Strains of *Pseudomonas putrefaciens* from clinical material

B. HOLMES, S. P. LAPAGE, AND H. MALNICK

From the Computer Trials Laboratory and National Collection of Type Cultures, Central Public Health Laboratory, Colindale, London

SYNOPSIS Eight strains of *Pseudomonas putrefaciens* have been received from among 466 strains of *Pseudomonas* submitted to the Computer Trials Laboratory for identification over the last eight years. Two of the strains of *P. putrefaciens* from patients with otitis media and otitis externa respectively appear to have played a pathogenic role. The biochemical characteristics of these eight strains were compared with those of seven culture collection strains.

Pseudomonas putrefaciens was first described under the name *Achromobacter putrefaciens* by Derby and Hammer (1931) who isolated the organism from tainted butter. Long and Hammer (1941) examined strains of this taxon which they isolated from dairy produce, streams, lakes, and moist soil and transferred the species to the genus *Pseudomonas*. *P. putrefaciens* is a well known fish-spoilage organism having been isolated from fresh, frozen, and stored cod fillets (Castell, Richards, and Wilmot, 1949) and from haddock (Levin, 1968; Chai, Chen, Rosen, and Levin, 1968). Two strains of *P. putrefaciens* from oil brines were described by Iizuka and Komagata (1964), and, according to Hugh (1970), these strains were two of the four strains of *P. putrefaciens* examined by Iizuka and Komagata (1963a and b) and by Komagata, Iizuka, and Takahashi (1965).

Reports of the isolation of strains of *Pseudomonas putrefaciens* from clinical material have come mainly from the United States of America. King (1964) placed 22 strains of *P. putrefaciens* she examined in one of her unnamed groups designated Ib; two of these strains were isolated from the ear. Thirteen strains of group Ib were isolated and described by von Graevenitz and Simon (1970); five were from ear discharges of patients with chronic otitis media of which two were isolated several times; four were from chronically infected leg ulcers; three from sputa of patients with pneumonia; and one strain from urine. Gilardi (1972) reported seven strains of *P. putrefaciens* isolated between September 1965 and June 1971; these included three from wounds, one from urine, and two isolated from

the ear, of which one was isolated in pure culture and judged to be responsible for otitis media. Riley, Tatum, and Weaver (1972) examined 109 cultures of *P. putrefaciens* from clinical specimens, the sources varying from the mouth and throat to various body wounds; these authors did not state, however, if any of their strains were isolated from the ear. Minagawa (1963) isolated strains belonging to this taxon from the stools of patients with acute enteritis suffering from severe abdominal pain and diarrhoea.

The present account reports two cases, one of otitis media and one of otitis externa, caused by *Pseudomonas putrefaciens*, six additional field isolates of this taxon from clinical material, and the biochemical results obtained from an examination of all these strains and the seven reference strains maintained in the National Collection of Type Cultures, Colindale.

Materials and Methods

ORGANISMS

Fifteen strains of *Pseudomonas putrefaciens* were examined (table I) and comprised eight field strains sent to the Computer Trials Laboratory for identification and seven NCTC strains of which one (NCTC 10695) is a strain of *Pseudomonas rubescens*. Citing a personal communication with Komagata, Hugh (1970) stated that NCTC 10695 (ATCC 12099) was one of the four strains of *P. putrefaciens* described by Iizuka and Komagata (1963b) and also that the latter authors recognized *P. putrefaciens* as an older name for *P. rubescens* (Pivnick, 1954, 1955). On our results NCTC 10695 could not be

Strain No.	Original Strain Designation	Received from	Source
Field Strains			
1	E631	C. E. D. Taylor, London	Sputum
2	E988	M. Magus, Toronto	Nasal swab
3	T111/69	G. I. Barrow, Truro	Oyster
4	CL 27/72	J. D. Abbott, Manchester	Ear swab, otitis media
5	CL 432/72	I. Ivanov, Dar es Salaam	Pleural fluid
6	CL 256/73	I. Ivanov, Dar es Salaam	Cerebrospinal fluid
7	CL 549/73	G. A. Fenwick, Newcastle upon Tyne	Tap swab
8	CL 595/73	T. L. C. Dale, London	Ear swab, otitis externa
Culture Collection Strains			
NCTC ¹ 10695	ATCC ² 12099, <i>Pseudomonas rubescens</i>	National Collection of Industrial Bacteria, NCIB 8768	Cutting oil
NCTC 10735	63	K. Komagata, Tokyo	Oil brine
NCTC 10736	P-19X	K. Komagata, Tokyo	Haddock
NCTC 10737	I-5	K. Komagata, Tokyo	Cuttlefish
NCTC 10738	136-2	K. Komagata, Tokyo	Faeces
NCTC 10762	734	A. von Graevenitz, New Haven	Pus
NCTC 10763	B89	A. von Graevenitz, New Haven ³	Bottled blood

Table I *Strains of Pseudomonas putrefaciens examined*¹NCTC = National Collection of Type Cultures²ATCC = American Type Culture Collection³Strain received by A. von Graevenitz from R. E. Weaver, Atlanta

distinguished from the isolates of *P. putrefaciens* and hence we would similarly consider *P. rubescens* a junior synonym of *P. putrefaciens*.

CLINICAL DETAILS OF THE FIELD STRAINS

Strain 1 was isolated from a 60-year-old woman with a febrile chest infection and bronchospasm. A radiograph of the chest revealed a small opacity in the right mid-zone. The patient responded to tetracycline and antispasmodics. Twelve years previously she was suspected of having tuberculosis and chest radiographs at that time revealed calcified nodules in the left upper zone. The organism is not thought to have played a pathogenic role and was not isolated in pure culture.

Strain 2 was isolated from the nose. Further details are not available.

Strain 3 was isolated from an oyster during an investigation of these shellfish by Barrow and Miller (1969).

Strain 4 was isolated in 1972 from acute otitis media of the right ear of an adult male engineer who in 1966 had undergone submucous resection and bilateral antrostomy. The patient was immediately treated with tetracycline by his own doctor and an ear swab taken during treatment yielded a predominant growth of the organism in question. Sensitivity to tetracycline was not routinely determined for pseudomonads at the laboratory to which the swab was sent. The strain was found sensitive to colistin sulphate by the laboratory and the patient was treated with this antibiotic on their advice. In February 1972 he was referred to the ear, nose and throat specialist who stated that the patient had a

'cart-wheel' drum and a resolving suppurative otitis media. The condition has now resolved.

Strain 5 was isolated from pleural fluid in mixed culture with *Proteus* and considered to be a contaminant.

Strain 6 was isolated in mixed culture with *Klebsiella* from the cerebrospinal fluid of a 1-year-old girl with suspected meningitis and also thought to be only a contaminant.

Strain 7 was isolated from a tap swab taken in a hospital intensive care unit during the detection and elimination of reservoirs of infection in the unit.

Strain 8 was isolated from a recurrent otitis externa associated with an underlying chronic suppurative otitis media in a man of 27. The otitis externa had been treated with neomycin and hydrocortisone (neo-cortef) lotion locally and the otitis media with ampicillin.

BACTERIOLOGICAL STUDIES

The eight field strains were compared with the seven NCTC reference strains (table I) in a wide range of biochemical tests (table II). All tests were recorded at or up to five days except where stated in parentheses in the text below. When possible strains were incubated at their optimum growth temperature. The following tests were carried out mainly by the methods and media described by Cowan and Steel (1965); particular methods where several are given and modifications to the method or medium appear in parentheses: aesculin hydrolysis (agar), acid production from a range of ammonium salt sugars (New Zealand agar 13 g in place of Japanese agar 20 g, bromothymol blue 0.03 g in place of 4 ml of

All Strains Positive in

Catalase production	Growth on MacConkey's agar	Nitrate reduction	Tween 80 hydrolysis
Deoxyribonuclease production	Growth on β -hydroxybutyrate	Oxidase production	
Gelatinase production ¹	Hydrogen-sulphide production	Pigment production	
Growth at room temperature	Motility	Tween 20 hydrolysis	

Table IIa

All Strains Negative in

Acid from PWS ² glucose	Acid from ASS inositol	Acid from ASS xylose	KCN tolerance
Acid from glucose 10 g/100 ml	Acid from ASS lactose	Arginine desiminase	Lysine decarboxylase
Acid from lactose 10 g/100 ml	Acid from ASS mannitol	Arginine dihydrolase	Malonate utilization
Acid from ASS ³ adonitol	Acid from ASS raffinose	Fluorescence on King's B medium	Phenylalanine deamination
Acid from ASS dulcitol	Acid from ASS rhamnose	Gas from PWS glucose	Poly- β -hydroxybutyrate inclusion granules
Acid from ASS ethanol	Acid from ASS salicin	Gluconate oxidation	Starch hydrolysis
Acid from ASS fructose	Acid from ASS sorbitol	Growth on cetrimide	β -Galactosidase production (ONPG)
Acid from ASS glycerol	Acid from ASS trehalose	Indole production	3-Ketolactose production

Table IIb

Strains Differ in	Positive	Negative	Strains Giving the Less Common Result
Acid from ASS glucose	8	7	4, 8 and NCTC 10695, 10736, 10738, 10762, 10763
Acid from ASS arabinose	8	7	4, 8 and NCTC 10735, 10736, 10738, 10762, 10763
Acid from ASS cellobiose	2	13	NCTC 10735, 10737
Acid from ASS maltose	8	7	4, 8 and NCTC 10695, 10736, 10738, 10762, 10763
Acid from ASS sucrose	7	8	1, 2, 3, 6, 7 and NCTC 10735, 10737
Aesculin hydrolysis	1	14	NCTC 10735
Alkali production on Christensen's citrate	4	11	1, 3 and NCTC 10735, 10737
Casein digestion	12	3	NCTC 10736, 10737, 10763
Growth on NaCl 6 g/100 ml	5	10	4, 8 and NCTC 10738, 10762, 10763
Growth on NaCl 7 g/100 ml	5	10	4, 8 and NCTC 10738, 10762, 10763
Growth on Salmonella-Shigella (S.S.) agar	7	8	4, 5, 8 and NCTC 10695, 10738, 10762, 10763
Growth at 5°C	5	10	2, 3 and NCTC 10735, 10736, 10737
Growth at 37°C	12	3	NCTC 10735, 10736, 10737
Growth at 42°C	8	7	1, 2, 3 and NCTC 10695, 10735, 10736, 10737
Growth on Simmons' citrate	3	12	3 and NCTC 10735, 10737
Nitrite reduction	8	7	2, 3, 5, 6, 7 and NCTC 10738, 10762
Opalescence on LV agar	13	2	NCTC 10736, 10737
Ornithine decarboxylase	13	2	1, 3
Pigment production on tyrosine	8	7	1, 2, 8 and NCTC 10735, 10736, 10762, 10763
Tyrosine hydrolysis	8	7	2, 8 and NCTC 10735, 10736, 10737, 10762, 10763
Urease production	3	12	1 and NCTC 10695, 10763
	<i>Alkaline</i>	<i>Negative</i>	
Hugh and Leifson O-F test	13	2	2 and NCTC 10735

Table IIc

Table IIa, b, c Biochemical characters of *Pseudomonas putrefaciens*

¹One strain negative in nutrient gelatin stab but positive by plate method

²PWS = peptone water sugar

³ASS = ammonium salt sugar

a 0.2 g/100 ml solution of bromocresol purple), acid and gas production from glucose peptone water sugar (Andrade's indicator, final concentration of carbohydrate 0.75 g/100 ml), catalase production (method 1, one day), alkali production on Christensen's citrate (ferric ammonium citrate and Na₂S₂O₃ omitted, New Zealand agar 11 g in place of Japanese agar 20 g), dihydrolase for arginine and decarboxylases for lysine and ornithine (method 1), fluorescence on medium B of King, Ward, and Raney (New Zealand agar 15 g in place of Japanese agar

20 g), gelatinase production (methods 1 and 3), gluconate oxidation (method 2, two days), acid production from glucose 10 g/100 ml and lactose 10 g/100 ml (New Zealand agar 10 g in place of Japanese agar 20 g), reaction in O-F medium of Hugh and Leifson (6 ml of a 1 g/100 ml aqueous solution of bromothymol blue instead of 15 ml of a 0.2 g/100 ml aqueous solution), hydrogen-sulphide production (methods 1-Oxid, and 3), indole production (method 2, two days), KCN tolerance (two days), opalescence on lecithovitellin (LV) agar,

growth on MacConkey's agar, malonate utilization (two days), motility (method 1, one day), β -galactosidase production (ONPG, two days), oxidase production (one day), phenylalanine deamination (method 1, two days), starch hydrolysis (method 1), tyrosine hydrolysis (New Zealand agar 11 g in place of Japanese agar 20 g, production of brown melanin-like pigment also recorded), and urease production (method 1). Tests for nitrate and nitrite reduction were carried out as described by Cowan and Steel (1965) except that the reagents used were those of Crosby (1967) in which the non-carcinogen, Cleve's acid, is used in place of α -naphthylamine.

The following tests were carried out by the method given in the reference in parentheses which also enclose any modifications: presence of arginine desiminase (Thornley, 1960), casein digestion (prepared from Oxoid skim milk powder 50 g, New Zealand agar 25 g, and distilled water 1500 ml), growth on cetrimide agar (Brown and Lowbury, 1965), production of extracellular deoxyribonuclease (Oxoid DNase agar but with modification of Schreier, 1969), production of 3-ketolactose from lactose (Bernaerts and de Ley, 1963, two days), reduction of selenite, 0.4 g/100 ml (Lapage and Bascomb, 1968; Na_2HPO_4 anhydrous 9.5 g in place of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 4.3 g, $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ 0.565 g instead of 2.8 g, two days) and hydrolysis of tween 20 and tween 80 (Sierra, 1957). Growth with

β -hydroxybutyric acid 0.5 g/100 ml as a sole carbon source was tested on the basal medium of Owens and Keddie (1968) and if growth was present after two days' incubation the bacteria were stained with Sudan Black B to test for the presence of poly- β -hydroxybutyrate inclusion granules.

Growth on Simmons' citrate medium (Oxoid) was confirmed by serial subculture. Growth at 5°C, room temperature, 37° and 42°C, was recorded from ordinary nutrient broth, and pigment production on ordinary nutrient agar. Salt tolerance was tested on both 6 and 7 g/100 ml of NaCl incorporated in nutrient agar. Ability to grow on Salmonella-Shigella agar was tested on Oxoid SS agar CM99.

Sensitivity to the following antimicrobial agents was also determined, by the disc method, on Oxoid Diagnostic Sensitivity Test agar to which lysed horse blood (final concentration 0.25%) is added in our media laboratory (the concentrations are given in table III): optochin (Oxoid DD1); bacitracin (Oxoid DD2); erythromycin, chloramphenicol, tetracycline, penicillin G, oleandomycin, novobiocin, sulphafurazole (Oxoid multodisk 11-14C); streptomycin, colistin methane sulphonate, ampicillin, nalidixic acid, nitrofurantoin (Oxoid Multodisk U1); carbenicillin, gentamicin (Mast); neomycin sulphate (Mycifradin, Upjohn Co); clindamycin hydrochloride (Dalacin, Upjohn Co); lincomycin hydrochloride (Lincocin, Upjohn Co), and vibrio-

All Strains Sensitive to

Chloramphenicol (10 µg)	Gentamicin (10 µg)	Neomycin sulphate (30 µg)	Tetracycline (10 µg)
Erythromycin (10 µg)	Nalidixic Acid (30 µg)	Nitrofurantoin (200 µg)	

Table IIIa

All Strains Resistant to

Bacitracin ¹	Novobiocin (5 µg)	Penicillin G (1.5 units)	0/129 ^a
Lincomycin hydrochloride (2 µg)	Optochin ^a	Sulphafurazole (100 µg)	

Table IIIb

Strains Differ in	Sensitive	Resistant	Strains Giving the Less Common Result
Ampicillin (25 µg)	5	10	2, 7 and NCTC 10735, 10736, 10737
Carbenicillin (25 µg)	13	2 ^a	6 and NCTC 10738
Clindamycin hydrochloride (2 µg)	14	1	NCTC 10738
Colistin methane sulphonate (200 µg)	10	5	3, 8 and NCTC 10735, 10737, 10763
Oleandomycin (5 µg)	14	1	6
Streptomycin (25 µg)	13	2	5, 6

Table IIIc

Table IIIa, b, c Sensitivity of *Pseudomonas putrefaciens* to antimicrobial agents

¹Discs prepared from solution containing 1.8 units/ml

^aDiscs prepared from solution containing 0.45 mg/ml

^bDiscs prepared from solution containing 1 mg/ml

^cThese strains also resistant at 100 µg

static agent 0/129 (solution prepared using BDH 2, 4-diamino-6, 7-di-*iso*-propyl pteridine phosphate).

Results

The results are given in table II and arranged by tests in which all strains gave a positive or negative result and tests in which the strains differed. The strain number of those strains giving the less common result in each of the tests in which the strains gave different results is given so that the results for each individual strain can be reconstructed.

The organisms were motile Gram-negative rods with colonies on nutrient agar that produced a highly characteristic salmon-pink to reddish-brown pigment. Three of the four NCTC reference strains isolated from non-clinical material grew aerobically at 5°C and at room temperature but failed to do so at 37° and 42°C; two of these three were fishery isolates. Most of the remaining strains, from clinical material, failed to grow at 5°C but grew at room temperature, 37°, and 42°C. Although the strains could be divided into two groups on their growth temperature range and these showed good correlation with their source of isolation each group contained both saccharolytic and non-saccharolytic strains tested. Saccharolytic strains typically produced acid from glucose, arabinose, maltose, and sucrose ammonium salt sugars although two strains were able to produce acid from cellobiose and NCTC 10695 (*P. rubescens*) produced acid from arabinose only (table II).

Discussion

The eight *Pseudomonas putrefaciens* field strains examined in this study agree well in their characteristics with those of the seven strains deposited in the National Collection of Type Cultures (tables II and III) and with those given by Iizuka and Komagata (1964), Levin (1968, 1972), von Graevenitz and Simon (1970), Hugh (1970), Gilardi (1972, 1973), and Riley *et al* (1972). However, Iizuka and Komagata (1964) could not demonstrate cytochrome oxidase in their two strains but NCTC 10735, one of their strains, was found to produce oxidase in tests in this laboratory. Occasional strains of *P. putrefaciens* fail to decarboxylate ornithine (this series, table II; Levin, 1972) but von Graevenitz and Simon (1970) found all their 13 strains unable to decarboxylate ornithine by Falkow's (1958) method which is possibly unsuitable for pseudomonads since the test was devised for the separation of strains of *Citrobacter*, *Escherichia*, *Salmonella*, and *Shigella*. Further, NCTC 10762 is assumed to be one of the

13 strains described by von Graevenitz and Simon (1970) and was able to decarboxylate ornithine when tested by Møller's (1955) method. Gilardi (1972, 1973) found that none of his strains produced opalescence on LV agar, whilst 13 of our strains did so, and seven of 13 of his strains produced acid from fructose whilst none of our strains did so.

Strains of *Pseudomonas putrefaciens* are easy to identify since they have a distinct salmon pink to reddish-brown pigment, are the only commonly isolated non-fermentative organisms able to produce H₂S on triple sugar iron agar and, apart from occasional strains of *Pseudomonas cepacia*, usually to decarboxylate ornithine.

The sensitivity of our strains to antimicrobial agents (table III) agrees well with those given by Gilardi (1972) and von Graevenitz and Simon (1970). Two strains were resistant to streptomycin and came from different areas in Tanzania, one of which was the only strain in the present series resistant to oleandomycin and also one of the two strains resistant to carbenicillin.

Levin (1972) divided the 54 strains of *Pseudomonas putrefaciens* (including *P. rubescens* ATCC 19857) that he examined into two groups which he suggested had possibly evolved by mutation and divergent evolution with the temperature of the habitat acting as a selective mechanism. He distinguished the two groups (table IV) on the ability of the strains to grow in added NaCl at a concentration of 6 g/100 ml and on the guanine plus cytosine (G-C) content of their deoxyribonucleic acid (DNA). The first group comprised 37 isolates unable to grow in added NaCl and with a G-C content of their DNA within a range of 47.8 to 50.8%. This group included all isolates from fishery sources of which none grew at 35°C and other isolates from diverse sources capable of growth at 35°C. The second group, comprising 16 strains able to grow in added NaCl and with a G-C content of their DNA within a range of 55.9 to 59%, were mostly of human clinical origin and all isolates in the group grew at 35°C.

Riley *et al* (1972) were also able to divide into two groups, on the basis of salt tolerance, the 109 cultures of *P. putrefaciens* they examined (table IV). Strains of their first group were unable to grow in added NaCl at a concentration of 7 g/100 ml and in addition failed to grow on Salmonella-Shigella agar and produced acid from arabinose, dextrin, maltose, and sucrose in a modified oxidative-fermentative medium. Strains of their second group in addition to growing in added NaCl were able to grow on Salmonella-Shigella agar and failed to produce acid from carbohydrates except for 'variable' (*sic*) results with glucose and fructose. Strains of the

Character	Levin (1972)		Riley, Tatum, and Weaver (1972)		Present Study	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Growth NaCl 6 or 7 g/100 ml	—	+	—	+	—	+
G-C content	47.8-50.8 %	55.9-59 %	NT	NT	NT	NT
Growth SS agar	NT	NT	—	+	—	+
Saccharolytic activity	NT	NT	+	—	+	—
Growth at 35 or 37°C	d	+	+	+	d	+
Source	Mainly fishery	Mainly human clinical	Mainly human	clinical	Fishery and clinical	Human clinical

Table IV Separation of *Pseudomonas putrefaciens* isolates into two groups by various authors

+ = Majority of strains give a positive result
 — = Majority of strains give a negative result
 d = Some strains, generally fishery isolates, give a negative result
 NT = not tested

first group also gave 'variable' results with glucose and fructose. The results of our carbohydrate utilization tests agree well with those of Riley *et al* (1972) except that those strains in our series able to produce acid from arabinose, maltose, and sucrose also produced acid from glucose, two strains produced acid from cellobiose, and none produced acid from fructose. The majority of strains described by Riley *et al* (1972) grew at 35°C (possibly few or no fishery isolates were included in their series). These authors gave only the range of sources of all their isolates together and not for each group.

Our strains could also be divided into two groups like those of Riley *et al* (1972) on the basis of salt tolerance, growth on Salmonella-Shigella agar and saccharolytic activity (table IV). The first group comprised 10 strains unable to grow in added NaCl at a concentration of either 6 or 7 g/100 ml of which eight were also unable to grow on Salmonella-Shigella agar and nine were saccharolytic. Most isolates in the group, including some strains from clinical material, grew at 35°C but three failed to do so and two of these three were fishery isolates. The second group comprised five strains able to grow in added NaCl all of which also grew on Salmonella-Shigella agar and failed to produce acid from carbohydrates. All isolates in this group grew at 37°C and all were from human clinical sources. The 13 strains of *P. putrefaciens* described by Gilardi (1973) all tolerated NaCl at a concentration of 6.5 g/100 ml and grew on Salmonella-Shigella agar and most were non-saccharolytic; the isolates were mainly of clinical origin.

The unexpected finding of Levin (1972) that all his isolates from fishery sources were unable to grow in added NaCl was confirmed with the strains in our series. All our three fishery isolates (from oyster, haddock, and cuttlefish (table I) failed to grow in added NaCl and the five strains able to grow in added NaCl comprised the two isolates from ear infections and three others isolated from faeces,

pus, and bottled blood respectively. This may explain why Riley *et al* (1972) were unable to find a difference in geographical distribution of the two groups they found. These authors thought that because of the difference in salt tolerance between their two groups it would be reasonable to expect that isolates able to grow in added NaCl would be isolated from sources located near coastal areas.

Since two of the reference strains included in the present study were isolated from cutting oil and oil brine respectively (table I) and one of the field strains was isolated from the ear of an engineer (strain 4) it is possible that ear wax contains a substance also present in oil which promotes the growth of *P. putrefaciens* and this may explain why strains of this taxon appear to play a pathogenic role mainly in cases of otitis media.

We are grateful to the Department of Health and Social Security whose grant for the identification of bacteria using a computer enabled this work to be carried out. Our thanks are also due to all those who sent us strains of *Pseudomonas putrefaciens* for identification especially to Dr J. D. Abbott of Manchester Public Health Laboratory who kindly provided us with clinical details on one of the two cases of ear infection, and to Dr T. L. C. Dale of Beecham Products (UK) for information on the second case.

References

- Barrow, G. I., and Miller, D. C. (1969). Marine bacteria in oysters purified for human consumption. *Lancet*, 2, 421-423.
- Bernaerts, M. J., and Ley, J. de (1963). A biochemical test for crown gall bacteria. *Nature (Lond.)*, 197, 406-407.
- Brown, V. I., and Lowbury, E. J. L. (1965). Use of an improved cetrimide agar medium and other culture methods for *Pseudomonas aeruginosa*. *J. c. in. Path.*, 18, 752-756.
- Castell, C. H., Richards, J. F., and Wilmot, I. (1949). *Pseudomonas putrefaciens* from cod filets. *J. Fish. Res. Bd. Canad.*, 7, 430-431.
- Chai, T., Chen, C., Rosen, A., and Levin, R. E. (1968). Detection and incidence of specific species of spoilage bacteria on fish. II. Relative incidence of *Pseudomonas putrefaciens* and fluorescent pseudomonads on haddock filets. *Appl. Microbiol.*, 16, 1738-1741.

- Cowan, S. T., and Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. University Press, Cambridge.
- Crosby, N. T. (1967). The determination of nitrite in water using Cleve's acid, 1-naphthylamine-7-sulphonic acid. *Proc. Soc. Wat. Treat. Exam.*, 16, 51-55.
- Derby, H. A., and Hammer, B. W. (1931). Bacteriology of butter. IV. Bacteriological studies on surface taint butter. *Bull. Iowa St. agric. exp. Sta.*, 145, 385-416.
- Falkow, S. (1958). Activity of lysine decarboxylase as an aid in the identification of *Salmonellae* and *Shigellae*. *Amer. J. clin. Path.*, 29, 598-600.
- Gilardi, G. L. (1972). Infrequently encountered *Pseudomonas* species causing infection in humans. *Ann. intern. Med.*, 77, 211-215.
- Gilardi, G. L. (1973). Nonfermentative Gram-negative bacteria encountered in clinical specimens. *Antonie v. Leeuwenhoek*, 39, 229-242.
- von Graevenitz, A., and Simon, G. (1970). Potentially pathogenic, nonfermentative, H₂S-producing Gram-negative rod (Ib). *Appl. Microbiol.*, 19, 176.
- Hugh, R. (1970). A practical approach to the identification of certain nonfermentative Gram-negative rods encountered in clinical specimens. *Publ. Hlth Lab.*, 28, 168-187.
- Iizuka, H., and Komagata, K. (1963a). An attempt at grouping of the genus *Pseudomonas*. *J. gen. appl. Microbiol.* (Tokyo), 9, 73-82.
- Iizuka, H., and Komagata, K. (1963b). Taxonomy of genus *Pseudomonas* with special reference to their modes of metabolism of carbon compounds. *J. gen. appl. Microbiol.* (Tokyo), 9, 83-95.
- Iizuka, H., and Komagata, K. (1964). Microbiological studies on petroleum and natural gas. II. Determination of pseudomonads isolated from oil-brines and related materials. *J. gen. appl. Microbiol.* (Tokyo), 10, 223-231.
- King, E. O. (1964). *The Identification of Unusual Pathogenic Gram-Negative Bacteria*. National Communicable Disease Center, Atlanta, Georgia.
- Komagata, K., Iizuka, H., and Takahashi, M. (1965). Taxonomic evaluation of nitrate respiration and carbohydrate fermentation in aerobic bacteria. *J. gen. appl. Microbiol.* (Tokyo), 11, 191-201.
- Lapage, S. P., and Bascomb, S. (1968). Use of selenite reduction in bacterial classification. *J. appl. Bact.*, 31, 568-580.
- Levin, R. E. (1968). Detection and incidence of specific species of spoilage bacteria on fish. I. Methodology. *Appl. Microbiol.*, 16, 1734-1737.
- Levin, R. E. (1972). Correlation of DNA base composition and metabolism of *Pseudomonas putrefaciens* isolates from food, human clinical specimens, and other sources. *Antonie v. Leeuwenhoek*, 38, 121-127.
- Long, H. F., and Hammer, B. W. (1941). Distribution of *Pseudomonas putrefaciens*. *J. Bact.*, 41, 100-101.
- Minagawa, M. (1963). Studies on the strains closely related to *Vibrio parahaemolyticus* and reddish brown pigment-producing *Pseudomonas* isolated from the stools of patients of acute enteritis. *A. R. Inst. Food Microbiol.* (Chiba Univ.), 16, 9-23.
- Møller, V. (1955). Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. *Acta. path. microbiol. scand.*, 36, 158-172.
- Owens, J. D., and Keddie, R. M. (1968). A note on the vitamin requirements of some coryneform bacteria from soil and herbage. *J. appl. Bact.*, 31, 344-348.
- Pivnick, H. (1954). *Pseudomonas rubescens*, a new species isolated from soluble oil emulsions. *Bact. Proc.*, 42-43.
- Pivnick, H. (1955). *Pseudomonas rubescens*, a new species from soluble oil emulsions. *J. Bact.*, 70, 1-6.
- Riley, P. S., Tatum, H. W., and Weaver, R. E. (1972). *Pseudomonas putrefaciens* isolates from clinical specimens. *Appl. Microbiol.*, 24, 798-800.
- Schreier, J. B. (1969). Modification of deoxyribonuclease test medium for rapid identification of *Serratia marcescens*. *Amer. J. clin. Path.*, 51, 711-716.
- Sierra, G. (1957). A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. *Antonie v. Leeuwenhoek*, 23, 15-22.
- Thornley, M. J. (1960). The differentiation of *Pseudomonas* from other Gram-negative bacteria on the basis of arginine metabolism. *J. appl. Bact.*, 23, 37-52.