



## BATCH PRODUCTION OF CHITINOLYTIC ENZYMES ChiANIMA AND ChiA74 IN *ESCHERICHIA COLI* K12-JM109 TO GENERATE ANTIMICROBIAL CHITIN DERIVED OLIGOSACCHARIDES

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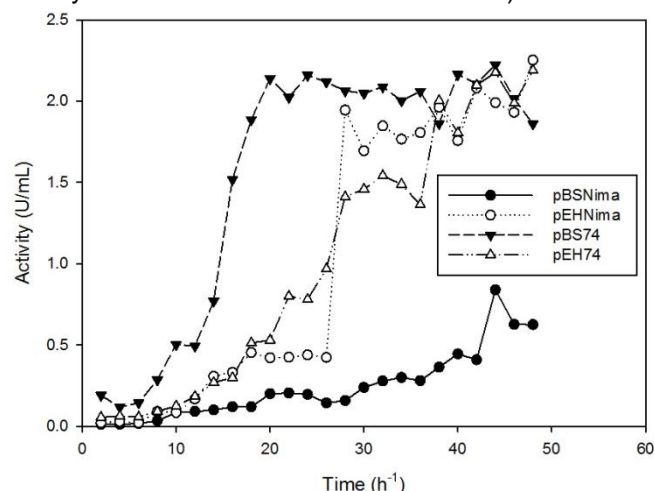
**Key words:** Chitinases, chitin-derived oligosaccharides, ChiA74.

**Introduction:** Large scales production of chitinases has gained great interest because of their ability to hydrolyze chitin for generating chitin-derived oligosaccharides (C-COS). The potential uses in biotechnology are: as biopreservatives, in biocontrol and biotherapeutics agents with activity against clinically significant food-borne pathogenic bacteria and etiological agents of diseases, including the bacteria that cause diarrhea and emetic syndromes in humans. Here we expressed endochitinase ChiANima of *Serratia marcescens* Nima using plasmid pEHNima and pBSNima, and ChiA74 of *Bacillus thuringiensis* subsp. *kenyae* using plasmid pEH74 and pBS74 for transforming *Escherichia coli* K12 JM109 (GRAS status).

**Methods: Biological material:** Strains *E. coli* K12-pBSNima and *E. coli* K12-pEHNima harbored the wildtype *chiANima* genes of *S. marcescens* Nima, and in *E. coli* K12-pBS74 and *E. coli* K12-pEH74 harbored the wildtype *chiA74* genes of *B. thuringiensis* subsp. *kenyae*. **Batch process:** First the process was performed in a 200 mL flask and then it was scaled-up in a 2.5 L Li-flux bioreactor with Luria Bertani broth. Enzymes production parameters were controlled digitally as follow: pH 7, dissolved oxygen (DO) 1, stirrer speed 180 rpm and 37°C during 50 hours to improve the enzyme activity and production. **Chitinase activity:** Every 2 h was determined the rate of growth by optical density (600nm) and the endochitinase activity by fluorescence (Turner fluorometer 450). Supernatants were centrifugated and concentrated [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 80% saturation]. Enzymes (concentrate) were dialysed [15 kDa cut-off Spectra/membrane] to obtain crude enzyme. Then, enzymes were denatured and fractioned by SDS-PAGE [12% (w/v)] and endochitinase activity *in situ* was determined by zymogram. **C-COS generation and activity:** Reactions of crude ChiA74 and ChiANima (~1U/ml) were mixed with 0.1 mL of colloidal chitin (10% w/v), 0.2 mL of 120 mmol L<sup>-1</sup> phosphate buffer (pH 6.8), and the mix was incubated with constant agitation to avoid solid substrate precipitation at 55°C to generate C-COS. COS were analyzed by silica gel 60 (Merck) thin layer chromatography (TLC). Antibacterial activity of C-COS was determined using a modified well-diffusion method against hazardous and foodborne pathogenic bacteria.

**Results: Chitinase activity:** *E. coli* K12-pEHNima, *E. coli* K12-pEH74 and in *E. coli* K12-pBS74 the chitinolytic activity increased ~two fold (2.24, 2.12 and 2.22 U/mL)

with respect to the activity (0.5, 0.85 and 0.9 U/mL) obtained by DH5 $\alpha$  strain with the same constructions in flasks (200 mL), whereas in *E. coli* K12-pBSNima the activity was the same as in flask (0.8 and 0.83 U/mL). The zymogram analysis show that *E. coli* K12-pBS74 and *E. coli* K12-pEHchiA74 endochitinase produced has a molecular mass ~70 kDa, whereas for *E. coli* K12-pEANima and *E. coli* K12-pBSNima was ~60 kDa (no activity was detected in control *E. coli* K12).



**Fig.1** Endochitinase activity of recombinant strains of *E. coli* K12. The endochitinase activity (in units per milliliter) was detected in the supernatant with 4-MU-(GlcNAc)<sub>3</sub> as fluorogenic substrate.

Enzyme samples generated C-COS (2, 3, 4, 5 and 6 degrees of polymerization) with activity against pathogenic bacteria.

**Conclusion:** Here we confirmed that batch production of chitinases using *E. coli* K12JM109 is an excellent strategy to obtain highest activity in chitinases, and its status GRAS allow us considered a next stage in their production: optimize the parameters for mass-production by Response Surface Methodology to improve the generation of C-COS.

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### References:

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