THE PRODUCTION OF YEAST-GROWTH STIMULANTS
BY THE MOLDS

I. ASPERGILLUS NIGER, TRICHODERMA LIGNORUM, AND
ASP. CLAVATUS

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During the investigation of the growth of yeast on synthetic media it had been observed that occasionally a greatly enhanced growth was shown in media which had been accidentally contaminated by molds. This phenomenon was so striking as to warrant a systematic study of the production of yeast growth stimulants by several molds. The studies here reported include the molds Aspergillus niger, Trichoderma lignorum, and Asp. clavatus1 and the yeast Saccharomyces cerevisiae (No. 4226 American Type Culture Collection). Further studies are in progress.

The subject of yeast growth stimulants known under the name of bios has been adequately reviewed up to 1925 by Tanner (1925) while later developments have been considered by Buchanan and Fulmer (1930).

Two media were used for the growth of the molds, one containing sucrose as a substrate and the other glycerol. The sucrose medium, medium C (Fulmer, Nelson and Sherwood 1921, Fulmer and Nelson, 1922) contained per 100 cc.: 0.188 gram \( \text{NH}_4\text{Cl} \), 0.100 gram \( \text{K}_2\text{HPO}_4 \), and 10 grams sucrose. The glycerol medium used was a modified Czapek's medium as developed by Naylor, Weisbrodt-Smith, and Collins (1930) and contained per liter: 0.5 gram \( \text{MgSO}_4 \), 1 gram \( \text{K}_3\text{HPO}_4 \), 0.5 gram \( \text{KCl} \), 0.01 gram \( \text{FeSO}_4 \), 5.3 grams \( \text{NH}_4\text{Cl} \) and 10 grams glycerol.

1 The molds were kindly furnished by Dr. J. C. Gilman of the Department of Botany.
The molds were allowed to grow for two weeks at about 20° in 500 cc. of medium in a 2-liter flask. The mats were removed and the medium passed through Berkfeld filters. The filtrates from the sucrose medium were used unaltered for the growth of the yeast except for the adjustment of the pH, while sucrose (100 grams per liter) was added to the glycerol medium filtrates. Growth of the yeast in these media was compared with that in similar media in which no mold had grown. The media were sterilized by filtration. This procedure was adopted in view of the findings of Fulmer and Huesselman (1927) that yeast growth stimulants may be formed during the sterilization of medium C under pressure. The media were inoculated with yeast to a count of one (250,000 cells per cubic centimeter) and incubated for forty-eight hours at 30° when the count was again determined.

In table 1 are given the results obtained by use of the media in which Aspergillus niger had been grown. It is at once evident that this mold produces yeast growth stimulants whether acting on sucrose or on glycerol.

Six liters of the filtrate from the glycerol medium which had supported the growth of the mold were evaporated to dryness at

<table>
<thead>
<tr>
<th></th>
<th>SUCROSE MEDIUM</th>
<th>GLYCEROL MEDIUM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Initial pH</td>
<td>6.9</td>
<td>6.9</td>
</tr>
<tr>
<td>86</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>97</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Final pH</td>
<td>2.7</td>
<td>5.5</td>
</tr>
</tbody>
</table>

I, III, V = media had supported mold growth.
II, IV, VI = media had not supported mold growth.
About 20 grams of a gray powder resulted. This material is not entirely soluble in water. A suspension of this residue was tested on the growth of yeast in medium C. The results are shown in table 2. These data indicate that the stimulating material is highly concentrated in this residue and is heat-stable.

<table>
<thead>
<tr>
<th>MILLIOMGAMS PER 100 CC. MEDIUM C</th>
<th>COUNT</th>
<th>MILLIOMGAMS PER 100 CC. MEDIUM C</th>
<th>COUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14</td>
<td>.</td>
<td>45</td>
</tr>
<tr>
<td>0.5</td>
<td>16</td>
<td>50</td>
<td>74</td>
</tr>
<tr>
<td>1.0</td>
<td>16</td>
<td>100</td>
<td>64</td>
</tr>
<tr>
<td>5.0</td>
<td>18</td>
<td>125</td>
<td>101</td>
</tr>
<tr>
<td>10.0</td>
<td>21</td>
<td>150</td>
<td>94</td>
</tr>
<tr>
<td>20.0</td>
<td>31</td>
<td>175</td>
<td>115</td>
</tr>
<tr>
<td>30.0</td>
<td>26</td>
<td>200</td>
<td>102</td>
</tr>
<tr>
<td>40.0</td>
<td>29</td>
<td>250</td>
<td></td>
</tr>
</tbody>
</table>

In table 3 are listed the products reported as the result of the action of Aspergillus niger upon glycerol or sucrose together with those chemicals reported as due to the action of the mold on the primary products listed from glycerol or sucrose. The data are summarized from the review by Fulmer and Werkman (1930).
To none of the products listed may be attributed the stimulation of the yeast growth.

The stimulant above reported was an extracellular product. It seemed of interest to test the mold itself. A 200-gram sample of the dried mold was extracted for ten hours with 3 liters of water at 50 to 60°. The suspension was filtered and evaporated to a thick syropy mass in vacuum and dried in a desiccator over CaCl₂.

### TABLE 4

Yeast-growth stimulating properties of extracts of Aspergillus niger

Basal medium C for yeast. Count taken after forty-eight hours. Concentration of extract in milligrams dry material per 100 cc.

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>Count*</td>
<td>Concentration</td>
<td>Count</td>
<td>Concentration</td>
</tr>
<tr>
<td>0</td>
<td>41</td>
<td>0</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>35</td>
<td>0</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>37</td>
<td>0</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>2</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>74</td>
<td>4</td>
<td>49</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>119</td>
<td>10</td>
<td>52</td>
<td>20</td>
</tr>
<tr>
<td>30</td>
<td>116</td>
<td>20</td>
<td>56</td>
<td>40</td>
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<tr>
<td>50</td>
<td>162</td>
<td>40</td>
<td>56</td>
<td>60</td>
</tr>
<tr>
<td>60</td>
<td>141</td>
<td>60</td>
<td>55</td>
<td>80</td>
</tr>
<tr>
<td>80</td>
<td>159</td>
<td>80</td>
<td>55</td>
<td></td>
</tr>
</tbody>
</table>

* Count = cells per cubic centimeter

I. Water-soluble fraction, pH of 1 per cent solution = 4.4.
II. Alcohol-insoluble fraction, pH of 1 per cent solution = 4.7.
III. Alcohol-soluble fraction, pH of 1 per cent solution = 3.3.
IV. Material removed from alcohol-soluble fraction by cooling to −10°C. pH of 1 per cent solution = 3.7.

The resulting material was quite black and dough-like in consistency. About 30 grams of residue were obtained. This is designated as fraction I. A 25-gram sample of this fraction was continuously extracted for four days with 95 per cent ethyl alcohol. The extract in contact with the mold was greenish in color while that in the reservoir was reddish brown. On cooling the alcohol extract to −10° a considerable quantity of needle-like
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crystals separated out together with some tarry material. The alcohol-insoluble material is designated as fraction II. The alcohol-soluble material not removed by the cooling is fraction III and the material separated by cooling, fraction IV. The effects of these fractions upon the growth of yeast at 30° in medium C are shown in table 4. Fractions I, III, and IV are very rich in stimulant.

In table 5 are given data showing the production of yeast growth stimulant by *Trichoderma lignorum* and by *Aspergillus clavatus*.

### TABLE 5

*Effect upon the growth of yeast of media which had supported the growth of Trichoderma lignorum and of Aspergillus clavatus*

<table>
<thead>
<tr>
<th></th>
<th><strong>TRICHOSTERMA LIGNORUM</strong></th>
<th><strong>ASPERSILLUS CLAVATUS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sucose medium</td>
<td>Glycerol medium</td>
</tr>
<tr>
<td><strong>I</strong></td>
<td>6.9</td>
<td>6.9</td>
</tr>
<tr>
<td><strong>II</strong></td>
<td>90</td>
<td>45</td>
</tr>
<tr>
<td><strong>III</strong></td>
<td>83</td>
<td>42</td>
</tr>
<tr>
<td><strong>IV</strong></td>
<td>97</td>
<td>57</td>
</tr>
<tr>
<td><strong>V</strong></td>
<td>88</td>
<td>46</td>
</tr>
<tr>
<td><strong>VI</strong></td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td><strong>VII</strong></td>
<td>83</td>
<td>13</td>
</tr>
<tr>
<td><strong>VIII</strong></td>
<td>97</td>
<td>18</td>
</tr>
<tr>
<td><strong>Initial pH</strong></td>
<td>6.9</td>
<td>6.9</td>
</tr>
<tr>
<td><strong>Final pH</strong></td>
<td>3.10</td>
<td>5.85</td>
</tr>
</tbody>
</table>

I, III, V, VII had supported growth of mold; II, IV, VI, VIII, had not supported growth of mold.

*clavatus* on the sucrose and the glycerol media, using the same procedure as that employed in the studies on the production of the extracellular stimulant by *Aspergillus niger*.

### SUMMARY

It has been shown that the growth of the molds *Aspergillus niger*, *Trichoderma lignorum* and *Aspergillus clavatus* either on glycerol or sucrose substrate produces in the medium a growth stimulant for *Saccharomyces cerevisiae*. Preliminary studies are reported on the bios content of *Aspergillus niger*. 
REFERENCES


Fulmer, E. I., and Werkman, C. H. 1930 Index to Chemical Action of Microorganisms, etc. C. C. Thomas.
