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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-OR23-1A), only the 30,000 form of the enzyme was produced in sufficient quantity to be detectable when induced with cellobiose rather than filter paper. - - -Department of Genetics, Leeds University, Leeds LS2 9JT, United Kingdom.