Identification and visualization of cellulase activities from Neurospora crassa

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Abstract
To elucidate the nature of cellulase activities found in Neurospora crassa, we analyzed the "induced" spent culture medium for the components of the cellulase enzyme complex.
Identification and visualization of cellulase activities from *Neurospora crassa*

To elucidate the nature of cellulase activities found in *Neurospora crassa*, we analyzed the "induced" spent culture medium for the components of the cellulase enzyme complex. This was the prerequisite of work on the isolation of the enzymes, their purification and N-terminal sequencing.

Supernatant from a 3-4 day culture grown on Vogel's sucrose minimal medium overnight was subjected to PAGE (10% polyacrylamide). Using replicate gels, one was stained in Coomassie blue, and the other was overlaid on an agar gel containing 0.1% carboxymethyl cellulose at 25° C overnight. The exposed CMC agar gel was then stained with 0.1% Congo red, which stains the undigested CMC. Volumes of 1 ul to 50 ul of supernatant containing less than 1 ug of protein gave detectable zones of clearing of CMC, detected visually on a light box after differential destaining with 1M sodium chloride.

With the cell-1 (T11, FGSC# 4335 and 4336) mutant or wild type (74-OR23-IA), three zones of CMC clearing were visible after exposure to filter paper-induced but not uninduced supernate. The major band of activity was stable in SDS-PAGE, and its Mr was between 60,000 and 70,000. The band migrated to the same position in PAGE without SDS, suggesting that the active enzyme is a simple monomer. Two other bands of clearing were circa 50,000 (the weakest) and 30,000. With wild type (74-OR23-IA), only the 30,000 form of the enzyme was produced in sufficient quantity to be detectable when induced with cellulbiose rather than filter paper...

A rapid method for DNA extraction is described. It is equally efficient with small or large quantities of mycelium, produces readily restricted DNA, and is comparable with that produced by the method of Case et al. (1979, PNAS 76:5259) in concentration of DNA obtained and average fragment size.

Mycelium from an overnight culture was harvested through a Whatman no. 1 filter, washed and freeze-dried. The mycelium (~50-100 mg) was placed in a 50 ml Sorvall tube, and an equal volume of 6M urea containing 2% SDS was added. The mixture was left on ice for 10-15 minutes, after which it was centrifuged at 10,000 rpm. The supernatant was transferred and extracted with phenol 2-3x. The DNA was precipitated from the aqueous phase by the addition of 2 vol of cold ethanol, collected by centrifugation, and dissolved in 100-300 ul of TE buffer, pH 8. RNA may be removed at this stage with RNase.

This method appears to be generally applicable to unicells, mycelium and other filamentous organisms. It has been successfully used also on the moss *Physcomitrella patens*...

Recently we found what appeared to be a new auxotroph in one of our stocks. It was eventually traced to the formate (for) locus. Later we found that it was not a new allele, but was the original C24 for allele which had gotten into the stock by an ancient error in stockkeeping.

In the course of determining the auxotroph's growth requirements, we found that it responded strongly to three combinations of supplements not previously reported for for. The for mutant had previously been reported to grow weakly on adenine alone, strongly on adenine plus methionine, and strongly on formate or formaldehyde. On our auxanograms the response to adenine alone is very weak, but the response to adenine combined with histidine, tryptophan or ascorbic acid is very strong. Neither histidine nor tryptophan is effective without adenine. Ascorbic acid gives a definite response without adenine but gives a stronger response when adenine is present.

All tests were done auxanographically. The supposed new auxotroph and a standard stock of for (an f1 of FGSC 133) behaved identically in all tests, and both grew well on formate.