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Purification and characterization of alkaline Lipase from *Pseudomonas aeruginosa* strain

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Abstract

The activities of a lipase produced by fourteen lipases producing bacterial strains (Aj-1, Aj-2, Aj-4, Aj-5, Aj-6, Aj-9, Aj-11, Aj-14c, Aj-15, Aj-18, Aj-24a, Aj24b, Aj32, Aj-38) isolated from the soil of the oil spilled places of Hubli-Dharwad, Karnataka (India) were analyzed and compared. Isolate Aj-4 showed the outstanding and higher lipolytic activities than those of other isolates.

Partial purification of crude enzyme produced from Pseudomonas aeruginosa sp. was carried out by ammonium sulphate precipitation and dialysis. The lipase purification steps involved are, ammonium sulphate saturation, gel filtration chromatography using Sephadex G-50 and ion exchange chromatography with DEAE cellulose.

Characterization studies indicated that the enzyme showed highest activity at pH 9.0 and 55ºC. It was stable at temperatures between 40ºC and 60ºC. More than 80% of activity was retained between 8-11 pH range and a temperature of 40-55ºC. The molecular mass of purified lipase was found to be about 58 kDa by SDS-PAGE. The sequence obtained by 16S rDNA was analyzed the analysis confirmed that the present strain was P. aeruginosa. The lipase sequence (816 bp) obtained was also analyzed. The protein was found to be possessing esterase superfamily domain, confirming the lipase.

Key words: *Alkaline Lipase characterization, partial purification, 16S rRNA*

1. Introduction

Lipases [EC 3.1.1.3] catalyze the hydrolysis of triglyceride at the interface between the insoluble substrate and water. They are ubiquitous in nature and are produced by various animals, plants, fungi, bacteria and archia. Among them, extracellular bacterial lipases are of considerable commercial importance, because of their diverse substrate specificity, stereo-specificity, and tolerance against heat and various organic solvents [1,2]. Therefore, they are widely used in food technology, in the detergent and chemical industries, and in biomedical sciences [3, 4, 5].

Study on lipase can be traced back to 100 years ago, while the lipases from microbe have gained enough attention in the last decades [6]. In recent years, the screening of lipase producer has been fixed at two aspects: on one hand, some scientists have devoted themselves to detecting some lipases with novel and specific properties such as alkalophile, halophile, psychrophile and thermophile from some adverse environments [7,8]; on the other hand, some microbiologists have paid enough attention to screening some productive strains from some oil sludge areas or industrial wastes [9,10].

At present, lipases originated from *Pseudomonas* and *Burkholderia* are the ones most commonly used in household detergents and in the trans-esterification process in the fine chemical industry and are involved in Group I.1 and Group I.2 [1, 2, 11]. The

subfamily I.1 consists of lipases with a molecular weight of approximately 30 kDa and a single disulfide bond in the molecule.

The challenge for the soil microbial ecologist is to identify the populations and guilds of microorganisms that have key functional roles in specific soil processes. Polymerase chain reaction (PCR) amplification of 16S rDNA genes [12,13] and enterobacterial repetitive intergenic consensus (ERIC)-PCR amplification [14,15,16] using consensus bacterial primers and separation of the resultant PCR amplifications by agarose gel electrophoresis (AGE) constitutes one of the popular techniques, which is used to describe soil bacterial ecology [17,18,19]. Bands on the gel can be sequenced and the resultant information can be used to infer something about the diversity of the original sample. There is a proliferation of these studies applied to soils, as molecular techniques have been systematically applied to many diverse environments. The diversity of Dhapa landfill at the East Kolkata (India) is investigated in 2007 with the usage of PCR amplification of 16S rDNA genes [20].

2. Materials and Methods:

2.1 Sample collection and processing:

Soil samples were collected at 4-5 cm depth with the help of sterile spatula in a sterile plastic bag from different petrol bunks in the vicinity of Hubli-Dharwad. After collection, samples were brought to the laboratory and 1 g of sample was suspended in 100 mL of sterile distilled water, agitated for 30 min on a shaker at 35ºC and kept as stock solution for further isolation of the micro organisms.

2.2 Screening of micro organism

The obtained colonies were then isolated and cultured in to different tubes containing Nutrient medium. The isolates obtained were screened for the lipolytic activity using lipid hydrolysis in nutrient broth containing 1% of Tributyrin. One isolate OCR-4, which showed maximum activity was selected and maintained on tributyrin agar slant at 4ºC Figure 1a & b. The culture was examined for various morphological and biochemical characteristics as per Bergey's Manual [21] of determinative Bacteriology.

2.3 Partial purification of enzyme lipase

The 200ml broth was centrifuged and the supernatant was saturated to 80% (w/v) with Ammonium sulphate. The saturation was carried by adding Ammonium sulfate slowly into the crude extract with constant stirring. The stirring was continued for 2 hours after Ammonium sulfate was completely dissolved to aid the complete precipitation. Whole process was carried out in cold and lasted for 8hrs. The precipitated protein was separated by centrifuging at 10,000g for 10mins. The protein pellet was dissolved in minimal volume of 25mM Tris buffer (pH 8.0). The precipitated proteins were further purified by dialysis. The protein samples were dialyzed using the low cut off membrane against 500ml of 5mM dialysis buffer containing 5mM Tris buffer (pH 8.0) for 8 hrs with 2 changes in buffer Figure 2.

2.4 DNA Preparation and PCR Amplification

DNA was isolated and purified according to Sambrook *et al.*, 1989 [22]. Each genomic DNA used as template was amplified by PCR with the aid of 16S rDNA primers (The forward primer were 5'-CTACGGGAGGCAGCAGTGG-3' and the reverse primer were 5'-TCGGTAACGTCAAAACAGCAAAGT-3'). The programme consisted of denaturation at 94ºC for 2 min and subsequent 35 cycles of denaturation at 94ºC for 1 min, annealing at 62ºC for 1 min, and extension at 72ºC for 1 min followed by final extension at 72ºC for 1 min. The presence of PCR products was determined by electrophoresis of 20 μL of the reaction product in a 0.8% agarose gel Figure 3a & b.

2.5 16S rRNA Sequencing and Data Analysis

Amplicon was cut and submitted for the sequencing at Bioserve Biotechnologies, (India) Pvt. Ltd, Hyderabad. The sequenced product obtained was analyzed, to check the homology with those sequences in the NCBI databases using BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST). The sequencing data was obtained utilizing this strategy indicated that the isolate under study was *P. aeruginosa*. Further the hit sequences were multiple aligned using Clustal X along with the Phylogenetic tree development Figure 4. The tree was visualized using Treeview software. The sequence was further translated to protein to know coding sequences by ORF finder (www.bioinformatics.org/sms/orf_find.html).

3. Results

3.1 Colony Morphology, Biochemical characterization, Comparative Lipolytic Activity of Lipase Producers

The 14 isolates obtained were grown in selective medium and were found to produce lipase which were identified as Aj-1, Aj-2, Aj-4, Aj-5, Aj-6, Aj-9, Aj-11, Aj-14c, Aj-15, Aj-18, Aj-24a, Aj24b, Aj32, Aj-38. When the lipases produced by these isolates were evaluated, the isolate Aj-4 showed outstanding results compared to the other isolates (Fig 1a and 1b).

3.2 Thermo and pH stability of Crude Lipase of Aj-4c

The enzyme has shown a very good stability up to 55ºC for one hour. Further it decreased considerably at 60ºC followed by a sharp decline in enzyme activity after 60ºC (Graph 1). The enzyme has an optimum pH of 9.0. Moreover, the enzyme was stable from pH 8.0 – 11.0 respectively (Graph 2).

3.3 Purification of Extracellular Lipase:

The enzyme was purified to homogeneity by ammonium sulfate precipitation and dialysis, Sephadex column, ion-exchange chromatography. The lipase activity assayed during ammonium sulphate precipitation was found to be 0.997 and dialyzed extract was found to be 1.354 (Table 1).

Table 1. Purification of lipase enzyme, protein concentration, enzyme activity, lipase yield and purity

Purification step	Protein Concentratio (mg/ml)	Activity (µmoles ml^{-1} min^{-1})	Specific Activity (U/g)	Yield (%)	Fold purity (%)
Crude	0.14	45.6	325.7	100	1
Amm. Sul. precipitation	0.83	199.4	240.24	592.85	73.76
Dialysis	0.3	270.8	902.66	214.28	277.14
Gelfiltration	0.59	39	65.5	368.75	22.99
Ion-exchange	0.005	401	2864.28	3.5	879.42

The eluted protein and lipase activity was detected in all the sephadex purification column fractions except in 1,3,14,17,18,19,22,23,24 and 25, numbered fractions. The highest protein concentration and lipase activity was found in the fraction tube 5,6,7,8 (Graph 3). The eluted DEAE fractions were screened for lipase activity by performing the lipase assay method the enzyme was eluted in to all the 30 fractions. The highest O.D of 0.492 was noticed in 11^{th} fraction (Graph 4). The molecular mass of purified lipase was found to be about 58 kDa by SDS-PAGE (Figure 2).

3.4 Effect of Metal salts on enzyme activity:

Lipase activity was examined by incubating various metal salts with enzyme extract in 50Mm Tris buffer (pH 8.0 at 40°C) for 1 hour. The PMSF has found to stimulate enzyme activity (Table 2).

3.5 PCR amplification*:*

The sequence obtained by 16S rDNA (1007 bp) was analyzed by nBLAST at NCBI. The analysis confirmed that the present strain was *P.aeruginosa.* The lipase sequence (816 bp) obtained was also analyzed by nBLAST, the results of which confirmed that the sequence was of lipase (Fig 3a, 3b & 4).

Table 2. Effect of Metal salts on lipase enzyme activity

Metal Inhibitors	Residual Activity
MgCl2	0
NaCo3	0
SDS	8.493
NaCl2	33.95
HgCl2	38.03
PMSF	43.2
MgSo4	0
CuSo4	3.32
FeCl 3	0
ZnSo4	0
MnSo4	0

4. Discussion

In the present study the isolated organism from the soil sample was characterized as *P. Aeruginosa* it formed yellowish-white, smooth, mucoid and large colonies. Similar results were reported that staining of *P. aeruginosa* produces green pigment and characteristic odour on cetrimide agar; *P. aeruginosa* reacted positively to catalase and oxidase tests, while it was negative for methyl red, Voges Proskauer and indole. Bacteria slowly hydrolyzed urea, utilized Simon's citrate the biochemical properties of the organism recorded by [23]. Taxonomical studies on lipase-producing strain showed that the strain is gram-negative, rod-shaped, aerobic, catalase and oxidase-positive. Similar results were reported by [24]. From these results, the strain classified into the genus of *Pseudomonas* according to Bergey's manual combined with 16S rDNA sequence analysis. *P. Aeruginosa.* Group III Pseudomonas lipases are larger containing about 475 amino acids and a molecular weight of 50,000 Dalton.

In the present study the isolated organism produced lipase and has shown lipase activity at different temperature and pH. It has been reported and reviewed that all bacterial lipase done by [2], states that maximum activity of lipases at pH values higher than 7 has been observed in many cases [Figure 6]. It is reported that Bacterial lipases have a neutral or alkaline optimum pH. With the exception of lipase from *P. fluorescens* SIK W1 that has an acidic optimum pH 4.8. Also, the lipase retained over 65% of its activity at pH 8.0. Lipases from *P. pseudomalei* 12 [25] and *P. aeruginosa* YS-7 [26] both isolated from *Pseudomonas* growing in different water-restricted environments are stable within the pH ranges of 7–10.5 and 6.5–7.5, respectively. Similar reports have been reported that the optimum temperature of the lipase from *P. aeruginosa* EF2 was reported to be 50°C [27]. The *P. aeruginosa* MB 5001 lipase has an optimum temperature of 55°C [28] but other *Pseudomonas* lipases, such as those from *P. fluorescens* 2D [29], *P. fluorescens* HU380

[30], *P. fragi* (Mencher JR, Alford JR.) and *P. mendoncina* [31] were found to be optimally active at 35–45°C. *P. aeruginosa* lipases seem to be more thermostable than others from this genus.

The Crude lipase obtained was first subjected to ammonium precipitation followed by sephadex and ion exchange chromatography and purification was achieved. Similar results were reported by [*32*] with 8.6 fold purification for from *P. aeruginosa* Pse A. Additional step of purification by ion exchange chromatography using DEAE A-50 increased it to 42.99 fold. Similar results were reported that using sephadex G-100 and DEAE–A50 chromatography simultaneously, [33] reported 5.3 fold purification of lipase from *Pseudomonas putida* 3SK. The molecular weight of lipase on SDS-PAGE was found to be approximately 60.0 kD. A single non smearing band was observed on native PAGE confirmed by activity staining. It has been reported that the molecular weights of lipases from *P. aeruginosa* vary considerably. This was similar to the lipases isolated from *P. aeruginosa* S5 [34] and *P. aeruginosa* Pse A [32]. Zymography in SDS- PAGE was not visible. This may be due to the inhibitory effect of SDS on enzyme under experimental conditions. The lipase from *P. aeruginosa* BN-1 showed alkaliphilic character as it retained more than 70% of its activity for 1h at 37°C and 9.5 pH. However, it is lower when compared to that reported for *Pseudomonas* sp. PK-12 CS lipase [35].

Thermostable lipases have been isolated from many sources, including *P. fluorescens* [36]; *Bacillus* sp. [37, 38, 39]; *B. coagulans* and *B. cereus* [40]; *B. stearothermophilus* [41]; *Geotrichum* sp. and *Aeromonas sobria* [42,43]; and *P. aeruginosa* [44]. The latter enzyme was significantly stabilized by Ca^{2+} and was inactivated by EDTA. This inactivation could be overcome by adding $CaCl_2$, suggesting the existence of a calcium-binding site in *P. aeruginosa* lipase. Chartrain *et al*., (1993) [45] observed that an extracellular lipase of *P. aeruginosa* MB5001 was strongly inhibited by 1 mM ZnSO4 (94% inhibition) but was stimulated by adding 10 mM $CaCl_2$ (1.24-fold stimulation) and 200 mM taurocholic acid (1.6-fold stimulation). Sharon *et al*., (1998) [44] reported a lipase of *P. aeruginosa* KKA-5 that retained its activity in presence of Ca^{2+} and Mg^{2+} but was slightly inhibited by Mn^{2+}, Cd^{2+}, and $Cu^{2+}$and salts of heavy metals (Fe^{2+}, Zn^{2+}, Hg^{2+}, Fe^{3+}) strongly inhibited the lipase, suggesting that they were able to alter the enzyme conformation. Ca^{2+} and Ba^{2+} increased the lipolytic activity while in the presence of Na^{+}, K^{+} and Mg^{2+} metal ions, the activity was similar to that of the control. Fe^{3+}, Al^{3+}, Co^{2+}, Hg^{2+}, Mn^{2+} and Ni^{2+} on the other hand, markedly decreased the enzyme activity. Metal ions like Hg^{2+}, Zn^{2+} and Cu^{2+} have been reported to have inhibitory effect on *Pseudomonas* lipases [46, 47]. *Pseudomonas* sp lipase has also been reported to be inhibited in the presence of Al^{3+}, Mn^{2+}, Ni^{2+} and Fe^{3+} [48].

The sequence obtained by 16S rDNA (1007 bp) was analyzed by nBLAST at NCBI. The analysis confirmed that the present strain was *P.aeruginosa*. The lipase sequence (816 bp) obtained was also analyzed by nBLAST, the results of which confirmed that the sequence was of lipase. The dendrogram generated by BLAST. The lipase virtual protein sequence contained 272aa. 16S rRNA gene sequence offered a useful method for the identification of bacteria. It had long been used as a taxonomic method in determining the phylogenies of bacterial species [49]. The almost complete 16S rRNA gene was sequenced and the (1406 bp) analysis clearly demonstrated that strain KM110 was a member of the genus *Pseudomonas* and exhibited maximum similarity with the 16S rRNA sequence of *Pseudomonas aeruginosa* LMG 1242T(Z76651) (98.94% sequence similarity) Figure 3b.

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References

[1] Jaeger, K.E., Dijkstra., B.W. Reetz., M.T., 1999. Bacterial biocatalysts: molecular biology, three-dimensional structures, and biotechnological applications of lipases. Annu. Rev. Microbiol. 53, 315-351.

[2] Gupta, R., Gupta, N., Rathi., P. 2004. Bacterial lipases: an overview of production, purification and biochemical properties. Appl. Microbiol. Biotechnol. 64, 763-781.

[3] Pandey, A. S., Benjamin, C.R., Soccol, P., Nigam, N., et al., 1999. The realm of microbial lipases in biotechnology. Biotechnol. Appl. Biochem. 29, 119-131.

[4] Jaeger, K.E., Eggert., T. 2002. Lipases for biotechnology. Curr. Opin. Biotechnol., 13, 390-397.

[5] Reetz, M.T., 2002. Lipases as practical biocatalysts. Curr. Opin. Chem. Biol., 6, 145-150.

[6] Ciafardini, G., Zullo, B.A., Iride, A., 2006. Lipase production by yeasts from extra virgin olive oil. Food. Microbiol., 23, 60–67.

[7] Klibanov, A.M., 2001. Improving enzymes by using them in organic solvents. Nature, 409, 241–246.

[8] Sorokin, D.Y., Jones, B.E., 2009. Improved method for direct screening of true lipase–producing Microorganisms with particular emphasis on alkaline conditions. Mikrobiologiia, 78,144-149.

[9] Ertugrul, S., Donmez, G., Takaç. S., 2007. Isolation of lipase producing Bacillus sp. from olive mill wastewater and improving its enzyme activity. J. Hazard. Mater., 149, 720–724.

[10] Kiran, G.S., Shanmughapriya, S., Jayalakshmi, J., Selvin, J., Gandhimathi, R., Sivaramakrishnan, S., 2008. Optimization of extracellular psychrophilic alkaline lipase produced by marine Pseudomonas sp. (MSI057). Bioproc. Biosyst., Eng., 31, 483–492.

[11] Kim, H.K., 2003. Molecular structures and catalytic mechanism of bacterial lipases. Kor. J. Microbiol. Biotechnol. 31, 311-321.

[12] Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol., 173, 697.

[13] Stackebrandt, E., Goebel, B.M., 1994. Taxonomic Note: A place for DNA-DNA reassociation and 16s rRNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Bacteriol., 44, 846-849.

[14] Hulton, C.S.J., Higgins, C.F., Sharp, P.M., 1991. ERIC sequences: a novel family of repetitive elements in the genomes of Escherichia coli, Salmonella typhimurium and other enterobacteria. Mol. Microbiol., 5, 825-834.

[15] Bruijn, F. De., J. 1992. Use of repetitive (repetitive extragenic palindromic and enterobacterial repetitive intergeneric consensus) sequences and the polymerase chain reaction to fingerprint the genomes of Rhizobium meliloti isolates and other soil bacteria. Appl. Environ. Microb., 58, 2180-2187.

[16] Gillings, M., Holle, M., 1997. Repetitive element PCR fingerprinting (rep-PCR) using enterobacterial repetitive intergenic consensus (ERIC) primers is not necessarily directed at ERIC elements. Lett. Appl. Microbiol., 25, 17-21.

[17] Versalovic, J., Koeuth, T., Lupski, J.R., 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic. Acids. Res., 19, 6823–6831.

[18] Cullen, D.W., Hisch, P.R., 1998. Simple and rapid method for direct extraction of microbial DNA from soil for PCR. Soil Biol. Biochem., 30, 983-993.

[19] Pennanen, T., Paavolainen, L., Hantula, J., 2001. Rapid PCR-based method for the direct analysis of fungal communities in complex environmental samples. Soil. Biol. Biochem., 33, 697-699.

[20] Ghosh, A., Maity, B., Chakabarti, K., Chattopadhyay, D., 2007. Bacterial diversity of east Calcutta wet land area: possible identification of potential bacterial population for different biotechnological uses. Microbial. Ecol., 54, 452-459.

[21] Claus, D., Berkeley, R.C.W., 1986. In Bergey's Manual of Systematic Bacteriology. Williams & Wilkins, Baltimore.

[22] Sambrook, J., (Ed) 1982. Molecular Cloning A Laboratory Manual Cold Spring Harbor Laboratory. New York

[23] Abro, S. H., Wagan, R., Tunio, M. T., Kamboh, A. A., et al., 2009. Biochemical Activities of Bacterial Species Isolated from the Frozen Semen of Cattle. Journal. of Agriculture. and Social. Sciences., 5, 109–113

[24] Arpigny, J. L., and Jaeger, K. E., 1999. Bacterial lipolytic enzymes: classification and properties. Biochem. J., 343, 177-183.

[25] Kanwar, L., Goswami, P., 2002. Isolation of Pseudomonas lipase produced in pure hydrocarbon substrate and its application in the synthesis of isoamyl acetate using membrane immobilized lipase. Enzyme. Microb. Technol., 31, 727–735.

[26] Shabtai, Y., Daya-Mishne, N., 1992. Production, purification and properties of lipase from a bacetrium (Ps. aeruginosa YS-7) capable of growing in water-restricted environments. Appl. Environ. Microbiol., 58, 174 -180.

[27] Gilbert, E.J., Cornish, A., Jones, C., 1991b. Purification and properties of extracellular lipase from Pseudomonas aeruginosa EF2. J. Gen. Microbiol., 137, 2223-2229.

[28] Chartrain, M., Katz, L., Marcin, C., Thien, M., et al., 1993. Purification and characterization of a novel bioconverting lipase from Pseudomonas aeruginosa MB 5001. Enzyme. Microb. Technol., 15, 575-580.

[29] Kojima, Y., Shimizu, S., 2003. Purification and characterization of the lipase from Pseudomonas flurescens HU380. J. Biosci. Bioeng., 96, 219-226.

[30] Mencher, J.R., Alford, J.R., 1967. Purification and characterization of the lipase from Pseudomonas fragi. J. Gen. Microbiol., 48, 317-328.

[31] Makhzoum, A., Owusu-Apenten, R.K., Knapp, J.S. 1996. Purification and properties of lipase from Pseudomonas fluorescens strain 2D. Int. Diary. J., 6, 459-472.

[32] Gaur, R., Gupta, A., Khare, S.K., 2008. Purification and characterization of lipase from solvent tolerant Pseudomonas aeruginosa PseA. Process. Biochemistry., 43(10), 1040-1046.

[33] Lee, S. Y., Rhee, J. S., 1993. Production and Partial Purification of a Lipase from Pseudomonas putida 3SK. Enzyme. and Microbial. Technology., 15, 617-623

[34] Rahman, R., Baharum, S.N., Basri, M., Salleh, A.B., 2005. High-yield purification of an organic solvent-tolerant lipase from Pseudomonas sp. strain S5. Anal. Biochem., 341, 267-274.

[35] Jinwal, U.K., Roy, U., Chowdhury, A.R., Bhaduri, A.P., et al., 2003. Purification and characterization of an alkaline lipase from a newly isolated Pseudomonas mendocina PK-12CS and chemoselective hydrolysis of fatty acid ester. Bioorg Med Chem 11,1041–1046.

[36] Kojima, Y., Yokoe, M., Mase, T., 1994. Purification and characterization of alkaline lipase from Pseudomonas fluorescens AK102. Biosci. Biotechnol. Biochem., 58, 1564–8.

[37] Wang, Y., Srivastava, K.C., Shen, G.J., Wang, H.Y., 1995. Thermostable alkaline lipase from a newly isolated thermophilic Bacillus strain, A30-1 (ATCC 53841). J. Ferment. Bioeng., 79, 433–8.

[38] Sidhu, P., Sharma, R., Soni, S.K., Gupta, J.K., 1998a. Production of extracellular alkaline lipase by a new thermophilic Bacillus sp. Folia. Microbiol., 43, 51–4.

[39] Sidhu, P., Sharma, R., Soni ,S.K., Gupta, J.K., 1998b, Effect of cultural conditions on extracellular lipase production by Bacillus sp. RS-12 and its characterization. Indian. J. Microbiol., 38, 9–12.

[40] El-Shafei, H.A., Rezkallah, L.A., 1997. Production, purification and characterization of Bacillus lipase. Microbiol. Res., 52, 199–208.

[41] Kim, H.K., Park, S.Y., Lee, J.K., Oh, T.K., 1998. Gene cloning and characterization of thermostable lipase from Bacillus stearothermophilus L1. Biosci. Biotechnol. Biochem., 62, 66–71.

[42] Lotrakul, P., Dharmsthiti, S., 1997. Lipase production by Aeromonas sobria LP004 in a medium containing whey and soybean meal. World. J. Microbiol. Biotechnol., 13,163–6.

[43] Macedo, G.A., Park, Y.K., Pastor, G.M., 1997. Partial purification and characterization of an extracellular lipase from a newly isolated strain of Geotrichum sp. Rev. Microbiol., 28, 90–5.

[44] Sharon, C., Furugoh, S., Yamakido, T., Ogawa, H., et al., 1998. Purification and characterization of a lipase from Pseudomonas aeruginosa KKA-5 and its role in castor oil hydrolysis. J. Ind. Microbiol. Biotechnol., 20, 304–7.

[45] Chartrain, M., Katz, L., Marcin, C., Thien, M., et al., 1993. Purification and characterization of a novel bioconverting lipase from Pseudomonas aeruginosa MB 5001. Enzyme. Microb. Technol., 15, 575–80.

[46] Iizumi, T., Nakamura, K., Fukase, T., 1990. Purification and characterization of a thermostable lipase from newly isolated Pseudomonas sp. KWI-56. Agric. Biol. Chem., 54, 1253-1258.

[47] Kumura, H., Mikawa, K., Saito, Z., 1993. Purification and characterization of lipase from Pseudomonas fluorescens No. 33. Milchwissenschaft., 48(8), 431- 434.

[48] Dong, H., Gao, S., Han, S., Cao, S., 1999. Purification and characterization of a Pseudomonas sp. lipase and its properties in non-aqueous media. Appl. Microbiol. Biotechnol., 30, 251–256

[49] Drancourt, M., Bollet, C., Carlioz, A., Martelin, R., et al., 2000. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J. Clini. Microbiol., 38, 3623-3630.

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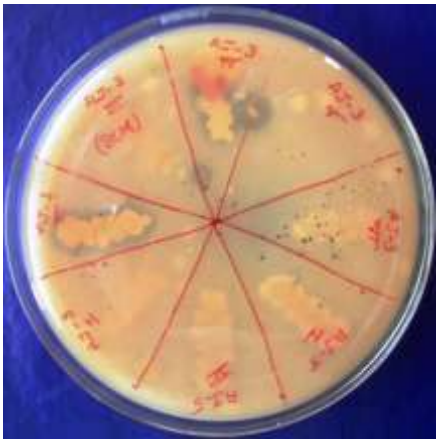

Fig 1a. Soil isolated microorganisms with lipase activity

Fig 1b. Screened soil isolated microorganism with lipase activity

Fig 2. Silver staining of Purified lipase enzyme by Diethylaminoethyl and Sephadex column

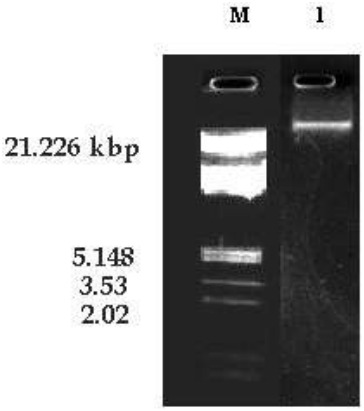

Fig 3a. Lane 1 showing restricted fragments of DNA showing different Molecular markers and Lane 2 showing isolated plasmid DNA from Pseudomonas aeruginosa

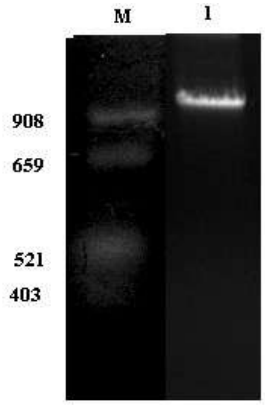

Fig 3b. Lane 1 showing molecular Marker DNA
and Lane 2 showing amplified fragment of lipase gene

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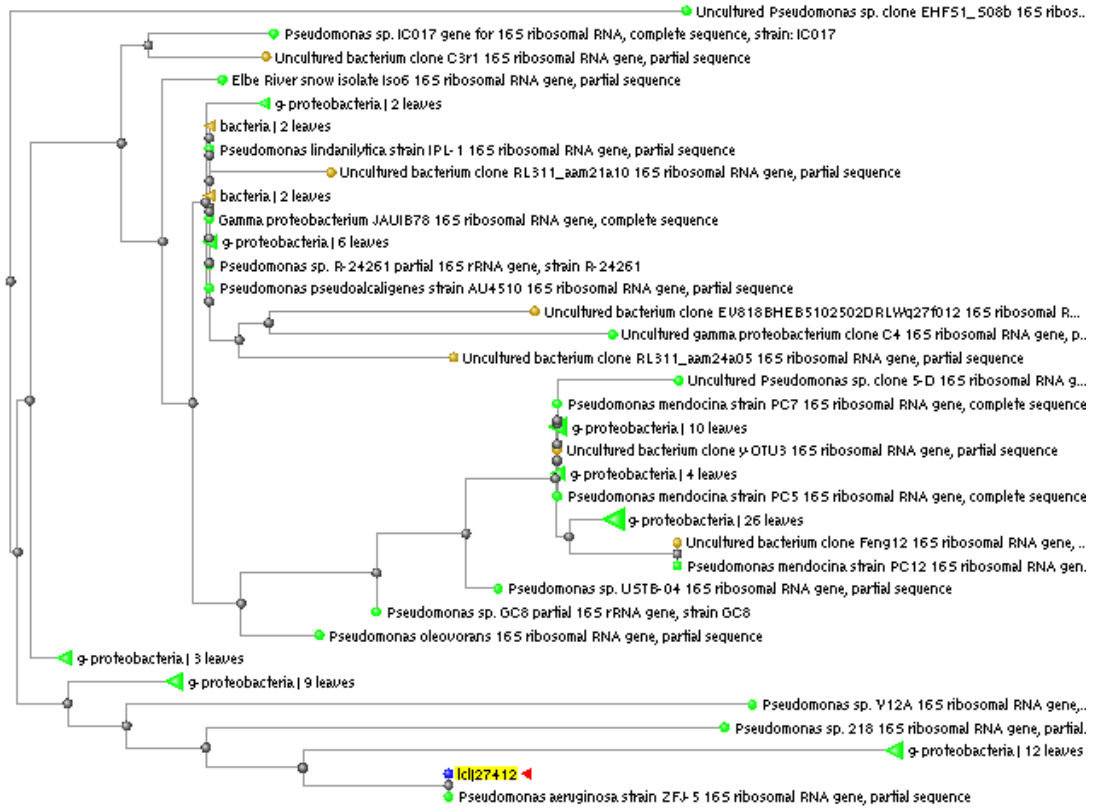
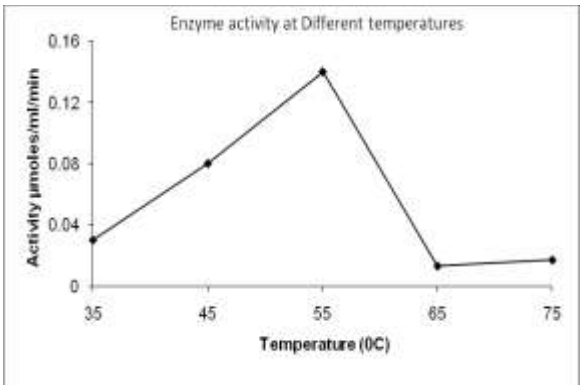
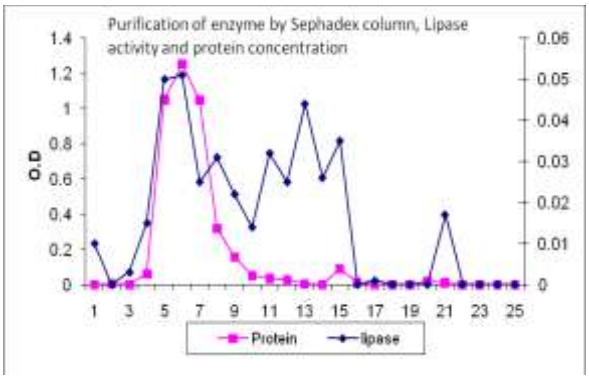


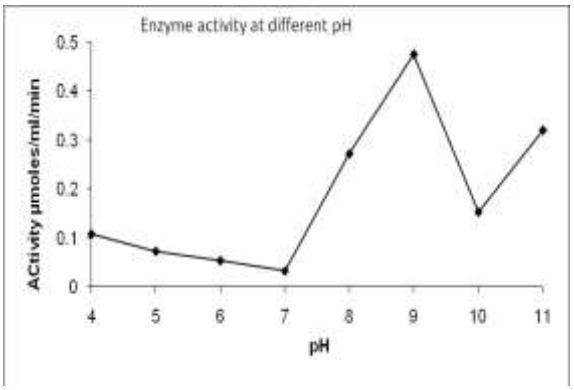
Fig 4. Phylogenetic tree construct showing isolated microorganism characterization of Pseudomonas aeruginosa



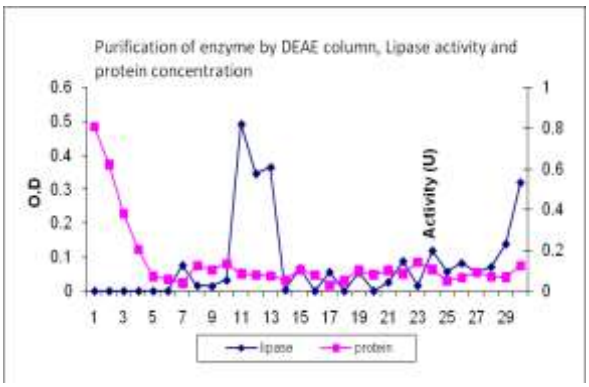
Graph 1. Lipase Enzyme activity at Different temperatures



Graph 3. Purification of enzyme by Sephadex column, Lipase activity and protein concentration



Graph 2. Lipase Enzyme activity at Different pH



Graph 4. Purification of enzyme by DEAE column, Lipase activity and protein concentration