SHORT COMMUNICATIONS

A Comparison of Methods for the Detection of Phenylalanine Deamination by *Moraxella* Species

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INTRODUCTION

Among the enterobacteria deamination of phenylalanine to phenylpyruvic acid is limited to species of *Proteus* and *Providencia* and various tests for this character are commonly used, particularly the combined malonate/phenylalanine medium of Shaw & Clarke (1955). Bøvre & Henriksen (1967) described a new species of *Moraxella*, *M. phenylpyruvica*, which characteristically is able to deaminate phenylalanine. The type strain of this species, deposited in the National Collection of Type Cultures (NCTC) as NCTC 10526, failed to produce phenylpyruvic acid from phenylalanine using the medium of Shaw & Clarke (1955) during routine tests in the NCTC. A number of field strains of *M. phenylpyruvica*, sent to the NCTC for identification, also gave negative results in this medium.

Therefore, 34 strains of *Moraxella* were tested by four methods for the detection of phenylalanine deamination in order to determine the most suitable method for use with *Moraxella* strains.

METHODS

Strains of bacteria used. Of the 34 strains used, 13 were NCTC cultures and 21 were field strains. The NCTC strains comprised one strain of *Moraxella lacunata*, one of *M. osloensis*, one of *M. kingii*, one of *M. phenylpyruvica*, three of *M. bovis*, three of *M. liquefaciens*, two of *M. nonliquefaciens* and one unnamed *Moraxella* strain. Of the field strains, 7 had been identified as *M. phenylpyruvica* on the basis of a comparison of their characters with the type strain of this species. The remaining 14 strains had been assigned to the genus *Moraxella* after examination in the NCTC, but the tests carried out were not detailed enough to identify them to species level. A strain of *Proteus vulgaris*, NCTC 401, was included in the study as a control.

Detection of phenylalanine deamination

Four methods were used:

(1) *Combined malonate/phenylalanine medium* (Shaw & Clarke, 1955). This test depends on bacterial growth in the medium, unlike the other methods described here in which heavy initial bacterial suspensions are used for the tests. The nutritionally more demanding of the *Moraxella* strains gave poor growth in the medium of Shaw & Clarke (1955). Therefore all

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the strains were also tested in medium supplemented with 10 % (v/v) horse serum. (a) *Basal medium*: $(\text{NH}_4)_2\text{SO}_4$, 2.0 g.; K_2HPO_4 , 0.6 g.; KH_2PO_4 , 0.4 g.; NaCl, 2.0 g.; sodium malonate 3.0 g.; DL-phenylalanine, 2.0 g.; yeast extract, 1.0 g.; distilled water, 1000 ml.; bromothymol blue, 12.5 ml. of a 0.2 % (w/v) aqueous solution. The solids were dissolved in the water by heating, the pH adjusted to 7.0 and the indicator added. After filtration the medium was distributed in 100 ml. volumes and sterilized by autoclaving at 15 lb/in.² for 20 min. For use, the medium was distributed aseptically into sterile 125 × 12.5 mm. test tubes. (b) *Medium plus horse serum*: Seitz-filtered horse serum was added to the basal medium to a final concentration of 10 % (v/v), and the medium was distributed aseptically into sterile 125 × 12.5 mm. test tubes. (c) *Inoculation*. The test medium whether with or without serum was inoculated with a dense suspension of the organisms in saline prepared from overnight cultures grown on 10 % (v/v) serum Columbia agar slopes (Columbia agar, Oxoid, London S.E. 1). (d) *Reading of results*. The tests were read at 24 and 48 h. by withdrawing the same amount of medium on each occasion and testing for the presence of phenylpyruvic acid by acidification with 0.1 N HCl followed by the addition of five drops of 10 % (w/v) aqueous FeCl_3 . The development of a green colour indicated a positive result.

(2) *Blotting-paper strip method* (Goldin & Glenn, 1962). This method was used by Bøvre & Henriksen (1967) in the original characterization of *Moraxella phenylpyruvica*. Strips of Ford's 428 mill 38 lb blotting paper (cut into 60 × 7 mm. strips) were immersed in a 1 % (w/v) solution of DL-phenylalanine in Sørensen's phosphate buffer, pH 7.4. After drying at 37° the phenylalanine test strips were stored at room temperature in screw-capped bottles. A heavy suspension of each organism to be tested was made from an overnight culture grown on a 10 % (v/v) serum Columbia agar slope, and 0.2 ml. volumes of the suspension were placed in four sterile 75 × 12.5 mm. test tubes and a phenylalanine blotting-paper test strip added to each tube which was tilted momentarily to moisten the strips. The tubes were incubated at 37° and read at $\frac{1}{2}$, 1, 2, and 4 h. by placing three drops of 10 % (w/v) aqueous FeCl_3 on the phenylalanine test strip. The development of a green colour indicated a positive result.

(3) *Phenistix strip* (Ames Co., Stokes Poges, Slough, Buckinghamshire) (Smith & Free, 1962). The growth from an overnight slope culture on 10 % (v/v) serum Columbia agar was emulsified in 1 ml. of 0.4 % (w/v) DL-phenylalanine and the tubes were sloped so that the suspension formed a layer over the agar surface. The results were read after incubation at 37° for $\frac{1}{2}$, 1, 2, 4 and 24 h. by dipping a Phenistix strip into the suspension. The development of a green colour indicated a positive result.

(4) *Agitation method*. The growth from an overnight culture on a 10 % (v/v) serum Columbia agar slope was emulsified in 2 ml. of 0.4 % (w/v) DL-phenylalanine in distilled water. The suspension was transferred to a sterile 150 × 15.5 mm. test tube. The tubes were then agitated on a mechanical flask shaker, the arms of which were projected over a 37° water bath thus allowing the bottoms of the tubes to be immersed during agitation; 0.5 ml. amounts of the suspension were removed at $\frac{1}{2}$, 1, 2 and 4 h. and tested for the presence of phenylpyruvic acid which was indicated by the development of a green colour after the addition of five drops of 10 % (w/v) aqueous FeCl_3 .

RESULTS

The four methods showed considerable difference in sensitivity, and Table 1 shows the number of positive results in each test and the time of incubation required. For the detection of phenylalanine deamination by *Moraxella phenylpyruvica*, both the agitation method and

the blotting paper strip method of Goldin & Glenn are suitable. The agitation method requires incubation for 1 h. and the paper strip method 4 h. to obtain positive results for these bacteria. The combined malonate/phenylalanine medium of Shaw & Clarke (1955) was positive in eight out of the nine cultures, but the green colour was often faint, being generally stronger in the serum-enriched medium; also 48 h. incubation was required and the inoculum used was heavier than would normally be used with *Proteus* strains. The Phenistix method was positive in only three of these strains.

Table 1. *Distribution of phenylpyruvic acid production detected by the four methods*

Taxon	Number of strains	Number of strains positive* in				
		Shaw & Clarke (1955) medium		Goldin & Glenn (1962) method	Smith & Free (1962) method (Phenistix)	Agitation method (this paper)
		Without horse serum	With 10% horse serum			
<i>Proteus vulgaris</i>	1	1 ²⁴	1 ²⁴	1 [‡]	1 ⁴	1 [‡]
<i>Moraxella phenylpyruvica</i>	8	7 ⁴⁸	7 ⁴⁸	8 ⁴	3 ⁴	8 ¹
Other <i>Moraxella</i> species	26	2 ⁴⁸	5 ⁴⁸	8 ⁴	1 ⁴	11 ²

* Time in hours, within which all the strains which were to yield a positive result achieved it, is given in superscript.

In the case of the *Moraxella* strains not considered to be *Moraxella phenylpyruvica*, the agitation method gave the greatest number of positive results (11 strains in 2 h. incubation) and the method of Goldin & Glenn gave eight positive results within 4 h. incubation. The strains of field isolates in the U.K. that were proved to be *M. phenylpyruvica* were from human sources as were the strains described by Bøvre & Henriksen (1962).

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