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ORIGINAL ARTICLE

Application of lipase extracted from *Pseudomonas aeruginosa* SJ2 as a detergent additive

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ABSTRACT
Partially purified lipase produced by Pseudomonas aeruginosaSJ2 was studied for its potential application in detergents. Five commercial detergents were supplemented with the partially purified enzyme and its washing performance was investigated. Lipase supplemented Patanjali Herbal Wash detergent showed the highest increase in percent oil stain removal while buffer plus lipase showed 14% stain removal. Results here showed that enzyme may also be employed in commercial formulations.
Keywords*: Detergent, Lipase, Pseudomonas aeruginosa SJ2.*

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INTRODUCTION

Humans have been employing enzymes from different micro-organisms to a number of processes across a wide range of fields from industry, medicine, and academia [10]. These enzymes are eco-friendlyand are more economical when compared to synthetic chemicals. In the recent years, ease of genetic manipulation has increased the use of microbial enzymes exponentially [6]. In the textile industry, enzymes have been used in detergent formulations and as detergent additives. Lipases from microbes are one of the prominent biocatalysts that act on carboxylic ester bonds. They are, therefore, used in detergent formulations to remove oil stains, which are difficult to remove under mild washing conditions [1].

A good detergent enzyme should be stable at alkaline pH and active in the presence of surfactants [4]. It should withstand oxidizing and chelating agents, which are used in detergent formulations as active oxygen-based bleaches and detergent builders. The enzyme also needs to be effective at a lower concentration and have broad substrate specificity. Traditionally, clothes are washed in hot or warm water. Use of synthetic fibers, which cannot tolerate temperatures above 50−60 °C,is on the rise. This coupled with the energy crisis has shifted the washing habits towards the use of lower washing temperatures of 30−40 °C [7]. Also, the components of the detergent may contain enzyme inhibitors. Therefore, an effective enzyme should be active at the desired washing temperatures and should be compatible with the detergent components [2].

Microbial lipases have been isolated from bacteria, fungi, and yeast. The commonly used lipases that are marketed commercially have been extracted from *Termomyces* sp., *Aspergillus* sp., and *Pseudomonas* sp. [2]. In this paper, we report the efficiency of a lipase extracted from *Pseudomonas aeruginosa*SJ2 (Genbank accession number is MN700061) as a detergent additive. Purified lipase combined with commercially available detergents was used to remove oil stains from fabric and the percent oil removal achieved was calculated.

MATERIAL AND METHODS

Preparation of soiled fabric, washing solutions, and enzyme extract

Cotton fabrics (5 cm × 10 cm) were defatted in boiling chloroform for 4 h and each piece was soiled by spotting 1 ml of olive oil in benzene (100 mg/ml) [3, 8]. Washing solutions (buffer (B), buffer + lipase (BL), buffer + detergent (BD), and buffer + detergent + lipase (BDL)) were prepared using components as shown in Table 1. Five commercial detergent powders available in the local market (Nirma, Surf Excel,Rin, Wheel, and Patanjali Herbal Wash) were used in the study. Purified lipase adjusted to contain 10 U/ml,was used as lipase solution in the washing experiment. Tris buffer solution, with and without lipase, was used as control [9].

Table 1: Constituents of the washing solutions used

Constituents	Volume (ml)			
	B	BL	BD	BDL
0.05MTris buffer (pH 8)	40	40	40	40
Detergent solution (1%)	-	-	50	50
Lipase solution (10 Units/ml)	-	10	-	10
Distilled water	60	50	10	-

*Table footnotes:*B, Buffer; BL, Buffer + Lipase; BD, Buffer + Detergent;BDL, Buffer + Detergent + Lipase

Estimation of olive oil:

The pieces of the soiled fabric were put into flasks containing the detergent solutions and washed at 30 °C using an orbital shakerat 100 rpm for 30 min. The pieces of fabric were then removed, rinsed thrice using 100 ml water with a soak time of 2 minutes, and then air-dried. The remaining olive oil from the soiled fabrics was extracted using petroleum ether for 6 h in a Soxhlet extractor; petroleum ether completely evaporated and the olive oil was weighed. All experiments were performed in triplicates and data are presented here as mean ± SD (Table 2).

Olive oil removed during washing was calculated using the following equation based on weight of the oil before and after washing.

Oil removal (%) = [(Wb−Wa) / Wb] x 100

Where, Wb is weight of olive oil before washing and Wa is the weight of olive oil after washing.

RESULTS

Oil-removal efficiency of different detergent solutions:

Efficiency of the extracted lipase as a detergent additive was investigated by simulating actual oil-stain removal experiments. Buffer containing Tris showed a modest mean oil removal of 7% without lipase and 14% with lipase. The solutions containing commercially available detergents showed oil removal in the range of 26-34% without lipase and 35-39% with lipase. It was evident that lipase increased oil removal in all the cases (Table 2).

Table 2: Mean oil removal achieved using the washing solutions

Sr. No.	Detergent	Mean oil removal(%)	
		BD	BDL
1	Nirma	28	35
2	Surf Excel	34	39
3	Rin	31	38
4	Wheel	30	36
5	Patanjali Herbal Wash	26	35
6	Buffer	7	
7	Buffer + Lipase	14	

Each value presented here is an average of triplicates of three independent trials. Mean standard deviation for all the values is <+/- 5.0%.

*Table footnotes:*BD, Buffer + Detergent;BDL, Buffer + Detergent + Lipase

DISCUSSION

An overall improvement in oil removal was observed with the addition of the lipase. There was a 100% improvement in the control buffer and an average 23% improvement in case of the commercially available detergents with addition of lipase. A recent study showed a similar efficiency of the lipase

extracted from another strain of *Pseudomonas aeruginosa* [5]. Out of the detergents used, Surf excel showed the highest amount of oil removal in both with and without lipase groups. However, Surf excel showed the lowest improvement out of all the washing solutions used.

The highest improvement was observed in case of Patanjali Herbal Wash after addition of the lipase. This differential improvement across the detergents and the control buffer might be due to some level of resistance conferred by the components of the commercial detergents against the lipase. Accordingly, Patanjali Herbal Wash seemed to confer least resistance towards the activity of the lipase. This might be due to the herbal nature of the detergent. However, further oil stain-removal studies using individual components of the Patanjali Herbal Wash are required to reach to a conclusion.

Only a few naturally-available enzymes are reported to be surfactant, bleach, and oxidant stable. Improving the stability of the lipase and to clarify its interactions with other components of the detergent is of high industrial importance. Separate studies investigating the interactions of the lipase with oxidizing agents, surfactants, and other inhibitory agents from the detergents are warranted. Elucidation of the mechanism of this inhibition should offer improved detergent formulations including lipases. Furthermore, studies investigating concentration-dependent effects of the lipase as well the detergent need to be performed [2].

Not all strains of *Pseudomonas aeruginosa* produce lipases with similar activity. A recent study reported that the lipase showed lipolytic activity *in vitro*, but did not improve oil-stain removal in the simulated washing experiment [1]. Ours is the first report of the application of the lipase extracted from *Pseudomonas aeruginosa*SJ2. Its efficiency in the washing experiment showed that it may also be effective in commercial formulations. Further studies in this direction are currently at the experimental stage in our lab.

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