Primary Plating Medium for Differentiating the Colonies of the Genus Proteus from Certain Other Enteric Bacteria

G. NOGRADY

Department of Bacteriology of the Faculty of Medicine, University of Montreal, Montreal, Canada

Received for publication March 2, 1959

The rapid differentiation of lactose-negative saprophytes from the lactose-negative pathogens is a significant problem in the diagnosis of Enterobacteriaceae. Particularly important is the recognition of the urease-positive group. With the presently used urea-containing solid and liquid media (Rustigian and Stuart, 1941; Ferguson and Hook, 1943; Stuart et al., 1945; Christiansen, 1946; Ewing and Bruner, 1947) one can determine the urease activity of a microorganism within a time of from 5 min to 48 hr depending on the composition of the medium, the size of inoculum, and the urease activity of the given strain. These methods delay the final bacteriological diagnosis because their application requires the inoculation of one or more media for the determination of urease activity after the use of an ordinary primary plating medium. Therefore, we wished to investigate the possibility of preparing a primary plating medium on which one could differentiate simultaneously the urea-splitting microflora from the lactose fermenting colonies of entero bacteria. In so doing, the detection of urease activity becomes a primary criterion in selecting colonies.

As far as we could ascertain, only two similar solid media are mentioned in the literature. The first is too sensitive for alkalisation (Preuss, 1949), the second (Zarett and Doetsch, 1949), however, is selective for the genus Proteus but does not permit the simultaneous detection of other species. The urea-containing medium of Singer (1950a, b; 1951) marks some similarities in composition but, as with other differential media, one can use it only after the primary plating media.

MATERIALS AND METHODS

The composition of the medium is as follows:

Sörensen's Na₂HPO₄/KH₂PO₄ (0.067 M), pH 6.45..................450 ml
Bacto-agar .................................. 15 g
Bacto-peptone .................................. 10 g
Bacto-yeast extract ................................. 6 g
Bacto-lactose .................................. 12.5 g
Sodium desoxycholate (Difco)² ................. 2 g

¹ This work was supported by the National Research Council of Canada, Grant M.A. 729. Presented at the eighth annual meeting of the Canadian Society of Microbiologists, Halifax, June, 1958, and demonstrated at the exhibition of the VIIth International Congress for Microbiology, Stockholm, August, 1958.

² Difco Laboratories, Inc., Detroit, Michigan.

REFERENCES


Sodium chloride (Merck)\(^2\)..............5 g
Brom-thymol-blue (Merck)..................0.125 g
Urea (Merck).............................2.5 g
Bacto-neutral red................................0.03 g
Distilled water................................to 1000.0 ml

Final pH: \(\sim 6.35\)

Solution A. Add 15 g agar in 350 ml of distilled water to 450 ml of Sörensen's phosphate buffer mixture (0.067 M, pH 6.45). Boil until the agar is dissolved.

Solution B. Add the following substances to 100 ml distilled water: 10 g peptone, 6 g yeast extract, 12.5 g lactose, 5 g sodium chloride, 2 ml of a 10 per cent solution of sodium desoxycholate, 20 ml of a 0.5 per cent solution of brom-thymol-blue (see below). Dissolve by heating. Mix solutions A and B and boil for 2 min. Add sterile distilled water to give a final volume of 1000 ml. Cool to 80 C and add aseptically the following: 5 ml of a 50 per cent solution of urea (sterilised by filtration), 10 ml of a 0.3 per cent solution of neutral-red (sterilised by autoclaving). Mix thoroughly, pour 17 ml quantities to each Petri plate. Dry for 20 min at 37 C. The colour of the medium should be light yellowish-brown. After incubation with a faecal dilution the inoculated medium turns to light orange.

The brom-thymol-blue solution is prepared by heating 0.5 g of brom-thymol-blue in 2 ml of normal sodium hydroxide and adding 5 ml of distilled water, shake and add normal hydrochloric acid until a green colour appears. Adjust the volume to 100 ml with distilled water.

The freshly prepared medium gives the best results, but one can store the medium at room temperature for 5 days with little change. It is not susceptible to contamination by many of the usual contaminants. After 7 to 10 days, a growth of mould sometimes appears. The inoculum should be small and the inoculated medium should be incubated at 37 C for 15 hr.

The recognition of colonies of various colours is facilitated by using light reflected from a white paper. The trials of this medium were made using pure cultures of fresh or old laboratory strains mixed with dilutions made from the faeces of healthy persons. With pure cultures, the differential characteristics of strains of Proteus become evident only in the presence of acidifying strains of Escherichia coli because they produce overalkalisation of the medium when alone. This overalkalisation could cause false results.

**RESULTS**

Table 1 shows the characteristic morphology of some of the enterobacteria studied on this new medium. It also indicates changes produced by them in this medium and specifies the reason for the change. This corresponds, generally, to the production of acid or alkali. The brownish-orange colonies with a transparent zone are characteristically those of Bacillus shigae (Shigella dysenteriae). In the case of thick colonisation, the neighbouring colonies turn bluish. The colonies of Salmonella typhosa are small, brownish-yellow with a narrow and transparent zone. Raised, brownish-yellow colonies are characteristic of Salmonella paratyphi B. The abundant production of alkali in the case of thick colonisation results in the bluish colouration of neighbouring colonies. The colonies of Proteus are small or medium, convex and of a green or deep-blue colour because of the liberation of urea alkali. Around the colonies there is a transparent zone which is blue in almost all instances. The colonies of Proteus are especially

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Morphology of Isolated Colonies</th>
<th>Change of Medium Around the Colonies</th>
<th>Reason for Change Noticed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shigella dysenteriae</td>
<td>Medium Circular Brownish-orange Transparent Convex</td>
<td>Broad transparent zone, sometimes bluish</td>
<td>Alkali production</td>
</tr>
<tr>
<td>Salmonella typhosa</td>
<td>Small Circular Brownish-yellow Transparent Convex</td>
<td>Thin transparent zone</td>
<td>Moderate alkali production</td>
</tr>
<tr>
<td>Salmonella paratyphi B</td>
<td>Large Irregular Brownish-yellow Transparent Raised</td>
<td>Broad transparent zone, sometimes bluish</td>
<td>Strong alkali production</td>
</tr>
<tr>
<td>Proteus species</td>
<td>Small or medium Circular Deep-blue or green Transparent, centre opaque Convex</td>
<td>Broad transparent zone, blue or colourless</td>
<td>Strong alkali production from urea by urease</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Large Circular Red Opaque Raised</td>
<td>Thin or broad red-zone, precipitated</td>
<td>Adsorption of neutral-red on the acid precipitated desoxycholate</td>
</tr>
</tbody>
</table>

\(^2\) Merck & Co., Inc., Rahway, New Jersey.
striking in the presence of colonies of *Escherichia coli*. The colonies of *Escherichia coli* are large, raised and red. In contrast with the other mentioned species, the colonies are always opaque. Around the colonies there is usually a red precipitated-zone.

The results show that this medium is suitable for the differentiation of colonies of urease-producing *Proteus* from colonies of the other enterobacteria which are not ureolytic. The characteristics of colonies of *Shigella dysenteriae* differ from those of *Salmonella paratyphi* B and *Salmonella typhosa*. This difference is not so definite on other primary plating media. The *Escherichia coli* which usually abounds in faecal specimens does not interfere with the identification of the other enterobacteria investigated.

The medium seems to be useful for the differentiation of the five different enterobacteria that were investigated under the given conditions of these trials, when the members of the genus *Proteus* are placed in one group.

Table 2 shows what interference effects might occur between the colonies of these five different enterobacteria particularly when one is in excess of the other. The object of this experiment was to determine the effects that overgrowth of certain species might have on the differential recognition of colonies for diagnostic purposes.

Five individual species are found in the first column of table 2, whereas the second column contains separately, but grouped, the four remaining enterobacteria that may be influenced by the single species in the first column. In the further columns are found the changes produced in the character of each type of colony and the reasons for the change.

The results show that only a thick colonisation of *Proteus* species influences unfavourably the differentiation of other colonies. The overgrowth of *Escherichia coli* merely produces a partial and pale decolouration of the colonies of *Proteus*, but not enough to interfere with differentiation.

**Discussion**

The ability of this medium to differentiate colonies of the species studied depends primarily on the buffer system. This ensures that the pH of the medium is influenced only by the high acidity produced by *Escherichia coli* or by the strong alkali produced by *Proteus*. The pH changes brought about by the colonies of the other species are limited and do not interfere with the differentiation of colonies. The indicator mixture used does not inhibit the species investigated, but will visualise marked changes in the pH to the alkaline or acid side. Its colour at neutrality differentiates both acid or alkali changes. The Bacto-peptone assures a moderate growth of *Proteus* without decolourisation of the brom-thymol-blue, which is often seen with other peptone preparations that yield a more vigorous growth. The Bacto-yeast extract assures the optimum growth of *Shigella dysenteriae* and *Salmonella paratyphi* B and the differentiation of these organisms from the others is definitive. We lost this possibility by substituting the yeast-extract for Bacto-beef extract or by its simultaneous utilisation. The increased quantity of lactose assures the recognition of colonies of *Escherichia coli* and is advantageous in the recognition of other colonies because the volatile acid products (CO₂) cause a slight acidity in the medium. This turnover is marked by the brom-thymol-blue. An other important role of this dye is the visualisation of the alkali production by *Proteus* caused by the splitting of urea. The surplus of volatile ammonia is bound by the phosphate buffer. The desoxycholate controls the gram positive intestinal organisms. This salt produces a fine precipitate in the medium which absorbs the neutral-red indicator. The acid accumulating around acid producing colonies of *Escherichia coli* caused an increase in the precipitation of sodium desoxycholate and simultaneously there appears the change in colour of the adsorbed neutral-red due to this acid. The concentration of agar controls the swarming of *Proteus* and also limits the diffusion of alkali compound produced.

**Table 2**

<table>
<thead>
<tr>
<th>Influence on the Colonies of</th>
<th>On the Neighbouring Colonies of</th>
<th>Changes in the Colony Size and Color</th>
<th>Reason for the Change Noticed</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td><em>Salmonella typhosa</em></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella paratyphi</em> B</td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Proteus</em> species</td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhosa</em></td>
<td><em>Shigella dysenteriae</em></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella paratyphi</em> B</td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Proteus</em> species</td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em> B</td>
<td><em>Salmonella typhosa</em></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Proteus</em> species</td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td><em>Proteus</em> species</td>
<td><em>Shigella dysenteriae</em></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella typhosa</em></td>
<td>Smaller</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em> B</td>
<td><em>Escherichia coli</em></td>
<td>Pale pink</td>
<td>Alkali production</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td><em>Shigella dysenteriae</em></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella typhosa</em></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella paratyphi</em> B</td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Proteus</em> species</td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>Pale pink</td>
<td>Alkali production</td>
</tr>
<tr>
<td></td>
<td><em>Shigella dysenteriae</em></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella typhosa</em></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella paratyphi</em> B</td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Proteus</em> species</td>
<td>Smaller, pale green or blue</td>
<td>Acid production</td>
</tr>
</tbody>
</table>
from urea. Intensive reduction of the concentration of agar produces an increase in the zone around the colonies of Proteus. An optimum concentration of urea is important because this regulates the amount of alkali produced by Proteus. An excessive amount of urea leads to overalkalisation of the medium. An important point is to avoid sterilising this medium by steam under pressure which can cause the formation of many inhibitory factors. Finally, the proper adjustment of the neutral-red indicator followed by immediate pouring of the medium assures a uniform dispersion of the easily precipitated indicator. Good drying of the surface of the medium is necessary to control swarming of Proteus (H form).

ACKNOWLEDGMENTS

The presentation (Halifax, June 1958) and the demonstration (Stockholm, August 1958) of this work was kindly supported by the Institute of Microbiology and Hygiene of the University of Montreal (Director: Dr. Armand Frappier).

I am indebted to Dr. Victorien Fredette, associate-professor of the Department of Bacteriology of the Faculty of Medicine, University of Montreal, and to Mr. Jean-Marc Desranleau, chief of the Laboratory of Bacteriology of the Province of Quebec, Ministry of Health at Montreal, as well as to Mr. Lawrence Wilson, Office of Biology, University of Montreal, for their kind cooperation during the progress of this work and for helpful suggestions in the revision of the English text.

The able technical assistance of Miss Michelle Morin is gratefully acknowledged.

SUMMARY

A buffered, urea-containing primary plating medium is described which permits differentiation of the urease-active members of the genus Proteus (mirabilis, morganii, retgeri, vulgaris) after 15 hr incubation at 37 C from other urease negative enterobacteria (Shigella dysenteriae, Salmonella paratyphi B, Salmonella typhosa, and Escherichia coli). The medium expedites orientation as early as first isolation because it allows elimination of the ureolytic genus Proteus. This action is unlike other media, which require further identification because of the similarity of these colonies. The medium is also suitable for the differentiation of some urease-negative enterobacteria (Shigella shigae, Salmonella typhosa, Salmonella paratyphi B) and one can clearly differentiate the colonies of Escherichia coli from others. The important component of the medium is the phosphate buffer which fixes the surplus of ammonia released from urea by Proteus species. The characteristic strong alkalisation is apparent only in the immediate neighbourhood of the colonies of Proteus. The volatile acids released by the fermentation of lactose by Escherichia coli favour this influence. The growth of gram positive organisms and the swarming of Proteus species is completely controlled.

REFERENCES

Christensen, W. B. 1946 Urea decomposition as a means of differentiating Proteus and Paracolon cultures from each other and from Salmonella and Shigella types. J. Bacteriology 53, 461-466.


