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Research Article

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Detergent compatible alkaline protease from various *Aspergillus* isolates from soil.

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Abstract

Proteases are extracellular enzymes with the capability to hydrolyze large proteins to smaller peptide fragments or amino acids. Fungal proteases are indeed one of the most important group of industrial enzymes. Protease is the main enzyme of detergent. Through the combination of protease and detergent additives there may be improvement in washing effects.

In the present study alkaline protease activity in various commercially available laundry detergents was studied. Compatibility of alkaline protease produced by various *Aspergillus* isolates from soil, with detergent was seen.

Keywords: Alkaline protease, Detergent compatibility, detergent

Introduction

Protease is mixed with detergents to decompose stains during laundry [Mukherjee et al., 2008, Vojcic et al., 2015]. The protease hydrolyses peptide bond of a polypeptide chain, the macromolecular protein is broken down into small polypeptides or amino acids, and it is peeled from the fabric under the action of surfactant and external force [Giri et al., 2011]. The common protease used for washing was trypsin, which is only needed in a small amount to achieve good washing results. But trypsin is mainly extracted from animal materials [Al-Ghanayem and Joseph 2020, Esposito et al., 2010], and the raw materials and processes have certain limitations and cost effective also. Thus, it has been gradually replaced with microbial alkaline protease, which can be produced by large-scale fermentation.

In the present study we examined the efficiencies of protease recovered from different strains of *Aspergillus*, in the presence of commercial detergents. The main objective of this work is to search for new proteases with novel properties from as many as sources possible, which could have application in detergent industry.

Materials and Methods

Fungal strain- *Aspergillus* strains were isolated from soil on Potato dextrose agar medium and screened for protease production on Yeast Extract Agar Medium.

Enzyme Production- The enzyme was produced in conical flasks containing 100 ml of protease production medium (Yeast Extract broth containing 1% casein), incubated at 28^0C for 7 days in an orbital shaker (BOD incubator, MAC, India). Cell free cultural filtrate was used as a crude enzyme solution.

Partial purification of enzyme- All operations were performed at 40° C. The enzyme was precipitated from crude extract by the gradual addition of solid ammonium sulphate with gentle stirring to 75% saturation and the pellet was collected by centrifugation at 10,000 rpm for 10 min. The protein pellet obtained after saturation was dissolved in 0.1M Tris- HCl buffer, and loaded on to prepacked desalting column of cross linked dextran with epichlorohydrin, equilibrated with Tris-HCl buffer, pH 7.8. The protease was eluted at 1.0M NaCl concentration. The desalted sample was analyzed further for compatibility with laundry detergents.

Protease assay- The protease activity was determined by the method of Anson (1938) and Folin Ciocalteu(1928). The Protease activity was determined in a reaction mixture of 1 ml enzyme, 5 ml (0.65mM) casein solution (pH 7.5) and incubated at 37^0 C for 10 minutes. The proteins were precipitated by adding 5 ml of TCA and free amino acids released by protease from casein hydrolysis were estimated. One protease unit is defined as the amount of enzyme that releases 1.0 micromol (181 micrograms) of tyrosine per minute at pH 7.5 at 37 ^{0} C Colour by Folin Ciocalteu Reagent).

Detergent Stability- The compatibility of partially purified protease with local laundry detergent was studied. Detergents used were Nirma (Nirma Limited India): Active wheel Gold (Hindustan Unilever Ltd.): Rin(Hindustan Unilever Ltd., India): Tide (Procter and Gamble products);Ariel(Procter and Gamble, Indian) and Ghari (M/S Rohit Surfactants private Ltd.). The detergents were diluted in distilled water (0.7%wt/vol) and incubated with crude protease from all the five strains of *Aspergillus* for 30 mins at 45^0 C, and the Residual activity was determined. Endogenous protease present in these detergents was inactivated by incubating the detergent solutions at 65°C for 1 h prior to the addition of the exogenous protease obtained from various strains of *Aspergillus* (Samal et al., 1990). The enzyme activity of a control sample (without any detergent) was taken as 100%.

Results and Discussion

For the possible commercial exploitation of protease in detergent industry, the protease obtained from various strains of *Aspergillus* was tested for its compatibility with six detergents of common use. Protease from all the strains of *Aspergillus* showed excellent compatibility in the presence of locally available detergents (Nirma, Active wheelgold, Rin, Ghari, Ariel, Tide) (Banik and Prakash, 2004) (Niyonzima and More 2015).

Protease from isolate No. 3 and 11 showed good stability in the presence of Tide (Table-1-3). As showed in table-2, protease stability of isolate no 10 was better in Rin, where 100% activity was present.

On the other hand, protease from isolate no. 14 showed good stability in presence of Ariel. Protease from isolate No. 10 and 11 showed 75% of its original activity in presence of Nirma.

It is concluded from the above study that Isolate No. 3, 10 showed excellent compatibility with various laundry detergents tested and the stability of the enzyme in detergents was good for longer time period. This study indicates that the *Aspergillus* strains produces protease which is compatible and can be used in manufacture of laundry detergents. The isolated protease can thus be a choice option in detergent industry. Further

study is needed to ascertain that among these proteases obtained from 3 fungal strains which protease is more suitable for detergent industry.

Table-1 Compatibility of protease activity from isolate no 3 with commercial detergents

Commercial Detergents (0.7% wt/vol)	**Relative residual enzyme activity(%)**
Control	100
Rin	82
Active Wheel Gold	83
Tide	100
Ariel	82
Ghari	83
Nirma	82

Table-2 Compatibility of protease activity from isolate no 10 with commercial detergents

Commercial Detergents (0.7% wt/vol)	**Relative residual enzyme activity(%)**
Control	100
Rin	100
Active Wheel Gold	96
Tide	88
Ariel	100
Ghari	84
Nirma	73

Table-3 Compatibility of protease activity from isolate no 11 with commercial detergents

Commercial Detergents (0.7% wt/vol)	**Relative residual enzyme activity(%)**
Control	100
Rin	96
Active Wheel Gold	90
Tide	97
Ariel	95
Ghari	97
Nirma	72

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