Mannitol-motility Medium In Routine Diagnostic Enteric Bacteriology

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Mannitol-motility medium (MMM), in conjunction with triple sugar iron agar and peptone water, has been found more useful than ordinary semisolid agar motility medium for screening and rapid differentiation of non-lactose fermenting members of the enterobacteriaceae and aerobic non-fermenting Gram-negative rods, in that it reduces the number of further biochemical tests that must be done prior to the final biochemical and/or serological identification of the organisms isolated. A description of the medium with interpretation of reactions and other pertinent details is presented. Results obtained in the study of 1846 stool specimens, from which 484 strains belonging to enterobacteriaceae and 103 strains of aerobic non-fermenting Gram-negative rods were isolated, provide evidence of the value of MMM.

Introduction

Tittsler and Sandholzer (1936) were the first to propose the use of semisolid agar medium for detecting the motility of bacteria. The value of adding mannitol to such a medium for the identification of enterobacteriaceae was demonstrated by Roland and Bourbon (1949) though their medium had the disadvantage of non-suppression of the gas produced during fermentation. Le Minor (1967) introduced a modification whereby gas production during fermentation was inhibited by the addition of potassium nitrate, the presence of which salt also facilitated detection of nitrate reduction. The mannitol-motility medium (MMM) as described by Le Minor (1967) was devised primarily to screen cultures for the presence of non-lactose fermenting members of the family enterobacteriaceae.

MMM has been employed in our laboratory in the place of ordinary semisolid agar since October 1968, and a report on its value in routine diagnostic bacteriology is presented below.

Material and Methods

MMM was prepared as described by Le Minor (1967). It contains 2% peptone, 0.4% agar, 0.2% mannitol, 0.1% potassium nitrate, and phenol red as the indicator. The pH of the medium is adjusted to 7.4 to 7.6.
The medium is inoculated by stabbing a needle through it to the bottom of the tube. Following overnight incubation at 37°C, the test for nitrate reduction is done by layering about 4-6 drops of sulphanilic acid over the medium and then adding alphaphenylalanine. The immediate development of a red colour indicates a positive test due to reduction of nitrate to nitrite. In the absence of a colour change initially zinc dust is added. If a red colour develops it indicates the presence of unreduced nitrate. If this does not produce a change of colour to red, further reduction of nitrate to nitrogen is indicated.

Stool specimens, received as faecal mass, faecal swabs or rectal swabs, were examined according to the methods described for previous studies (Bhat and Myers 1962). In brief, they were inoculated on to sheep blood agar (BA), MacConkey agar (MA), desoxycholate citrate agar (DCA) and bismuth sulphite agar (BSA). Following this, swabs were twisted thoroughly in a tube of selenite-F enrichment broth (SB), or a bit of the faecal mass was added to SB. After 18-20 h incubation at 37°C, the SB was subcultured onto Salmonella-Shigella agar (SSA). All the agar plates were incubated aerobically at 37°C and were examined following overnight incubation. In the absence of 24-h growth suggestive of pathogenic enterobacteria, DCA, SSA and BSA plates were further incubated and examined again after approximately 48 h incubation.

Non-lactose fermenting colonies from MA, DCA and SSA, and suspicious looking colonies from BSA were inoculated into MMM, triple sugar iron agar (TSI) which incorporates glucose, lactose and sucrose, and peptone water (PW). These constituted the primary differential media for rapid screening prior to testing further for the biochemical and serological reactions required for final identification.

After overnight incubation, changes found in MMM were recorded and tests for nitrate reduction were carried out where indicated. TSI reactions were recorded and interpreted as described in previous studies (Bhat and Myers 1962). PW cultures were tested for the presence of indole.

The changes found upon examination of the MMM culture and the activities they indicate, are shown in Table I.

Results

From October 1968 to January 1970, a total of 1846 stool specimens was bacteriologically studied. Non-lactose fermenting colonies isolated from these stool specimens had been tentatively classified as one or the other of the organisms listed in Tables II and III and were subsequently identified as such by either serological or further biochemical tests. No Arizona or Vibrio strain were isolated from the stool specimens studied; nor was Bordetella bronchiseptica found. Hence, stock strains of these were included in the study.

A summary of MMM, TSI and indole reactions typical of the commonly encountered non-lactose fermenting Gram-negative rods is given in Table II.
Table I. Interpretation of changes observed in inoculated MMM

<table>
<thead>
<tr>
<th>Appearance of medium</th>
<th>Interpretation of reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red in colour, clear</td>
<td>Uninoculated medium</td>
</tr>
<tr>
<td>(a) Turbidity confined to the stab-line</td>
<td>Non-motile organism</td>
</tr>
<tr>
<td>(b) Turbidity confined to the aerobic top layer; not extending to the anaerobic lower layers</td>
<td>Non-motile; aerobic organisms unable to use nitrate as hydrogen acceptor (Jensen, 1965)</td>
</tr>
<tr>
<td>Lateral spreading of the turbidity from the stab-line, in varying degree</td>
<td>Motile organism-degre of spreading, indicative, of sluggish to highly active motility</td>
</tr>
<tr>
<td>Slight deepening of the original red colour, with turbidity</td>
<td>Non-fermentation of mannitol</td>
</tr>
<tr>
<td>Yellow throughout except at the surface which usually remains deep red, with turbidity</td>
<td>Fermentation of mannitol</td>
</tr>
<tr>
<td>Slight deepening of the original red colour, with trapping of gas bubbles along the stab-line, sometimes with breaking up of the medium</td>
<td>Non-fermentation of mannitol with reduction of nitrate to nitrite and further reduction to N₂</td>
</tr>
</tbody>
</table>

It may be seen that MMM reactions are useful for differentiation between organisms which are alike in their reactions in TSI and in production, or otherwise, of indole. Reactions of atypical E. coli in comparison with those of P. morganii should be noted, as well as those of the various Shigellae; and those of the H₂S-producing Salmonellae, Arizona and Bethesda-Ballerup strains in contrast to the H₂S-producing species of Proteus and Edwardsiella tarda; similarly P. retigeri and Providence strains.

In Table III a summary of the findings for non-fermenting strictly aerobic Gram-negative rods is presented. All of these organisms, which were studied, were indole-negative, and all showed identical reactions in TSI, though growth of Pseudomonas species was often characterized in PW by production of pigment at the surface and in TSI by a pigmented metallic sheen and a fruity odour. These results show that the combination of media studied was of differentiating value for these organisms also.

Discussion

When Le Minor (1967) described MMM, he stressed mainly the value of detection of mannitol fermentation for preliminary identification of non-lactose fermenting enterobacteria, since it minimized the number of tests for biochemical activities that had to be carried out by dividing the organisms into the important broad groups.

The results reported here show that the introduction of this medium proved of further value in the preliminary identification of non-fermenting aerobic Gram-negative rods.

Though potassium nitrate had been incorporated in the medium by Le Minor primarily to suppress gas formation by aerogenic mannitol fermenters, we found it to be
## Table II. MMM, TSI and indole test reactions of non-lactate fermenting Gram-negative rods

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates studied</th>
<th>MMM</th>
<th>TSI</th>
<th>Indole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical E. coli</td>
<td>222</td>
<td>A+</td>
<td>Alk</td>
<td>Ag</td>
</tr>
<tr>
<td>P. morganii</td>
<td>45</td>
<td>A</td>
<td>Alk</td>
<td>Ag</td>
</tr>
<tr>
<td>Sh. dysenteriae group</td>
<td>6</td>
<td>A</td>
<td>Alk</td>
<td>A</td>
</tr>
<tr>
<td>Sh. flexneri group</td>
<td>33</td>
<td>A/-</td>
<td>Alk</td>
<td>A</td>
</tr>
<tr>
<td>Sh. boydii group</td>
<td>6</td>
<td>A/-</td>
<td>Alk</td>
<td>A</td>
</tr>
<tr>
<td>Sh. somniel</td>
<td>4</td>
<td>A</td>
<td>Alk</td>
<td>A</td>
</tr>
<tr>
<td>Alkaligenes-Dispar</td>
<td>11</td>
<td>A</td>
<td>Alk</td>
<td>A</td>
</tr>
<tr>
<td>Salmonellae</td>
<td>14</td>
<td>A+</td>
<td>Alk</td>
<td>Ag</td>
</tr>
<tr>
<td>Arizona</td>
<td>12</td>
<td>A+</td>
<td>Alk</td>
<td>Ag</td>
</tr>
<tr>
<td>Bethesda-Ballerup*</td>
<td>27</td>
<td>A+</td>
<td>Alk</td>
<td>Ag</td>
</tr>
<tr>
<td>Edwardsiella tarda</td>
<td>2</td>
<td>A+</td>
<td>Alk</td>
<td>Ag</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>2</td>
<td>A</td>
<td>Alk</td>
<td>Ag</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>86</td>
<td>A+</td>
<td>Alk</td>
<td>Ag</td>
</tr>
<tr>
<td>P. rettgeri</td>
<td>13</td>
<td>A+</td>
<td>Alk</td>
<td>A</td>
</tr>
<tr>
<td>Providence group</td>
<td>12</td>
<td>A</td>
<td>Alk</td>
<td>A+</td>
</tr>
<tr>
<td>V. cholerae and other</td>
<td>10</td>
<td>A+</td>
<td>Alk</td>
<td>A</td>
</tr>
<tr>
<td>biochemically active</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vibrios</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Key:**
- **+ Positive**
- **- Negative**
- **+/− Generally positive, though occasionally negative**
- Ag = Acid with gas
- A = Acid only
- Alk = Alkaline
- A/Alk = Generally acid, though occasionally alkaline
- V = Variable
- *Bethesda-Ballerup subgroup belonging to the Chromobacter group*
- †The amount of gas produced in glucose following fermentation is very little
- ‡As no isolation was made, stock cultures of these organisms were studied

of great value also in preliminary differentiation in the identification of the non-fermenting aerobic Gram-negative rods. Thus, organisms like strains of *Pseudomonas*, *Alcaligenes*, etc., which grow anaerobically in the presence of nitrate as described by Sellar (1964) and Jessen (1965) may be quickly differentiated from organisms, such as *B. anitratum* and *Mima* species which are unable to grow anaerobically. Growth of the former occurs throughout, or along the stab, to the bottom of the tube, of the latter at the surface, or very slightly below.

MMM following overnight incubation divides organisms also into those capable of reducing nitrate to nitrite, and those incapable of this; and further detects the ability of organisms like *Pseudomonas* species to reduce nitrite to nitrogen gas.

Hence, though the nitrate reduction test is not of differential value for organisms within the family enterobacteriaceae, since all of these reduce nitrate to nitrite, it is a very useful test for differentiation of the non-fermenting aerobic Gram-negative rods.
Table III. MMM and TSI reactions, and indole test results of aero-obic non-fermenting Gram-negative rods

<table>
<thead>
<tr>
<th>Organism</th>
<th>MMM</th>
<th>TSI</th>
<th>Indole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Material</td>
<td>Morbidity</td>
<td>Surface and slug growth</td>
</tr>
<tr>
<td>Pseudomonas group</td>
<td>84</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>A. faecalis</td>
<td>86</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B. bronchiseptica</td>
<td>34</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>B. trifurcata and</td>
<td>31</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mima species</td>
<td>33</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Key: + Positive — Negative +/— Generally positive though occasionally negative
     Alk—Alkaline

* Cultures of the strains which produce gas in MMM after overnight incubation, generally fail to show colour change, thus indicating the necessity of addition of zinc dust. See text.
† As no isolation was made, stock cultures were studied.

The ease with which the MMM can be prepared and handled and the rapidity with which reactions can be determined make the medium a very valuable one for routine use in a diagnostic laboratory.

Acknowledgment

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References


