

Entomopathogenic fungus *Conidiobolus coronatus* as a model for studying stress adaptation

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INTRODUCTION. Naturally occurring entomopathogenic soil fungus *Conidiobolus coronatus* have the ability to directly penetrate the insect cuticle, so it is an "ecological" alternative to chemical agents reducing the insect pest population. However, *C. coronatus* shows different virulence depending on the host: almost 100% virulence against the wax moths *Galleria mellonella*, while the larvae of the fly *Calliphora vicina* are resistant to fungal infection. In addition, this fungus can cause human infections. It is believed that, diverse virulence may be different fungal adaptation to stress conditions. An excellent model for studying this phenomenon is the insecticide fungus-insect system. Barriers that must overcome the fungus are cuticle and hemolymph of insects. Previously performed analyzes using GC-MS showed that the composition of cuticle differs depending on the species and stage of development of the insect, and LC-MS/MS analyzes indicated that the fungus produces different proteins depending on culture conditions (unpublished data).

THE AIM OF THE STUDY was to check if the cuticle and/or hemolymph of susceptible insects species (*G. mellonella*) and resistant (*C. vicina*) to infection by *C. coronatus*, can cause the changes in: activity of *C. coronatus* antioxidant enzymes, activity of ATPases and GTPase, concentration of stress metabolites and toxicity.

METHODS. After termination of *C. conidiobolus* cultivation in minimal medium, the fungus was incubated with the addition of cuticle and hemolymph of the larvae of both insect species. Positive control was the culture of the fungus in which oxidative stress was artificially induced by the addition of hydrogen peroxide. The activity of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase), activity of ATPase and GTPase, as well as concentration of stress metabolites (trehalose and mannitol) was studied in the hyphal homogenates. The presence of fungal toxin (aflatoxin) was also tested in post-culture filtrates.

Results: High activity of glutathione peroxidase and superoxide dismutase in a fungal culture subjected to the hydrogen peroxide, indicated on a synergistic acting of these enzymes in the antioxidant protection of fungus against oxidative stress. In contrast, addition of hemolymph and cuticle of both insect species decreased the activity of glutathione peroxidase. Quite high activity of superoxide dismutase and catalase in presence of most insect's additives, suggests that these enzymes can be a standard "equipment" of the *C. coronatus* fungus as a tool to overcome an unfriendly environment. The decrease in the concentration of trehalose and the production of aflatoxin in contact with the insect's cuticle suggest that the cuticle components are a less available source of C and N than the hemolymph. A drastic decrease in activity of ATPase and GTPase caused oxidative stress.

CONCLUSIONS. Changes in most cases of enzymatic activity as well as different concentration of trehalose and mannitol in fungus just after 1 hour from contact of *C. coronatus* with oxidative stress and insect-derived additives, suggest that *C. coronatus* reacts very quickly to environmental changes. Moreover, the fungus responds differently depending on the environmental conditions. A significant decrease in activity of ATPase and GTPase in the fungus subjected to oxidative stress, indicate that oxidative stress affect on fungal signaling pathways. The production of aflatoxin in mycelium after contact with cuticle of both insect species may indicate that the cuticle components are a less available source of C and N than the hemolymph. Research are pioneering for the Entomophtorales order. However, the explanation of the reasons for the diverse ability of the fungus *C. coronatus* to overcome the protective barriers of insects requires further comprehensive research at the genetic level.

Work supported by the grant 2017/X/NZ3/01020 (NCN).