Extraction of mycelial protein: some specific comparisons

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Abstract
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Various methods have been described for extraction of soluble mycelial proteins of 
N. crassa, and none of these methods were compared by Stone, Strickland and Bartlett (1964 Con. J. Microbiol. 10: 29). These, and other methods in use in various laboratories, allow studies. We have found that certain combinations of extraction methods are especially suited to efficient and convenient extraction of both β-glucosidase and both β-galactosidase enzymes of 
N. crassa. We have also compared the extraction of these enzyme activities with extraction of alkaline phosphatase and of total protein.

Induced mycelium was obtained by growing the "L5D" isolate on 1.5% lactose for 5 days at 30°C with rotary agitation, with cellobiose added at 2 days to a concentration of 0.001 M. Mycelium was harvested on a Buchner funnel and a 10.0 g portion was lyophilized, yielding 1.89 g dry weight. The remainder was sealed in Saran wrap and frozen. The ratio of dry to wet weight allows comparison of extraction of wet and dry mycelium.

The following extraction procedures were compared:
I. Wiley Mill: lyophilized mycelium was ground in a 50 ml chamber. Extraction was for 10 min at 60 volts input.
II. Omni-Mixer: Sorvall (Ivan Sorvall, Inc.): 3.18 g wet mycelium was extracted with 45 ml buffer with 15 g acid-washed fine glass beads; 10 min at 68 volts using a serrated impeller.
III. Virtis: 45.4.24 g (wet) + 60 ml buffer; 10 min at a setting of 68 volts with a 250 ml chamber, using sharp cutting blades.
IV. Virtis: 45.4.24 g (wet) + 60 ml buffer + 20 g fine glass beads; 10 min at 68 volts using a serrated impeller.
V. Sonifier (Branson): 1.03 g (wet) + 15 ml buffer + 5 g glass beads; or, 15 ml of samples I, II, III or IV. Sonified 11/2 min at 6.3 amperes.

All extraction procedures were carried out with samples immersed in ice on ice water both, with the exception of the Wiley Mill procedure. The buffer used for all extractions was 0.01 M phosphate (Na), pH 7.4, containing 5 x 10⁻⁵ M dithiothreitol. Note that the ratio of buffer to mycelium was the same in all extractions. Immediately after the final step of each procedure, samples were sealed in screw capped tubes and shaken horizontally at approximately 70 cycles per minute while resting in ice on a reciprocal shaker. Samples were then centrifuged for 30 minutes at 27,000 x g in a refrigerated centrifuge. The resulting supernatant crude extracts were assayed using p-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-β-D-galactopyranoside and p-nitrophenyl phosphate and using the protein assay method of Lowry, et al. (1951 J. Biol. Chem. 193: 265). Enzyme activities are presented in arbitrary units.

The results of extraction of both β-glucosidase and β-galactosidase enzymes, and of alkaline phosphatase and total protein were summarized in Table 1. From the data presented, specific activities may also be calculated. For all enzymes studied, the most effective total extraction is achieved by combining procedures II and V. The most effective single procedure is II. Expression of the results as specific activities greatly reduces the differences observed with different extraction procedures. Although differences remain, the relatively constant specific activities suggest that valid comparisons may be made between wet or dry samples.

Table 1. Effectiveness of various extraction procedures for the extraction of several enzymes.

<table>
<thead>
<tr>
<th>Extraction procedure</th>
<th>Aryl β-glucosidase (units/ml)</th>
<th>Cellobiose (units/ml)</th>
<th>β-galactosidase (units/ml)</th>
<th>Alkaline phosphatase (units/ml)</th>
<th>Protein (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>45</td>
<td>50</td>
<td>201</td>
<td>123</td>
<td>7.5</td>
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<tr>
<td>II</td>
<td>74</td>
<td>66</td>
<td>232</td>
<td>187</td>
<td>140</td>
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<tr>
<td>III</td>
<td>58</td>
<td>58</td>
<td>239</td>
<td>151</td>
<td>90</td>
</tr>
<tr>
<td>IV + V</td>
<td>69</td>
<td>91</td>
<td>244</td>
<td>191</td>
<td>152</td>
</tr>
<tr>
<td>III + IV</td>
<td>21</td>
<td>21</td>
<td>57</td>
<td>180</td>
<td>27</td>
</tr>
<tr>
<td>V</td>
<td>49</td>
<td>48</td>
<td>178</td>
<td>154</td>
<td>90</td>
</tr>
<tr>
<td>v</td>
<td>40</td>
<td>43</td>
<td>173</td>
<td>139</td>
<td>90</td>
</tr>
<tr>
<td>IV + v</td>
<td>66</td>
<td>44</td>
<td>201</td>
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<td>55</td>
<td>48</td>
<td>166</td>
<td>156</td>
<td>120</td>
</tr>
</tbody>
</table>

These results demonstrate the value of procedure II, alone or in combination with V, in comparative studies of soluble proteins of 
N. crassa. It should be emphasized, however, that use of this and certain other extraction procedures, has yielded erratic results if not followed by the gentle reciprocal agitation prior to centrifugation.

The origins of many extraction procedures are very difficult to trace, and we have not attempted to provide a summary of appropriate references. However, we suggest that, wherever possible, original descriptions be cited for each procedure.

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