Pseudomonas maltophilia, an *Alcaligenes*-like Species

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SUMMARY

Pseudomonas maltophilia is frequently encountered in specimens submitted to the clinical laboratory for bacteriological examination. This report describes morphological, physiological and serological attributes of this species. Photomicrographs show the presence of polar multitrichous flagella in stained preparations. These pseudomonads do not produce acid from glucose but readily produce acidity from maltose oxidation. A historical review of the epithet *Alcaligenes bookeri* is presented.

INTRODUCTION

'Bacterium bookeri' NCTC 6572 was isolated in 1943 by J. L. Edwards (Public Health Department, County Bacteriology Laboratory, Stafford, England) from a specimen of pleural fluid and was reported as being non-motile and probably a skin contaminant. It was later found (Hugh, 1953) to have multitrichous flagella. Hugh (1953) recorded the morphological and physiological characteristics of six strains that are now classified as *Pseudomonas maltophilia* including NCTC strain 6572, in a study of *Alcaligenes*. It became apparent that *P. maltophilia* had been unwittingly misidentified as *Alcaligenes faecalis* by Ulrich & Needham (1953); see their strains 249 and 282. An additional 28 strains of *P. maltophilia* have since been isolated from the following sources: river water, well water, raw milk, frozen fish, rabbit and human faeces, blood, pericardial fluid, ascitic fluid, pleural fluid, spinal fluid, oropharyngeal swabs, and contaminated tissue culture. Twenty-six of the above strains have now been restudied and the detailed results are recorded here. An abstract (Hugh & Ryschenkow, 1960) has already been published. The present report serves to amend the previously published description; the name dates from May, 1960 and should be cited as *Pseudomonas maltophilia* Hugh & Ryschenkow, 1960.

METHODS

Morphology. Leifson's (1960) technique was employed to stain flagella.

Physiology. The effects of the bacteria on carbohydrates were determined by the OF (oxidative-fermentative) principle of Hugh & Leifson (1953) and Leifson (1958). The medium used had the following composition: Difco Casitone (pancreatic digest of casein), 5 g.; agar, 3 g.; bromthymol blue (2%, w/v, aqueous solution), 4 ml.; carbohydrate, 10 g.; distilled water, 1000 ml.; final pH 7.1.

Seitz-filtered 10% (w/v) carbohydrate solution was added aseptically to the cool autoclaved (15 min. at 121°) melted basal medium. The medium was then dispensed

aseptically into 13 × 100 mm. sterile tubes to a depth of 50 mm. Ethanol medium was prepared by adding 3 ml. Seitz-filtered absolute ethanol to 97 ml. of the above melted OF basal medium. The ethanol medium was dispensed into sterile 13 × 100 mm. tubes to a depth of 50 mm. and immediately cooled. Ability to grow in an acid medium was studied in yeast extract broth adjusted to pH 4·5 with HCl (Rhodes, 1959) as described by Shimwell, Carr & Rhodes (1960).

Modified Kovacs's reagent (Gadebusch & Gabriel, 1956) was used to detect indole in 1 % (w/v) tryptone broth. Simmons's citrate, Christensen's urea, Møller's (1954, 1955) KCN broth, Kligler's iron agar for hydrogen sulphide detection, Kohn's (1953) charcoal gelatin, Carlquist's (1956) test for lysine decarboxylase activity, phenylalanine deaminase activity (Ewing, Davis & Reaves, 1957), the cytochrome oxidase slope test of Ewing & Johnson (1960), and the 2-ketogluconate test of Moore & Pickett (1960) were used. Physiological tests were incubated at 37°; time of incubation and criteria for the tests being positive are given in the references cited and are described by Ewing (1960).

Serology

Preparation of antigen for production of O antisera. Brain heart infusion broth cultures, incubated at 37° for 24 hr., were placed in flowing steam (100°) for 2 hr. and preserved by adding 0·3 ml. of commercial 40 % (w/v) formaldehyde to 100 ml. of the broth.

Preparation of antigen for production of H antisera. Actively motile organisms, picked from spreading growth in semi-solid agar Gard (1938) plate incubated at room temperature, were inoculated into brain heart infusion broth. After incubation at 22° for 18–24 hr., the broth was diluted with an equal volume of saline containing 0·6 % (v/v) formalin.

Schedule of immunization. Rabbits were given four intravenous injections of H or O antigen at intervals of 4 days. The volume administered was increased each time as follows: 0·5, 1·0, 2·0 and 4·0 ml. The animals were bled on the sixth day after the last injection. Normal serum for control was obtained from each rabbit before immunization. The sera were preserved by the addition of an equal volume of glycerol and stored at 4°.

Preparation of antigens for O and H agglutination. Antigen for O slide-agglutination tests was prepared from an infusion agar slope incubated at 37° for 24 hr. The growth was emulsified in 0·5 ml. saline to form a dense homogeneous suspension. The suspensions were placed in a boiling water bath for 15 min., cooled and tested. Antigen for H tube agglutinations was prepared in the same manner as antigen used for the production of H antisera.

Serological technique. The somatic agglutination of an organism was determined by slide agglutination with a 1/10 dilution of the O antisera. The technique described by Edwards & Ewing (1955) for the somatic agglutination of salmonellas was followed. A twelve tube serial dilution of H antiserum was used to determine flagellar agglutination of the organisms. Tubes were examined for flocculent agglutination after incubation at 45° for 1 hr. followed by 18 hr. at 10°.

RESULTS

Morphology

The organism in peptone broth was a Gram-negative rod about $0.5 \times 1.5 \mu$. Capsule and spore formation was not demonstrable. All the strains were motile and showed a tuft of polar flagella. Plate 1 illustrates the polar multitrichous morphology of *Pseudomonas maltophilia*. The flagella appear to be similar in wavelength and amplitude to the flagella of a typical strain of *P. aeruginosa*.

Physiology

This strictly aerobic organism produced a dense turbidity in peptone broth in 18–24 hr. The strains studied did not produce a distinctive pigment in ordinary media. Smooth glistening growth with an entire margin readily appeared on nutrient agar and blood agar in 24 hr. at 37°. All strains produced grey to white colonies on Leifson's deoxycholate agar. A few strains required 48 hr. to grow out on this medium. Sheep erythrocytes in infusion agar base around well-isolated surface colonies of all 26 strains were not haemolysed. The tube haemolysis test with sheep erythrocytes, as performed in the study of cholera vibrios (Pollitzer, 1959), was negative for all strains. None of the strains grew in peptone broth at pH 4.5.

The following physiological reactions were negative for all 26 strains: acidity from arabinose, glucose, galactose, lactose, mannitol, rhamnose, sucrose, xylose; the indole, methyl red, and Voges-Proskauer tests; urea hydrolysis, nitrate conversion to nitrogen gas, hydrogen sulphide production, phenylalanine deaminase, and 2-ketogluconate production.

The following physiological reactions were positive for all 26 strains: acidity from maltose (acidity was produced in the open tube, but not in the closed tube), motility, gelatin, catalase and lysine decarboxylase. The gelatin test generally became positive before the fourth day. Acidity from maltose generally became evident on the first or second day. Freshly isolated strains of *Pseudomonas maltophilia* inoculated into the basal medium and basal medium containing glucose or other carbohydrates which were not metabolized, produced a strong alkaline reaction at the surface of the medium after 24–48 hr. of incubation. Some old strains which have been kept alive in the laboratory for 8–10 years by periodical transfer in semi-solid medium now produce a very weak acid reaction after prolonged incubation in glucose medium. Some strains produced a positive reaction, while others produced a negative reaction in the tests listed in Table 1.

Antigenic structure

O antigens. Fifteen distinct somatic serotypes were encountered among the 26 strains of *Pseudomonas maltophilia* (Table 2). Organisms of one somatic serotype did not appear to share major antigens with organisms of other serotypes, since cross-reactions between the various O groups were not pronounced. Slow and weak minimal agglutination reactions, which occurred infrequently, were interpreted to be due to minor antigens and were recorded as negative (–) in Table 2. The serum with which the antigen first reacted established the O group to which the organism was assigned. These positive (+) reactions were prompt and strong.

Table 1. *Physiological variability found in 26 Pseudomonas maltophilia strains*

	Number of strains		Time required for the strains to become positive at 37°
	Positive	Negative	
Acidity from fructose (aerobic)	3	23	Positive on the 1st day, after 2nd day positives began to become alkaline
Acidity from mannose (aerobic)	23	3	Generally positive on the 1st or 2nd day
Citrate utilization	6	20	Generally positive on 2nd day
KCN resistance	23	3	Positive on 1st or 2nd day
Nitrate reduction to nitrite	11	15	Test performed after 48 hr. incubation
Cytochrome oxidase slope test	16	10	Test performed after 24 hr. incubation
Acidity from ethanol (aerobic)	1	25	4 days

Living suspensions of *Pseudomonas maltophilia* and suspensions treated with ethanol and heat were also used in the O slide-agglutination test. Of the three different O antigen preparations tested, the boiled suspensions were most suitable. The ethanol treated suspensions and the living suspensions frequently produced a slow and weak agglutination, or no agglutination, with homologous antisera. The information recorded in Table 2 was obtained with boiled suspensions of *P. maltophilia*. Undiluted glycerol-preserved normal control serum did not agglutinate the corresponding organism used for immunization.

Table 2. *O agglutination of Pseudomonas maltophilia with antisera prepared against the corresponding organism*

Antigen	Antiserum														
	560	810-2	294	555	609	601	557	447	558	653-4	788-3	556	109-4	363-4	873-3
560, 430, 559, 661-1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
810-2, 104-2, 245-3	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
294	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
555	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
609, 229, 194-1, 457-1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
601, 611	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
557, 751-4	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
447	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
558, 483-2	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
653-4	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
788-3	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
556	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
109-4	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
363-4	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
873-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

+ = strong agglutination reaction.

- = no agglutination or weak and slow agglutination.

The 15 *Pseudomonas maltophilia* O antisera did not agglutinate O antigens prepared from 2 strains of each of the following 8 organisms: *Aeromonas hydrophila*, *Alcaligenes faecalis* (peritrichous), *Bordetella bronchiseptica*, *Herellia vaginicola*,

Lophomonas alcaligenes, *Pseudomonas aeruginosa*, *Mima polymorpha*, *Pseudomonas diminuta*. The 16 O antigens of the above organisms were prepared in the same manner as were the *P. maltophilia* O antigens.

There was no evidence of an O antigen common to the following three groups of *Pseudomonas maltophilia*: 12 strains of oropharyngeal origin, 5 strains which apparently were the cause of natural infections, and 2 strains of faecal origin.

Two of the O antigen groups were examined by absorption to confirm that the O antigens within each were serologically similar. The homologous somatic antibodies in antiserum produced by immunization with a boiled suspension of strain 560 were removed by absorption with a live suspension of strain 430 or 559 (see Table 3). The homologous somatic antibodies in antiserum produced by immunization with a boiled suspension of strain 810-2 were removed by absorption with a live suspension of strain 104-2 or 245-3 (see Table 4).

Table 3. *Agglutination following absorption of homologous O antibodies to Pseudomonas maltophilia strain 560 with heterologous organisms*

Living suspension (strain number)	Unabsorbed O antiserum to strain 560	O antiserum to strain 560 absorbed with strain	
		430	559
560	+	—	—
430	+	—	—
559	+	—	—
661-1	+	—	—

Table 4. *Agglutination following absorption of homologous O antibodies to Pseudomonas maltophilia strain 810-2 with heterologous organisms*

Living suspension (strain number)	Unabsorbed O antiserum to strain 810-2	O antiserum to strain 810-2 absorbed with strain	
		104-2	245-3
810-2	+	—	—
104-2	+	—	—
245-3	+	—	—

H antigens. The reciprocal of the homologous titres of each of the six H antisera are tabulated in Table 5. All 26 strains of *Pseudomonas maltophilia* were agglutinated by one or more of the six H antisera. The H antiserum prepared with *P. maltophilia* strain 560 agglutinated 23 of the 26 strains (88%). Eighteen of the strains (69%) appear to have a very similar flagellar antigenic structure. There appear to be at least 4 distinct flagellar antigens.

Glycerol-preserved normal serum in dilutions of 1/20, 1/40 and 1/80 did not agglutinate the corresponding organisms used for immunization.

A fine granular agglutination reaction occurred in some of the H agglutination tests in antiserum dilutions of 1/80 or less. This was best seen with a $\times 10$ hand lens. This phenomenon was interpreted to be due to O antibodies in the H antiserum reacting with the cell bodies of the formalinized flagellar antigen. Such reactions are recorded as negative (—) in Table 5.

Table 5. *Flagellar agglutination of Pseudomonas maltophilia strains with antisera prepared against the corresponding organism*

Suspension (strain no.)	Antisera against strains					
	555	560	810-2	104-2	294	557
	Reciprocal of dilutions					
555	20,480	10,240	2,560	—	—	—
560	20,480	10,240	2,560	—	—	—
447, 556*	5,120 to 20,480	2,560 to 10,240	1,280 to 5,120	—	—	—
810-2, 245-3	10,240	5,120	20,480	20,480	—	—
104-2	—	—	10,240	40,960	—	—
294	—	—	—	—	5,120	—
611	20,480	10,240	2,560	—	5,120	—
229	20,480	2,560	—	—	—	—
430	—	10,240	—	—	—	—
557	—	—	—	—	—	10,240

— = less than 80.

* These organisms and the following were agglutinated over the stated range: 558, 559, 601, 609, 109-4, 194-1, 363-4, 457-1, 483-2, 653-4, 661-1, 751-4, 788-3 and 873-3.

DISCUSSION

The 26 strains of *Pseudomonas maltophilia* examined were morphologically and biochemically much alike, but serologically highly diverse. The following characteristics generally serve to identify the species:

Flagellation	Polar multitrichous
Acid from glucose (aerobic)	No acid
Acid from maltose (aerobic)	Acid
Acid from maltose (anaerobic)	No acid
Acid from mannose (aerobic)	Acid
Citrate utilization	Negative
Potassium cyanide resistance	Positive
Nitrate reduced to nitrogen gas	No gas
Charcoal gelatin	Hydrolysed
Lysine decarboxylase (Carlquist)	Positive
2-Ketogluconate production	Negative

Although no haemolysis was seen around well-isolated colonies, blood agar medium around colonies in the heavily inoculated areas showed a greenish discoloration. This discoloration was interpreted to be the result of excessive accumulation of alkaline metabolic by-products.

The oxidase test for this species of *Pseudomonas* is often positive, delayed and generally difficult to interpret. The positive reactions, obtained with some strains of *Pseudomonas maltophilia*, are generally not as intense as those seen with cholera vibrios and *Aeromonas*. The value of the oxidase test as the sole criterion for the recognition of species of *Pseudomonas* has marked limitations since some species do not produce a positive test and *Vibrio comma* and aeromonads produce a positive test. On the basis of these observations it does not appear judicious to incorporate a positive oxidase test as part of the description of the genus *Pseudomonas*.

The physiological variability found among the 26 strains of *Pseudomonas maltophilia* studied (see Table 1) is interpreted to be variation within the concept of species. No attempt was made to designate physiological varieties of the species in view of the limited number of strains studied and the apparent absence of a practical reason for such division.

Serological reactions did not serve the authors as a useful tool to screen unknown organisms in search of *Pseudomonas maltophilia*. cursory studies on a strain of *P. maltophilia* might result in confusion with *Alcaligenes faecalis*, *Bordetella bronchiseptica* or *Lophomonas alcaligenes*, since all are motile and produce an alkaline reaction in glucose peptone broth. *A. faecalis* and *B. bronchiseptica* have peritrichous flagella. *L. alcaligenes* has lophotrichous flagella and does not attack carbohydrate, hence it differs from *P. maltophilia* which has polar multitrichous flagella and oxidizes maltose and mannose. Clinical bacteriologists frequently fail to differentiate the above organisms because the identification is erroneously thought to be based on a single character or at most only a few characters, and often these are not necessarily the most important for recognition of the taxon. Before the identity of an aerobic, asporogenous Gram-negative, glucose-nonfermenting rod should be regarded as established, the complete morphological and physiological reaction pattern should be determined and compared with the complete pattern of the species in the genus. If the reaction patterns have not been suitably worked out the unknown organism must be compared with recognized authentic type or neotype cultures.

The authors recognize that other species of *Pseudomonas* might be closely related *P. maltophilia*. However, it is difficult to state how other species are related or how they may be precisely differentiated at this stage of our understanding. Ninety-four differently named species of *Pseudomonas* deposited in the American Type Culture Collection were studied; not one of the strains possessed the characteristics of *P. maltophilia*.

Pseudomonas maltophilia 810-2 is designed the type strain. Strains 560, 611, 661-1 and 873-3 may also be useful in future comparative studies. These five strains have been placed in various repositories and have the following corresponding accession numbers:

RH number	Canadian National Research Council number	American Type Culture Collection number
560	—	13636
611	727	13843
661-1	728	—
810-2	729	13637
873-3	730	—

The species name *Alcaligenes bookeri* has been inadvertently assigned to strains included in the above described taxon; hence it would appear appropriate to review the history of this name. Booker (1887) isolated an organism from the faeces of children with diarrhoea. He labelled the organism Bacillus A and described it as actively motile; milk coagulated, alkaline and peptonized; gelatine liquefied. No mention was made of the Gram reaction, carbohydrate reaction or flagellation. Booker (1890) stated that his Bacillus A might have been *Proteus vulgaris*. Ford

(1903) proposed the name 'Bacillus bookeri' for an organism he isolated from the intestine of a child and considered to be like Booker's Bacillus A. He described the organism as: rod shaped; no spores, no effect on glucose, lactose or sucrose; aerobic; yellow or yellow-brown growth on agar slope; litmus milk alkaline and reduced; nitrate to nitrite reduction negative; gelatin, casein and coagulated serum liquefied; indole not produced. There is no statement of Gram reaction or flagellar morphology in Ford's report. It does not seem to us that Ford's 'Bacillus bookeri' was adequately enough described to enable one to recognize the species today with certainty. Levine & Soppeland (1926) proposed the name 'Bacterium bookeri'. Weldin (1927) and Kutscher (1937) did not describe the flagellar morphology of their strains of *Alcaligenes bookeri*. Subsequent literature reviews, such as *Bergey's Manual* (1923, 1957) also used the name *A. bookeri*. *A. bookeri* is described as an organism with the general properties of alcaligenes and specifically with peritrichous flagella and ability to liquefy gelatin; however, the source of this description has not been made evident. It is generally agreed that the type species *Alcaligenes faecalis* is a peritrichous organism. It follows that any other flagellated species included in the genus must also be peritrichous. In view of these comments we consider that *A. bookeri* is a species *incertae sedis* and therefore the epithet *bookeri* should not be applied to *Pseudomonas maltophilia*. In addition, *P. maltophilia* is a polar flagellated organism; hence it is not appropriate to place the taxon in the genus *Alcaligenes*. A review of the literature has not as yet been productive in establishing an earlier epithet for the taxon described in this report.

The specific epithet and noun *maltophilia* is derived from the old Anglo-Saxon word *malt* and the Greek work *philia*. It has the literal word meaning 'malt friend' or 'friend of malt'. The etymology of the specific epithet *P. maltophilia* makes it clear that it is a hybrid epithet. This is a subsidiary point and does not affect the acceptance of the name.

The utilization by bacteria of disaccharides without utilization of either of its constituent monosaccharides has been observed repeatedly. Pelczar & Doetsch (1949) described a *Neisseria* sp. which utilized maltose with acid production while glucose was not utilized. The authors can confirm the existence of such an unnamed *Neisseria* sp. in the oropharyngeal region. *Pseudomonas maltophilia* is yet another example of an organism with this type of physiology.

Liu (1961) concluded that identification of pseudomonads is difficult because of the lack of a biochemical reaction pattern for each species and that identification of species is dependent upon unstable pigment production and unstable pathogenicity. The observations presented in this study of *Pseudomonas maltophilia* do not offer support for these conclusions. The following redefinition of the genus *Pseudomonas* has been found useful: Gram-negative straight or curved rods, polar-flagellated when motile; strict aerobes which do not produce acid from glucose under anaerobic conditions in a glucose peptone medium, glucose may be oxidized to acid end products in the presence of oxygen; indole, methyl red and Voges-Proskauer tests negative; pigments when present are usually water soluble.

During the later stages of preparation of this manuscript in 1961 Dr J. Tannenbergh (Genessee Laboratory, Batavia, New York, U.S.A.) informed us that *Pseudomonas maltophilia* strain 1144 was isolated in pure culture at a necropsy from granulomatous lesions in the lung and heart musculature. Bacterial colonies appeared to be present

within small granulomata at the boundary between necrosis and granulomatous cells. Strain 1144 was agglutinated by O antiserum 560. Strains 560, 430, 559 and 661-1 were isolated from spinal fluid, human oviduct, chest fluid and buccal cavity of a normal adult, respectively. Dr J. Tannenberg will amplify these observations and prepare a report later.

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EXPLANATION OF PLATE

- Fig. 1. *Pseudomonas maltophilia* strain 611 showing a rod with a polar tuft of seven short flagella; $\times 2000$.
- Fig. 2. *P. maltophilia* strain 611 showing a rod with a polar tuft of five flagella; $\times 2000$.
- Fig. 3. *P. maltophilia* strain 611 showing a rod with a polar tuft of four flagella; $\times 2000$.
- Fig. 4. *P. maltophilia* strain 611 undergoing binary fission with one of the two daughters showing a polar tuft of three flagella; $\times 2000$.
- Fig. 5. *P. maltophilia* strain 611 showing a rod with a polar tuft of three flagella; $\times 2000$.
- Fig. 6. *P. maltophilia* strain 611 showing a rod with a polar tuft of two flagella; $\times 2000$.
- Fig. 7. *P. maltophilia* strain 611 undergoing binary fission with both daughters showing a polar tuft of two flagella at the distal ends; $\times 2000$.
- Fig. 8. *P. maltophilia* strain 611 showing a rod with a single long polar flagellum; $\times 2000$.