PHOSPHINOTHRICIN AS A NEW SELECTABLE MARKER FOR THE ENTOMOPATHOGENIC FUNGUS *Lecanicillium lecanii*

Zaizy Rocha-Pino[1], Israel Padilla-Guerrero[2], Keiko Shirai[1], Michael J. Bidochka[2].
[1]Universidad Autonoma Metropolitana, Department of Biotechnology, Lab. Biopolymers. Av San Rafael Atlixco No. 186 Col. Vicentina, Mexico City 09340, Mexico,
[2]Brock University Department of Biological Sciences, St. Catharines, On L2S 3A1, Canada;
mbidochka@brocku.ca, smk@xanum.uam.mx
Key words: Lecanicillium lecanii, phosphinothricin, green fluorescent protein (GFP)

Introduction. Fungus used as biological pesticides are object of great interest because these represent an alternative as environmentally friendly technologies for the pest control. For this reason is important to know more about its impact on the ecosystem where they are applied. The transformation technique is indeed useful tool for studies of morphology, genetic even for development of microorganisms with increased benefits [1, 2]. *Lecanicillium lecanii* is an entomopathogenic fungus able to infect insects which are causing economic losses in agriculture,[3] however little is known about its mechanisms and genes involves.

The aim of this study was to apply a method to transforming *L. lecanii* via *Agrobacterium*-mediated to obtain an analysis tool for future studies.

Methods. *L. lecanii* strain 313 transformation via *Agrobacterium tumefaciens* AGL-1 was performed according to Fang et al[4] with modifications. It was used phosphinothricin (PPT) resistance gen (BAR) and green fluorescent protein gen (eGFP) cassette as a selectable marker (Padilla-Guerrero, unpublish). 100 µg/mL cloramphenicol was used to kill remaining *Agrobacterium* and 250 µg/mL of PPT to select fungal transformants. The transformant method was carried out by triplicate. *L. lecanii* transformants were cultured on selective medium by 3 generations.

Results. In this work, *L. lecanii* was transformed via *Agrobacterium*-mediated using the cassette BAR-GFP. BAR gene offered resistance to PPT, which is an irreversible inhibitor of glutamine synthetase activity and active component of herbicides (5). Thus, the dual property of this new selectable marker makes it a quick and easy tool for selection of colonies by targeted gene integration. The transformant efficiency was 17 ± 2 colonies for 5 x10^{-5} target conidia. The *L. lecanii* transformants were visualized after 5 to 6 days on cultured on selectable medium. The transformants showed mitotic stability in absence of PPT after 3 generations on selective medium. All *L. lecanii* transformants obtained were able to express GFP (Figure 1).

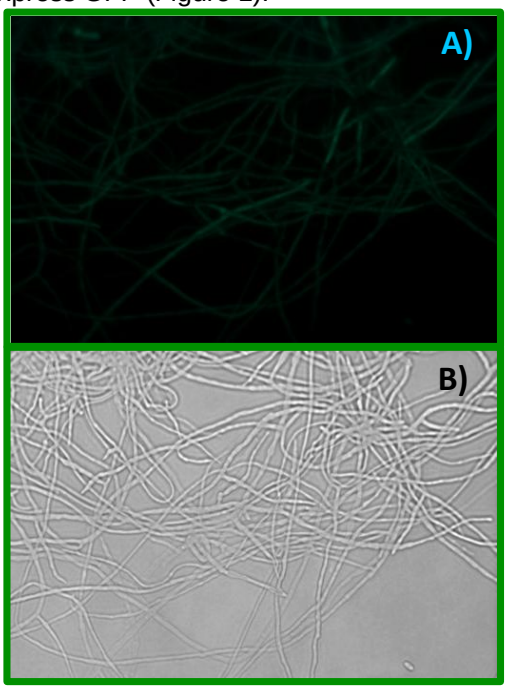

Fig.1 Micrographs of *L. lecanii*:pBAR-GFP grown in M-100 at 27°C,10 days. A) Fluorescence, B) Bright-field. 40X objective, exposure time 2s.

Conclusions. *Agrobacterium*-mediated transformant system was successfully applied to *L lecanii*. The PPT resistance and GFP expression represent a suitable tool for future studies about distribution and dynamics of proteins of *L. lecanii* in order to learn more about its development and environmental interaction.

Acknowledgements. The research was supported by NSERC Discovery Grant to MJB. The authors acknowledge the funding provided by CONACYT (No. 105628) and the PhD scholarship of ZRP, as well as scholarships for fellows CONACYT national mixed: abroad that allow the training of ZRP in Brock University, Canada.

References.

1. Donzelli BG, Krasnoff SB, Churchill AC, Vandenberg JD, Gibson DM. (2010). *Curr Genet* 56(2): 151-62.
2. Tseng MN, Chung PC, Tzean SS. (2011). *Appl Environ Microbiol*. 77(13): 4508-19.
3. Goettel MS, Koike M, Jun Kim J, Aiuchi D, Shinya R, Brodeur J. (2008). *J Invertebr Pathol* 98:256-226.
4. Fang W, Pei Y, Bidochka MJ. (2006). *Can J Microbiol*. 52(7): 623-6.
5. Lea PJ and Ridley M. (1989). Soc*. Exp. Biol. Semin. Ser*. 38:137-170.