Effect of nitrogen source and pH on the growth of a glutamine requiring strain (glm)

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Abstract
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Figure 2. Viability of a microconidial suspension stored in distilled water (A), or in Fries minimal minus a carbon source (B). Viability determined as in Figure 1 above.

Under the experimental conditions used, microconidiation begins about the third day after inoculation. Figure 2 summarizes the viability of microconidia, harvested from a 5 day old culture, and stored at 4°C as a suspension in water (curve A), or in Fries minimal, minus a carbon source (curve B). In the light of recent data of Brockman and deSerres (1963 Am. J. Bot. 50: 709) on the "sorbore toxicity" effect of sucrose vs a mixture of glucose and fructose, it is possible that much higher initial viabilities of microconidia can be obtained. Further, microconidia are known to be susceptible to desiccation, and higher viability is known to be associated with growing microconidial strains in humid atmospheres.

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Reich and Silagi (1963 Proc. Intern. Congr. Genet. 11th, The Hague, 1:49) reported a number of allelic mutants of independent origin which require L-glutamine (500 mg/l) for growth. glm strains are not leaky on minimal, are very sensitive to L-amino acids, especially methionine, and lack the enzyme glutamine synthetase (Reich, personal communication).

The results reported below were obtained on glm allele 1015 (FGSC#1115). FGSC #1115 is the double mutant glm, inos, carrying inos allele 89601. All media were supplemented with inositol (25 mg/l). L-glutamine was sterilized by filtration. Wild type strain STA4 (FGSC#262) was used for comparative purposes. During routine testing it was observed that the glm strain grows on minimal synthetic agar slants with little or no delay either in growth or conidiation, and grows especially well on Neurospora Culture Agar (Difco Laboratories, Detroit, Michigan). Neurospora Culture Agar contains proteose peptone, yeast extract, maltose and agar and has a final pH of 6.7. Reich and Silagi reported delayed growth on all media tested; no such delay was observed on Neurospora Culture Agar. Thus, it would appear that this medium would be ideal for the routine culture of glm strains. Reich and Silagi used Vogel's medium N throughout their investigations. Our data confirm that the glm strain fails to grow in minimal medium N even after long periods of incubation (see Figure 1 and Table ).
Nitrogen in synthetic cross medium is entirely in the form of nitrate ions, while medium N contains both ammonium and nitrate ions (supplied as NH₄NO₃ at 2 g/l). Medium N also contains citrate ions at a concentration of 3 g/l. In flask assays substantial growth of the glm strain was obtained in media free from ammonium ions. Mycelial growth on a modified minimal synthetic cross (containing only 0.2 g/l MgSO₄ instead of 0.5 g/l), and adjusted to an initial pH of 6.5 was equal to that obtained in medium N supplemented with L-glutamine (1 g/l) (compare Figure I, point B with minimal only in the absence of...
Further, growth of the glm strain was found to be progressively inhibited by increasing concentrations of NH₄Cl (Figure 1, lower curve). Ammonium ion inhibition does not occur in media supplemented with L-glutamine (Figure 1, upper curve). Either in NH₄Cl supplemented (2 g/l) or unsupplemented media, increasing amounts of citrate were without effect except at the highest concentration (4 g/l). It is perhaps worthy of note that the glm strain grown in media supplemented with 4 g/l of citrate plus L-glutamine showed evidence of a colonial growth habit and altered carotenoid pigmentation. The dry weight of glm mycelia grown in ammonium ion-free media supplemented with L-glutamine is over twice that when grown on medium N supplemented with L-glutamine (Figure 1, point B), approaches that of wild type grown on medium N supplemented with L-glutamine (Figure 1, point D), and exceeds that of wild type grown on the ammonium ion-free medium supplemented with L-glutamine (Figure 1, point F). When grown in medium N, even the wild type strain is markedly stimulated by glutamine or asparagine (Figure 1, points C, D, E). Apparently additional inhibitory components other than ammonium ions exist in medium N for the glm strain.

Experiments using flask cultures to investigate the effect of pH on the glm strain were inconclusive because it was impossible to control the pH changes which occur during growth. However, using the growth tube technique in which the growing mycelial frontier is constantly exposed to fresh media, results were obtained (Figure 2 and Table I). Under these conditions the growth rate of the glm strain on minimal, arbitrarily plotted 50 hours after inoculation, shows a marked pH dependence. This effect is largely obliterated in the presence of added L-glutamine. In contrast, the wild type strain is relatively insensitive to pH.
Table 1

Effect of pH on 'lag' period. Experimental conditions given in legend to figure 2.

<table>
<thead>
<tr>
<th>pH of media</th>
<th>Hours for initial 20 mm growth</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>glm minimal + L-glutamine</td>
<td>glm minimal + L-glutamine</td>
<td>wild type</td>
</tr>
<tr>
<td>3.3</td>
<td>63.0</td>
<td>33.0</td>
<td>19.0</td>
</tr>
<tr>
<td>3.75</td>
<td>36.5</td>
<td>25.5</td>
<td>20.5</td>
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<tr>
<td>4.2</td>
<td>38.5</td>
<td>25.0</td>
<td>19.5</td>
</tr>
<tr>
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<td>24.0</td>
<td>19.5</td>
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<td>5.2</td>
<td>35.0</td>
<td>23.5</td>
<td>20.0</td>
</tr>
<tr>
<td>5.65</td>
<td>35.0</td>
<td>24.0</td>
<td>20.0</td>
</tr>
<tr>
<td>6.1</td>
<td>30.5</td>
<td>18.5</td>
<td>15.5</td>
</tr>
<tr>
<td>medium N</td>
<td>&gt;103.0</td>
<td>22.5</td>
<td>19.0</td>
</tr>
</tbody>
</table>

All cultures were sampled at the end of the growth period and showed no change in requirement.

over the range tested, and identical results were obtained with or without added L-glutamine. The effect of pH on the glm strain was manifested not only in growth rates but also in the duration of the 'lag' period prior to linear growth after inoculation (Table I). The effect was less marked in the presence of L-glutamine but persisted at pH 3.3. No 'lag' was noted for the wild type strain either with or without added L-glutamine (Table I).

In summary, the glm strain is inhibited by components in minimal medium N. One of these is ammonium ions. Ammonium ion inhibition can be overcome by L-glutamine. Growth in ammonium ion-free minimal synthetic cross medium equals that in medium N supplemented with L-glutamine. However, even in ammonium ion-free medium L-glutamine is markedly stimulatory for growth. Addition of L-glutamine to this medium results in growth of the glm strain nearly equal to wild type on medium N containing L-glutamine, and somewhat greater than wild type on synthetic cross medium supplemented with L-glutamine. In growth tubes the glm strains shows a sensitivity to low pH which is largely overcome by L-glutamine. Over the pH range investigated, the growth rate of the glm strain is less than that of wild type, but the final mycelial weight is equal. The mechanism of action of these phenomena has not been established. For optimal growth glm strains should be grown on Neurospora Culture Agar. To score isolates from crosses segregating for glm, medium N should give the clearest results. - - - Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire.