# INTRACELLULAR EXPRESSION OF THE ENDOCHITINASE CHIA74 OF BACILLUS THURINGIENSIS TO CRYSTALIZE WITH CRY PROTEINS

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*Key words: Bacillus thuringiensis, endochitinase, biological control*

**Introduction.** It has been shown that homologous and heterologous chitinases act synergistically with the insecticidal Cry proteins of *Bacillus thuringiensis* (*Bt*) (1). As chitinases (Chi) are secretion proteins, they have to be obtained from supernatants of a bacterial culture and be mixed with Cry (2), procedure that could be avoided whether chitinases and Cry were occluded together and form crystals. Recently, it was reported the intracellular expression in *Bt* of a homologous chitinase under the control of pcry1Ac3 an also the formation of chimeric crystals of Cry1Ac-Chi, but they were not stables (3, 4). Alternatively, we were able to hyperexpress ChiA74 in *Bt* by using the pcytA-STAB (strong promoter-mRNA stabilizer), which allows an increment in the stability of the mRNA-*chiA74*, although sporulation and crystal size was affected (3).

Here, we engineered different constructions by deleting the ChiA74 signal peptide, and the expression of the enzyme was up-regulated by the pcytA-STAB system or under the control of the wild promoter of ChiA74. Results and perspectives of the intracellular expression of ChiA74 and its potential use in the biological control will be shown.

**Methods.** Specific oligonucleotides were designed to amplify *chiA74* without the signal peptide (ChiA74Δsp). Amplicons were ligated into shuttle vectors to left the mutants under the regulation of the pctA-STAB system or wild promoter. Also a fusion of ChiA74Δsp with the green fluorescent protein (GFP) (5) was performed. Acrystalliferous strains of *Bt* were transformed with those constructions and transformation was corroborated by PCR using specific oligonucleotides. Recombinant bacteria were cultivate in NB plus salts (2) and monitored at different times by contrast phase microscopy until lyisis. Strains were also characterizated by fluorescent microscopy, SDS-PAGE-zymograms with fluorogenic derivates to detect chitinase activity and by qPCR.

**Results.** Constructions harboring the *chiA74*Δ*sp* under the control of the wild *chiA74* promoter (pEH*chiA74*Δ*sp*) or the pcytA-STAB system (pEB*chiA74*Δsp) were sequenced and Δ*sp* was confirmed. Those constructions were introduced in 4Q7, an acrystalliferous strain of *B. thuringiensis*. When 4Q7-pEH*chiA74*Δ*sp* and *B. thuringiensis* 4Q7-pEB*chiA74*Δsp were cultivated, small bodies were observed inside the cells. Interesting, occlusion bodies were bigger when *chiA74* was under the control of the wild promoter (Fig. 1a) than gene controlled by the pcytA-STAB system (Fig 1b). It is probably that the later expression of pcytA (i.e. sporulation stage) compared with the wild promoter (i.e. vegetative state), affects the formation of ChiA74 bodies, as small bodies were observed before lysis. No occlusion bodies were observed in 4Q7 used as negative control. We demonstrated that occlusion bodies were formed by ChiA74 as they were obtained from cells, solubilized and detected with a fluorogenic derivates and molecular mass confirmed by zimograms. In addition, when GFP was ligated to the C-terminal of ChiA74, intracellular fluorescence was observed. Currently we are performing qPCR to compare the *mRNA-chiA74*Δsp in the recombinant strains and engineered different constructions with Cry-ChiA74 to show the utility of chimeric crystals to control pest.

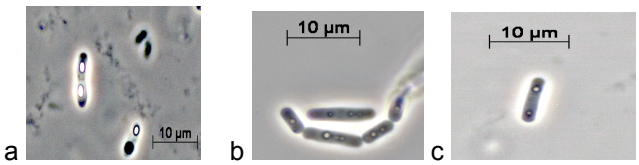


**Fig.1** Bacterial cultures observed at 90 h under contrast phase microscopy. (a) *B. thuringiensis* 4Q7-pEH*chiA74*Δ*sp,* (b) *B. thuringiensis* 4Q7-pEB*chiA74*Δsp,(c) *B. thuringiensis* 4Q7

**Conclusions.** This is the first time that occlusion bodies of different sizes, constituted by an endochitinase are formed by expressing the enzyme with the wild promoter and also by using a strong promoter with the STAB region. It was detected the formation of bigger bodies expressing *chiA74* under the control of the wild promoter than with the pcytA-STAB system. It will be necessary to carry out additional studies, (e.g. protein stability) to explain why smaller bodies were obtained using a weak rather a strong promoter. Chimeric constructions of ChiA74-Cry will be necessary to demonstrated the utility of crystallized endochitinase in the biological control.

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

**References.**

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