STUDIES ON AN INTERESTING
SACCHAROMYCES CARLSBERGENSIS MUTANT

by

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INTRODUCTION

CHAKRABERTY (1962) came across a mutant single-spore culture, 26a, during his studies of a local Baker's yeast strain 60 C92 belonging to Saccharomyces carlsbergensis. He was unable to determine the nature of its nutritional deficiency. This mutant failed to respond to the members of vitamin B complex and also to the amino acids. It was therefore decided to carry on further biochemical and genetical studies on this mutant.

MATERIAL AND METHODS

Organisms: The single spore culture 26a from strain 60 C 92 was redesignated as Hla for the sake of brevity. Two uracilless single-spore cultures, 96E 6 ch×4, or Jl, obtained by X-ray treatment by JALIL (1958) and XVI/3 or Dl, obtained by U.V. treatment by DEY (1961), were also used in these investigations.

Media and methods: Investigations on nutritional deficiency were conducted by appropriately supplementing a synthetic medium made after BURKHOLDER (1943) and BURKHOLDER, MCVEIGH, & MOYER (1944). It had the following composition per litre; Dextrose, 20 g; recrystallised asparagine, 2 g; MgSO₄·7H₂O, 2.0 g; CaCl₂·2H₂O 0.33 g; KH₂PO₄, 1.5 g; (NH₄)₂SO₄, 2.0 g; KCl, 0.1 g. Trace elements were added to this medium in parts per million as follows: B, 0.01; Mn, 0.01; Zn, 0.07; Cu, 0.01; Mo, 0.01; and Fe, 0.05. Sodium borate, manganese chloride, zinc sulphate, copper sulphate, sodium molybdate and ferrous sulphate were the actual forms in which these trace elements were used.

Vitamin supplements were added in gamma per litre of medium as follows: thiamine, 200; riboflavin, 100; pyridoxine, 200; niacin, 200; biotin, 2; pantothenic acid, 200; and inositol, 1000. Thiamine
hydrochloride, pyridoxine hydrochloride, biotin methyl ester, and calcium pantothenate were the actual forms of these vitamin compounds used.

Tests for aminoacid deficiencies were conducted by supplementing the synthetic medium with 3.2 mg of each of the following 25 aminoacids per 100 ml: l-valine, dl-isoleucine, l-leucine, dl-threonine, dl-phenylalanine, dl-methionine, l-lysine, l-arginine, l-histidine, l-tryptophan, glycine, dl-alanine, dl-serine, β-alanine, dl-norvaline, dl-norleucine, dl-ornithine, dl-citruline, l-asparagine, l-glutamic acid, l-hydroxyproline, l-proline, dl-cystine, dl-tyrosine, and dl-aspartic acid.

Tests for deficiencies in DNA or RNA synthesis were done by supplementing 100 ml of synthetic medium with 20 mg of DNA or RNA. DNA and RNA hydrolysates were added at the rate of 20 cc per 100 ml of the synthetic medium. The DNA and RNA hydrolysates were prepared following the procedures of MARSHAK & VOGEL (1951) and WYATT (1951) respectively. The utilisation of the five bases adenine, guanine, thymine, cytosine, uracil and of 5-methyl cytosine was investigated by adding 20 mg of each of the bases per 100 ml of the synthetic medium. Tubes in each test were incubated at 25° C for 72 hours and then observed for the growth of the mutant. All tests were made in duplicate.

Stocks were maintained on yeast-glucose-peptone medium with the following composition:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Yeast extract</td>
<td>1 g</td>
</tr>
<tr>
<td>Bacto peptone</td>
<td>2 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>2 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

All media were sterilized at 15 pounds pressure for 15 minutes.

**Experiments and Results**

In order to determine the nature of nutritional deficiency in the mutant Hla, it was inoculated in (1) Basal Synthetic medium (BSM). (2) BSM plus 7 members of vitamin B complex, (3) BSM plus 25 aminoacids, (4) BSM plus DNA, (5) BSM plus RNA and (6) BSM plus 5 bases namely, adenine, guanine, cytosine, thymine, and uracil. All the tubes were incubated at 25° C for 72 hours. Hla failed to grow in all the six media.

The lack of response to members of vitamin B complex, amino-acids, as well as nucleic acids was a bit puzzling. It was thought that possibly the concentrations of supplements used were toxic to the growth of this mutant. The concentrations of the supplements were therefore brought down to one fourth and one tenth and the mutant was reinoculated. It again failed to grow in any of the supplemented media and it became obvious that the concentrations of supplements used in previous experiments were not likely to be inhibitory to the growth of this mutant.