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EVALUATION OF PROTEASE AND LIPASE ACTIVITIES OF INDIGENOUS ENTOMOPATHOGENIC FUNGAL ISOLATES

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Introduction

The whitefly, *Bemisia tabaci* (Gennadius), is one of the most economically important insect pests affecting tomato, cotton, and numerous other agricultural crops. The repeated use of chemical insecticides against this pest may promote the development of insecticide resistance and cause a range of environmental problems. Therefore, the use of entomopathogenic fungi as biological control agents represents a promising alternative approach.

Entomopathogenic fungi constitute an important group of microorganisms with considerable potential for the biological control of insect pest populations. Fungal infection involves a sequence of processes, including adhesion of conidia to the insect cuticle, conidial germination, formation of penetration structures, and enzymatic degradation of the cuticular barrier. Since the insect cuticle is composed of complex structural components, including proteins, lipids, and chitin, extracellular hydrolytic enzymes produced by entomopathogenic fungi are considered important factors involved in the infection process.

Purpose

The aim of this study was to qualitatively evaluate the proteolytic and lipolytic activities of indigenous *Beauveria* and *Metarhizium* isolates, compare their enzymatic indices, and screen isolates with promising enzymatic potential for further investigation.

Materials and Methods

Entomopathogenic fungi were isolated from soil samples collected from a cotton agroecosystem in the Syrdarya region and from insect sources. Larvae of *Galleria mellonella* L. and *Leptinotarsa decemlineata* Say were used as insect sources. After removal of the surface soil layer, samples were collected from a depth of approximately 5 cm and stored at 4 °C until laboratory examination.

Preliminary identification of the fungal isolates was based on their macro- and micromorphological characteristics. Colony growth characteristics and morphology, as well as microscopic structures, were examined. Based on preliminary screening of their morphological and cultural characteristics, four *Beauveria* sp. isolates and one *Metarhizium* sp. isolate were selected for the assessment of enzymatic activity.

Proteolytic activity of the isolates was qualitatively assessed on casein-containing agar medium. The hydrolysis zone formed around the fungal colony as a result of substrate degradation was used as an indicator of protease activity. Lipolytic activity was evaluated on Tween 80-containing

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medium. Following incubation, the diameters of the fungal colonies and the surrounding reaction zones were measured and recorded.

To compare the enzymatic characteristics of the isolates, the enzymatic index (EI) was calculated based on the diameters of the fungal colony and the surrounding hydrolysis zone as follows:

The enzymatic index (EI) was calculated as the ratio of the total diameter of the fungal colony and the surrounding hydrolysis zone to the diameter of the fungal colony.

The resulting EI values were used for the preliminary qualitative or semi-quantitative comparison of the proteolytic and lipolytic potential of the fungal isolates.

Results and Discussion

Enzymatic screening demonstrated variation among the indigenous entomopathogenic fungal isolates in their ability to produce proteases and lipases. Positive proteolytic and lipolytic activities were detected in 80% of the five isolates **examined**, indicating that most of the isolates possessed the potential to hydrolyze protein- and lipid-based substrates.

The enzymatic index for lipolytic activity ranged from 1.07 to 2.81, demonstrating a relatively broad variation in lipolytic activity among the isolates.

For proteolytic activity, EI values ranged from 1.03 to 1.30. Compared with lipolytic activity, the protease EI values varied within a narrower range. Nevertheless, the combined assessment of proteolytic and lipolytic activities allowed differentiation among the isolates according to their overall enzymatic potential.

Based on the combined evaluation of protease and lipase activities, *Beauveria* sp. 2 was identified as one of the promising isolates because of its overall enzymatic profile. Although *Beauveria* sp. 1 exhibited high lipolytic activity, no detectable proteolytic activity was observed. In contrast, *Beauveria* sp. 3, *Beauveria* sp. 4, and the *Metarhizium* sp. isolate showed comparatively lower proteolytic and lipolytic activities. These findings support the selection of *Beauveria* sp. 2 for further quantitative evaluation of enzyme production and more detailed investigation of its biological characteristics.

The enzymatic index is primarily a screening parameter; therefore, the results obtained should be interpreted as indicators of the qualitative or semi-quantitative enzymatic potential of the isolates rather than as direct measurements of enzyme concentration or catalytic activity.

Because several hydrolytic enzymes contribute to the penetration of entomopathogenic fungi through the insect cuticle, protease and lipase activities alone are insufficient to draw definitive conclusions regarding isolate virulence. Nevertheless, these enzymes may serve as useful physiological indicators for the preliminary screening of promising isolates. Further assessment of chitinase activity would provide a more comprehensive characterization of the cuticle-degrading enzymatic potential of the selected isolates.

Conclusion

The present study demonstrated variability in proteolytic and lipolytic characteristics among indigenous *Beauveria* and *Metarhizium* isolates. Positive protease and lipase activities were detected in 80% of the isolates examined. The enzymatic index ranged from 1.07 to 2.81 for lipase and from 1.03 to 1.30 for protease, indicating greater variability in lipolytic activity than in proteolytic activity among the isolates.

Based on the combined evaluation of protease and lipase activities, *Beauveria* sp. 2 was identified as an isolate with promising enzymatic characteristics. These findings can be used for the preliminary characterization of the enzymatic potential of indigenous entomopathogenic fungi and for the selection of promising isolates for subsequent biological studies.

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