



PURIFICATION AND CHARACTERIZATION OF PHENAZINE-1-CARBOXYLIC FROM *Pseudomonas aeruginosa* STRAIN LV AGAINST *Botrytis cinerea*

Simionato, A.S.; Navarro, M.O.P.; Vivan, A.C.P.; Gionco, B.; Barazetti, A.R.; Freitas, V.F.; Conter, J.A.N.; Nandi, R.; Simões, G.C.; Silva, C.S.; Santos, I.M.O.; Luciano, E.A.; Balbi-Peña, M.I.; Andrade, G.; de Oliveira, A.G.

State University of Londrina, Londrina, Brazil; anessimionato@gmail.com

Abstract: One of the most important post-harvest plant pathogens that affect strawberry cultivars is *Botrytis cinerea*, known as grey mold [1]. The fungus remain in latent form until the activation conditions are favorable, making it difficult to control the infection [1], which causes several losses in all productive chain. The challenge is to find alternatives to control the fungus in the post-harvest such as the use of antagonistic bacteria or their metabolites as fungicide with low toxicity for consumers. The different microorganisms producing bioactive compounds, and the genus *Pseudomonas* spp. produces a range of broad-spectrum antimicrobials, as phenazine derivatives [2]. This study aimed to purify and identify the phenazine-1-carboxylic (PCA) produced by *P. aeruginosa* (LV strain) and their antifungal activity against *B. cinerea*. After cultured, the cells were pelleted by centrifugation and the supernatant passed through purification processes. The compounds produced were extracted with ethyl acetate, resulting in the organic fraction called EAP (ethyl acetate phase). The EAP passes through a liquid column vacuum and fractions with phenazines compounds went through the flash column chromatography for purification of PCA. The purity level of phenazine compound was determined by reverse phase high performance liquid chromatography semi-preparative (RP-HPLC, Agilent®, USA). The structure of PCA was confirmed by nuclear magnetic resonance (NMR) and electrospray ionization mass spectrometry (ESI-MS). The antifungal was checked by filter paper disc assay and minimum inhibitory concentration technics. The PCA showed satisfactory results for inhibitory activity, with MIC of 25 µg/mL showing a significant activity *in vitro* against *B. cinerea* (Figure 1). These results are promising as alternative to control *B. cinerea*, and new studies are needed to assess the behavior of this compound *in vivo* assays.

References:

- [1] Vallejo, I., Muñoz, F., Carbu, M., Rebordinos, L., Fernandez-Acero, F.J., Cantoral, J.M. 2002. Study on fungicide resistance of *Botrytis cinerea* – isolaten an *Erkrankten erdbeerpflanzen*. Arch. phytopathol. plant prot. 36: 1-7.
- [2] Pierson III, L.S. and Pierson, E.A. 2010. Metabolism and function of bacteria in the environment and biotechnological processes. Appl. microbiol. biotechnol. 86: 1659-1670.

Figure 1: Graphic of Minimum Inhibitory Concentration (MIC) PCA x *Botrytis cinerea*

