> World J Microbiol Biotechnol. 2015 Jan;31(1):85-94. doi: 10.1007/s11274-014-1767-0. Epub 2014 Nov 1.

Purification and characterization of secretory serine protease from necrotrophic oomycete, Pythium myriotylum Dreschler

Ravindranathan Aswati Nair [1], Chellappan Geethu

Affiliations + expand

PMID: 25367417 DOI: 10.1007/s11274-014-1767-0

Abstract

Progressive increase in extracellular proteolytic activity with respect to growth was detected in cultures of necrotrophic oomycete Pythium myriotylum Dreschler with maximum activity detected at stationary phase of growth. The secretory protease from P. myriotylum designated, spPm1 was purified to homogeneity giving a single band of 47 kDa molecular mass on non-reducing SDS-PAGE and exhibiting caseinolytic activity in the zymogram. Under reducing conditions, an additional band of 27.0 kDa molecular size observed was subjected to matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF-MS) analysis. Resulting peptide identified as an autolytic product and generated under reducing conditions on SDS-PAGE, showed homology to domains of oomycete effector molecules. spPm1 retained proteolytic activity over broad pH (5.0-12.0) and temperature (10-80 °C) ranges with optimal pH and temperature at 8.0 and 60 °C respectively. spPm1 was identified as a serine protease following experiments with inhibitors specific to various protease groups. spPm1 displayed high stability to surfactants, organic solvents, oxidizing agents and to metal-ion chelator, EDTA. Kinetic parameters, Km and Vmax were determined as 0.04 mM and 7.52 U min(-1) mg(-1) respectively. Preferential hydrolysis of synthetic fluorogenic substrate, SAAPPPA (N-succinyl-L-alanyl-alanyl-proline-phenylalanine-p-nitroanilide) confirmed spPm1 as belonging to subtilisin serine protease family. The excellent stability of spPm1 protease characterized from P. myriotylum is discussed with respect to its potential industrial applications.