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# Identification and characterization of *Moraxella phenylpyruvica*

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*From the National Collection of Type Cultures, Central Public Health Laboratory, London*

SYNOPSIS Eight strains of *Moraxella phenylpyruvica* have been isolated from clinical material in the United Kingdom, the first to be reported from this country. They were characterized, together with three strains of *M. phenylpyruvica* of the National Collection of Type Cultures (NCTC), and compared with NCTC strains of eight other *Moraxella* species. The strains of *M. phenylpyruvica* formed a homogeneous group which is readily distinguishable from other *Moraxella* species. Deamination of phenylalanine is not restricted to *M. phenylpyruvica* which, however, is urease positive and is stimulated by bile, in contrast to other *Moraxella* spp.

Flamm (1957) described a strain of *Moraxella* which he named *M. polymorpha*, isolated from the cerebrospinal fluid of a child with meningitis. Bøvre and Henriksen (1967) examined this strain and found it to agree in a number of characters with eight other *Moraxella* strains from such clinical sources as the urogenital tract, blood cultures, and pus. They considered that Flamm's strain belonged to the same species as the other eight strains and suggested that the epithet 'polymorpha' in *Moraxella polymorpha* was illegitimate and would cause confusion with De Bord's (1939, 1942) *Mima polymorpha*. The strains were found to deaminate phenylalanine, a property which was thought to be unusual in the genus *Moraxella*, and Bøvre and Henriksen proposed renaming this species *M. phenylpyruvica* (see also Judicial Commission of the International Committee on Systemic Bacteriology, 1971).

Eight strains of *M. phenylpyruvica* isolated in the UK from clinical material (urogenital tract, pleural fluid, nasal swabs, and various lesions) have been examined in the National Collection of Type Cultures (NCTC), together with the three NCTC strains of this species. During a comparison of these strains with eight other *Moraxella* species, it was found that the ability to deaminate phenylalanine is not limited to *M. phenylpyruvica* (Snell and Davey, 1971). However, *M. phenylpyruvica* can be distinguished from other *Moraxella* species by several other characters as well as deamination of phenylalanine. The purpose of the present work is to record the isolation of this little-known organism in the UK, and to record a number of hitherto undescribed characteristics which help to distinguish it from other *Moraxella* species.

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## Materials and Methods

### ORGANISMS

Eleven strains of *M. phenylpyruvica* were examined (Table I): eight field strains sent to the NCTC for

| *Strain No.* | *Original Strain Designation* | *Received from* | *Source* |
|---|---|---|---|
| 1 | A152/68 | M. P. Jevons, London | Vulva |
| 2 | A12/70 | R. F. Alexander, London | Urine |
| 3 | A30/70 | E. G. Gordon, Worcestershire | Pleural fluid |
| 4 | A58/70 | E. G. Gordon, Worcestershire | Vagina |
| 5 | A80/70 | J. C. Burne, Dartford | Nasal swab |
| 6 | A81/70 | K. B. Rogers, Birmingham | Pressure sore |
| 7 | A84/70 | W. E. Rimington, Doncaster | Bartholin cyst |
| 8 | A100/71 | I. R. Poulter, Warwick | Leg ulcer |
| NCTC 10526 | 2863 | American Type Culture Collection, ATCC 23333 | Blood |
| NCTC 10750 | 5542 | American Type Culture Collection, ATCC 23334 | Blood |
| NCTC 10752 | — | American Type Culture Collection, ATCC 17958 | Cerebrospinal fluid |

Table I *Strains of* Moraxella phenylpyruvica *examined in the present work*

identification; three NCTC strains including the neotype of *M. phenylpyruvica* (NCTC 10526) and Flamm's strain of *M. polymorpha* (NCTC 10752). Strains of eight other *Moraxella* species were examined for comparison and are listed in Table II.

| *Name* | *Number* |
|---|---|
| *M. bovis* | NCTC 8561, 9425, 9426, A52/69[1] |
| *M. kingii* | NCTC 10526, 10746 |
| *M. lacunata* | NCTC 7985, 10747, 10748 |
| *M. liquefaciens* | NCTC 7911, 10358, 10359 |
| *M. nonliquefaciens* | NCTC 7784, 10464, A33/67, A9/69 |
| *M. osloensis* | NCTC 10465, 10749, 10755, 10756 |
| *M. saccharolytica*[2] | NCTC 10753 |
| *M.* sp.[3] | NCTC 10717 |

Table II *Strains of other* Moraxella *spp. examined in the present work*

[1]Strains with designations prefixed by A are field strains identified in the NCTC.
[2]Described by Flamm (1956).
[3]Described by van Bijsterveld (1970, 1971).

## CLINICAL DETAILS OF THE FIELD STRAINS

*Strain 1*
A 4-year-old girl, admitted to hospital for investigation of enuresis, had a mild vulvo-vaginitis. The vaginitis cleared spontaneously.

*Strain 2*
A primigravida with preeclamptic toxaemia was found to have excess albumin in the urine. The strain was isolated on one occasion only and a normal delivery took place two weeks later.

*Strain 3*
This strain was isolated from pleural fluid of a 67-year-old man, admitted to hospital with diffuse pulmonary fibrosis.

*Strain 4*
This strain was present in large numbers in a vaginal discharge. The normal flora was very scanty.

*Strain 5*
A 14-month-old girl with gastroenteritis yielded this strain from a nasal swab. The gastroenteritis cleared up rapidly and spontaneously within two days.

*Strain 6*
An 11-year-old boy, born with a myelocoele and spina bifida, had both faecal and urinary incontinence. A pressure sore developed in which this strain was present in large numbers together with *Proteus* sp. and non-haemolytic and viridans type streptococci.

*Strain 7*
A 26-year-old woman had for three years a Bartholin cyst which became infected and from which this strain was isolated. After excision of the cyst, she fully recovered.

*Strain 8*
A man with a severe ankle injury developed a leg ulcer after seven weeks in plaster. The strain was isolated from the ulcer on two occasions before and during treatment with first fucidin, then ampicillin and finally ultraviolet light.

## BACTERIOLOGICAL INVESTIGATIONS

The media and methods used were mainly those described in Cowan and Steel (1965). The media were supplemented where indicated with horse blood or serum. The microscopic appearance was examined after 24 hours' growth at 37°C on 5% (v/v) blood nutrient agar. Tests for motility were carried out by the hanging drop method from overnight cultures in 10% (v/v) serum nutrient broth. The colonial appearance was observed after 24 and 48 hours' growth at 37°C on 5% (v/v) blood nutrient agar. Growth at 5°C, 22°C, and 42°C was tested for on blood agar and anaerobic growth on blood agar at 37°C in an atmosphere of 90% (v/v) hydrogen and 10% (v/v) carbon dioxide.

The strains were tested for growth on nutrient agar, on MacConkey agar, on O/F medium (Hugh and Leifson, 1953), solidified with 1% agar, and in Koser's and Christensen's citrate media. Growth with 0·5% (w/v) $\beta$-hydroxybutyric acid as a sole carbon source was detected on the basal medium of Owens and Keddie (1968). If growth was obtained, the bacteria were stained with Sudan Black B to test for the presence of poly-$\beta$-hydroxybutyrate inclusion granules. Those strains failing to grow on this medium were grown on 10% (v/v) serum nutrient agar containing $\beta$-hydroxybutyric acid and then stained as above. The effect of the following substances or additives on growth was recorded: 10% (v/v) serum in nutrient agar; brilliant green incorporated in blood agar at a concentration of 1 in 500,000 (w/v); ox bile in concentrations of 10% (w/v) and 40% (w/v) and sodium taurocholate in concentrations of 0·5% (w/v) and 2% (w/v) incorporated as ditches in blood agar plates. The strains of *M. phenylpyruvica* were also tested for stimulation of growth by stearate, oleate, Tween 80, butyrate, propionate, valerate, acetate, pelargonate, and lecithin, each at a concentration of 0·1% (w/v) in blood agar.

Minimum inhibitory concentrations (MICs) of penicillin were determined by subculture on blood agar containing the antibiotic in a range of concen-

trations between 0·0001 and 100 IU/ml. The Oxford strain of *Staphylococcus aureus* (NCTC 6571) was used as a control and was inhibited at a concentration of 0·05 IU penicillin/ml. Tests for the production of penicillinase were made by the method of Parker and Lapage (1957), using two strains of *Staphylococcus aureus* as controls (NCTC 4136, positive control and NCTC 10443, negative control).

Acid production from glucose was recorded in the O/F medium of Hugh and Leifson (1953), both with and without the addition of 10% (v/v) serum, and also on 10% (v/v) serum nutrient agar slopes containing 1% (w/v) glucose and 0·01% (w/v) phenol red. Catalase production was tested on cultures grown on 10% (v/v) serum nutrient agar, and production of oxidase was detected by Kovacs' (1956) method using tetramethyl-*p*-phenylenediamine on cultures grown on blood agar.

The following characters were determined in media both with and without 10% (v/v) serum: nitrate reduction, urease activity (Christensen's method, 1946), opalescence on egg yolk agar, phosphatase production (phenolphthalein phosphate incorporated in nutrient agar), indole production (Kovacs' and Erhlich's reagents), $\beta$-galactosidase (ONPG), and arginine desimidase (Thornley's medium, 1960). Proteolytic activity was demonstrated by the following tests: liquefaction of gelatin in nutrient gelatin stabs both with and without 10% (v/v) serum, and on nutrient agar gelatin plates; serum liquefaction on Loeffler's serum slopes; casein digestion on milk agar plates and in purple milk broth. Decarboxylation of arginine, lysine, and ornithine was tested using Møller's medium (1955) and starch hydrolysis using nutrient agar starch plates. Deamination of phenylalanine was tested for by the method of Shaw and Clarke (1955) and also by agitating a heavy suspension of bacteria in 0·4% DL-phenylalanine at 37°C for one hour followed by the addition of 10% (w/v) ferric chloride (Snell and Davey, 1971).

### SELECTIVE MEDIUM FOR *M. phenylpyruvica*

For the attempted isolation of *M. phenylpyruvica* from faeces, a selective medium was devised, containing the following constituents expressed as percentages (w/v) of nutrient agar base: potassium tellurite 0·001% and sodium taurocholate 1% as selective agents; glucose 1%, neutral red 0·01%.

### DNA BASE COMPOSITIONS

Deoxyribonucleic acid was extracted by the method of Marmur (1961) and base compositions were estimated by the 'melting temperature' (Tm) method of Marmur and Doty (1962). The Tm determinations were made in either saline-phosphate buffer (SP: 0·1 M NaCl + 0·01 M phosphate, *p*H 7·0) or standard saline-citrate buffer (SSC: 0·15 M NaCl + 0·015 M sodium citrate, *p*H 7·0). The equations relating Tm to the percentage of guanine + cytosine/total bases (% GC) for these two buffers were: for SP buffer: % GC = 2·352 Tm-153·49 (Owen, Hill, and Lapage, 1969), and for SSC buffer: % GC = 2·44 Tm-169·0 (Marmur and Doty, 1962).

## Results

Microscopically, the 11 strains of *M. phenylpyruvica* consisted of non-motile ovals and rods, measuring 0·7$\mu$ × 1·5-2·0$\mu$, which were Gram negative, although all strains showed some cells which retained the crystal violet dye as intracellular granules. After overnight incubation, the strains formed convex, translucent colonies on blood agar, about 0·5 mm in diameter, which enlarged to 1·0 mm diameter after 48 hours. The colonies were butyrous in consistency and were easily emulsified in saline. No haemolysis was observed on 5% blood agar but a green discolouration of the blood was noticed around the mass growth of cultures incubated anaerobically. The strains grew at 22°C, 30°C, and 37°C after 24 hours' incubation, at 5°C after five days' incubation, and eight of the strains grew at 42°C after 48 hours' incubation. All the strains grew anaerobically, although growth was slight with some strains. Growth was obtained on nutrient agar and was improved by the addition of 10% serum.

The biochemical characters of *M. phenylpyruvica*, summarized in Table III, agree in general with those described by Bøvre and Henriksen (1967) except for the four tests discussed below:

### 1 ANAEROBIC GROWTH

All strains in the present series showed varying degrees of anaerobic growth, ie, in a hydrogen + carbon dioxide atmosphere, whereas Bøvre and Henriksen (1967) described their strains as failing to grow anaerobically. Possibly this discrepancy is due to different test conditions.

### 2 GROWTH ON SOLID O/F MEDIUM

Bøvre and Henriksen (1967) reported that their strains of *M. phenylpyruvica* grew on this medium. In the NCTC, growth was either poor or absent and the results were not reproducible. This may have been due to differences in inoculum size and carry over of nutrients from the medium on which the bacteria were first grown. Serial subculture of the strains on the O/F medium failed to improve the reliability of this test.

| Tests Positive | Tests Negative | Tests Variable | | |
|---|---|---|---|---|
| | | | Positive | Negative |
| Catalase production | Haemolysis | Nitrate reduction | 10 | 1 |
| Oxidase production | Casein digestion | Urease production | 9 | 2 |
| Phenylalanine deamination | Serum liquefaction | Opalescence on egg yolk | 8 | 3 |
| Growth on MacConkey's agar | Gelatin liquefaction | Growth 42°C | 8 | 3 |
| Growth stimulated by bile (10%) | Growth on β-hydroxybutyrate (basal medium) | | | |
| Resistance to brilliant green (1/500 000) | Poly-β-hydroxybutyrate inclusion granules (serum agar medium) | | | |
| Retention of crystal violet (Gram) by some cells | Citrate utilization | | | |
| Growth 5°C | Indole | | | |
| | Acid from glucose | | | |
| | Arginine desimidase | | | |
| | Lysine decarboxylase | | | |
| | Ornithine decarboxylase | | | |
| | β-galactosidase (ONPG) | | | |
| | Starch hydrolysis | | | |
| | Phosphatase production | | | |

Table III *Biochemical characters of* Moraxella phenylpyruvica

### 3 PENICILLIN SENSITIVITY

Bøvre and Henriksen (1967) reported MICs to range from 0·0005 to 0·04 IU penicillin/ml for their *M. phenylpyruvica* strains. They calculated MICs from inhibition zone diameters and regression equations. With our different technique (see Bacteriological Investigations), eight of our strains were inhibited within a range of 0·05 to 0·5 IU penicillin/ml incorporated in the agar. Three strains grew in the presence of 100 IU penicillin/ml and were subsequently found to produce penicillinase. The MICs of the strains are given in Table IV.

| Strain | MICs (IU ml) |
|---|---|
| NCTC 10752 | 0·05 |
| 3 | 0·10 |
| 1, 2, 4, 8 and NCTC 10526, 10750 | 0·50 |
| 5, 6, 7 (penicillinase positive) | >100 |

Table IV *Minimum inhibitory concentrations (MICs) of penicillin for* M. phenylpyruvica

### 4 DEAMINATION OF PHENYLALANINE

All the strains of *M. phenylpyruvica* produced phenylpyruvic acid from phenylalanine both by the sensitive agitation method of Snell and Davey (1971) and in the medium of Shaw and Clarke (1955). In this latter medium, hower, incubation for 48 hours was required before a colour reaction could be observed, which was often faint but usually stronger if the medium was enriched with serum. Bøvre and Henriksen (1967) found that deamination of phenylalanine was restricted to *M. phenylpyruvica*, but, in the present study, several strains of some other *Moraxella* spp were also found to deaminate phenylalanine when tested by the 'agitation' method (see Table V).

| Species | Positive Strains | Negative Strains |
|---|---|---|
| *M. osloensis* | 10749, 10756 | 10465, 10755 |
| *M. lacunata* | — | 7985, 10747, 10748 |
| *M. saccharolytica* | 10753 | — |
| *M. kingii* | — | 10529, 10746 |
| *M.* sp. (Van Bijsterveld) | 10717 | — |
| *M. bovis* | — | 8561, 9425, 9426 A52/69 |
| *M. liquefaciens* | 10358, 10359 | 7911 |
| *M. nonliquefaciens* | — | 7784, 10464 A33/67, A9/69 |

Table V *Deamination of phenylalanine by* Moraxella *spp. other than* M. phenylpyruvica *as determined by the method of Snell and Davey (1971)*

### STIMULATION OF GROWTH BY BILE

The growth of strains of *M. phenylpyruvica* was found to be improved on MacConkey agar. Ox bile and unpurified sodium taurocholate were then found to stimulate growth, while strains of other *Moraxella* spp were unaffected or inhibited by these substances. The stimulation of *M. phenylpyruvica* was presumably due to impurities known to be present in bile salts (Leifson, 1935; Society for General Microbiology, 1956), because Analar grade sodium taurocholate did not exert a stimulatory effect.

Fatty acids are likely to be present in bile salts and have variable effects on bacterial growth (Leifson, 1935; Wildy and Hare, 1953). Baumann, Doudoroff, and Stanier (1968) found that oleic acid stimulated some strains of *M. lacunata*. In the present study, growth of *M. phenylpyruvica* was stimulated by stearate, oleate, and Tween 80, but not by butyrate, propionate, valerate, acetate, pelargonate, or lecithin.

### DNA BASE COMPOSITIONS

The DNA base compositions determined in the

present study, and previously published data for the same strains, are expressed as the percentage of guanine and cytosine/total bases present (%GC) and are given in Table VI. The values found for *M. phenylpyruvica*, NCTC 10526 (42·2% GC) and *M.* sp. NCTC 10717 (48·0% GC) closely agree with those given by Bøvre and Henriksen (1967) and van Bijsterveld (1970), who made their determinations from buoyant densities (Schildkraut, Marmur, and Doty, 1962). The previous discrepancy between the value of 49·0% GC for *M. kingii* NCTC 10529 (Hill, Snell, and Lapage, 1970) and 44·5% GC (Bøvre, Fiandt, and Szybalski, 1969) was checked again with a fresh preparation which gave 48·7% GC. Discrepancies between laboratories are not uncommon (Hill, Leach, and Andrews, 1970), though not usually as large as this.

## DISTRIBUTION OF *M. phenylpyruvica* STRAINS

The conjunctiva, skin, and mucous membranes are usually considered to be the natural habitat of *Moraxella* spp. *M. phenylpyruvica* has been isolated, however, from the urogenital tract, blood cultures, cerebrospinal fluid, and pus from various lesions (Flamm, 1957; Bøvre and Henriksen, 1967; Pedersen, Marso, and Pickett, 1970), and from pleural fluid, nasal swabs, and pressure sores (this paper). Some of these sites are liable to faecal contamination. Faeces have not been previously considered as a possible reservoir of this species which, moreover, is stimulated by bile. This possibility was therefore investigated.

The selective medium devised was successful for the recovery of all strains of *M. phenylpyruvica* added to faeces. The selective agents inhibited the majority of the faecal flora except some streptococci. The colonies of *M. phenylpyruvica* on this medium were colourless, whereas other organisms produced acid from the glucose and thus formed red colonies. One hundred specimens of faeces from different patients were cultured on this medium, but no strains of *M. phenylpyruvica* were found.

| *NCTC Numbers and Names* | *Percentage GC (Present Study) from Melting Temperatures* | *Percentage GC (Other Published Data) from* | |
|---|---|---|---|
| | | *Melting Temperatures* | *Buoyant Densities* |
| 10526 *M. phenylpyruvica* | 42·2 | — | 43·0[1] |
| 10717 *M.* sp. (Van Bijsterveld) | 48·0 | — | 49·0[2] |
| 10529 *M. kingii* | 48·7 | 49·0[3] | 44·5[4] |
| 10753 *M. saccharolytica* | 38·4 | — | — |

Table VI *DNA base compositions determined in the present work*

[1]Bøvre and Henriksen (1967)
[2]Van Bijsterveld (1970)
[3]Hill Snell, and Lepage (1970)
[4]Bøvre, Fiandt, and and Szybalski (1969)

| | *Moraxella phenyl-pyruvica* | *M. oslo-ensis* | *M. lacu-nata* | *M. sac-charo-lytica* | *M. kingii* | *M. sp. (Val Bijster-veld)* | *M. bovis* | *M. lique-faciens* | *M. non-lique-faciens* |
|---|---|---|---|---|---|---|---|---|---|
| Number of strains | 11 | 4 | 3 | 1 | 2 | 1 | 4 | 3 | 4 |
| Catalase | + | + | + | + | − | ± | ± | + | + |
| Growth on MacConkey agar | + | + | − | − | − | − | − | 2 | − |
| Phenylalanine deamination (agitation method) | + | 2 | − | + | − | ± | − | 2 | − |
| Nitrate reduction | 10 | 2 | 2 | + | − | − | − | + | + |
| Retention of crystal violet (Gram) | + | 2 | 1 | − | − | − | − | 2 | − |
| Urease | 9 | − | − | − | − | − | − | − | − |
| Opalescence on egg yolk | 8 | − | − | − | − | − | 3 | ± | − |
| Growth stimulation by bile | + | − | − | − | − | − | − | − | − |
| Casein digestion | − | − | + | − | ± | ± | ± | + | − |
| Serum liquefaction | − | − | ± | − | − | − | ± | + | − |
| Resistance to brilliant green 1/500 000 | + | 2 | − | − | − | − | 1 | + | − |
| Phosphatase | − | 1 | 1 | + | + | + | − | − | − |
| Poly-β-hydroxybutyrate granules | − | + | − | − | − | − | − | − | − |
| Haemolysis | − | − | − | − | + | − | + | − | − |
| Acid from glucose | − | − | − | + | + | + | − | − | − |
| Growth 5°C | + | − | − | − | − | − | − | − | − |
| Growth 42°C | 8 | − | − | − | − | − | − | − | − |
| DNA base composition, % GC (selected strains) | 42-43·5[1] | 43·5[2] | 42[2] | 38·3 | 49·0[4] | 48-49[3] | 42·5[2] | 41·5[2] | 42·2[2] |

Table VII *Differentiation of* Moraxella phenylpyruvica

[1]Bøvre and Henriksen (1967), Bøvre, Fiandt, and Szybalski (1969), and Table VI
[2]Bøvre, Fiandt, and Szybalski (1969).
[3]Van Bijsterveld (1970), and Table VI.
[4]Hill, Snell, and Lapage (1970), and Table VI.

## Discussion

The eight field strains and three NCTC strains of *Moraxella phenylpyruvica* form a homogeneous group differing from other *Moraxella* spp in a number of characters (see Table VII). Although deamination of phenylalanine is not limited to *M. phenylpyruvica*, nonetheless this species can be readily distinguished; in particular, it is urease positive (nine of 11 strains), stimulated by bile and grows at 5°C, which are features not shown by other moraxellas. Considered in pairs, *M. phenylpyruvica* differs from each of the other *Moraxella* spp in Table VII in six (*M. osloensis*, *M. liquefaciens*) to 14 characters (*M. kingii*).

The DNA base composition of *M. phenylpyruvica* (42·0-43·5% GC) lies within the range of base compositions of other nonsaccharolytic moraxellas (41·5-43·5% GC, see Table VII). Saccharolytic moraxellas lie slightly outside this range: *M. kingii* and *M.* sp. NCTC 10717 have higher values (48-49% GC) and *M. saccharolytica* a lower value (38·5% GC). The saccharolytic moraxellas should perhaps be excluded from the genus *Moraxella*; Henriksen and Bøvre (1968), in fact, considered *M. kingii* to be only 'distantly related' to other *Moraxella* spp, but saw no other alternative genus for its inclusion.

Bøvre and Henriksen (1967) and Bøvre (1970) found no genetic transformation between *M. phenylpyruvica* and *M. nonliquefaciens*, and only low similarity in DNA-RNA *in vitro* molecular hybridization experiments, despite the similarity in base composition. *M. nonliquefaciens*, on the other hand, had varying degrees of compatibility in transformation experiments with other *Moraxella* spp.: *M. bovis* *M. lacunata*, *M. liquefaciens*, and *M. osloensis*. *M. phenylpyruvica* thus appear to be genetically isolated. The taxonomic utility of DNA base composition is limited to where there are differences in composition, indicating dissimilarity in base sequences, ie, genetic messages, which, in turn, indicate unrelatedness. The contrary finding of a similar base composition does not necessarily imply similar base sequences. The genetic evidence above and the low phenetic similarity with other *Moraxella* spp. suggests that *M. phenylpyruvica* is only distantly related to other members of the genus.

Baumann *et al* (1968) amended the definition of the genus *Moraxella* to include sensitivity to 1 IU penicillin/ml, although the moraxellas they studied did not include saccharolytic strains. The discovery of three strains of *M. phenylpyruvica* producing penicillinase and growing in the presence of 100 IU penicillin/ml shows that the criterion of penicillin sensitivity for inclusion in the genus *Moraxella* should not be applied in a monothetic fashion (Sokal and Sneath, 1963).

The pathogenicity of *M. phenylpyruvica* is probably low, although it may possibly be an opportunist pathogen. It is difficult to assign a causative role of the clinical conditions to infection by any of the strains described here. However, two strains of this organism were isolated from blood cultures by the late Dr E. O. King (*fide* Bøvre and Henriksen, 1967) and Flamm's original strain was from cerebrospinal fluid in a case of infant meningitis. Despite our failure to demonstrate the presence of *M. phenylpyruvica* in faeces, it remains a possibility that this organism is present in the intestinal tract but only in small numbers in faeces or else that the carrier rate is low. Examination of faeces from patients with infections due to *M. phenylpyruvica* would be interesting and might be more successful. It would be useful to devise an enrichment medium, although it might prove difficult as these bacteria grow poorly in liquid media. Many bacteria grow in the presence of bile, but stimulation is rare. Stimulation of *Bacteroides fragilis* by bile has been reported (Loesche, Socransky, and Gibbons, 1964).

Reports of the isolation of *M. phenylpyruvica* are rare, but the isolation of eight strains in the UK over a period of three years indicates that this species may be more widely distributed than the paucity of reports would suggest. The relative ease of identification, using the characteristics described in this paper, may lead to the identification of further strains.

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