

STUDIES ON THE COMPATIBILITY OF LIPASE ON DETERGENTS SYNTHESISED FROM WILD AND MUTANT STRAINS OF *Pseudomonas aeruginosa* BDG-6

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Abstract : This study has been undertaken to study the compatibility of lipase on detergents synthesised from microorganisms. The lipases produced from the microorganisms *Pseudomonas aeruginosa* BDG-6 which was isolated from the oil spilled soil and the UV mutated strain *Pseudomonas aeruginosa* MBDG were used for studying the compatibility with various commercially available detergents (Tide plus, Surf excel, Rin matic, Ariel matic, Triple Power and Henko matic) of Indian market. More than 95% of lipase activity was observed in all the detergents even after 1 hr of incubation of lipases from wild and mutant bacterial strains. Tide plus showed the maximum % increase in lipase activity and maximum oil removal of 76.32 ± 1.1 in *Pseudomonas aeruginosa* MBDG.

IndexTerms - *Pseudomonas aeruginosa*, lipase, detergents, oil.

I. INTRODUCTION

The use of enzyme-mediated processes can be traced to ancient civilizations. Among the industrial important enzymes, lipases constitute an interesting class of enzymes with wide biotechnological applications. This enzyme belongs to the major class of hydrolases having the ability to hydrolyze triacylglycerol to glycerol and free fatty acids over oil - water interface. The ability of lipases to perform very specific chemical transformation (biotransformation) have make them increasingly popular in the food, detergent, cosmetic, organic synthesis and pharmaceutical industries (Park et al 2005; Gupta et al 2007; Franken et al 2009). Although a number of lipase-producing bacterial sources are available only a few are commercially exploited as wild or recombinant strains (Gupta et al 2004).

The industrial demand for new sources of lipases with different catalytic characteristics stimulates the isolation and selection of new strains. Potential microorganisms which produce lipases are found in different habitats, including wastes of vegetable oils, dairy product industries and soils contaminated with oils, seeds, and deteriorated food (Sharma et al 2001). This indicates that nature offers a tremendous potential for identifying new sources of lipases with novel properties. The search is always on microorganisms from natural sources with desired lipolytic properties. Different techniques have been used to enhance the production of lipases through the optimization studies involving substrate composition, fermentation parameters and culture conditions (Salihu et al 2012, Sujatha & Dhandayuthapani 2013). A focus should be made on getting the right microbial strain through screening and modification, selection of appropriate medium components, identification of physical parameters and intended applications of the produced lipases (Salihu & Alam 2012).

The cleaning power of detergents seems to have peaked; all detergents contain similar ingredients and are based on similar detergency mechanisms. To improve detergency, modern types of heavy duty powder detergents and automatic dishwasher detergents usually contain one or more enzymes such as protease, amylase, cellulase and lipase (Ito et al 1998). Because of their ability to hydrolyze fats, lipases find a major use as additives in industrial laundry and household detergents (Fujita et al 1992). Detergent lipases with the desired properties are obtained through a combination of continuous screening (Wang et al 1995; Cardenas et al 2001) and protein engineering (Kazlauskas & Bornscheuer 1998).

II. MATERIALS AND METHODS

2.1 Production of lipase by microorganisms

The potential lipase producing bacterial strain was isolated from oil spilled soil sample by serial dilution and plating techniques (Collins et al 1995). A total of eight different colonies were isolated and designated the name as BDG1-BDG8. Out of the eight bacterial colonies isolated the isolate BDG-6 showed maximum lipase production of 24.22U mL^{-1} by screening techniques with oil supplemented basal medium. Lipase assay was performed by spectrophotometer method using 4 nitrophenol as substrate (Krieger et al 1999). For identification of the selected isolated strain morphological and biochemical tests were carried out according to Bergey's Manual of Determinative Bacteriology (Holt et al 1998). From the morphological, biochemical

and the phylogenetic tree derived from the 16S rRNA gene sequences from Genbank database, the isolates BDG-6 was identified as *Pseudomonas aeruginosa* and it was deposited in the genbank (**Accession number- KT865646**). Mutation has improved the productivity of industrial cultures (Parekh et al 2000). Strain improvement of the selected parent strain was done by mutating it by UV irradiation at varying time intervals. A total of five isolates of *P. aeruginosa* BDG-6 were selected and screened for maximum lipase production. The isolate three *P. aeruginosa* BDG-6 showed maximum lipase production. Then it was selected as mutant bacterial strain and designated as *P. aeruginosa* MBDG. Classical method of optimization studies were undertaken to improve lipase production in basal media by *P. aeruginosa* BDG-6 and mutated strain *P. aeruginosa* MBDG. The extracellular lipases produced by the bacterial strains were purified by ammonium sulphate and gel filtration chromatography. The purity of lipases was confirmed by 10% SDS-PAGE. The molecular weight determination was carried out by comparing the value of the band with the standard obtained by SDS-PAGE. The molecular weights of lipases of *P. aeruginosa* BDG-6 and *P. aeruginosa* MBDG were found to be 56 and 55 kDa respectively. The purified lipase was characterised.

2.2 Compatibility of Lipase in Various Commercial Detergents

The compatibility of purified lipase from wild and mutant strains in different commercially available detergents of Indian market was studied. The commercial detergents tested includes Tide plus (Procter & Gamble, USA), Surf excel (Hindustan Lever Ltd., Mumbai, India), Rin matic (Hindustan Lever Ltd., Mumbai, India), Ariel matic (Procter & Gamble, USA), Triple Power (Power soap Ltd., Tamil Nadu, India) and Henko matic (Jyothy Lab., Mumbai, India) were purchased from local market.

The endogenous lipases contained in these detergents were inactivated by heating the diluted detergents for 1h at 65°C prior to the addition of the enzyme preparation. To determine the stability, an aliquot of enzyme sample (50U/mL) was incubated with equal volume of detergent solution (7mg/ml of respective detergent) in Tris-HCl buffer (0.1M, pH 8.0) for 1h at 28±2°C (Room Temperature (RT)). Samples were withdrawn for every 15 min and the residual activity was determined by assayed under standard assay conditions and expressed as percent relative activity. The relative activity (%) of each sample was determined and compared with the control without detergent. The relative activity of control was defined as the enzyme activity without detergent, incubated under the similar conditions and was taken as 100%. The experiments were carried out in triplicate for each study. All values are represented as mean ± SD of three replications. The standard deviation did not exceed 5% of the average values.

2.3 Wash performance studies

Wash performance of purified lipase from wild and mutant bacterial strains were performed with different detergents. In this study four set of washing solution were prepared for each detergent as follows and finally made the volume into 100mL. The endogenous lipase contained in the detergent was inactivated by heating the detergent solution for 1h at 65°C prior to the addition of the enzyme solution.

- Set 1: 50mL of distilled water + 50 mL of 0.5% detergent.
- Set 2: 40mL of distilled water + 50 mL of 0.5% detergent + 10mL of lipase enzyme (50U/mL⁻¹)
- Set 3: 90mL of distilled water + 10mL of lipase enzyme (50U/mL⁻¹)
- Set 4: 100 mL of distilled water

A white cotton cloth piece (10 cm x 10 cm) was stained with oily stain. Washing of oil soiled cloth by maintaining the above four different conditions was performed for 20min at 28±2°C (Room Temperature (RT)) and 100 rpm of stirring. After incubation the cloth pieces were taken out from each set, rinsed with water, dried and visual examination was done to check the effectiveness of stain removal and also the percentage of oil removal by extracting the oil from the cloth with petroleum ether for six hours in Soxhlet extractor. Controls were consisted of soiled cloth pieces without enzyme treatment. After completely evaporating the petroleum ether from the extract, the oil weight was determined. The % of oil removal was calculated using the following formula, based on the weight of the total fatty acids on the cloth before and after washing.

$$\text{Oil removal (\%)} = \frac{W_i - W_f}{W_i} \times 100$$

Where, W_i – initial weight of oil before washing (mg) W_f - final weight of oil after washing (mg).

III. RESULT AND DISCUSSION

3.1 Compatibility of Lipase in Various Commercial Detergents

In the present investigation, six different commercial detergent powders which include Tide plus, Surf excel, Rin matic, Ariel matic, Triple Power and Henko matic were purchased from local market and used to test the compatibility of lipases from wild and mutant strains. As shown in the Fig 3.1, more than 95% of activity was observed in all the detergent even after 1h of incubation of lipases from wild and mutant strains. But the lipase from mutant strain showed maximum activity than wild strains.

Among six different detergents Tide plus showed the maximum % increase in lipase activity. This detergent was used for further studies. Both the wild and mutant lipases were 100% compatible to all the detergents tested in the present investigation, since there was no decrease in enzyme activity. The results indicated that the lipases from wild and mutant strains were highly stable in all the detergents tested as it retained more than 95% of activity in all the detergents even after 1h of incubation, since all these detergents have an alkaline pH which may be supported for high stability. Similarly Chauhan et al (2013) also reported that the lipase from *Staphylococcus arlettae* JPBW-1 was an ideal candidate for use in laundry detergent formulations, since it possessed better stability with surfactants, commercial detergents, and oxidizing agents. Lipases which are stable and work at alkaline pH (8 to 11), suitable wash conditions for enzymated-detergent powders and liquids have good potential for use in detergent industry (Hasan et al 2006). Hasan et al (2007) reported 100% stability of lipase produced by *Bacillus* sp. FH5, at pH 10. Hence this enzyme showed promising results when used in combination with different commercially available conventional detergents.

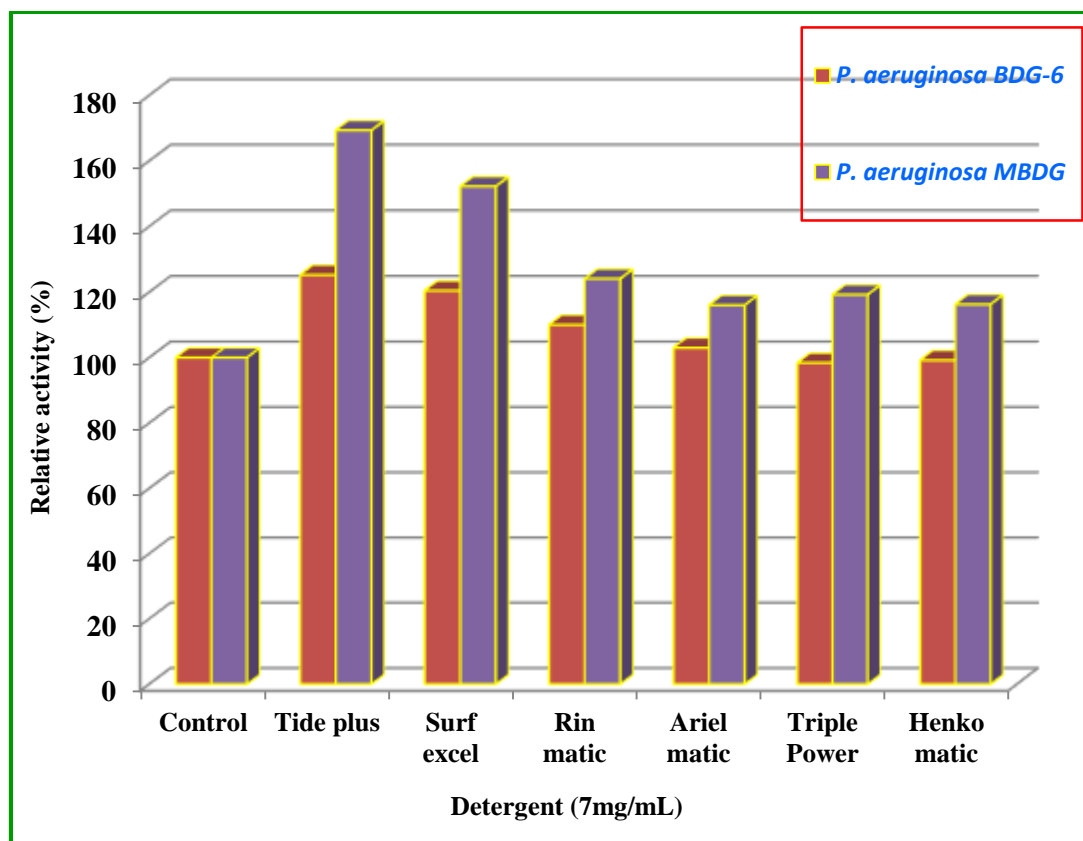


Figure 3.1 Compatibility of lipases from wild and mutant bacterial strains with different commercial detergents

3.2 Effect of lipase on oil removal with detergent

The effect of lipases on oil removal from the soiled cloth was studied by wash performance studies with different detergents such as Tide plus, Surf excel, Rin matic, Ariel matic, Triple Power and Henko matic at concentration of 0.5%. All the detergents showed a significant % of oil removal with combination of lipase from mutant bacterial strain than wild one. Among all detergents, the maximum percentage of oil removal of 76.32 ± 1.1 was in *P. aeruginosa* MBDG and it was chosen for further studies. This result indicates that the lipase from mutant strain was more suitable for detergent than the lipases from wild strains. The results are tabulated in Table 1 & 2. The lipase was more effective with nonionics than with anionics which attributes to the higher enzyme activity inhibition by anionic surfactants (Chauhan et al 2013, Flipsen et al 1998, Sajna et al 2013).

Table 3.1 Effect of *P. aeruginosa* BDG-6 lipase on oil removal from cotton cloth with different detergents (All values are represented as mean \pm SD of three replications)

Detergent	Lipase from <i>P. aeruginosa</i> BDG-6			
	% of oil removal			
	Set 1	Set 2	Set 3	Set 4
Tide plus	35.31 \pm 2.1	42.12 \pm 3.2	2.14 \pm 1.2	-
Surf excel	31.21 \pm 1.3	39.21 \pm 1.2	2.21 \pm 0.1	-
Rin matic	31.23 \pm 2.4	40.12 \pm 4.3	2.11 \pm 1.1	-
Ariel matic	28.41 \pm 3.2	38.41 \pm 3.1	2.13 \pm 2.1	-
Triple Power	23.21 \pm 3.1	40.12 \pm 3.1	2.21 \pm 2.2	-
Henko matic	26.11 \pm 2.2	35.21 \pm 2.2	2.13 \pm 1.3	-

(Set 1- Wash solution with detergent only, Set 2- Wash solution with detergent and enzyme, Set 3-Wash solution with enzyme only, set 4- Wash solution without enzyme and detergent)

Table 3.2 Effect of *P. aeruginosa* MBDG lipase on oil removal from cotton cloth with different detergents (All values are represented as mean \pm SD of three replications)

Detergent	Lipase from <i>P. aeruginosa</i> MBDG			
	% of oil removal			
	Set 1	Set 2	Set 3	Set 4
Tide plus	35.12 \pm 2.1	76.32 \pm 1.1	4.01 \pm 1.1	-
Surf excel	31.23 \pm 1.0	74.21 \pm 3.1	4.10 \pm 1.1	-
Rin matic	31.33 \pm 3.0	74.21 \pm 1.1	4.01 \pm 1.0	-
Ariel matic	28.21 \pm 3.1	71.32 \pm 1.2	4.00 \pm 1.1	-
Triple Power	23.22 \pm 2.2	71.02 \pm 1.0	4.01 \pm 1.2	-
Henko matic	26.12 \pm 2.3	70.21 \pm 1.3	4.01 \pm 1.1	-

(Set 1- Wash solution with detergent only, Set 2- Wash solution with detergent and enzyme, Set 3-Wash solution with enzyme only, set 4- Wash solution without enzyme and detergent).

IV. CONCLUSION

The current study revealed the detailed understanding on the compatibility of purified lipases with various detergents. Both the wild and mutant lipases were 100% compatible to all the detergents tested in the present investigation, since there was no decrease in enzyme activity. More than 95% of activity was observed in all the detergents even after 1h of incubation of lipases from wild and mutant strains. The maximum percentage of oil removal was also observed when it was mixed with detergents. The lipase from mutant strain showed maximum lipase activity than wild strain in detergents. Among the various detergents used, Tide plus showed the maximum % increase in lipase activity and maximum % of oil removal (76.32 \pm 1.1 for *P. aeruginosa* MBDG and 42.12 \pm 3.2 for *P. aeruginosa* BDG-6). The lipases produced were highly compatible with detergents and it can be used in the detergent industries. Definitely in the near future lipases will dominate the global enzyme market.

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