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CHITINASE PRODUCTION FOLLOWING CO-IMMOBILIZATION OF *MICROMONOSPORA CHALCAE* WITH CHITIN IN CALCIUM ALGINATE.

A. O'Riordan, M.L. McHale, J. Gallagher[+] and A.P. McHale[+]*

Dept. of Biological Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland.

+ Dept. of Microbiology, Trinity College, Dublin 2, Ireland.

SUMMARY: The actinomycete *Micromonospora chalcae* produces a chitinolytic system following growth on chitin containing medium. When the organism was co-immobilized in calcium alginate, the amount of chitinase produced was 2-fold higher than the levels produced by the free system. When the immobilized organism was used in a batch fed reactor system it was capable of producing much more enzyme than the free system.

INTRODUCTION

Chitin, a B-linked polymer of N-acetyl-glucosamine, as distinct from cellulose, represents a vast, renewable fermentation feedstock of both carbohydrate and nitrogen. Global estimates of chitinous waste materials accessible as potential fermentation feedstock are in excess of 150 kilotonnes per annum (Knorr, 1986). Many organisms have been shown to produce chitinase systems following growth on chitin containing media (Jeniaux, 1966; Beyer *et al.*, 1979; Trimble *et al.*, 1970) The actinomycete *Micromonospora chalcae* has the ability to grow with chitin as the major carbon source and produces a chitinolytic system (Gallagher *et al.*, 1989). However, as in cellulase production, the most commonly used inducers are insoluble and the development of continuous enzyme production systems is more difficult. To reduce enzyme production costs (Dale, 1987) it would be advantageous to develop a continuous or at least semi-continuous system for enzyme production. We have previously shown that the filamentous fungus *Talaromyces emersonii* CBS 814.70, when co-immobilised with cellulose in calcium alginate, is capable of increased cellulase production in both batch and fed-batch fermenter systems (McHale, 1988). Here we demonstrate that *Micromonospora chalcae* produces increased levels of chitinase in a microorganism/substrate co-immobilised system and that this system is

capable of producing increased yields of chitinase in both batch and batch fed systems. On the basis of results reported here and elsewhere (McHale, 1988) such systems may be generally suitable for the development of semi-continuous systems in fermentations where insoluble inducers are utilized.

MATERIALS AND METHODS

Organism: *Micromonospora chalcae* from the National Collection of Marine Bacteria (UK) was maintained on 1.5% (w/v) agar plates containing 0.5% (w/v) bactotryptone, 1% (w/v) tryptone and 1% (w/v) chitin (from crab shells, Sigma, UK) at 30°.

Submerged Culture of M.chalcae *on chitin containing media.*: The organism was cultured in 100mls of medium in 250ml conical flasks in a shaking incubator at 30°C. The liquid medium consisted of L broth containing 2% (w/v) chitin.

Co-immobilisation of M. chalcae *with chitin in Ca alginate*: 2.5g of washed cells were mixed with 75mls of 2%(w/v) sodium alginate (Kelco, UK) containing 1g chitin. The mixture was then added drop-wise to 150ml of 50mM $CaCl_2$ and the resulting beads, ca. 1.5mm, were allowed to stand in the $CaCl_2$ solution for 1h. The beads were then transferred to 250ml flasks containing 100ml of medium without chitin.

Chitinase assay: 20ul aliquots of culture filtrate culture filtrate were incubated with 0.5 ml 0.2M phosphate buffer, pH 7.0 containing 2uM p-nitrophenyl-B-D-chitobiose at 30°C for various lengths of time in order to ensure linearity. Assays were stopped by the addition of 0.5ml of 0.4M glycine buffer, pH 10.4 and the absorbance at 430nm was determined. Activity is expressed as moles of p-nitrophenol released per min. per ml of culture filtrate.

RESULTS AND DISCUSSION

Production of chitinase activity by the co-immobilised and free systems in batch culture. The immobilised and free systems were set up as described above and sampled daily. From the results (Fig.1) it is clear that enzyme production by both systems is similar up to about 36h. Production by both systems reached a peak at approximately 3.0 days, and while the free system produced 0.6U/ml of culture filtrate, the immobilized system produced significantly greater amounts of activity, 0.9U/ml of culture filtrate (Fig.1). When fermentation was allowed to continue production by the free system decreased to approximately 0.25U/ml of culture supernatent while the immobilised system, though there was a slight decrease in

production, continued to produce relatively higher amounts of chitinase activity (Fig.1). Very similar observations were previously made for production of cellulase by the thermophilic fungus *Talaromyces emersonii* CBS 814.70 co-immobilised with cellulose (McHale, 1988).

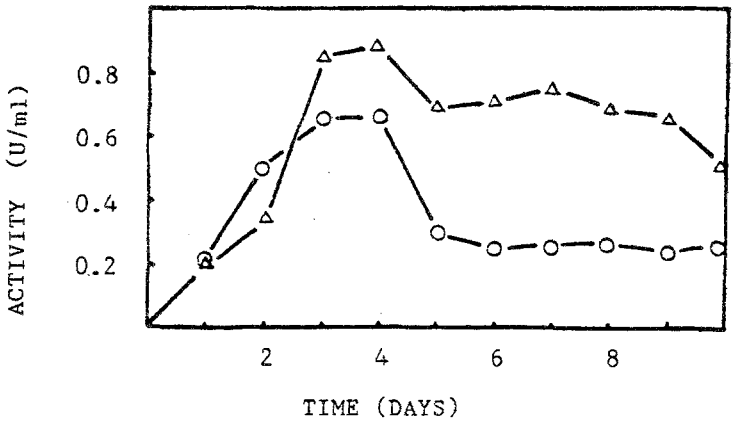

Fig. 1. Chitinase production by immobilised (-) and free (-) *M.chalcae* in batch culture.

Chitinase production in batch-fed culture. Fermentation were continued for a period of 16 days in a batch-fed manner. Both the free and immobilised systems were re-fed on days 6 and 12. The free system was fed by harvesting cells and residual chitin by centrifugation and resuspending in medium without chitin while the immobilised system was re-fed by decanting the old medium from the beads and resuspending them in fresh medium in the absence of chitin. The results (Fig.2) show that as previously (Fig.1) production of chitinase up to day 3 was lower in the free system than in the immobilized system. Following refeeding at both days 6 and 12 production of enzyme by the immobilised system was much higher than that produced by the immobilised system.

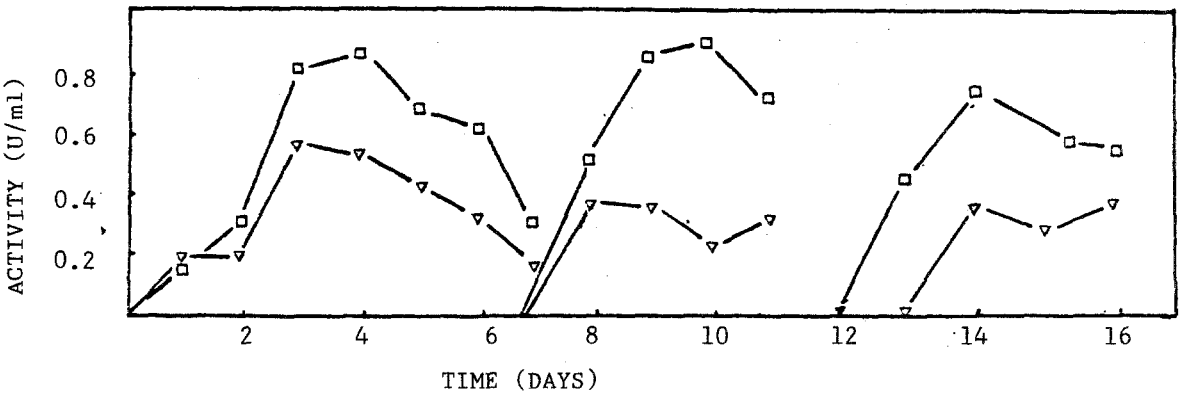

Fig. 2. Chitinase production by batch-fed immobilised (-) and free (-) *M.chalcae*

These results are similar to those obtained previously by co-immobilising the fungus *T.emersonii* with cellulose and examining cellulase production (McHale, 1988), and confirm the advantage of co-immobilisation of the inducer substrate. Such a method could potentially be used in all systems which utilise solid substrates as inducers in order to reduce handling problems in the development of continuous production processes.

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