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Research Article

# Wash Performance of Crude Alkaline Proteases from *Bacillus halodurans* RSCVS-PF21 and *Fermenti bacillus* sp. RSCVS-HS3 for Detergent Industry

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## Abstract

The study's goal was to check wash performance potential of crude alkaline protease filtrates from *Bacillus halodurans* RSCVS-PF21 and *Fermenti bacillus* sp. RSCVS-HS3 both isolated from alkaline soil from vindhya region (Rewa division) of Madhya Pradesh of Central India. It was found that when mixed with detergents these alkaline protease crude filtrates enhanced the washing efficacy of detergents. Thus, both are potential candidates to be used in detergent industry.

**Keywords:** *Bacillus halodurans* RSCVS-PF21; *Fermenti bacillus* sp; RSCVS-HS3; Alkaline Proteases; Bacteria

## Introduction

Alkaline proteases have high commercial potential accounting for 25% of the world global enzyme market (Kumar., *et al*. 2004) [11]. This has applications in various sectors such as detergent, food and feed, pharmaceutical, leather, paper & pulp industries, textile, for silver recovery from photographic plates and in waste treatment. Alkaline proteases have been used widely at commercial scale in the detergent industry.

The various products have been used for cleaning of laundry, dentures and contact lenses. In 1913, "Brunus", the very first enzymatic preparation, was prepared consisting of crude pancreatic extract and sodium carbonate. This preparation was first introduced in market in 1956 with a trade name of BIO-40. Novo industry A/S introduced into the market "Alcalase" in 1960 with a trade name of BIOTEX produced by *B. licheniformis* (Jacobson., *et al*. 1985) [10]. Protease produced by *B. cereus* BM1 was reported as a good detergent ingredient. It showed stable activity in a solution of 10% (w/v) commercial detergent, which suggests its commercial consumption (Varela., *et al*. 1997) [15], Illanes 2008) [9]. Iso-electric point is crucial for the selection of proteases for use in detergent. Proteases exhibit strong results when pH and PI points of these enzymes are concomitant. Compatibility with surfactants, bleaches and perfumes (Bayoudh., *et al*. 2000) [3] optimum temperature and pH (Kumar., *et al*. 1998) [12] (Gupta., *et al*. 1999) [7] ionic strength and removal potential of stain have also been regarded as the choice of detergent proteases (Bas and Boyaci 2007) [2]. Current interest has been to search and identify new alkaline proteases working in a wide range of temperature. Recently, rDNA technology has been used to produce bioengineered detergent proteases with greater shelf life and stability. The replacement of active amino acid residues has been studied for bleach and oxidation stability of protease enzymes using enzyme engineering (Oberoi., *et al*. 2001) [13] (Haddar., *et al*. 2009) [8]. Proteases have been used not only as laundry detergent but also as normal washing and cleaning detergents in many sectors (Shanlin., *et al*. 1997) [14] (Bornscheuer., *et al*. 2012) [4].

Many alkaline proteases producing bacterial isolates were isolated from soils of different habitats of different localities of Vindhya region, Madhya Pradesh, India. Out of several proteolytic isolates, two promising new bacterial strains were identified as *Bacillus halodurans* RSCVS-PF21 (Genbank accession no. MT279908) from poultry farm soil and *Fermenti bacillus* sp. RSCVS-HS3 (Genbank accession no. MT279752) from agriculture field soil, based on phenotypic, biochemical, and molecular characterizations (Chauhan and Mishra 2020) [5] (Chauhan and Mishra 2023) [6]. In this study, wash performance potential of crude alkaline protease filtrates from both isolates has been checked out.

## Materials and Methods

### Crude alkaline protease filtrates

Crude alkaline protease filtrates were obtained from *Bacillus halodurans* RSCVS-PF21 and *Fermenti bacillus* sp. RSCVS-HS3 on CPYA solid media (Chauhan and Mishra 2020) [5] (Chauhan and Mishra 2023) [6].

### Effect of crude alkaline protease on wash performance of detergent

The cleaning efficiency of crude enzyme was studied on piece of white cotton cloth (5 cm × 5 cm). These cloths were stained with blood (1 ml). Further, following wash experiment was conducted: (a) blood stained cloth piece with 30 ml water (control), (b) blood stained cloth piece with 20 ml detergent (10 mg/ml) and 10 ml water, (c) blood stained cloth piece with 20 ml detergent (10 mg/ml) and 10ml crude enzyme, (d) blood stained cloth piece with 20 ml water and 10 ml enzyme. All the plates were incubated at 50°C for 30 min, rinsed in the plain water and dried. Visual examination of cloth pieces was done to figure out the effect of enzymes in removal of blood stains (Agrawal., *et al*. 2016) [1].

## Results

Result of the effect of crude alkaline protease filtrate from *Bacillus halodurans* RSCVS-PF21 in the removal of blood stain from cotton cloth is shown in Figure 1. It clearly illustrated the cleaning action of *Bacillus halodurans* RSCVS-PF21 crude protease. After an incubation of 30 min, blood stain was not removed completely with detergent alone, while the mixture of crude enzyme with commercial detergent, removed the blood stain more efficiently.

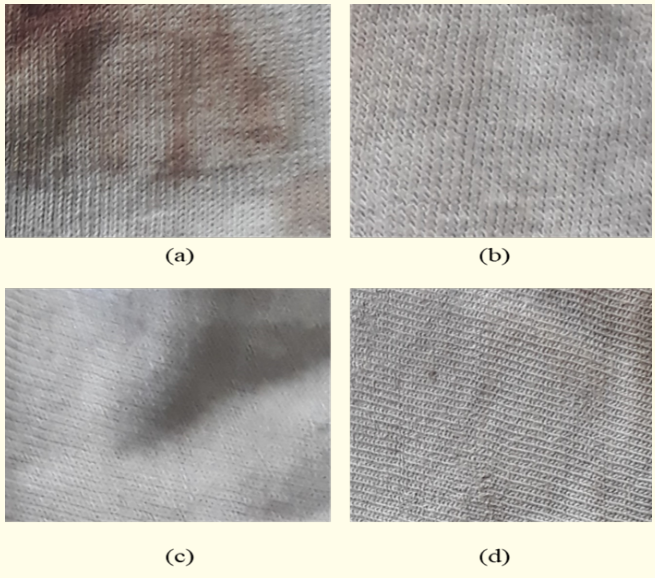


**Figure 1:** Evaluation of wash performance of crude alkaline protease from Bacillus halodurans RSCVS-PF21 in the removal of blood stain cotton cloth. (a). blood stained cloth piece + 30 ml water (control) (b). blood stained cloth piece + 20 ml detergent (10 mg/ml) + 10 ml water (c). blood stained cloth piece + 20 ml detergent (10 mg/ml) + 10ml crude enzyme, (d). blood stained cloth piece + 20 ml water + 10 ml enzyme.

The effect of crude alkaline protease filtrate of *Fermenti bacillus* sp. RSCVS-HS3 in the removal of blood stain from cotton cloth was investigated. Wash performance test shown in Figure 2, clearly illustrated the cleaning action of *Fermenti bacillus* sp. RSCVS-HS3 crude protease. After an incubation of 30 min, blood stain was not removed completely with detergent alone, while the mixture of enzyme with commercial detergent. removed the blood stain more efficiently.

## Discussion

The crude enzyme filtrates of both *Bacillus halodurans* RSCVS-PF21 and *Fermentibacillus* sp. RSCVS-HS3 was tested separately for their wash performance in the removal of blood stain from cotton cloth (Figure 1 and Figure 2). Wash performance test of both crude enzyme filtrates revealed that after an incubation of 30 min, blood

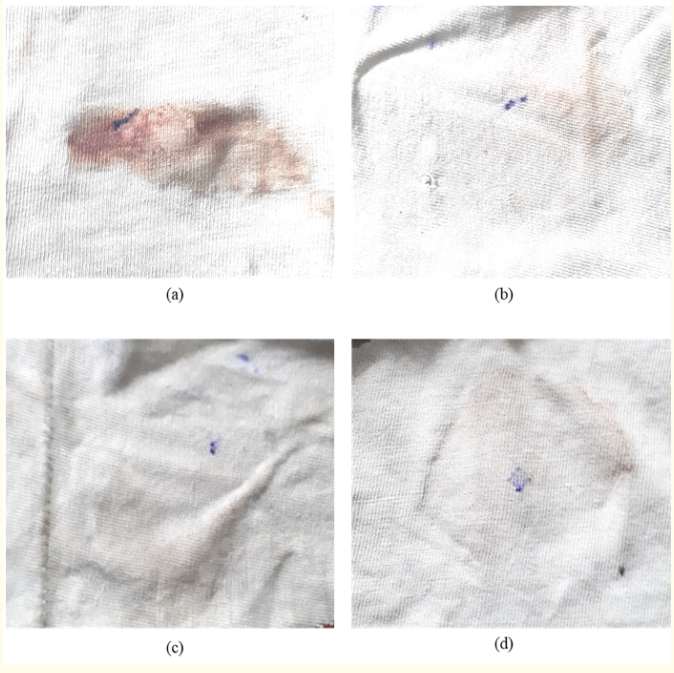


**Figure 2:** Evaluation of wash performance of crude alkaline protease of Fermenti bacillus sp. RSCVS-HS3 in the removal of blood stain from cotton cloth. (a). blood stained cloth piece + 30 ml water (control), (b). blood stained cloth piece + 20 ml detergent (10 mg/ml) + 10 ml water, (c). blood stained cloth piece + 20 ml detergent (10 mg/ml) + 10ml crude enzyme, (d). blood stained cloth piece + 20 ml water + 10 ml enzyme.

stain was not completely removed with detergent alone, but the mixture of crude enzyme filtrate and commercial detergent, removed the blood stain more efficiently. Vishalakshi., *et al*. (2009) [16] also reported the similar effect of alkaline protease obtained from *Streptomyces gulbargensis* in removal of blood stain from surgical instruments and cotton cloth.

## Conclusion

Wash performance test of crude enzyme filtrates from *Bacillus halodurans* RSCVS-PF21 and *Fermenti bacillus* sp. RSCVS-HS3 conducted separately, revealed that when crude enzyme filtrates and commercial detergent are mixed then removal of the blood stain on cotton cloth was more efficient. Thus, from above it is clear that both novel alkaline proteases producing bacterial strains investigated from Vindhya region, M.P., India can be potential candidates in detergent industry as well as in many other similar industrial applications which require protease activity in extreme conditions.

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## Declaration of Competing Interest

The authors declare no conflict of interest.

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