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 pEH74 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 [(NH₄)₂SO₄, 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⁻¹ 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 DH5α 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-pEH74 endochitinase produced has a molecular mass ~70 kDa, whereas for *E. coli* K12-pEHNima and *E. coli* K12-pBSNima was ~60 kDa (no activity was detected in control *E. coli* K12).

**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.

Acknowledgements: This work was partially supported by Grant 156682 from SEP-CONACYT, México to JEB-C.

References: