PURIFICATION PARTIAL OF A LYSINE AMINOPEPTIDASE FROM *Sporisorium reilianum*

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Introduction.

Sporisorium reilianum is a phytopathogenic fungus, causal agent of head smut a worldwide disease affecting maize (1). This penetrates soil saprophytic plant during germination of the seed, resulting in a systemic infection which occurs after flowering (2).The study the biology of *S. reilianum* has been limited to few reports focused on the determination to its life cycle. However, the role of intracellular proteolysis in this fungus has been little studied, taking into account that this process is a vital mechanism by which the cell can remain viable in situations that could compromise their survival (3).

The present study was conducted the partial purification of the intracellular lysine aminopeptidase produced by this plant pathogen, which may have an important role in the life cycle of the fungus, in addition to possible biotechnological applications.

Methods.

Aminopeptidase activity was measured by the hydrolysis of L-Lysine *p*-nitroanilide. For partial purification of lysine aminopeptidase we used a crude extract intracellular, obtained from a 48 h. culture in YEPD medium (1% yeast extract, 2% peptone and 2% dextrose). Submitted to a process ammonium sulfate fractionation to 60%. The active fraction was applied to a hydrophobic interaction column with a linear gradient from 1.7 to 0.0 M of $(NH_4)_2SO_4$. The fraction obtained was desalted in a desalting column. The sample was loaded on ion exchange column with a linear NaCl 1 M gradient at 0-100%. Cromatographic procedures were performed with the FPLC system. Partial purification was monitored by SDS-PAGE and silver staining (4).

Results.

Lysine aminopeptidase was partially purified from *S. reilianum* achieved by fractionated precipitation with amonium sulfate followed by three chromatographics steps.

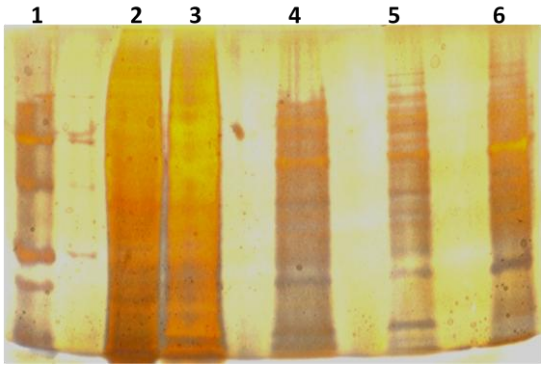

Fig.1 Gel electrophoresis stained with silver. 1. Molecular weight markers. 2. Intracellular enzymatic crude extract (I.E.C.E.). 3. Ammonium Sulfate fractionation. 4. Hydrophobic interaction chromatography. 5.Desalting fraction. 6. Ion exchange chromatography

Table 1. Table of purification

Purification step	Total Protein (mg)	Total activity (U)	Specific activity (U mg^{-1})	Yield (%)	Purif (fold)
I.E.C.E.	292.85	11591.06	39.58	100	1
Amonnium sulfate fractionation	24.26	1270.13	52.34	10.95	1.32
Hidrophobic interaction	14.16	973.89	68.74	8.40	1.73
Desalting	5.31	657.70	123.84	5.67	3.12
Ion exchange	1.29	292.98	227.06	2.52	5.73

Conclusions.

A lysine aminopeptidase was purified partially from the plant pathogen *S. reilianum.*

References.

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