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RESEARCH ARTICLE

LIPASE PRODUCTION FROM *MYROIDES ODORATIMIMUS* (SKS 05) AND HPTLC ANALYSIS OF PURIFIED LIPASE

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ABSTRACT

Background: Microbes play an important role in the area of biodegradation. In order to reduce the damages caused due to oil spills, preliminary study on the role of *Myroides odoratimimus* obtained from chicken in the biodegradation of diesel hydrocarbons. **Objectives**: Production of Lipase from *Myroides odoratimimus* (SKS 05) through solid state fermentation using different substrates and characterization of Lipase enzyme produced from fermentation. Checking its purity through HPTLC analysis. **Methods:** Lipase enzyme production through submerged and solid state Fermentation. Selection of substrate to increase lipase production and check the effect of various parameters in lipase activity like pH, temperature, incubation period, carbon source and moisture level. Partial purification of lipase using column chromatography and quantification of partially purified lipase using HPTL. **Results:** The present study reports that the production of lipase by *Myroides odoratimimus* (SKS 05) using different oil cakes. Groundnut oil cake was found suitable for maximum lipase production and its activity increases at temperature 35 to 40 °C, pH8, moisture level from 50 to 60% and glucose used as a sole carbon source. **Conclusions:** Lipase production from *Myroides odoratimus* can be enhanced using substrates and maintaining optimium conditions. The produced lipase can be used for industrial as well as biodegradation purpose

INTRODUCTION

Enzymes or microbial cells are used as biological catalysts due to their high specificity and economic advantages without any environmental impact. The hydrolysis and synthesis of long-chain acylglycerols are catalyzed by Microbial lipases. With the rapid development of enzyme technology they are given much attention. Compared to plants and animals, microorganisms have been found to produce high yields of lipases. Solid State Fermentation is a well-adapted and cheaper process than Submerged Fermentation for the production of a wide spectrum of bio-products (animal feed, enzymes, organic acids, bio-pulp, aroma compounds, antibiotics, compost, bio-pesticide, bio-fertilizer etc). It is a high recovery method for the production of industrial enzymes and has been reported that in many bio-productions, the amounts of products obtained by Solid State Fermentation are many fold higher than those obtained in submerged cultivations. In addition, the products obtained have slightly different properties (e.g., more thermo-tolerance) when produced in Solid State Fermentation. Therefore, if Solid State Fermentation variables are well controlled and the purity of the product is defined, this technology may be a more competitive process than is commonly thought.

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The free water is indispensable to the microorganism's growth and is adsorbed on a solid support or complexed into the interior of a solid matrix (Soccol, 2003). This method has economic value for countries with abundance of biomass and agro industrial residues, as these can be used as cheap raw materials (Tunga, 2003). Cheap agricultural by products which have been gaining a great interest as suitable substrates in Solid State Fermentation for fungi like gingelly oil cake (Kamini *et al*., 1998) and olive oil cake (Kademi *et al*., 2003). Enzyme production can be enhanced by improving the availability of the substrates where gum arabic used to emulsify lipid substrates (Mahler *et al*., 2000). Lipase production is influenced by the type and concentration of carbon and nitrogen sources, the culture pH, the growth temperature and the dissolved oxygen concentration (Elibol and Ozer, 2001). The choice of a purification method largely depends on its use in a particular stage of the total purification scheme. To purify a protein to homogeneity, with an overall yield of 30% and a purification factor of 320, four or five purification steps are usually required. HPTLC, a chromatographic technique that can separate a mixture of compounds used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. The objective in this study was to focus on the purification and partial characterization of extracellular Lipase obtained from *Myroides odoratimimus* (SKS 05). The present research was undertaken to optimize condition for the production of Lipase from *Myroides odoratimimus* (SKS 05)

by Solid State Fermentation using different oil cakes and confirm its lipase activity by comparing it with the standard using HPTLC.

MATERIALS AND METHODS

Myroides odoratimimus (SKS05) is a strain isolated from chicken and maintained in Dr. GRD college of Science, Coimbatore. In order to prepare the inoculum, a loopful of cells from a freshly grown slant of *Myroides odoratimimus* (SKS05) was transferred into a 250 mL conical flask containing 100 ml of Food Flavobacterium Broth (Beef extract - 0.1 g, Peptone - 0.1 g, Sodium chloride - 0.5 g, Magnesium chloride - 0.5 g, Dextrose - 0.5 g). Dissolved in 100 ml of distilled water and incubated at 37ºC in a shaking incubator at 180 rpm for 24 h with pH 7.5.

Lipase Enzyme Production

***Materials required* (Lipase assay (Titrimetric determination of Lipase Activity)**

- Emulsifying Reagent (NaCl -1.7g, KH_2PO_4 - 0.14g, Glycerol - 54 ml
- Gum Arabic - 1g, Distilled water - 100ml, 0.2 M citrate buffer (pH 7.5)
- Crude Enzyme , Ethanol (95%),Substrate emulsion

Procedure: 100 ml of emulsifying reagent was prepared and used as stock. From the 100 ml of emulsifying reagent, 17 ml was taken and mixed with 7 ml of olive oil and stirred properly and used as substrate emulsion. 1 ml of substrate emulsion, 0.8 ml of 0.2 M Citrate buffer (pH 7.0) and 0.2 ml of the enzyme was added into a 100 ml Erlenmeyer flask and incubated at 37^{o}C for 60 min. The reaction was terminated by adding 1 ml of 95% ethanol. The amount of fatty acid liberated was calculated by titration using 0.05 N NaOH, by addition of 2 to 3 drops of Phenolphthalein. Blank reagent was prepared by dissolving 1 ml of substrate emulsion and 0.8 ml of Citrate buffer and titrated using 0.05 N NaOH, to determine the amount of fatty acid liberated. The quantity of fatty acids liberated in each sample was calculated based on the equivalents of NaOH, as per the following equations. Lipase activity: One unit of Lipase activity was defined as the amount of enzyme releasing one µmole of free fatty acid in one minute under standard assay condition.

Enzyme extraction and Protein estimation: After 48 h the biomass with the substrate was agitated with 50 ml distilled water on a magnetic stirrer for 30 min, then the contents were filtered through muslin cloth and then the filtrate was centrifuged at 10,000 rpm for 15 min. The supernatant obtained after centrifugation was used as enzyme source. The total protein concentration was estimated by Lowry *et al*., (1951) method.

Submerged Fermentation: *Myroides odoratimimus* (SKS 05) was inoculated in the Food Flavobacterium Broth and incubated at 37ºC for 48 h. The medium was then centrifuged and the supernatant was collected and assayed for the protein content and enzyme activity.

Solid State Fermentation: *Myroides odoratimimus* (SKS 05) was subjected to Solid State Fermentation in different substrates like Cotton seed, Neem, Coconut, Groundnut and Gingely oil cakes.10 g of coconut, groundnut, gingely, neem and cottonseed oil cake was suspended in 90 ml of minimal media in a 250 ml flask. It was then autoclaved at 15 lbs pressure, 120ºC for 20 min and cooled. Then 5 ml of inoculum was added to the 90 ml of Minimal media, kept for incubation at 37ºC for 48 h. (Composition of Minimal media- KH_2PO_4 - 0.3 g, Na_2HPO_4-0.6 g, NaCl -.5 g, NH_4Cl-0.2 g, $MgSO_4$ -0.1 g, pH-8 , Disilled water- 100 ml

Substrate selection: Coconut, Groundnut, Gingelly, Neem, Cottonseed oil cake (Fig 1) were used as substrates for lipase production and the lipase activity showed in presence of different substrates was checked through titrimetric method. The substrate showing maximum activity was then used for the characterization of lipase from *Myroides odoratimimus* (SKS 05) for different parameters to increase the lipase activity.

Effect of Incubation Time/ pH/Temperature/carbon source/moisture level: *Myroides odoratimimus* (SKS 05) inoculum was incubated in the minimal medium for 24, 48, 60, 72 and 96 h in presence of different substrates (coconut, groundnut, neem, cottonseed and gingely oil cakes) at 37^{o}C. Similarly at different pH (6, 7, 8, 9, and 10) and at different temperature (30oC, 35oC, 40oC, 45oC and 60oC) lipase activity was estimated. Effect of various carbon sources such as glucose, fructose, sucrose, lactose and starch in the minimal medium at the concentration of 0.01 g/g of different substrate and also at different moisture level (30 to 60%) was checked. After 48 h the enzyme is extracted and protein is estimated as discussed above.

Partial purification through column chromatography: The crude enzyme was first applied to a column (20 x 2.5 cm) of CM- cellulose (Sigma Chemical Co.), which was pre-equilibrated with 10 mM Sodium Phosphate buffer (pH 7.5). The Lipase was allowed to bind to the gel for 2 h at 4°C and was eluted with a linear gradient of Triton X-100 (0 to 1%). The flow rate was 60 ml/h and fractions of 5 ml were collected.

HPTLC analysis of Partially purified and Standard Lipase : HPTLC quantification of Lipase enzyme obtained from *Myroides odoratimimus* (SKS 05) was carried out. Standard Lipase (HiMedia) was loaded in a 100 µl CAMAAG syringe and placed in CAMAAG Linomet 5 applicator. 2 µl and 5 µl of Standard lipase were applied to the (10 x 10) HPTLC aluminium sheet silica 60 F 254, as a first and second band respectively by using CAMAAG WinCats software. Syringe was washed thoroughly and partially purified lipase enzyme was loaded, 2 µl and 5 µl were applied in third and fourth band respectively. The plate was air dried after application and developed in Chloroform: Acetonitrile: Water (7:3:4) in Twin trough chamber. The developed plate was detected at 254 nm in 5.00 x 0.45 mm slit dimension by using Scanner 3. Test sample (partially purified lipase (1 ml) and the standard lipase (0.1 g/ml) was used for the HPTLC analysis. For HPTLC the absorbance of standard lipase and partially purified lipase was checked at variable wavelength using HACHS spectrophotometer.

Characterization of Partially Purified Lipase produced from Myroides odoratimimus (SKS 05): Alterations in Lipase activity was measured by first adding 1ml of partially purified Lipase to the reaction mixture containing 1 ml of Citrate buffer (7.5) and 1 ml of olive oil, which was incubated at 37°C for 1

h. After 1 h the reaction was stopped, by the addition of 1ml of ethanol (95%) and Lipase activity was assayed by titrating against 0.01N NaOH with 2 to 3 drops of phenolphthalein and it was used as control for further assay.

Optimum pH and temperature for the activity and stability of Partially Purified Lipase: The effect of pH on the activity of Lipase was measured at various pH from 4 to 9.The pH of the reaction mixture was varied using Citrate buffer (0.2M of Sodium Citrate and 0.2M Citric Acid for 200ml) from 4 to 9 and the partially purified Lipase along with 1 ml of olive oil was incubated for 60 min at 37^{o}C. Similarly the effect of temperature on Lipase activity was measured by incubating the reaction mixture (1 ml citrate buffer, 1 ml olive oil and 1 ml partially purified lipase) at different temperature (20, 30, 40, 50 and 60^{o}C) for 60 min. After the incubation period the reaction was stopped by the addition of 1 ml ethanol (95%) and Lipase activity was assayed. To check the Lipase stability, a portion of purified Lipase was held at 4^{0}C for 2 h in citrate buffer at different pH 4 to 10. After 2 h the residual activity of purified Lipase at different pH was determined.

RESULTS

The *Myroides odoratimimus* (SKS 05) obtained from Research Group, School of Biotechnology Dr. GRD college of science showed yellow colony on Food Flavobacterium Medium. From the Table 1 the Lipase activity of *Myroides odoratimimus* (SKS 05) obtained from SmF (150 U/ml) was less compared to SSF (145.2 U/ml). *Myroides odoratimimus* (SKS 05) produced lipase by Solid State Fermentation using different oil cakes viz coconut, groundnut, gingelly, neem, cottonseed oil cake under different parameters like temperature, pH, incubation time , moisture content and carbon sources. Among the different substrates used, crude enzyme extracted from medium containing groundnut oil cake showed highest activity of enzyme.

Effect of Time of Incubation on Lipase production in different substrate: Out of the different subatrate used groundnut oil cake showed maximum lipase activity compared to other substrate at different incubation time. Highest was at 60 h with a lipase activity of 156 U/ml (Fig 2).

Effect of pH on Lipase production in different substrate: Both Coconut and groundnut oil cake at pH 8 showed maximum lipase activity than other substrates. There was marked increase in the yield till pH 8 and then there was very minimum activity at pH 10 in all the substrates used in SSF (Fig 3).

Effect of Temperature on Lipase production: *Myroides odoratimimus* (SKS 05) when inoculated in different substrates at different temperature, maximum activity of lipase was at 40°C in coconut oil cake and at 35°C in groundnut oil cake. There was decrease in lipase activity above 30°C in neem oil cake, gingely oil cake, and cotton seed oil cake medium (Fig 4).

Effect of Carbon Source on Lipase production: Addition of different carbon sources like glucose, galactose, lactose, and starch showed remarkable change in the activity of lipase. In presence of lactose, sucrose and starch the lipase showed maximum activity in gingely oil cake. Cotton seed and groundnut oil cake showed maximum activity of lipase in presence of glucose (Fig 5).

Effect of Moisture level on Lipase production: Maximum lipase activity was obtained in groundnut oil cake at 60% moisture level. Also Coconut oil cake showed maximum activity at different moisture level compared to other substrate, maximum at 50% of moisture level**.** Hence 50 to 60% of moisture level is required for maximum lipase activity (Fig 6).

Partial purification: Different fractions were obtained from purification steps which are tested for Lipase activity using titrimetric method. On the CM-cellulose column, the highest Lipase activity was eluted infractions ranging from 0% to 1%, maximum specific activity was found to be in 1%. Highly active fraction (1%) were pooled and applied to the column DEAE-cellulose and was eluted with linear gradient of NaCl (0.05M-1M) and the Lipase activity was found to be highest between 0.05M and 0.25M as shown in Table 3.

HPTLC analysis of partially purified and Standard Lipase: Hence, on comparison, the Rf values obtained for both the standard and the purified Lipase (5 and 2 µl), it can be concluded that the Standard Lipase showed Rf value from 0.81 to 0.86 in Chloroform: Acetonitrile: Water (7:3:4) and one of the peak of the purified Lipase were found in the same Rf as Standard (Fig 7 to Fig 10).

Characterization of Partially Purified Lipase obtained from *Myroides odoratimimus* (SKS 05): Alterations in Lipase activity was measured by first preparing reaction mixture containing 1ml of partially purified Lipase obtained after DEAE filtration using 1 ml of Citrate buffer (pH 7.5) preincubated at 37°C for 1 h. After 1 h, the reaction was stopped by the addition of 1 ml ethanol (95%) and Lipase activity was assayed by Titrimetric method and was used as control for further assay.The activity of Lipase was increased gradually with rise in temperature and after 50^{o}C, the activity of Lipase was decreased (Fig 11). The Lipase obtained from *Myroides odoratimimus* (SKS 05) showed 90% stabiltiy at 40^{o}C. The lipase lost about 50% of its activity at 20^{o}C. The effect of pH on activity of purified Lipase obtained from the culture showed maximum activity at 8. The purified Lipase showed maximum stability at pH 6 with 88% of activity. Below pH 8 the purified Lipase lost about 50% of its activity after holding at 4^{o}C for 24 h. Lipase was stable between pH 6 to 8 as shown in Fig 12.

DISCUSSION

The lipase enzyme produced by Solid State Fermentation showed maximum activity in comparison to the enzyme produced by Submerged Fermentation from *Myroides odoratimimus* (SKS 05). Similar results were given by Mohanasrinivasan *et al.,* in 2008 where they compared the lipase yield by Solid State and Submerged Fermentations using fungal species from biopharmaceutical oil waste and found that in most cases SSF gave higher enzyme productivity on comparison to SmF. Yet, in another comparative study on lipase production in SmF and SSF systems, Christen *et al*., (1995) observed a 5-fold increase in lipase productivity in SSF system. In contrast to this, Ohnishi *et al*., (1994) reported less lipase production from *Aspergillus oryzae* using SSF, when compared to SmF where high enzyme yields were obtained. Hence the use of soild state fermentation and submerged state fermentation depend on the species characteristics which are

Table 1. Lipase activity showed by enzyme produced from *Myroides odoratimimus* (SKS 05) by SSF and SmF

Enzyme sample	Lipase activity (U/ml)	Protein concentration (mg/ml)	Specific activity Of Lipase (U/mg)
Crude SSF (Groundnut cake)	150	6.2	24.1
Crude SmF	145.2	6.6	22

Table 2. The Lipase activity of different fractions obtained from CM and DEAE cellulose

CM Cellulose		DEAE Cellulose	
Fractions	Fatty acid liberated (µmol/ml)	Fractions	Fatty acid liberated µmol/ml)
0%	2000	0.05M	18900
0.2%	3000	0.25M	17700
0.4%	7000	0.45M	15600
0.6%	4000	0.65M	14000
0.8%	9000	0.85M	12000
1%	10000	1M	600

Fig. 1. Different substrates (Coconut, Groundnut, Cotton seed, Neem and Gingely oil cakes) used for Lipase production from *Myroides odoratimimus* (SKS 05) by Solid State Fermentation.

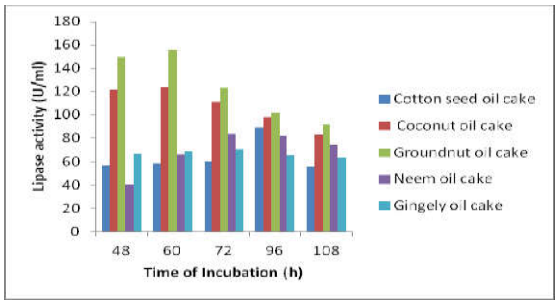

Fig. 2. Lipase production from *Myroides odoratimimus* (SKS 05) by SSF using different substrates at different Time of Incubation

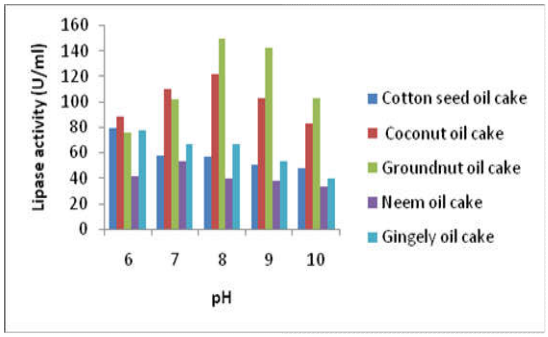

Fig. 3. Lipase production from *Myroides odoratimimus* (SKS 05) by SSF using different substrates at different pH

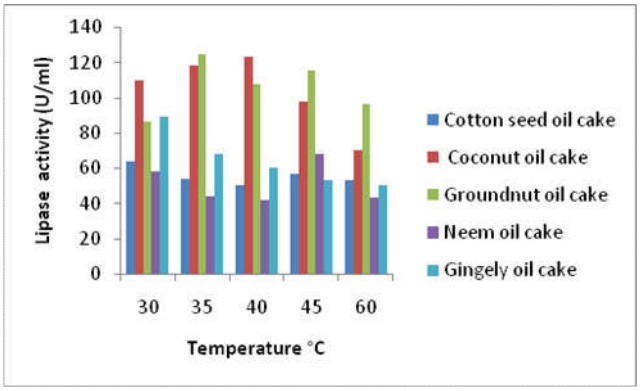

Fig. 3. Lipase production from *Myroides odoratimimus* (SKS 05) by SSF using different substrates at different pH

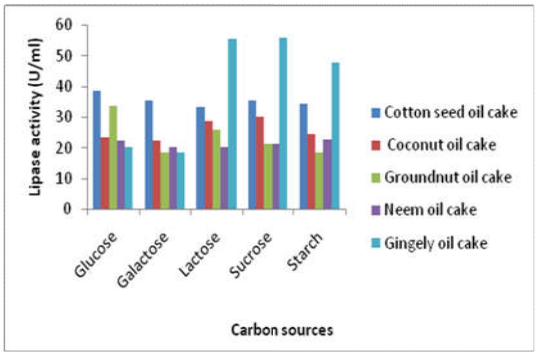

Fig. 5. Lipase production from *Myroides odoratimimus* (SKS 05) by SSF using different substrates in presence of different carbon sources

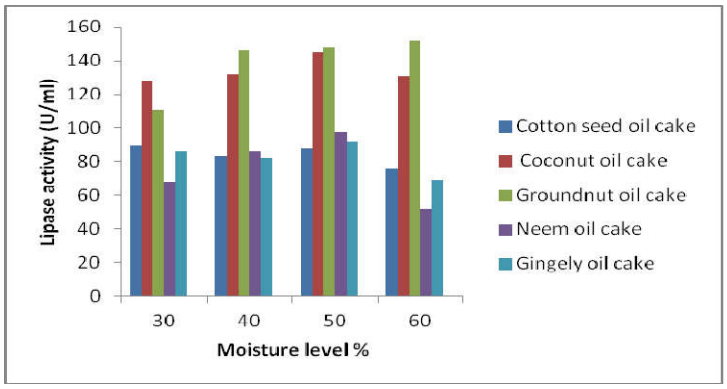

Fig. 6. Lipase production from *Myroides odoratimimus* (SKS 05) by SSF using different substrates at different Moisture levels

HPTLC profile of 2 µl Standard Lipase

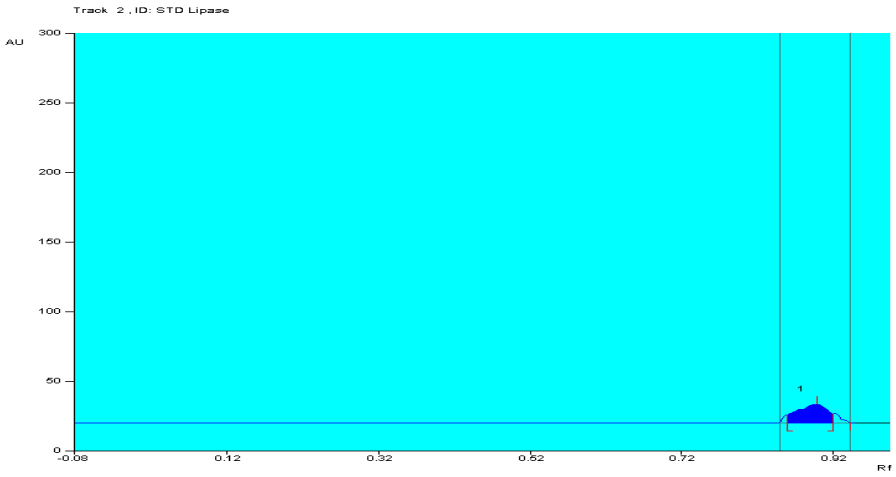

Fig. 7. Retention time showed by 2µl of Standard Lipase, with peak at 0.86 Rf

Peak	Start	Start	Max	Max	Max	End	End	Area	Area	Assigned
	Position	Height	Position	Height	%	Position	Height	-	%	Substance
1	0.81 Rf	0.0 AU	0.86 Rf	28.0 AU	100.00%	0.93 Rf	5.6 AU	1298.8AU	100.00%	Lipase *

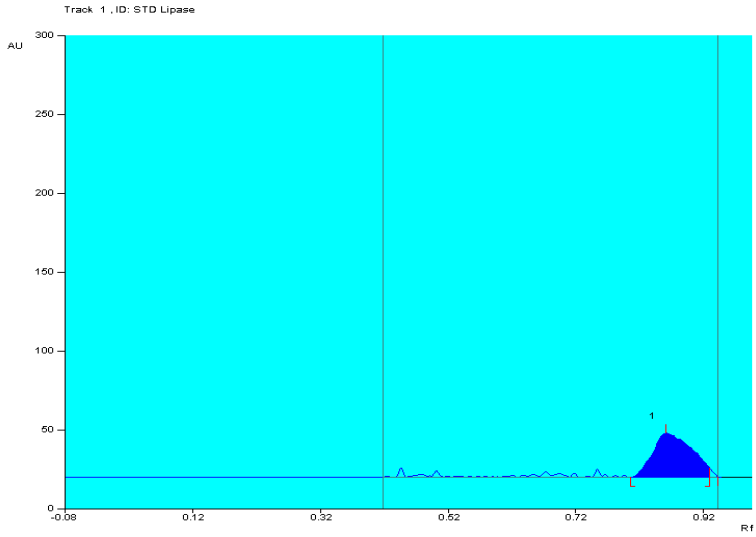

Fig 8: Retention time showed by 5µl of Standard Lipase, with peak at 0.86 Rf

Peak	Start	Start	Max	Max	Max	End	End	Area	Area	Assigned
	Position	Height	Position	Height	%	Position	Height		%	Substance
1	0.84 Rf	1.3 AU	0.86 Rf	14.4 AU	100.00%	0.89 Rf	9.8 AU	360.6AU	100.00%	Lipase *

HPTLC profile of 2 µl partially purified Lipase

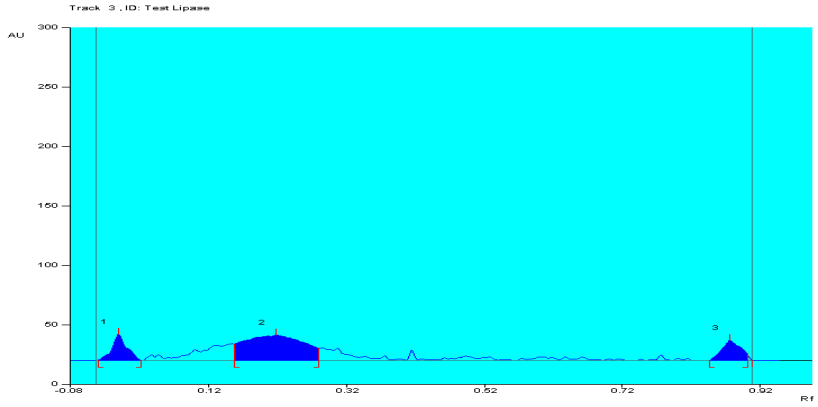

Fig 9. Retention time showed by 2µl of partially purified Lipase from *Myroides odoratimimus* (SKS 05) with maximum peak at 0.01 RF, 0.22 Rf, 0.88 Rf

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned Substance
1	-0.04 Rf	0.1 AU	-0.01 Rf	21.8 AU	36.51%	0.03 Rf	0.1 AU	354.8AU	17.00%	Unknown
2	0.16 Rf	14.0 AU	0.22 Rf	21.2 AU	35.50%	0.28 Rf	10.1 AU	1389.8AU	66.57%	Unknown
3	0.85 Rf	0.2 AU	0.88 Rf	16.7 AU	28.00%	0.90 Rf	5.5 AU	343.1 AU	16.44%	Lipase

HPTLC profile of 5 µl partially purified Lipase

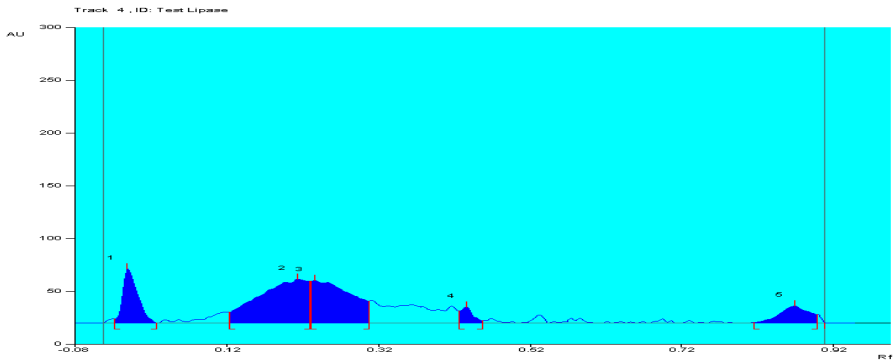

Fig 10: Retention time showed by 5µl of partially purified Lipase from*Myroides odoratimimus* (SKS 05) with Maximum peak at 0.01 RF, 0.22 RF, 0.240 RF, 0.44 Rf, 0.87 Rf

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned Substance
1	-0.02 Rf	4.1 AU	-0.01 Rf	51.1 AU	31.15%	0.03 Rf	0.2 AU	758.6 U	14.99%	Unknown
2	0.13 Rf	10.2 AU	0.22 Rf	41.5 AU	25.29%	0.23 Rf	39.4 AU	2024.0AU	40.00%	Unknown
3	0.23 Rf	39.5 AU	0.24 Rf	40.1 AU	24.43%	0.31 Rf	20.7 AU	1620.4 U	32.02%	Unknown
4	0.43 Rf	11.5 AU	0.44 Rf	15.2 AU	9.26%	0.46 Rf	2.5 AU	192.7 AU	%	Unknown
5	0.82 Rf	0.3 AU	0.87 Rf	16.2 AU	9.87%	0.90 Rf	8.1 AU	464.7 AU	9.18%	Lipase *

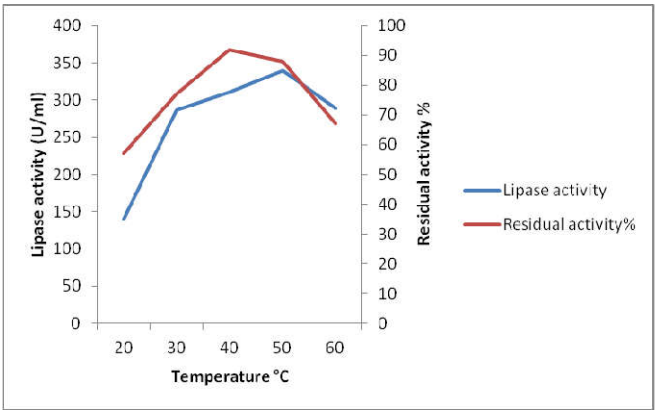

Fig. 11. Activity and stability profiles of partially purified Lipase from *Myroides odoratimimus* (SKS 05) in relation to temperature

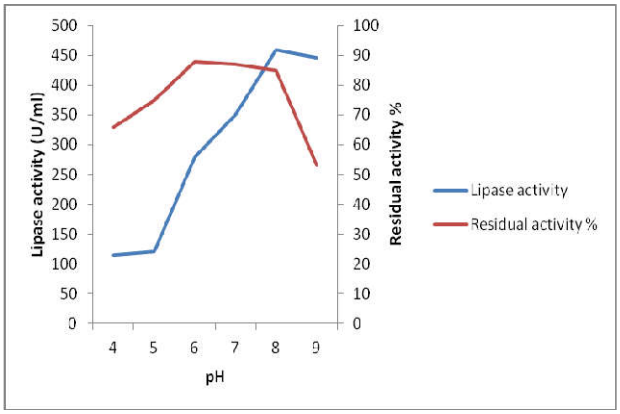

Fig. 12: Activity and stability profiles of Lipase from *Myroides odoratimimus* (SKS 05) at varied pH

used for the production of lipase. Selection of a suitable substrate for the production of enzyme is a primary-key factor and an extremely significant step. Groundnut oil cake produced maximum lipase activity from *Myroides odoratimimus* (SKS 05) in comparison to other substrate. Similar results were reported by Rajeswari in 2011, about the production of alkaline lipase from *Penicillium chrysogenum* using groundnut oil cake as substrate. In contrast to the result obtained, Coconut cake has been used as a potent substrate for production of Lipase by *Candida rugosa* in Solid State Fermentation (Benjamin and Pandey, 1997). Similarly substrate selection depends on the species which were taken for the research purpose.

The availability of different substrate for the production of lipase by *Myroides odoratimimus* (SKS 05) was influenced by the variation in the time of incubation, type and concentration of carbon and nitrogen sources, the culture pH, the growth temperature and the dissolved oxygen concentration and pH. Short incubation period offers potential for inexpensive production of enzyme (Sonjoy *et al.,* 1995). In the present study, at 60 h of incubation, lipase produced from *Myroides odoratimimus* (SKS 05) maintained maximum activity towards different substrate. Similar results were reported by Chaturvedi *et al*., (2010), about the influence of incubation period for lipase production from *Bacillus subtilis* using different oil cakes. In contrast to the result the lipase from *Bacillus subtilis* showed maximum activity at 48 h of incubation and after 48 h the activity decreased, which was probably due to catabolite repression by glucose released from starch hydrolysis (Joseph *et al.,* 2011). The maximum activity of lipase in different substrate was at pH 8. Similar results are shown by Yu and Chou in 2005, that the activity of lipase from *Amylomyces rouxii* was observed maximum at pH 8. Similary Chander (1980) discussed the role of pH on the activity of lipase from *B.licheniforms* and reported that maximum lipase activity was observed at pH 6.

Enzyme production in solid state is greatly affected by temperature as discussed in many research articles. Maximum yield of lipase was obtained between 35^{o}C - 40^{o}C. In 1996, Ghosh *et al* reported maximum Lipase production from *Rhizopus nigricans* using SSF at 30^{o}C and by *Talaromyces emersonii* at 45^{o}C. Varalakshmi *et al.,* (2009) reported that the glucose and sucrose supplementation in the medium for SSF resulted in the repression of enzyme production. But, in case of *Myroides odoratimimus* (SKS 05), high lipase production was observed in case of groundnut oil cake when glucose was used as a sole carbon source. According to Sumitra *et al*., 2007, the supplementation of the substrate with different carbon sources showed an increased production of the enzyme. The effect of moisture content was examined in the range from 30% to 60% for the analysis of lipolytic activity of *Myroides odoratimimus* (SKS 05) in different substrates. Maximum activity of lipase was observed at 50% of moisture level in different substrates. In contrast to present study, Rajeshwari (2001) reported that maximum enzyme was produced at 20% of moisture level in *P.chrysogenum*. An increase in moisture level is believed to reduce the porosity of the substrates, thus limiting oxygen transfer. Low moisture content causes reduction in the solubility of nutrients of the substrates and low degree of swelling. The eluent obtained from purification steps carried to purify the crude enzyme obtained from *Myroides odoratimimus* (SKS 05), was checked for lipase activity *i.e.* production of fatty acid per ml of eluent. Low yield of purified enzymes can be attributed to loss during Ammonium sulphate precipitation as well as tight binding of lipase to hydrophobic column as a significant amount of lipase activity never eluted from the column. The crude enzyme obtained from *Myroides odoratimimus* (SKS 05) showed higher lipase activity when purified using DEAE technique. The purified Lipase was found in the same Rf as standard. HPTLC is becoming a routine analytical technique by virtue of its advantages. Several samples can be run simultaneously using a small quantity of mobile phase. In 2011, Rajan used HPTLC for the quantification of alkaline Lipase obtained from *Aspergillus fumigatous* where micro quantities of Free Fatty Acids (FFA) was detected by TLC and quantified by HPTLC. The partially purified Lipase from *Myroides odoratimimus* (SKS 05) showed maximum activity at pH 8 and the Lipase lost about 50% of its activity. The fall on the alkaline side of pH 8 is primarily due to destruction of the enzyme, whereas the fall on the acid side of pH 6 is partly due to a decreasing affinity of the Lipase for its substrate and partly due to an irreversible destruction of the enzyme protein (Oterholm, 1970). At 50^{o}C retained 80% of its activity. The enzyme systems involved in lipase formation are repressed at higher temperatures, thus resulting in a lower productivity (Anderson, 1980).

Conclusion

Owing the potential, to use cheap nutrients for maximizing lipase production which can reduce the production cost. This is one of the prime objectives of industrial microbiology for large scale production of valuable metabolites. The utilization of biopharmaceutical oil waste on one hand provides alternative substrate and on the other, helps in solving pollution problems. Study gives an idea on utilization of waste oil cakes for enzyme production through SSF and adds value addition to oil mill wastes. The partially purified lipase showed optimal activity and stability in a wide range of temperatures and pH values. Moreover, because of its pronounced thermal stability as well as preservation of activity and stability in different pH, this enzyme could be of significant biotechnological potential, particularly in organo synthetic reactions carried out at higher temperatures. Future research will focus on structural characteristics of this enzyme and also its application in various industries as well as biodegradation process.

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