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Techniques for colonial growth and protoplast production in Humicola grisea var. thermoidea

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Abstract

Humicola grisea is a thermophilic, cellulolytic fungus, with significant biotechnological potential for protein secretion. For convenient growth on solid medium, 70% reduction in colony diameter was achieved by the addition of 20 mM sodium citrate to modified Aspergillus complete medium (Pontecorvo et al. 1953, Adv. Genet. 5:141-238).

Techniques for colonial growth and protoplast production in *Humicola grisea* var. *thermoidea*

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Spheroplasts were produced from mycelium by enzyme digestion with a 1:1 mixture of Novozyme 234 and cellulase CP. Spheroplast production and regeneration were studied using Mg_2SO_4, KCl, NaCl and sucrose and sorbitol as osmotica, but only the first of these gave detectable regeneration, and that at circa 1%. Because of this low regeneration, optimal conditions were determined for mycelial, growth time for mycelium, and enzyme concentration and time for digestion in order to produce on adequate number of viable conidia for potential transformation. This optimal protocol is given below.

Conidia were harvested from colonies grown for 7 days at 40 C by suspending in 0.85% NaCl, and inoculated at 10(5) conidia/ml into Aspergillus liquid minimal medium plus 0.5% yeast extract and 0.2% casein hydrolysate, incubated at 41 C and 150 rpm for 20.5 h. The mycelium was harvested by filtration and washed with 0.5 M Mg_2SO_4 in 0.02 M pH 5.6 phosphate buffer. To 125 mg wet weight of mycelium, 10.5 mg of enzyme mixture in 2.5 ml 0.5 M Mg_2SO_4 was added. This was incubated at 120 rpm for 180 min, then centrifuged at 500 rpm for 30 sec to remove mycelium. The supernatant containing spheroplasts was removed and centrifuged at 4000 rpm for 10 min. The spheroplast pellet was resuspended in buffered 0.5 M Mg_2SO_4 and recentrifuged at 4000 rpm for 10 min, repeated twice. Spheroplasts were resuspended in 1.0 ml of buffered 0.5 M Mg_2SO_4.

The spheroplast preparation was diluted in buffered Mg_2SO_4 and plated on solid Aspergillus complete medium supplemented with either sodium citrate or Mg_2SO_4 osmoticum, the latter adequately restricting colony size without addition of sodium citrate. Approximately 1% of spheroplasts regenerated, with more than 99% of them osmotically sensitive. This typically gave circa 10(6) regenerable spheroplasts from circa 10(8) total spheroplasts obtained from 125 mg wet weight of mycelium.