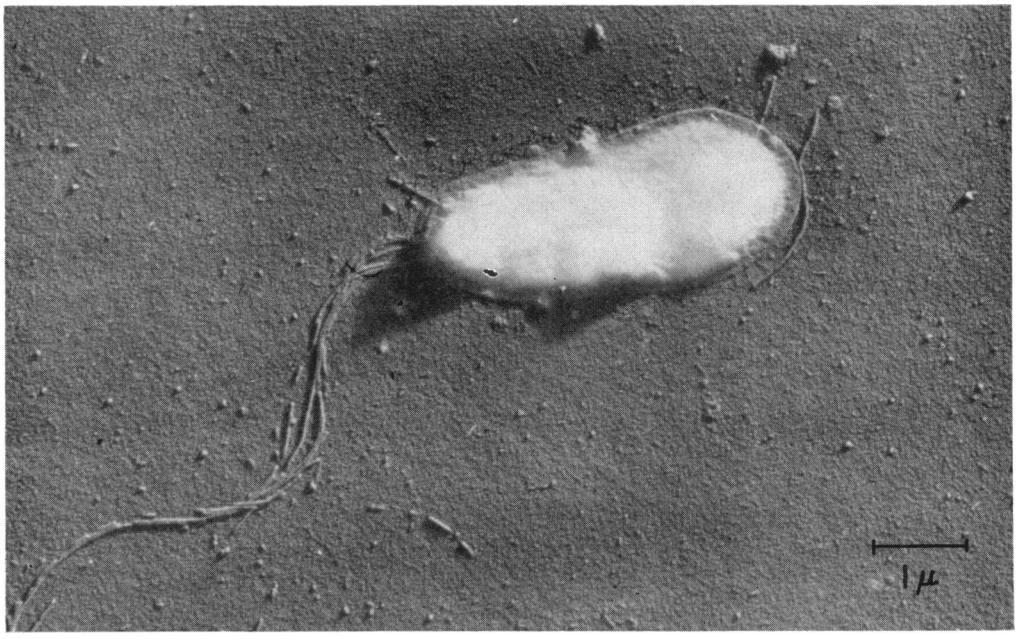

Figure 4. Malleomyces pseudomallei strain MP-J, osmic acid fixation. Magnification 24,700 ×

of breaking up into short fragments (figure 4). This fragmentation was also seen in a monotrichate cell of *M. pseudomallei* strain MP-D. Strains of *Pseudomonas stutzeri* were also both mono- and lophotrichate. Electron micrographs of a nonfixed preparation of *Pseudomonas ovalis* likewise demonstrated monotrichate and lophotrichate flagellation.

Oxidation of carbohydrates. Utilization of carbohydrates was observed to vary with the amount of nitrogen contained in the basal medium and with the time of incubation. Results

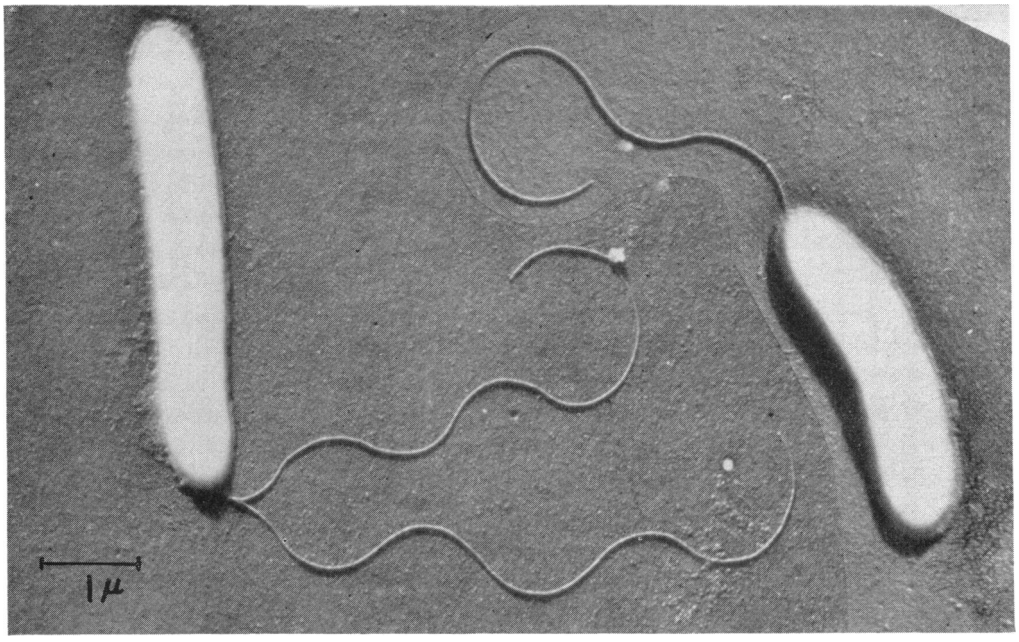

Figure 5. Pseudomonas stutzeri strain PS-2, osmic acid fixation. Magnification 21,000 ×

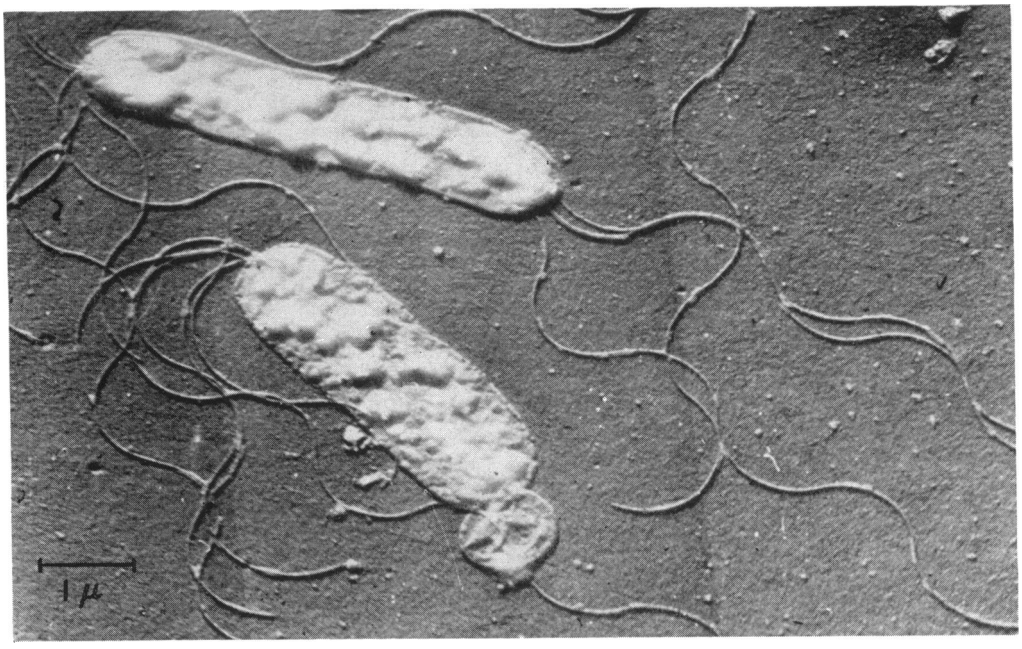

Figure 6. Pseudomonas ovalis strain PO-2, without fixation. Magnification 21,000 ×

obtained at 6 days incubation at 37 C, using 2 g $(NH_4)_2HPO_4$ per liter of medium are shown in table 3.

Proteolysis. Strains of *M. mallei* were slow (three weeks or longer) to liquefy gelatin. These strains were also slow in their reaction on milk (casein); slight acid production in a week or more was followed by slow coagulation. Usually there was no digestion of the clot, though it retracted considerably. *M. pseudomallei* and *P. aeruginosa* were indistinguishable in their rapid gelatin liquefaction, and also in their alkaline

TABLE 3

*Utilization of carbon compounds**

Carbon Source	Malleomyces		Pseudomonas			
	Mallei	Pseudomallei	Aeruginosa	Ovalis	Stutzeri	Fluorescens
Arabinose	−	+	+	v	v	+
Xylose	−	−	+	v	+	+
Glucose	+	+	+	+	+	+
Levulose	v	+	+	−	−	+
Galactose	v	+	+	v	v	+
Lactose	−	v	−	−	−	−
Sucrose	−	−	−	−	−	+
Maltose	−	v	−	−	−	−
Trehalose	−	v	v	−	−	+
Raffinose	−	−	−	−	−	−
Inulin	−	−	−	−	−	−
Glycerol	−	+	+	−	−	+
Mannitol	+	+	v	v	−	+
Dulcitol	−	−	−	−	−	−
Salicin	−	−	−	−	−	−

* Observations made at 6 days.

\+ = Acid production; − = no acid production; v = variable results.

peptonization of milk. Some strains of *P. aeruginosa* developed a slight green pellicle on the surface of the amber peptonized milk. *M. pseudomallei* developed a heavy yellowish sediment in milk with age, but none of the strains used coagulated milk. Similar results were reported by Lajudie and Brygoo (1953) who studied 41 strains of *M. pseudomallei*.

One strain (PA-28) of *P. aeruginosa* was slow to liquefy gelatin. Another, PA-34, did not liquefy gelatin even on prolonged incubation.

In general, the cultures which liquefied gelatin also digested Locke's egg medium and horse serum slants. Exceptions to this were noted in the slow gelatin liquefiers. No digestion of egg albumin or serum was found with any of the cultures of *M. mallei*. Of the strains of *P. stutzeri*, only PS-3 was nonproteolytic on gelatin. The other two cultures in this group, strains PS-1 and PS-2, liquefied gelatin within three weeks and softened egg albumin, but did not digest it. Aging cultures on this latter medium had a pinkish mucoid growth.

The reactions of *P. ovalis* and *P. stutzeri* in milk were alkaline within a few days in contrast to the slow acid production of *M. mallei*.

Hemolysis of red blood cells. *Malleomyces* species were nonhemolytic on rabbit and on sheep blood plates. Strains of *P. aeruginosa*, though isolated from pathological processes, gave varying and nonreproducible degrees of hemolysis within 24 hr in both rabbit and sheep cells. Some were greenish translucent after several days, a result of pyocyanin production.

Utilization of nitrate. All of the Malleomyces cultures produced nitrite from nitrate broth within 24 hr. A few also produced visible gas, but the nitrate was not completely utilized at the end of 5 days except by strain MP-Q. All but 7 of the *Pseudomonas* species gave a positive nitrite test at 24 hr. Four strains remained negative for the duration of the test. These constitute a fairly uniform group with the characteristics described by Haynes (1951) for *P. ovalis*. Three of the pseudomonad cultures evolved gas within a few hours. These strains were positive for nitrites at 4 hr and negative at 24 or 48 hr. Tests for residual nitrate were negative indicating complete utilization of both nitrate and nitrite. These 3 strains appeared to belong to a group similar to *P. stutzeri* (*nitrogens*) described by Van Neil and Allen (1952).

Urease activity tested in two media gave variable results. Generally Christensen's agar was more sensitive than BBL urease test medium. Seventeen of the Pseudomonad cultures were positive on this medium. All strains of *M. mallei* were negative and most of the strains of *M. pseudomallei* were negative. However, results were not always reproducible from day to day with any of these strains.

Hydrogen sulfide was not produced by any of the cultures in Kliger's iron agar.

Indole production, tested by the Kovac method at the end of 24 and 48 hr of incubation, was negative in all cultures.

Utilization of inorganic nitrogen and organic acid salts as a source of carbon. Koser's citrate medium and Simmon's citrate agar supported growth of all strains of *P. aeruginosa* and of all *M. pseudomallei*, except strain MP-E. This latter strain behaved like the *M. mallei* strains in showing faint growth in Koser's medium and a few yellow colonies after one week on Simmon's citrate agar.

The oxidation of gluconate. The first series of tests of oxidation of potassium gluconate was made on 1- and 3-day shaken cultures using the cupric reagent recommended by Haynes (1951) to test for reduction. Using two *P. aeruginosa*

cultures tested by Haynes, PA-32 and PA-35, and three other *P. aeruginosa* types and three strains of *M. pseudomallei*, a series of comparative tests in gluconate broth with static and shaken cultures was made using Benedict's copper sulphate reagent for determining oxidation. Qualitative tests for oxidation of the gluconate were made after 3 and 7 days incubation at 37 C. Results were the same in both static and shaken cultures. The remainder of the tests were made on static cultures only. For those cultures which showed no reduction, incubation was continued and tests were performed on these cultures at two weeks. None of the Malleomyces strains were able to oxidize the gluconate by this test. All but one (PA-22) of the strains of *P. aeruginosa* gave a positive test by this method. Tests on this strain after shaking 3, 7 and 18 days were also negative.

TABLE 4

Significant differentiating characteristics

Characteristics	Malleomyces		Pseudomonas			
	Mallei	Pseudomallei	Aeruginosa	Ovalis	Stutzeri	Fluorescens
Growth temperature (C)*						
42	−	+	+	p	+	−
37	+	+	+	+	+	−
21	−	−	+	+	+	+
10	−	−	−	p	p	+
Plating media*						
CTAB	−	−	+	−	−	p
Azide	−	−	+	−	+	p
SS	−	−	+	+	+	+
Desoxycholate	−	p	+	+	+	+
Brilliant green bile	−	+	+	+	+	+
NaCl concentrations†						
2%	−	+	+	+	+	+
3%	−	p	+	+	+	+
4%	−	−	+	p	+	+
5%	−	−	p	−	+	+
6%	−	−	−	−	+	−
pH 5.6*	+	+	+	+	−	+

* 24 hour observations.

† 48 hour observations.

+ = Growth; − = no growth; p = partial inhibition of growth.

Pigment production. Most of the strains of *P. aeruginosa* produced pigment in all four media employed. Two strains which produced pyocyanin in Burton's and on Gessard's slants at pH 7.6 were without color on Gessard's at pH 6.8. Strains of *P. ovalis* and *P. fluorescin* were fluorescinogenic in Georgia and Poe's medium (1931) and brown in old cultures on Gessard's medium at pH 7.6. In none of these four media, regardless of length of time or temperatures of incubation, was it possible to detect even traces of pigment in *M. mallei* and *M. pseudomallei* other than shades of brown or orange-brown in some strains of *M. pseudomallei*. No fluorescin could be detected in those cultures of *M. pseudomallei* which had exhibited a slightly fluorescent appearance in glycerol agar at pH 6.8. Meader *et al.* (1925) state that fluorescent pigment is oxidized to a stable amber color. If oxidized fluorescin is the source of the brown color in the aging cultures *M. pseudomallei*, it has been impossible to detect it in its earlier stages with the media we employed.

All *Pseudomonas* species except *P. stutzeri* produced some type of green or fluorescent pigment. The three strains of *P. stutzeri* produced only water-insoluble yellow pigments in old cultures.

Incubation temperatures, sodium chloride concentration, acid cultivation and *selective inhibition.* Results of these studies are summarized in table 4.

DISCUSSION

Studies of *M. pseudomallei* and pseudomonad strains using the electron micrograph did not disclose any marked morphological differences. Except for *M. mallei*, which was atrichous, members of both genera show monotrichous and lophotrichous flagellation. Both types of flagellation are presented in the same preparation in some strains (figure 5). Bartholomew (1949) studied 6 species of the genus *Pseudomonas* and found that the numbers of flagella and their attachment varied for individual cells in a single culture. The electron micrographs (figures 2, 3, and 6) suggest evidence of the cytoplasmic origin of flagella, affirming the observations of Houwink and Van Iterson (1950) that flagella are organs of the bacteria and as such may be used in classification of the species.

Reports in the literature for fermentative reactions for *M. mallei* are meager and conflicting. Minett (1930) reports no carbohydrates attacked other than dextrose and this character varies with the strain. Wilson and Miles (1946) report slow acid production from glucose and salicin. *M. pseudomallei* is more active on carbohydrates, but no two workers are in agreement on all sugars attacked (Legroux and Genevray, 1933; Lajudie and Brygoo, 1953).

There are many reports in the literature concerning the reaction of pseudomonads on carbon compounds (Stein, 1942; Bender and Levine, 1942; Elrod and Braun, 1942; Lockwood and Nelson, 1946; Liu, 1952). Sears and Gourley (1928) found differences in carbohydrate metabolism of *P. aeruginosa* by varying the peptone content of the media. Bender and Levine (1942) stressed the need for standardizing both the basal medium and the incubation time. The basal medium employed in our tests gave results with pseudomonads which compare favorably with those of other workers. The results obtained with *M. pseudomallei* in this medium are much more uniform than those of Lajudie and Brygoo (1953) who used a peptone-water base.

Differentiation of *Pseudomonas* species from the genus *Malleomyces* on the basis of chromogenicity is possible only when the typical phenazine pigments characteristic of *Pseudomonas* species can be demonstrated. Smith (1890) noticed the production of an orange pigment when the bacillus of glanders was grown on glycerol agar of slightly acid reaction. The fact that some strains of *M. pseudomallei* produce a slight fluorescence in glycerol agar suggested the possibility that media used to enhance pigment production in *Pseudomonas* species might likewise demonstrate some characters of value for defining more clearly the *M. pseudomallei* species.

Seven of the 28 pseudomonad strains when first studied exhibited either no chromogenicity or a weak fluorescence comparable in degree to that of *M. pseudomallei* in glycerol agar. By use of special media, chromogenicity was enhanced in all these pseudomonad strains, whereas pigments were not produced by any of the 15 strains of *M. pseudomallei*. Pigments may be a valuable means of identifying pseudomonads. However, pigment production has been shown to be inconstant and subject to discontinuous variation (Hadley, 1927, Gaby, 1946). Therefore, the basis of classification proposed by Tobie (1945) for the genus *Pseudomonas*, bacteria which produce water soluble phenazine pigments of any color or water soluble fluorescent pigments, cannot be used with certainty to separate this genus from other similar achromogenic species.

A classification of bacterial species based upon morphological, biochemical, or cultural characteristics must be based upon constantly reproducible criteria.

Gaby and Free (1953) named six characteristics which they considered sufficient to identify *P. aeruginosa* whether pigmented or non-pigmented. These are (1) typical colonies, (2) odor of trimethylamine, (3) motile bacilli, which produce a pellicle, (4) acid from glucose but not from lactose and sucrose, (5) liquefaction of gelatin, and (6) urease (test) negative. Except for the odor of trimethylamine, these characteristics may apply also to strains of *M. pseudomallei*. Ten of the strains of *M. pseudomallei* in this study produced acid from glucose, but not from lactose and sucrose. Most of them were urease negative. Ureolytic pseudomonads have been reported by Ishikawa (1928), White and Hill (1941) and Utzino *et al.* (1938).

Haynes (1951) defined three characteristics for identifying *P. aeruginosa*-growth at 42 C, ability to reduce potassium gluconate in shake cultures, and production of a characteristic slimy sediment in this latter medium in static cultures. *M. pseudomallei* grows at 42 C and also produces a slime when grown in potassium gluconate, but, on the basis of Haynes' characteristics, it differs from *P. aeruginosa* in being unable to reduce potassium gluconate in shake cultures. Nine strains of *Pseudomonas* species, four *P. ovalis*, three *P. stutzeri*, and one *Pseudomonas fluorescens* as well as one *P. aeruginosa* strain PS-22, failed to reduce potassium gluconate. Strain PS-22, which lost its ability to produce pyocyanin and pyorubin while being carried in our stock cultures, was unable on repeated trial to reduce gluconate either in static or shaken cultures and also failed to oxidize a number of the carbohydrates utilized by typical *P. aeruginosa*. In other respects it did not differ from 19 other *P. aeruginosa* strains studied. There is insufficient evidence to show that the ability of *P. aeruginosa* to reduce potassium gluconate

is not subject to the same discontinuous variation as the pigment production of this species.

The experience of our laboratory would indicate the necessity for intensive study of exotic isolates if pseudomonads are not to be erroneously reported as *M. pseudomallei*.

ACKNOWLEDGMENTS

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SUMMARY

A comparative study of the morphological, cultural, and biochemical activities of three strains of *Malleomyces mallei*, 15 strains of *Malleomyces pseudomallei*, 20 strains of *Pseudomonas aeruginosa* and eight other *Pseudomonas* species indicated:

(1) That members of the genus *Malleomyces* can be readily distinguished from *Pseudomonas aeruginosa* by their inhibition of growth on SS agar, desoxycholate agar, sodium azide agar, cetyltrimethyl ammonium bromide agar, and by incubation at 21 C.

(2) That oxidation of carbohydrates, proteolysis, and nitrate reduction are of little value in the differentiation of *M. pseudomallei* from *P. aeruginosa*.

(3) That within the genus *Pseudomonas*, speciation is possible on the basis of reactions on differential plating media, proteolysis, reduction of nitrate, and growth at pH 5.6.

(4) That it was not possible to induce phenazine pigment production in 18 strains of malleomyces using methods which enhanced fluorescin and pyocyanin production in 7 previously nonpigmented strains of pseudomonads.

(5) That the cephalotrichous nature of *M. pseudomallei* as revealed by the electron microscope indicates that it could be included in the family *Pseudomonadaceae*.

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