A SYNTHETIC MEDIUM FOR THE L TYPE COLONIES OF PROTEUS

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The minimal requirements for growth of many Proteus strains were found by Fildes (1938) to be satisfied by a medium containing minerals, ammonium salts, lactate, and nicotinic acid. L type colonies, however, have only been grown on natural media, usually containing 10 to 20 per cent animal blood serum or other body fluids. Dienes and Weinberger (1951) state that Proteus 3B L type colonies will grow on nutrient agar without serum. The serum in complex natural media has been replaced by bovine albumin plus an acetone insoluble lipid fraction of egg yolk (Edward, 1953) and by riboflavin (Tulans et al., 1950). Dienes (1953) states that Proteus L forms will not grow on a synthetic medium that supports growth of the rods.

The present paper deals with amino acid media which support rapid growth of Proteus rods and L forms. L type colonies will be formed and will grow on a synthetic medium solidified with agar. The poor growth on natural media without added serum seems to be due to the presence of an inhibitor(s) found in several natural products, rather than the absence of growth factors.

METHODS

Two strains of Proteus were used: P. mirabilis, strain K (Medill and Hutchinson, 1953), and Proteus, strain 52, kindly supplied by Dr. Louis Dienes. Both strains gave the same response, but the data reported are for strain K. The final composition of the basal synthetic medium is shown in table 1. When a solid medium was desired, a previously autoclaved 2.2 per cent solution of agar was added to an equal volume of sterile basal medium to yield a final concentration of 1.1 per cent. The amino acids added to the medium and their final concentrations are listed in table 2. Alternative nitrogen sources used were either 0.1 per cent (NH4)2SO4 or 0.5 per cent vitamin-free casamino acids (Difco). The basal natural medium was tryptose blood base (Difco) solidified with 1.1 per cent agar. For the growth of L type colonies 200 units per ml of Schenley crystalline penicillin G were added.

Inoculations were made with 18-24 hour washed cells harvested from 5 ml of brain heart infusion broth (B.B.L.) and resuspended in 2 ml of distilled water. Agar plates were inoculated on the surface with 0.1 ml of a 5 × 10⁻³ dilution for nutrition experiments and 0.1 ml of a 10⁻³ dilution for inhibitor experiments. Inocula for rod growth were spread with a glass rod, but for L type colony growth the plates were tilted until the inocula were spread. Tubes and flasks were inoculated with one drop of the concentrated suspension.

Growth of the rod form was studied under three different conditions of oxygen tension, i.e., stationary deep broth, agitated shallow broth, and agar surface. For measurement of stationary growth the media were prepared in 16 mm test tubes in 10 ml amounts, and turbidity was measured 24 hours after inoculation with the Evelyn colorimeter with a 660 filter. Turbidity is expressed as optical density. Growth of the rod forms on agar surfaces is confluent; and in order to provide some objective comparison between experiments and with the broth growth, the surface growth was suspended in 10 ml of distilled water for turbidity determinations. The general validity of this method was not determined, but it is considered adequate for the large differences dealt with here. Aerobic growth took place in 125 ml Erlenmeyer flasks with 20 ml of medium per flask. One hour after inoculation these flasks were shaken continuously on a New Brunswick Scientific Company shaker for 23 hours. The cultures were then poured into 16 mm test tubes.

for the measurement of turbidity. The formation and growth of L type colonies were determined by colony counts and estimation of colony size.

In order to obtain L type growth in liquid media, the method of Abrams (1954) was used. An agar block containing L type colonies was inoculated into a flask of broth and continuously shaken for four days. These L forms were washed and used as inocula for synthetic liquid media. Incubation of all cultures was at 37 C.

RESULTS

In the basal synthetic broth medium plus (NH₄)₂SO₄, the rods grow to visible turbidity in 48 hours. The addition of agar and penicillin permits the development of very small L type colonies after five days of incubation. However, the main interest of this investigation was in a synthetic medium which would support growth of the rods and L forms comparable to that obtained on natural media. As the medium is improved, both the number of L type colonies and the colonial size increase. The addition of vitamin-free casamino acids (Difco) greatly accelerates the growth of the rods and the formation and growth of L type colonies. On this medium L type colonies are visible after 24 hours, and by 48 hours many colonies reach a diameter of 0.5 mm. If the casamino acids are replaced by glutamic acid alone, the response in broth is not equal to that on casamino acids; and growth on agar of L forms and rods does not occur in 24 hours. The mixture of the amino acids listed in table 2 replaces the casamino acids completely for growth in broth and for agar growth of both the rods and L forms. The results of a representative experiment are presented in table 3.

It can be seen from the photographs in figures 1-4 that both the synthetic and nonsynthetic

TABLE 3

<table>
<thead>
<tr>
<th>Addtion to Basal Medium</th>
<th>Growth</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Rods</td>
<td>L forms</td>
</tr>
<tr>
<td></td>
<td>Aerated broth</td>
<td>Stationary broth</td>
</tr>
<tr>
<td>(NH₄)₂SO₄, 0.1%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Urea, 0.5%</td>
<td>0.886</td>
<td>0.297</td>
</tr>
<tr>
<td>Glutamic acid, 0.1%</td>
<td>0.276</td>
<td>0.131</td>
</tr>
<tr>
<td>Amino acid mixture</td>
<td>0.710</td>
<td>0.284</td>
</tr>
</tbody>
</table>

The growth of the rods was measured after 24 hours and that of the L forms after 48 hours.

* Urea, 35 mg/ml, was added to the stationary broth tubes.
† Growth of rods, to the extent of slight turbidity, appears in 48 hours; tiny L type colonies appear in 5 days.
‡ After 48 hours, growth equivalent to 0.465 appears.
§ After 72 hours, a small number of tiny L type colonies appear.
Figures 1-4. The growth of L type colonies on various media. The magnification is the same in all photographs, and all colonies are three days old.

Figure 1. Tryptose agar.

Figure 2. Tryptose agar with 10 per cent horse plasma.

Figure 3. Basal medium with 0.5 per cent casamino acids.

Figure 4. Basal medium with the amino acid mixture.

amino acid media solidified with agar support better growth of the L type colonies than do natural media with or without plasma.

The addition of one per cent PPLO serum (Difco) in the natural basal agar medium yielded less luxuriant growth than the addition of horse plasma, and the addition of 50 µg of riboflavin per ml to the natural basal agar supported poorer growth than the basal agar alone.

Granular growth of L forms, similar to that described by Abrams (1954), occurred in liquid synthetic medium.

The effect of several natural media ingredients on the growth of L forms and rods on agar media is presented in table 4. It can be seen that yeast extract, peptone, and hydrolyzed casein inhibit the growth of the L forms although the growth of the rods is stimulated. Yeast extract is at least as inhibitory to the L forms in 0.1 per cent concentration as in 1.0 per cent; thus the inhibitory effect is not caused by a high concentration of nutrients.

DISCUSSION

The nutritional requirements of L forms and the normal rod form of Proteus are essentially similar since both forms grow well in a defined amino acid medium. The main difference appears to be the sensitivity of the L forms to an inhibitor(s) in some natural products. The in-
TABLE 4
The effect of natural media ingredients on the growth of rods and L forms on agar

<table>
<thead>
<tr>
<th>Additions to Basal Medium</th>
<th>Growth</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Rods</td>
<td>L type</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optical</td>
<td>colonies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>density</td>
<td></td>
</tr>
<tr>
<td>0.5% casamino acids</td>
<td>0.894</td>
<td>1,736</td>
<td>1,681</td>
</tr>
<tr>
<td>0.5% casamino acids +</td>
<td>1.187</td>
<td>161</td>
<td>154</td>
</tr>
<tr>
<td>1% Difco yeast extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5% casamino acids +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1% Difco yeast extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5% casamino acids +</td>
<td>1.034</td>
<td>489</td>
<td>587</td>
</tr>
<tr>
<td>0.5% Difco peptone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5% casamino acids +</td>
<td>1.204</td>
<td>162</td>
<td>211</td>
</tr>
<tr>
<td>0.5% Sheffield N-Z-amine, B</td>
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Amino acid media are described which support rapid growth of Proteus rods and L forms. The formation and growth of L type colonies are better on synthetic media solidified with agar than on natural media. The poorer growth on natural media appears to be due to the presence of inhibitor(s) in several natural products.

REFERENCES


