

## LETTER TO THE EDITOR

***Bacillus thuringiensis*-based biopesticides, are they as effective as they should be?****¿Son los biopesticidas basados en *Bacillus thuringiensis* tan efectivos como deberían?**

Dear Editor:

*Bacillus thuringiensis* synthesizes a number of invertebrate toxins that are mainly active against insects and has demonstrated its potential and safety as a biocontrol agent for decades<sup>3</sup>. These proteins include crystal (Cry and Cyt) and vegetative (secretable) insecticidal proteins (Vip) that are highly toxic against insects<sup>3</sup>. To date, *B. thuringiensis*-based biopesticides represent a clear alternative to chemical insecticides and account for about 80% of all biopesticides marketed worldwide<sup>4</sup>. Chemical insecticides contaminate water and food sources, are harmful for non-target organisms and generate insect resistance. *B. thuringiensis*-based biopesticides are biodegradable and specific for their targets. In fact, their biodegradability becomes their main disadvantage since their active ingredients, the insecticidal crystal proteins, are susceptible to natural abiotic factors such as pH, temperature and sunlight. This disadvantage has stimulated the development of different encapsulation approaches intended to protect and extend the shelf life of sprayable formulations. The encapsulation of *B. thuringiensis* toxins into recombinant bacteria is a convenient tool for enhancing their field persistence, which deserves further investigations since it will allow not only to protect the active ingredient but also to concentrate secretable insecticidal proteins (e.g. Vip3 and Cry11)<sup>2</sup>. However, in order to be successful, the system of choice should meet the following requirements: (i) a GRAS (General Recognized As Safe) bacterium should be used. Several GRAS bacteria have been successfully used for the production of recombinant proteins (e.g. *Bacillus megaterium* and *Bacillus subtilis*), representing clear alternatives for the production of encapsulated insecticidal proteins; (ii) the bacterial cell wall should remain intact as a natural microcapsule. This will allow not only to protect the toxin but also the intracellular concentration of secretable insecticidal proteins; (iii) the protein should remain encapsulated into the cell maintaining its activity. Bioassays must be performed in order to rule out loss of activity and to determine the digestibility of the

bacterial cell wall by the insect and in comparison against non-encapsulated proteins; (iv) this system should be capable of being produced at industrial scale and be competitive in the biopesticide market; therefore, expensive inducers such as IPTG (isopropyl  $\beta$ -D-1-thiogalactopyranoside) must be avoided; (v) the recombinant strains should not be capable of transferring recombinant DNA to wild type strains. Some systems prevent 'leaks' of recombinant DNA by using chemical methods that kill recombinant bacteria after protein expression (e.g. fixation of recombinant cells with lugol)<sup>1</sup>. *B. thuringiensis* has been the most used bacterium for the control of insect pests and human-disease vectors during the last forty years by means of the production of formulated pesticides and transgenic crops. However, the active ingredients of formulated *B. thuringiensis* pesticides are susceptible to different environmental factors that diminish their activity, also limiting their shelf life after application. The improvement of their residual activity will not only allow to formulate secretable toxins but also stimulate their development and increase their presence in the global pesticide market, which is currently as low as 2%<sup>5</sup>.

**References**

1. Grace JK, Ewart DM. Recombinant cells of *Pseudomonas fluorescens*: a highly palatable encapsulation for delivery of genetically engineered toxins to subterranean termites (Isoptera: Rhinotermitidae). *Lett Appl Microbiol*. 1996;23:183–6.
2. Hernández-Rodríguez CS, Ruiz de Escudero I, Asensio AC, Ferré J, Caballero P. Encapsulation of the *Bacillus thuringiensis* secreted toxins Vip3 Aa and Cry1 Ia in *Pseudomonas fluorescens*. *Biol Control*. 2013;66:159–65.
3. Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR, Dean DH. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol Mol Biol Rev*. 1998;62:775–806.
4. Whalon ME, Wingerd BA. Bt: mode of action and use. *Arch Insect Biochem Physiol*. 2003;54:200–11.
5. Yang W, He K, Zhang J, Guo S. pH-controlled *Bacillus thuringiensis* Cry1 Ac protoxin loading and release from poly-electrolyte microcapsules. *PLoS One*. 2012;7:e45233.

Leopoldo Palma<sup>a,b</sup>

<sup>a</sup> Centro de Investigaciones y Transferencia de Villa María (CITVM-CONICET), Universidad Nacional de Villa María, (5900) Villa María, Córdoba, Argentina

<sup>b</sup> Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina

E-mail address: palma.leopoldo@gmail.com

29 April 2016 14 September 2016 14 October 2016

<http://dx.doi.org/10.1016/j.ram.2016.10.003>

0325-7541/© 2016 Asociación Argentina de Microbiología. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).