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Research Article

PARTIAL PURIFICATION AND CHARACTERIZATION OF DETERGENT COMPATIBLE LIPASE FROM MARINE *STREPTOMYCES FUNGICIDICUS* RPBS-A4 FOR APPLICATION IN OIL BASED STAIN REMOVAL

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A B S T R A C T

Lipase obtained from *Streptomyces fungicidicus* RPBS-A4 was partially purified using ammonium sulphate precipitation (80% w/v) followed by dialysis. Biochemical and physical properties of the purified enzyme was investigated and it showed molecular weight of 32.0 KDa and zymogram of yellowish band over a pink background. The purified enzyme was stable in the pH range of 8.0-11.0 and most lipase activity obtained at 12 hours of incubation in the pH value of 9.0. Thermo stability profile revealed that the enzyme showed 100% activity at 40°C and nearly 91.6% of the activity at 60°C. Lipase obtained was maximally stimulated by both the calcium and manganese ions. Among the organic solvents maximum activity was obtained in the presence of isoamyl alcohol followed by acetone. More than 63% of the enzyme activity was detected even in the existence of commercial detergents after 3 hours of incubation. The partially purified lipase removed the used motor oil spot more efficiently when coupled with detergents that were different than the detergents alone. Storage indicating that enzyme could be kept without a lot of reduction in its action for up to 20 days at room temperature.

INTRODUCTION

Lipases (EC 3.1.1.3) are hydrolases that, in biological systems, initiate the catabolism of fats and oils by hydrolyzing the fatty acyl ester bonds of acylglycerols. Microbial lipases recently have attracted considerable attention owing to their biotechnological potential, ranging from the use within laundry detergents to stereo specific biocatalysis (Jaeger *et al*., 1994). These lipases are secreted into the culture medium by many fungi and bacteria. They vary from each other within their physical and biochemical properties (Aires-Barros *et al*., 1994). Looking for new sources of lipases is justified by realizing many different future applications requiring not only enzyme-substrate specificity but in addition process stability such as wide pH tolerance and high thermal stability. Enzymes may be stabilized in the presence of organic solvents (DeSantis and Jones, 1999) however; having naturally stable and highly active enzymes in organic solvents are of greater advantage. Purification and characterization are the key procedures for discovering novel lipases (Saxena *et al*., 2003, Aisaka and Terada, 1980). Most of the lipase purification schemes described in the literature focused on purifying small levels of the enzyme to homogeneity to characterize it. Little information has been published on large scale processes for commercial purification of lipase. Most commercial applications of lipases don't require highly pure enzyme. Excess purification is expensive and reduces overall recovery. The detergent industry has remained the biggest market for industrial enzymes and new enzyme goods are constantly being developed for use (Ahuja *et al*., 2004). A major problem in laundries is the removal of adsorbed lipids from fabrics, which often contain oily, long chained and water-insoluble triacylglycerol like oils. This could be accomplished using detergent formulation containing lipase enzyme that degrade triacylglycerols into free fatty acids, di-and mono-acylglycerols, and possibly glycerol (Thirunavukarasu *et al*., 2008). The lipases enhance the washing capacity of detergents as well as removal of fatty food stains and sebum from fabrics, which are difficult to get rid of under normal washing conditions (Andree *et al*., 1980). The increasing demand for alkaline lipases as a soap additive is especially because affiliation with the nonphosphate detergents. Ideally, alkaline lipases in a soap formulation should be stable over a broad range of temperature, pH and compatible with surfactants and oxidizing agents at lower concentrations with broad substrate specificity (Jellouli *et al*., 2011). To use any lipase for industrial application, it is essential to characterize the

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enzyme and study its properties. Therefore, in this paper we report partial purification, characterization and application of alkaline lipase from a marine *Streptomyces fungicidicus* RPBS-A4. Further, we plan to use this lipase for additive in laundry detergents.

MATERIALS AND METHODS

Microorganism and production of lipase

Streptomyces fungicidicus RPBS-A4 was isolated from the Bay of Bengal near Chirala coast of Andhra Pradesh and identified as a lipase producer after culturing on agar plates containing tributyrin (Rajanikanth and Damodharam, 2017). The lipase production was carried out in shrimp waste based medium (SWM) with the same composition as used in our previous study (Rajanikanth and Damodharam, 2016). Briefly, 8mm disc of inoculum grown on starch casein agar medium was transferred to shrimp waste based media at 40°C and 120 rpm for a fermentation period of 96 hours. The initial pH was adjusted to 9.0. The fermented broth was centrifuged at 5000 rpm for 10 minutes and supernatant collected was used as crude extracellular enzyme.

Partial purification of lipase

Lipase produced was extracted and partially purified using ammonium sulphate precipitation followed by dialysis (Englard and Seifter, 1990). Precipitation was done at 10%, 10-20% and 20-30% saturation for 2-3 hours and at 40-50%, 50-60%, 60-80%, and 80-90% saturation of ammonium sulphate for overnight incubation, with magnetic stirrer at 4°C. After each and every precipitation step, the fraction was centrifuged at 12,000 rpm for 15 minutes at 4°C. All the precipitates obtained were re suspended in a small quantity of buffer (10 mM Tris-HCl, pH 8.0). Then it is dialyzed against large volume of the exact same buffer by successive change in buffer after 2 hours at 4°C. The process was continued before the last trace of ammonium sulphate was removed. All of the concentrated fractions were subjected to protein estimation (Lowry *et al*., 1951) and enzyme activity was assayed by titrimetry using olive oil as substrate (Jensen, 1983).

Determination of Molecular weight and detection of lipase activity by zymogram

The molecular weight of the partially purified enzyme was determined by SDS-PAGE using 10% separating gel (Laemmli, 1970). The gel was stained with silver staining method as described by (Bollag *et al*., 1996).Lipase activity of enzyme protein band was confirmed by native poly acrylamide gel electrophoresis (Davis, 1964) accompanied by chromogenic plate analysis (Singh *et al*., 2006). Ammonium sulphate precipitated (60%) fraction was subjected to native poly acrylamide gel electrophoresis in a 10% separating gel and 4% stacking gel. Proteins were permitted to stack at 60 mV and to split up at 105 mV. The gel was run for a period of about 3 hours before the dye front reached the end of the gel. The gel was rinsed 3 times with distilled water and equilibrated in 50mM Tris-HCl buffer for 30 minutes at room temperature. Chromogenic substrate plate was prepared by utilizing phenol red (0.01%) along with 1% tributyrin, 10mM $CaCl_2$, and 2% agar. The pH was adjusted to 7.3 to 7.4 by using 0.1M NaOH. The gel was overlaid with the molten chromogenic substrate (40°C), which was then permitted to solidify. The plate was incubated at room temperature until a yellow band was observed.

Characterization of partially purified enzyme

Lipase partially purified by ammonium sulphate precipitation and dialysis was further characterized for its biophysical and biochemical properties described as follows.

Effect of pH on enzyme activity and stability

The pH effect on enzyme activity and stability were studied by using various buffers in the pH range of 4.0 to 11.0 (Bora and Kalita, 2008). The reaction mixture containing 0.5 ml of 5% tributyrin substrate (prepared in 0.1 M buffers having different pH values), 0.3 ml buffer and 0.1 ml enzyme was incubated at 50°C, for 30 minutes, in shaking incubator at 200 rpm per min. After 30 minutes, the reaction was terminated by the addition of 1 ml of acetone: ethanol solution (1:1). Then the lipase activity was measured as described earlier. Enzyme + buffer mixtures (without substrate) were pre-incubated at room temperature. After 30 minutes, 0.5 ml of 5% tributyrin was put into these mixtures and the residual lipase activity was assayed to find out the stability.

Effect of temperature on enzyme activity and stability

The effect of temperature on enzyme activity was studied by incubating partially purified enzyme at different temperatures in the range of 10-80ºC (Bora and Kalita, 2008). The reaction mixture containing 0.5 ml of 5% tributyrin substrate, 0.3 ml of buffer and 0.1 ml enzyme was incubated at different temperatures (30-80°C), for 30 minutes, in a shaking incubator at 200 rpm per min. After 30 minutes, the reaction was terminated by the addition of 1 ml of acetone: ethanol solution (1:1). Then the lipase activity was measured as described earlier. The enzyme + buffer mixtures were being incubated at different temperatures (30-80ºC). After 30 minutes, 0.5 ml of 5% tributyrin was put into these solutions and the residual lipase activity was assayed to find out its stability.

Effect of organic solvents on lipase activity

The enzyme solution was combined with different solvent solutions to yield the specified final solvent concentration (10%). The solvents used were acetone, methanol, ethanol, propanol, butanol and isoamyl alcohol. The enzyme was incubated in the presence of organic solvents for 30 minutes and relative activity (%) was calculated (Lailaja and Chandrasekaran, 2013).

Effect of metal ions on enzyme activity

The effect of metal ions was tested on the activity of lipase made by *S. fungicidicus* RPBS-A4. The enzyme was incubated with several metal ions including $CaCl_2$, $MgSO_4$, KCl, NaCl, $COCl_2$, $MnCl_2$, $ZnSO_4$, $HgCl_2$ and $CuSO_4$ at a final concentration of 1 mM for 30 minutes at room temperature. After incubation, residual lipase activity was assayed (Lailaja and Chandrasekaran, 2013).

Compatibility of lipase with commercial detergents

Suitability of enzyme for use within detergents was determined when it comes to its compatibility in a variety of commercial detergents by directly incorporating them in to

the enzyme assay mixture (Chellappan, 2006). The stability of the enzyme in the presence of commercial detergents was determined using Ariel, Surf Excel, Tide, Nirma and Wheel. The detergent solution (7mg/ml) was prepared in double distilled water and the solutions were boiled at 100ºC for 10 min to destroy any enzyme already present and then cooled at room temperature. 2 ml of partially purified enzyme was put into 50 ml solutions of detergent and the assay mixture was incubated for different time intervals (0.5 to 3 hours) at 30ºC and the residual activity was determined.

Compatibility of lipase with oxidizing agents

Lipase compatibility in presence of oxidizing agents was determined in TrisHCl buffer (0.1M, pH 9.0) containing 0.5-2.0 % (v/v or w/v) of hydrogen peroxide, sodium perborate and sodium hypochlorite for one hour at 30°C and relative activity was estimated and compared with the control without oxidizing agent. The relative activity of control was defined as the enzyme activity without oxidizing agent, incubated under similar conditions and was taken as 100% (Chauhan and Garlapati, 2013).

Wash performance of lipase in oil-based stain removal

Destaining activity of lipase of *S. fungicidicus* RPBS-A4 was studied on white cotton cloth pieces (5x5 cm) pre-stained with used motor oil (Adinarayana *et al*., 2003). The stained cloth pieces were allowed to sit overnight and taken in separate flasks. The following sets were prepared and studied:

Flask with distilled water (100 ml) + cloth piece stained with used motor oil.
Flask with distilled water (100 ml) + cloth piece stained with used motor oil + 1ml Ariel detergent (7mg/ml).
Flask with distilled water (100 ml) + cloth piece stained with used motor oil + 2ml enzyme solution.
Flask with distilled water (100 ml) + cloth piece stained with used motor oil + 1ml Ariel detergent (7mg/ml) + 2ml enzyme solution.

The aforementioned flasks were incubated at 60ºC for 15 minutes. After incubation, cloth pieces were taken out and rinsed with water and dried. Visual examination of cloth pieces were made on the effect of enzyme in the removal of stains. Untreated cloth pieces stained with used motor oil was taken as control.

Shelf life of purified enzyme

Shelf life or storage is an essential parameter for commercial usage of industrial products. The purified lipase 2ml was stored within an Eppendorf tube at room temperature (30˚C) for 20 - 30 days to test the effect of storage of purified lipase on its activity.

RESULTS AND DISCUSSION

Production and partial purification of extracellular lipase

In the current study, the marine *Streptomyces fungicidicus* RPBS-A4 was grown in shrimp waste based medium for 4days. After incubation culture broth was centrifuged at 5,000 rpm for 10 minutes at 4° C. The supernatant collected was used as crude enzyme and partially purified further by ammonium sulphate precipitation followed by dialysis. On the list of precipitants used, 60% ammonium sulphate (enzyme: ammonium sulphate v/w) yielded maximum specific activity of 562.5 IU/mg with purification fold and percentage recovery of 11.01 and 85.28. Results obtained for purification of the crude enzyme are summarised in Table.1.

Determination of molecular weight and detection of lipase activity by zymogram

SDS-PAGE analysis of the partially purified enzyme, performed and it yielded an individual band, testifying the dipeptide nature of the enzyme (Fig. 1). The molecular weights of the lipases were reported to be in the range of 27 to 43 kDa (Kok *et al*., 1995, Pratuangdejkul and Dharmsthiti, 2000). In the current study, the molecular weight of the lipase was determined by SDS-PAGE and the gel was stained with silver nitrate. The molecular mass of lipase estimated in comparison of the electrophoretic mobility of marker proteins suggests that the *Streptomyces fungicidicus* RPBS-A4 lipase possesses an apparent molecular mass of - 32kDa. Native polyacrylamide gel was performed by conformation of lipase enzyme using zymogram assay.

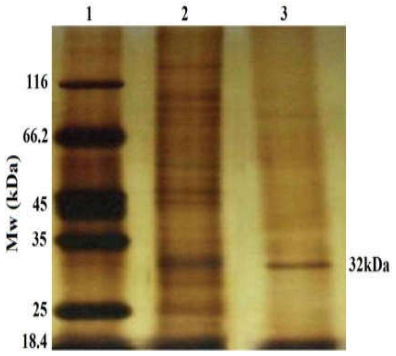


Fig 1 SDS-PAGE (10%) of partially purified lipase
Lane 1: unstained protein molecular weight markers
Lane 2: crude lipase.
Lane 3: partially purified lipase

Fig 2 Zymographic analysis of lipase.

Table 1 Effect of precipitants on purity and yield of Streptomyces fungicidicus lipase

Purification step	Volume (ml)	Enzyme activity (IU/ml)	Total activity (IU)	Soluble protein (mg/ml)	Total protein (mg)	Specific activity (IU/mg)	Yield of protein (%)	Yield of activity (%)	Purification fold
Culture filtrate	100	15.83	1583	0.31	31	51.06	100.00	100.00	1.00
Enzyme: $NH_4(SO_4)_2$ %)									
20	40	20	800	0.1	4	200	12.9	50.53	3.91
40	40	26.25	1050	0.12	4.8	218.75	15.48	66.32	4.28
50	40	28.75	1150	0.07	2.8	410.71	9.03	72.64	8.04
60	40	33.75	1350	0.06	2.4	562.5	7.74	85.28	11.01
70	40	18.75	750	0.07	2.8	267.85	9.03	47.37	5.24
80	40	15	600	0.26	10.4	57.69	33.53	37.90	1.12

Distinct yellow bands were observed against pink background within 30 minutes is shown in Fig. 2. However, it absolutely was observed that the yellow zone increases because of the spread of fatty acids on prolonged incubation; this problem was overcome by introducing $CaCl_2$ in the medium for quenching the fatty acids.

Characterization of purified enzyme

Effect of pH on enzyme activity and stability

Changes in pH will affect the protein structure and enzyme activities (Ohnishi *et al*., 1994a, 1994b). The effect of pH on lipase activity is shown in (Fig. 3a) where lipase showed activity in the pH range of 6.0-11.0.The activity was highest at pH 9.0 (0.1M Tris buffer). It had been also noted that the enzyme was totally inactive at pH below 6.0 and very less activity has been observed at pH 6.0 and 7.0 respectively. So, the enzyme was just active in the alkaline pH range. (Kumar *et al*., 2005) reported that the purified lipase from *B.coagulans* BTS-3 was stable within a pH range of 8.0 - 10.5 with optimum activity at 8.5. Stability of the enzyme at different pH values was determined by measuring the residual activity after incubating the enzyme in the pH range of 4.0-11.0 for 24 hours at 40ºC. *Streptomyces fungicidicus* RPBS-A4 showed residual activity of 100% at pH 9.0 for12 hour's incubation period (Fig. 3b).

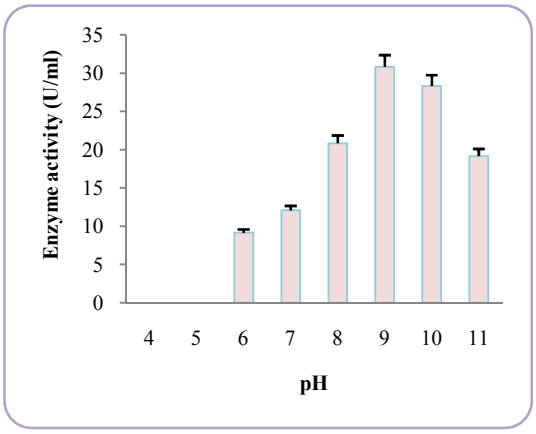
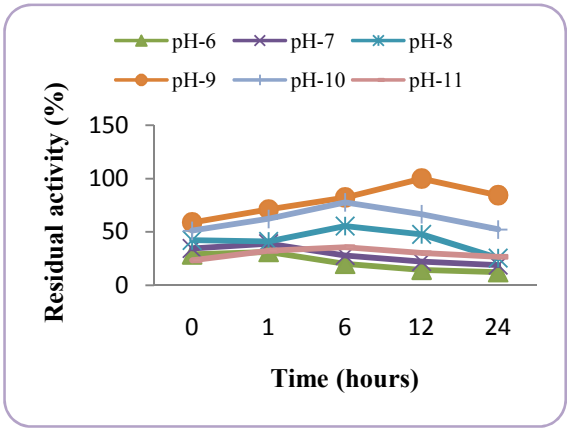


Fig 3 (a) Effect of pH on lipase activity



(b) Stability of lipase enzyme at various pH levels

Effect of temperature on lipase activity and stability

The effect of temperature on lipase activity was analysed by carrying out assays at different temperatures (10-80°C) at pH 9.0. With the rise in temperature, the enzyme activity started increasing and reached an optimum at 40°C. Thermal stability was investigated by incubating the enzyme at various temperatures (30-80°C) for one hour. Thermo stability profile indicated that the enzyme showed great stability as much as 60°C (Fig. 4). Since thermo stable lipases, which are active and stable in acidic and alkaline media, are extremely attractive and have a high potential for different industrial applications, this marine isolate *Streptomyces fungicidicus* RPBS-A4 lipase will be a potent and valuable enzyme for further applications. It has been reported that the drop in the percentage of residual activity at high temperatures results first in certain conformational changes in the tertiary structure, and then almost complete inactivation of enzymes (Ozen *et al*., 2004).

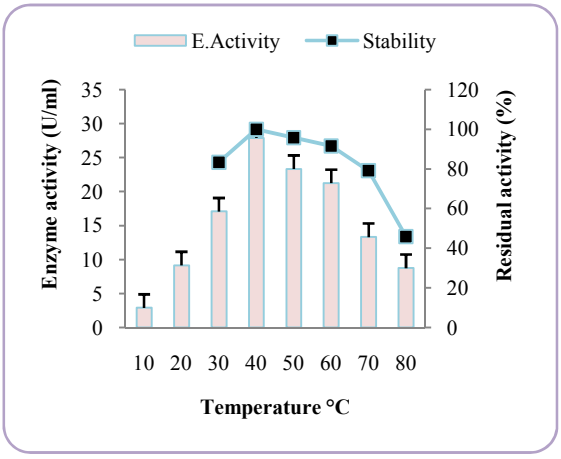


Fig 4 Effect of temperature on lipase activity and Stability

Effect of organic solvents on lipase activity

Stability and activity in organic solvents are very important characteristics of protein catalysts utilized in organic synthesis reactions. Generally, polar solvents were more destabilizing than their non-polar counterparts. The upsurge in lipase activity in the non-polar solvents might be due to the hydrophobicity of the solvents. There is a tendency for hydrophilic solvents to cause more significant enzyme inactivation than hydrophobic solvents and the significant deactivation in polar solvents is because of the stripping-off of crucial bound-water monolayer from the enzyme molecule required for its activity (Doukyu and Ogino, 2010, Ogino and Ishikawa, 2001).The results of various organic solvents on enzyme activity are presented in (Fig. 5).

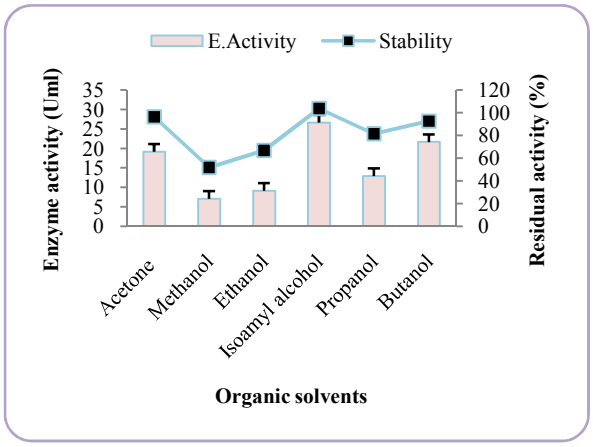


Fig 5 Effect of organic solvents on lipase activity and Stability

The enzyme was generally highly stable in the presence of organic solvents. In the experiments carried out with organic solvents, it had been seen that isoamyl alcohol (103.64%), acetone (96.26%), butanol (92.53%), propanol (81.42%), ethanol (66.66%), and methanol (51.82%) activated this enzyme.

Effect of various metal ions on enzyme activity

The metal ions are essential in maintaining cell wall rigidity, stabilizing oligomeric proteins and covalently binding protein peptidoglycan complexes in the outer membrane (Macció *et al*., 2002). Metal ions are reported to stimulate lipase-catalyzed hydrolysis of oil by detaching the fatty acids from the oil-water interface and allowing lipase to behave freely on oil molecules (Sharon *et al*., 1998). The effects of various metal ions on enzyme activity are presented in (Fig. 6). Thermostable lipase activity was enhanced by several metal ions (1mM) with highest relative activity achieved when the enzyme was pre-treated with $CaCl_2$ followed by NaCl and $MgSO_4$ with 103.28%, 96.66%, and 93.33%, respectively. (Kambourova *et al*., 2003) suggested that the positive effect of Ca^{2+} is because of formation of insoluble ion salts of fatty acids during hydrolysis, thus avoiding the product inhibition. (Rahman *et al*., 2005) stated that metal ions are likely to be bind to the enzyme and change the enzyme's conformation to counter better stability and hence greater activity.

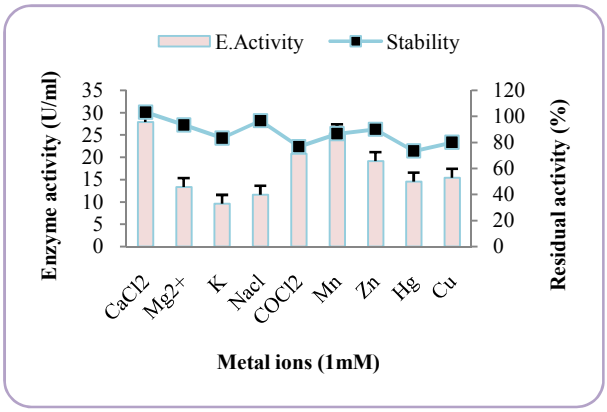


Fig 6 Effect of metal ions on lipase activity and Stability

Lipase compatibility with detergents

A problem in laundries is removing adsorbed lipids from fabrics, which frequently contain oily, long-chained and water-insoluble triacylglycerols like oils. This might be accomplished using detergent formulations containing lipases which degrade triacylglycerols into free fatty acids, di-and mono-acylglycerols, and possibly glycerol. The lipases increase the washing capacity of detergents in addition to removal of fatty food stains and sebum from fabrics, which are difficult to remove under normal washing conditions. For these applications, the current investigation provided a novel thermostable alkaline lipase for laundry and automatic dishwashing to degrade the residues of used motor oils such as for instance scooter engine oil, lorry engine oil, etc. and thereby greasy stains on clothes of mechanic shops can profitably be removed. The usage of enzymes in household laundry detergents is environment friendly since all enzyme goods are non-toxic completely biodegradable and helps reduce clothes-washing energy consumption (Gulati *et al*., 2005).

Detergent compatibility of the enzyme was studied to be able to confirm the potential of this alkaline lipase as a detergent additive, its compatibility and stability was examined at room temperature (28±2ºC) towards the most popular, commercial Indian detergents such as for instance Ariel, Surf excel, Tide, Nirma and Wheel. The lipase obtained was showed excellent stability and compatibility with a wide variety of locally available commercial detergents. It showed highest compatibility with 'Ariel 'detergent retaining 96.6% activity after 0.5 hour incubation at 30ºC. However, it retained 86.64%, 76.64% and 73.28% activity after 1.0, 2.0 and 3.0 hours of incubation at 30ºC, respectively (Table. 2). Subsequent to Ariel detergent the enzyme was more appropriate for 'Surf excel 'detergent exhibiting 90% activity after 0.5 hour incubation, and retained 83.28%, 73.28% and 66.64% activity after 1.0, 2.0 and 3.0 hours of incubation, respectively at 30ºC. Remaining activity of the enzyme was significantly more than 63% with rest of the tested detergents even after 3.0 hours of incubation. Therefore, it could be suggested that the lipase from *Streptomyces fungicidicus* RPBS-A4 is suitable for commercial detergents being an additive.

Table 2 Compatibility of lipase from *S.fungicidicus* RPBS-A4 with commercial detergents at 28±2ºC

Detergents	Relative residual lipase activity (%)			
	0.5 hour	1.0 hour	2.0 hour	3.0 hour
Ariel	96.6	86.64	76.64	73.28
Surf Excel	90	83.28	73.28	66.64
Tide	86.6	80	70	63.28
Nirma	80	76.64	66.64	63.28
Wheel	83.3	76.64	70	66.64

The maintenance of the initial activity of an alkaline lipase in the detergent could be related to the detergent ingredients that raise the accessibility of the lipase to the substrate by favouring the emulsification, maintaining the interfacial properties, and by solubilisation of the lipolysis products (Jurado *et al*., 2007). The alkaline lipase purified from *Bacillus sp*. DH4 was also stable in a variety of detergents (Bora and Kalita, 2008). A loss in less than 10% of initial activities in Ariel, Surf, Ujala, Tide, Sunlight and Henko for 3 hours at 50ºC was reported for the alkaline lipase of *Bacillus smithi* BTMS 11 (Lailaja and Chandrasekaran, 2013). The alkaline lipase of *Staphylococcus sp.* ESW maintained initial activity in Ariel and Axion, and retained more than 80% of the initial activity in Dixan and Nadhif for 1 hour at 50ºC (Cherif *et al*., 2011). The present stability and compatibility makes the alkaline lipase of *Streptomyces fungicidicus* RPBS-A4 a perfect choice for the detergent industry.

Lipase compatibility with oxidizing agents

Bleach stability of the enzyme was also checked in the presence of hydrogen peroxide, Sodium hypochlorite and Sodium perborate. The lipase was highly stable towards oxidizing agents at 0.5% concentration for 1hour at 30ºC and it retained 85.15% of activity even at 2.0% concentration of hydrogen peroxide, while activity was gradually decreased with upsurge in concentrations of sodium perborate and sodium hypochlorite from 1.0 to 2.0% (Fig.7). Remarkably, the current lipase exhibited better resistance towards strong oxidizing agents especially hypochlorite (92.53% activity at 0.5% concentration) compared to the relative activity of lipolase (Novozymes, 2007), which exhibited 43% activity

after 1hour treatment as reported by (Rathi *et al*., 2001). Therefore, the stability of oxidations is a significant characteristic necessary for an enzyme to be incorporated in to a detergent.

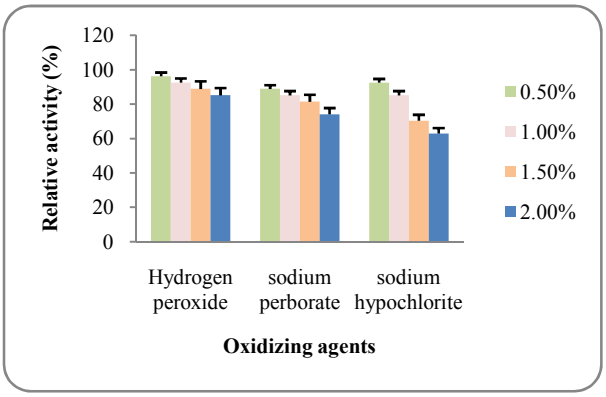


Fig 7 Compatibility of *S.fungicidicus* lipase with oxidizing agents

Wash performance of lipase in oil-based stain removal

The wash performance analysis was carried out to ascertain the efficiency of alkaline lipase for the removal of lipid stains from fabrics. Analysis was carried out with new white cotton cloth stained with used motor oil. After washing stained clothes in the detergent solution with and without enzyme and visual examination of the dried clothes after having a tap water rinse, it had been pointed out that the enzyme, on its own was equally or more efficient than the commercial detergent in removing the stains. When added with the detergent, the wash performance was much more superior. The outcomes of wash performance in test swatches are as showed in Fig. 8.

(a) **(b)** **(c)** **(d)** **(e)**

Fig 8 Wash performance (against used motor oil) of lipasefrom *S. fungicidicus* RPBS-A4 in combination with commercial detergent (Ariel) at 30°C. (a) Cloth stained with used motor oil, (b) Stained cloth washed with water only, (c) Stained cloth washed with detergent only, (d) Stained cloth washed with enzyme only and (e) Stained cloth washed with detergent and enzyme

Shelf life of detergent

The effect of storage of purified lipase on its activity was determined as it is an essential parameter for commercial usage of enzyme. The enzyme stored at room temperature (Table. 3) revealed that the enzyme was 92.33% active for 15 days and the activity is reduced to 88.45, 76.91and 53.83% after 20th, 25th and 30th day respectively. Storage suggests that enzyme may be stored for 20 days at room temperature without much loss in its activity. Similarly, retention in enzyme activity of 95% was seen at 32ºC, respectively, for the lipase made by *Bacillus licheniformis* VSG1 (Sangeetha *et al*., 2010).

Table 3 Effect of storage (shelf life) on purified lipase activity at room temperature

Shelf Life (Days)	Lipase relative activity (%)
0	100
15	92.33
20	88.45
25	76.91
30	53.83

CONCLUSION

In the present study, preliminary experiments on the destaining of used motor oil stains from test fabrics demonstrate satisfactory results. This research, therefore, is an effort to unravel the microbial diversity of local resources with regards to some functional attributes. The lipase made by *Streptomyces fungicidicus* RPBS-A4 owing to its alkaline nature and capability to work in the presence of various detergents and solvents, could show to be of significance for applications in industrial processes such as for instance detergent formulations, sewage treatment and leather processing.

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