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SOLID STATE FERMENTATION FOR CHITOSAN HYDROLYSING ENZYMES PRODUCING BIOACTIVE CHITOSAN OLIGOMERS

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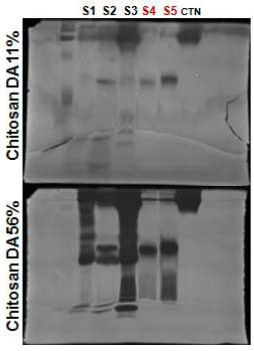
Solid state fermentation (SSF) holds tremendous potential for the production of enzymes. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source [1]. The process has some advantages over submerged fermentation among which are: lower cost, obtaining highly concentrated products, and lower risk of contamination. Among the several factors that are important for microbial growth and enzyme production using a particular substrate, particle size and moisture level/water activity are the most critical [2].

The goal of this project is to obtain bioactive amino-oligosaccharides through chitosanases produced by solid state fermentation of shrimp shells by *Trichoderma polysporum*. These wastes are sources of protein and chitin that can be used for other purposes such as cultivation of micro-organisms. The crude enzyme mixtures obtained were then used in the hydrolysis of chitosan for the production of potentially bio-active oligomers.

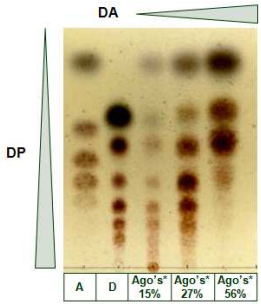
We analyzed the chitosanolytic enzymes produced by *Trichoderma polysporum* under different fermentation conditions and their hydrolysis products, using electrophoresis followed by activity staining and TLC of the oligomeric products. The chromogenic derivatives p-nitrophenyl-N-acetyl-β-D-glucosaminide (pNP-GlcNAc), p-nitrophenyl-β-D-N,N'-diacetylchitobiose [pNP-(GlcNAc)2] and p-nitrophenyl-β-D-N,N',N''-triacetylchitotriose [pNP-(GlcNAc)3](Sigma) were used as substrates for determination of chitinolytic activity. The enzyme cocktail produced by *T. polysporum* using different inducing substrates such as shrimp shell differed in terms of quantity and quality of iso-enzymes produced (Figure 1), and consequently, different oligomeric products were obtained when the crude enzyme mixtures were incubated with chitosan polymers (data not shown).

Figure 2 shows the TLC separation of the oligomeric products obtained when the crude enzyme mixture of SSF was incubated with polymeric chitosans with three different degrees of acetylation (DA).



Legend: S1 – Extract of Submerged Fermentation (SmF) from *Trichoderma* Complete Medium (TCM); S2 – Extract of SmF (TCM+shrimp shell); S3 – Extract of SmF (TCM+colloidal chitin); S4 – Extract of SSF (filtration in paper filter and centrifugation); S5 – Extract SSF; CTN – Chitosanase (standard).

Fig. 1. Evaluation of chitosanolytic enzyme production during SSF and SmF.



Legend: A: N-acetylglucosamine (DP 1,3,4,5,6); D: glucosamine (DP 1,2,3,4,5,6); *Ago's: Chitosan oligomers.

Fig. 2. Chitosan oligomer profiling.

The products obtained when different chitosans were incubated with the enzyme cocktail differed in their degree of polymerisation (DP): the lower the DA of the substrate, the higher the DP of the products, clearly indicating the presence of chitinase rather than chitosanase activity.

For the bioactivity assays, we will analyze the induction of an oxidative burst in plant cells, the induction of disease resistance in intact plants, the induction of an inflammatory response in human macrophages, and the antimicrobial activities.

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