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Enhancement of commercial detergent's wash performance by addition of enzymes of *Bacillus subtilis* FH1

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Abstract

Currently enzymatic detergents emerged as better option due to their cleaning and stain removal effectiveness in comparison to the synthetic surfactants. An extracellular alkaline protease produced by *Bacillus subtilis* strain FH1 was studied for its potential application as detergent additive. Enzyme was tested for its compatibility and stability by incubating it with several detergents like Surf Excel, Ariel, Express, Bonus and Brite and residual activity was checked by standard enzyme assay, it showed 92, 85, 100, 75 and 126% residual activity respectively. Its wash performance was checked by adding in these detergents while washing manually soiled cotton cloth pieces and showed excellent wash performance in combination with detergents at lower temperature. Due to stability and good wash performance the enzyme can be used as detergent additive.

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Introduction

Contemporary cleaning items are composite blends of many active ingredients which differ on detergents purpose. Aside from surfactants as principle ingredient, detergents contain builders, optical brighteners, and corrosion inhibitors, bleaching agents, froth controllers and enzymes along with other auxiliary additives. Detergent performance along with cleaning efficiency is due to combination of ingredients which act synergically on the dirt (Jurado *et al.*, 2007). Currently enzymes added detergents emerged as one of the most effective and better choice due to their higher cleaning and stain removing potential as compared to the synthetic chemical based detergents (Kirk *et al.*, 2002).

Detergent industries are the prime consumers of biological enzymes to develop "green chemicals" based efficient detergents (Mitidieri *et al.,* 2006).

Enzyme based formulations makes the detergents environment friendly and more potent to remove the stubborn stains. Detergents being used for laundry, nowadays comprise mixtures of enzymes like lipases, proteases, cellulases and amylases.

Statistically ~60% of the global annual sale of hydrolytic enzymes comprises of protease, which is significantly higher proportion in enzymes marketing. Biological proteases have wide range of applications in pharmaceutical, food, leather, silk industry, detergent sector, synthetic peptide formation, diagnostics, and waste management and to recover silver from old X-ray films (Kumar and Takagi, 1999).

To ensure the protein based stain removal alkaline protease also named as "green chemicals" are being added in detergents (Rai *et al.,* 2009). Staining of fabrics by hydrophobic proteins from environment or soil can also be prohibited by alkaline proteases. They also help to enhance the whiteness of the clothes (Beg and Gupta, 2003).

Microbes are the most common source of proteases, which are being directly secreted by them in the liquid media used during fermentation. Their extracellular secretion makes the enzymes downstream processing for purification easy as compared to other sources such as animals and plants (Lageiro *et al.*, 2007). A number of fungus spp. (mold and yeast) and bacteria are being used as alkaline protease producer for commercial usage. *Bacillus* species is one of the most prevalent source of alkaline protease at commercial scale (Priest, 1977; Gupta *et al.*, 2002).

The most specific and stable characteristics of biological origin enzymes such as tolerance to wide range of temperature, pH, metal ions concentration make them product of choice. These enzymes' high compatibility with other components of detergents stimulate the researchers and R&D departments of industries to explore some novel sources (Lageiro *et al.*, 2007).

High performance and stability of proteases in highly alkaline environment and temperature make them interesting and potential target for biotechnological and bioengineering applications. Their alkaline friendly nature makes them suitable and applicable in detergents (Priest, 1977; Gupta *et al.*, 2002).

In current study the stability and the activity of protease obtained from a strain of *B. subtilis* FH1 was evaluated by adding them in local and commercial solid detergents.

Material and methods

Microorganism used

Microorganism used in this study was a mesophilic bacterium isolated from tannery waste and identified as *Bacillus subtilis* FH1 and proved good producer of extracellular protease (Natt, 2000).

Media

Inoculum was grown in Nutrient Broth. Medium used for protease production was optimized by Natt (2000) and had the composition Gelatin 20g/l, Casein Hydrolysate 6g/l, Glycerol 12.5ml/l (autoclaved separately), pH adjusted to 8.0 then

autoclaved at 121°C for 20 mins. 10% inoculum of 24hr age was added to production media and placed in a rotary shaker at the agitation speed of 200 rpm at 37°C for 48 hrs, then centrifuged at 12,000 rpm for 15 minutes. Supernatant after centrifugation was collected as crude enzyme extract.

Enzyme assay

Protease activity of extracted enzyme was measured by using casein as substrate and method reported by Kembhvi *et al.,* (1993). 0.5 ml substrate (casein) solution (1% casein in Tris HCl buffer, pH 8.0) was mixed with 0.5 ml of enzyme extract, incubated at 37°C for 30 minutes. 1ml of 10% Trichloroacetic acid was used to stop the reaction. Mixture was placed at 4°C for 20 minutes then centrifuged at 10,000 rpm for 10 minutes. Supernatants' optical density was estimated spectrophotometer at 280 nm.

A standard calibration curve was generated by using (0-100mg/l) concentrations of Tyrosine. One unit of Protease activity is demarcated as amount of enzyme that liberates 1 µg of tyrosin per minute. Assay was done in triplicates and the mean was taken.

Effect of detergents on enzyme stability

Efficacy and compatibility of protease preparation from FH1 was evaluated in combination with commercially available detergents.

The solid detergents tested were Surf Excel, Ariel, Express, Bonus and Bright. Commercial detergents solution of 7mg/ml concentration was mixed and incubated with crude enzyme extract at 37°C for ½ hour and residual activity was calculated by standard assay. The activity of the crude enzyme extract, incubated under same experimental conditions without any detergent additive was taken as 100%.

Evaluation of wash performance

100µl of fresh human blood was used to stain the clean white colored cotton cloth pieces (2.5 cm^2) and air dried. These soiled cloth pieces were then washed with 7mg/ml concentration solution of commercial solid detergents (Surf Excel, Ariel, Express, Bonus and Bright solid detergents available in Pakistani market) supplemented with crude protease and without it. Experiment was conducted in two separate flasks one with commercial detergent plus protease extract from FH1 at 20 U/10 ml activity and other with only detergent solution. Final volume of each experimental flask was 10ml. Each flask was incubated at 4°C, 20°C, 37°C and 50°C for ½ hour in shaker incubators (150 rpm). After 30 min, cloth pieces were taken out, rinsed twice in 100ml water and air dried. Cloth pieces from both experiments were visually examined by 5 different individuals and efficacy of FH1 enzyme in protein stain removal was analyzed.

Results and discussion

Activity with detergents

In this study, the protease of *B. subtilis* FH1 showed residual activity in presence of Surf Excel, Ariel, Express, Bonus and Brite 92, 85, 100, 75 and 126% respectively (figure 1). It shows that protease has good stability with commercial detergents. Maximum stability was observed in case of Bright.

Washing performance

The wash performance of the crude protease produced from FH1 in the present study was evaluated by its potential to get out the human blood stain from cloth pieces. Enzyme in combination with several commercial detergents was studied.

The combinations of (7 mg/ml) concentration solution of detergents and crude enzyme (10%, v/v) showed equally good results (figure 2). The detergent supplemented with protease preparations significantly enhanced the efficacy of detergent to get out the blood stains from cloth at 4°C. So this protease could be used in enzyme based detergent.

The results indicated the synergistic effect of detergent and protease in efficient removal of protein based stains. Stability and significant activity of FH1 produced protease at as low temperature as 4°C make it a better emerging choice for alkaline protease addition in detergents in recent times.

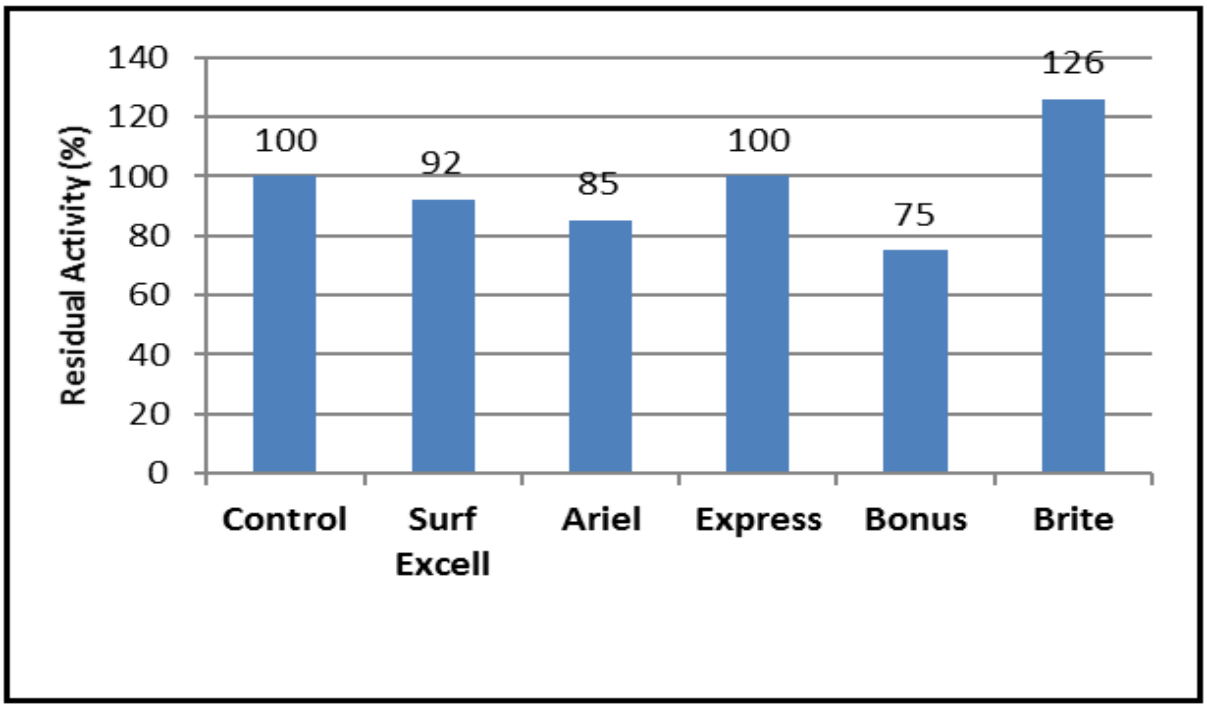

Fig. 1. Residual Activity of Protease with commercial detergents.

This low temperature functional protease facilitate washing at ambient temperatures and preserve the fabric quality and lower down the energy consumption. In literature only a handful studies that reported the low temperature active proteases (Kitayam, 1992; Tamiya and Nakamura, 1996).

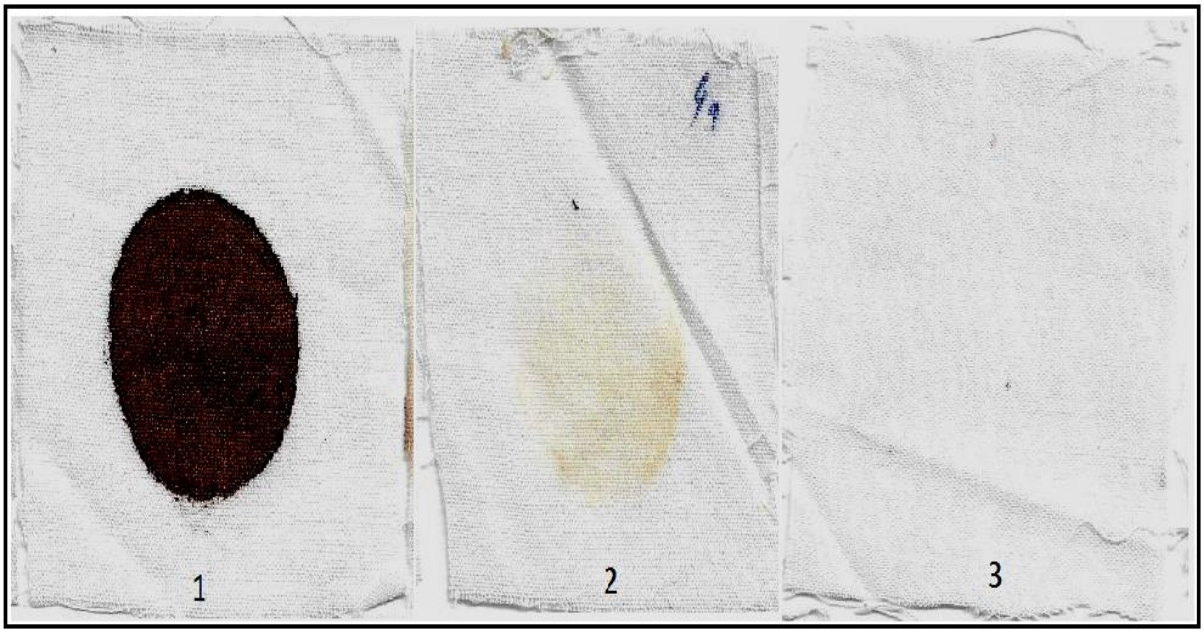

Fig. 2. 1 Cloth prior to wash, 2 Washed with 0.7% Ariel at 4°C, 3 Washed with 0.7% Ariel and 10% Enzyme Crude Extract at 4°C.

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