

PURIFICATION OF A SPORE SURFACE LIPASE FROM *METARHIZIUM ANISOPLIAE*.

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The fungus *Metarhizium anisopliae* is a well-characterized, broad host-range arthropod pathogen employed in biological control of agricultural pests. Entomopathogenic fungi actively invade their hosts through the cuticle by mechanical pressure, via a prothorium formation, and enzymatic degradation by synergistic action of hydrolases, as chitinases and proteases. The present work describes the purification and characterization of a spore surface lipase from *M. anisopliae*. Spores from *M. anisopliae* were immersed and strongly shaken in extraction buffer. The resulting supernatant was filtrated and applied on to a DEAE-Sepharose column. Elution of proteins was done with a NaCl linear gradient (0-1.0 M). The fractions with lipolytic activity were applied on to a Phenyl Sepharose column and elution of proteins was made with a Na₂SO₄ linear gradient (1.0-0 M). As the last purification step a gel filtration column Superose 12 was used. For all chromatographic steps, FPLC purification system and Tris-HCl buffer 50mM, pH 8.0 were used. The purification fractions were analyzed by lipolytic activity assays using pNP-Palmitate as substrate. The last step of purification showed only one fraction with lipolytic activity containing one protein band visualized in a SDS-PAGE electrophoresis. Characterization of the spore surface lipase from *M. anisopliae* and elucidation of its function are under way.

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