Bioresource Technology 115 (2012) 228–236

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Purification and characterization of haloalkaline thermoactive, solvent stable and SDS-induced protease from *Bacillus* sp.: A potential additive for laundry detergents

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ARTICLE INFO

Article history:
Received 24 August 2011
Received in revised form 20 October 2011
Accepted 22 October 2011
Available online 29 October 2011

Keywords:
Haloalkaline
Serine protease
Solvent stable
Thermotolerant
Wash performance

ABSTRACT

An extracellular haloalkaline, thermoactive, solvent stable, SDS-induced serine protease was purified and characterized from an alkali-thermo tolerant strain *Bacillus* sp. SM2014 isolated from reverse osmosis reject. The enzyme was purified to homogeneity with recovery of 54.4% and purity fold of 64. The purified enzyme was composed of single polypeptide of molecular mass about 71 kDa. The enzyme showed optimum activity at alkaline pH 10 and temperature 60 °C. The km and Vmax for the enzyme was 0.57 mg/ml and 445.23 U/ml respectively. The enzyme showed novel catalytic ability at high pH (10), temperature (60 °C) and salinity (3 M). Moreover, the stability of enzyme in organic solvents (50% v/v) of logP ⩾ 2 signified the prospective of this enzyme for peptide synthesis. The compatibility of the enzyme with surfactants and various detergent matrices together with wash performance test confirmed its potential applicability in laundry industry.

1. Introduction

Global concern for the environment has attracted the researchers to investigate enzymes as replacement of chemical catalysts in various biochemical processes. The continual exploration of enzymes and their utilities have expanded their industrial market with the growth of 7.6% per year (David et al., 2009). Of the various industrial enzymes, proteases alone contribute approximately 60% of the total sales in the world (Banik and Prakash, 2004) and bacteria belonging to genus *Bacillus* produce most commercial proteases used today (Maurer, 2004). Proteases are the most versatile enzyme with a long history of catalytic applications in food and pharmaceutical industries. Recently, their roles in synthesis of bioactive peptides and as additive in commercial detergents are gaining attention. The composition and nature of the surfactants in detergents greatly manifest the stability of the enzyme. The other prerequisites for the utilizing proteolytic enzymes in detergent formulations include their functional ability and stability at alkaline pH in presence of various surfactants and detergents. The natural proteases in general are not stable under these conditions (Gupta et al., 2005). However several physiochemical methods such as chemical modification, immobilization, entrapment, protein engineering and directed evolution have been employed for their stabilization at various extreme conditions (Ogino and Ishikawa, 2001). Even the gene-shuffling techniques have not resulted in successful products. There have been extensive studies on screening of microbes to obtain proteases with functional ability under the required extreme conditions (Maurer, 2004) so that the overall process becomes environment friendly, feasible and economic. In this context, alkaline proteases of microbial origin have been extensively studied however detergent stable proteases were characterized from a few bacterial species (Deng et al., 2010). Since it is known that extremophiles could be the potent producers of enzymes with functional ability at extreme physico-chemical conditions, Shivanand and Jayaraman (2009) characterized salt stable protease from halotolerant bacteria. Similarly organic solvent tolerant bacteria were reported to produce solvent stable proteases (Xu et al., 2010; Shah et al., 2010). In this way it would be further interesting to investigate polyextremotolerant microbes and their extracellular enzymes with an assumption that they would have wider adaptability in various environmental conditions (Jain et al., 2010). However, the information on polyextremotolerants has been less explored in comparison to extremotolerant microbes.

In our previous study, a strain belonging to genus *Bacillus* was isolated from RO reject that showed characteristic features of sustaining the three extremities such as pH (10), temperature (60 °C) and pressure tension of 450 psi under absolute oxygen atmosphere (Jain et al., 2010). The present study has been designed to extrapolate the potential of this strain as a source of enzyme protease

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doi:10.1016/j.biortech.2011.10.081