[PS2.64]

Coronatin-1 Isolated from Entomopathogenic Fungus *Conidiobolus coronatus* Kills *Galleria mellonella* Hemocytes *in vitro* and Forms Potassium Channels in Black Lipid Membrane

W. Wieloch[1], M.I. Bogus*[1], M. Ligeza[1], I. Koszela-Piotrowska[2], A. Szewczyk[2]
[1]*Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warszawa, Poland,*
[2]*Institute of Experimental Biology, Polish Academy of Sciences, Pasteura 3, 02-093 Warszawa, Poland*

Entomopathogenic fungi are important natural regulatory factors of insect populations and have potential as biological control agents of insect pests. The cosmopolitan soil fungus *Conidiobolus coronatus* (Entomopthorales) easily attacks *Galleria mellonella* (Lepidoptera) larvae. Prompt death of invaded insects is attributed to the action of toxic metabolites released by the invader. Effect of fungal metabolites on hemocytes, insect blood cells involved in innate defense response, remains underexplored to date.

C. coronatus isolate 3491 inducing 100% mortality of *G. mellonella* larvae exposed to sporulating colonies, was cultivated according to Bania et al (2006). Post-incubation filtrates were used as a source of fungal metabolites. A two-step HPLC allowed to isolate coronatin-1, an insecticidal 36 kDa protein showing both elastolytic and chitinolytic activities.

Addition of coronatin-1 (50 µg/ml) into primary *in vitro* cultures of *G. mellonella* hemocytes resulted in rapid disintegration of spherulocytes, shrinkage of plasmatocytes, pseudopodia atrophy, and disintegration of nets formed by cultured hemocytes.

After incorporation of coronatin-1 (1.9 mg /ml, 1-2 µl) into planar lipid bilayers (black lipid membranes) we observed ion channels selective for K^+ ions in 50/450 mM KCl solutions. Potassium current flows were recorded in nearly 70 % of experiments and were divided into two groups – with small or large amplitude. All observed channels were active at both positive and negative membrane potentials. Under experimental conditions incorporated coronatin-1 exhibited a reversal potential (E_{rev}) of 47.7 mV, what indicates K^+-selectivity of this protein.

The success of the purification of coronatin-1 will allow research on the biochemical aspects of the mode of action of this molecule to be initiated including ability of coronatin-1 to form potassium channels in immunocompetent hemocytes.

Reference: Bania, J., Samborski, J., Boguś, M.I., Polanowski, A., 2006. Arch. Insect Biochem. Physiol. 62, 186–196.

Acknowledgments: Work supported by the MNiSW grants 2P04C 002 28; N303 341035.

Keywords: mycotoxin, Conidiobolus coronatus, Galleria mellonella, hemocytes