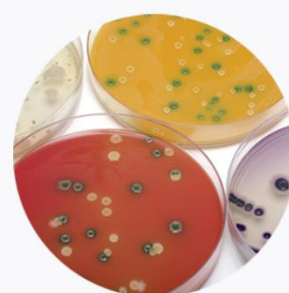


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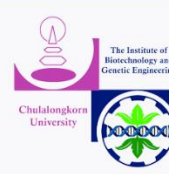


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Content

O-BB-02	Soil eukaryotic microbiome around the Antarctica Great Wall Station	1
O-BB-08	Autotrophic Ammonia Oxidation of Ammonia Oxidizing Archaea in Recirculating Aquaculture System of Marine Shrimp Production	8
O-BI-01	Continuously Bio-Hydrogen Production from Palm Oil Mill Effluent using Dark Fermentation	15
O-BI-02	Value added of acid oil from biodiesel process	23
O-BI-04	Feasible pretreatment of <i>Rhizoclonium</i> spp. for butanol production by <i>Clostridium beijerinckii</i>	28
O-BI-08	Over-production of cellobiohydrolase I from <i>Trichoderma</i> sp. XST1 in <i>Aspergillus aculeatus</i> (BCC17849)	36
O-EB-05	Competitive adsorption between chitosan - <i>Escherichia coli</i> DH5 α network and hydroxypropyl methlycellulose at the oil/water interface	42
O-EB-06	Effect of heterotrophic bacteria on nitrification by entrapped nitrifier under p-nitrophenol toxic shock	49
O-FA-03	ACE-inhibitory and antioxidative activity of riceberry variety's bran protein hydrolysate: identification of its peptides activity	57
O-FA-04	Comparison of volatile flavor compounds in acid-hydrolyzed vegetable protein neutralized by sodium hydrogen carbonate and sodium hydroxide	62
O-FA-05	Expression of phosphate transporter 1 (<i>PHT1</i>) in Thai cassava under drought condition	66
O-FA-06	Production of xylooligosaccharides from grass and weed xylans using xylanase from <i>Aureobasidium pullulans</i> AP 46	71
O-Me-03	Characterization of genomic variants in Thai lung cancer patients by using whole-exome sequencing	79
O-MB-01	Shape the reconstructed metabolic pathway of cassava carbon assimilation into a cell	87
O-MB-04	Identification of non-coding RNAs in microalgae using Random Forest algorithm	98
O-MB-05	A hybrid computational approach for predicting the intergenic microRNA promoters	104
O-MB-06	Development of bi-cistronic DNA vaccines expressing GP5 and M proteins of Porcine Reproductive and Respiratory Syndrome Virus	113
O-MB-07	Expression and production of soluble recombinant diphtheria toxoid (CRM197EK) in <i>Escherichia coli</i>	121
O-MB-08	Isolation and characterization of inhibitory substance from Thai <i>Streptomyces</i> PB007 against NS2B-NS3 two-component protease of dengue virus	126
O-MB-09	Generation of recombinant <i>Bacillus subtilis</i> displaying Porcine Epidemic Diarrhea Virus (PEDV) S1 protein on spore surface	131
O-MB-10	Cordi pep: Bioactive peptide discovery pipeline from <i>Cordyceps sinensis</i> (<i>Ophiocordyceps sinensis</i>) using <i>in silico</i> digestion	139
O-Na-02	Biopolymer beads for release of retinoid	146
O-Na-03	Preparation of bacterial nanofibrillated cellulose by using high pressure homogenization	153
P-BB-01	Screening of zinc and cadmium tolerance and plant-growth promoting rhizobacteria from <i>Zea mays</i> L. rhizosphere	159
P-BB-03	Influence of Culture Media on Growth and Laccase Production of <i>Panus neostrigosus</i>	167
P-BB-04	Isolation and identification of chitinolytic bacteria from mangrove forest soil	173
P-BB-05	Molecular identification of fungi contaminating art works at archeological sites in upper central provinces, Thailand	179

P-BI-02	Use of floating plants as oxygen provider in a sediment microbial fuel cell for bioelectricity generation	185
P-BI-03	Production of polyhydroxyalkanoate (PHA) from various carbon sources by <i>Pseudomonas</i> sp. PSU	192
P-BI-04	Production, characterization and film formation of exopolysaccharides from <i>Aureobasidium pullulans</i> YTP6-14	200
P-BI-05	Effects of preculturing conditions on growth of <i>Sporolactobacillus kofuensis</i> SB7-2 to increase productivity of D-lactic acid fermentation	207
P-BI-07	Optimization of lipopeptide biosurfactant production by <i>Bacillus subtilis</i> BBK-1 in 5-L batch bioreactor	214
P-BI-09	Comparison of the growth kinetics and fatty acid composition of <i>Chlorella</i> sp. T12 cultivated in BG 11 and Chu no.10 media	222
P-BI-10	Recovery of poly (3-hydroxyalkanoate) produced from <i>Azohydromonas lata</i> DMS 1123 using environmental friendly solvents	228
P-BI-11	Isolation and screening of ionic liquid-tolerant cellulolytic bacteria for the production of cellulosic biofuels	233
P-BI-12	Total lipid and unsaturated lipid contents in cyanobacterium <i>Synechocystis</i> sp. PCC 6803 with overexpressing Aas gene	239
P-BI-13	Effect of starch supplementation on 1,3-propanediol production by <i>Clostridium butyricum</i> ISI5-42	246
P-BI-16	Characterization of a thermostable xylanase from <i>Thermobifida fusca</i> PA 1-1	253
P-BI-17	Intracellular levels of total lipid and unsaturated lipid of <i>Synechocystis</i> sp. PCC 6803 with overexpressing <i>sll1848</i> gene	259
P-BI-19	Antifungal efficiency of essential oils and crude sophorolipids against the growth of filamentous fungal isolate from moldy clothes and leathery products	266
P-BI-20	Characterization of a novel alkaline lipase from <i>Pseudomonas</i> sp. HCU2-9 for detergent formulation	273
P-BI-21	Characterization of ionic liquid tolerance cellulase produced from an osmotolerance bacterium for biorefinery application	281
P-BI-22	Partitioning of protease from <i>Bacillus licheniformis</i> 3C5 in polyethylene glycol/salt aqueous two-phase systems	286
P-BI-23	The production of mevastatin from <i>Monascus</i> sp. SS14 and its' mutants	292
P-BI-24	Exopolysaccharide production by submerged culture of wild mushroom, <i>Lentinus</i> sp.	298
P-BI-25	Screening and characterization of exopolysaccharide from EPS producing Lactic acid bacteria isolated from fermented foods	305
P-BI-28	Effect of effluent recirculation on the performance of anaerobic plug flow reactor for digestion of food waste to biogas	312
P-EB-02	Efficiency of crude oil-degrading bacterial consortia from sediments of Gulf of Thailand	318
P-EB-05	Efficiency of bacterial consortia from sediments of upper Gulf of Thailand for PAH biodegradation	326
P-EB-06	Development of a dried emulsion stabilizer consisted of bacterial cells and chitosan for treatment of oil spill	334
P-EB-07	Biodegradation of profenofos by <i>Pseudomonas</i> sp. under presence of oxygen and nitrate	339
P-EB-08	Screening of glyphosate resistance bacteria from agricultural soils with different plantations	345
P-EB-09	Screening of biosurfactant-producing bacteria using low-cost substrates	350
P-EB-10	Immobilization of bacterial consortia on cockle shell for diesel bioremediation	361

P-EB-11	Isolation and characterization of xylenes degrading-bacteria, <i>Pseudomonas citronellolis</i> PC1	367
P-EB-12	First isolation of heavy metal resistant actinomycetes from zinc mine soils in Thailand	373
P-EB-13	Isolation and molecular identification of heavy metal resistant bacteria from zinc mine soils	381
P-EB-16	Production of biosurfactant from chitosan-immobilized <i>Bacillus</i> sp. GY30	389
P-EB-17	Photosynthetic purple non-sulfur bacteria from activated sludge for diesel degradation	397
P-EB-19	The enhancement of toluene dioxygenase to degrade 4-chloroaniline by error prone PCR technique	404
P-EB-23	Characterization of recombinant methyl parathion hydrolase-glutathione-S-transferase capable of degrading methyl parathion pesticide	411
P-EB24	Benzene degradation kinetics of <i>Acinetobacter</i> sp. strain B18 isolated from industrial wastewater	416
P-EB-26	Optimization of conditions for protease production by <i>Bacillus pumilus</i>	421
P-EB-27	Isolation of exopolysaccharide producing bacteria and the application of exopolysaccharide as biofloculant	425
P-EB-31	Effects of Biofilm on Consumption and Adsorption of Disinfection By-Product and Natural Organic Matters in Tap Water	431
P-FA-02	Development of paper made from kraft pulp mixed with ash from power plant in order to absorb moisture of straw mushroom to extend its shelf life	438
P-FA-03	Optimization on response surface models for the optimal production condition of fermented chicken tendon	443
P-FA-05	Responses of 'KDML 105' and salt-tolerant 'UBN 02123-50R-B-2' rice <i>Oryza sativa</i> L. under menadione-induced oxidative stress	449
P-FA-06	Characterization of bacteriocin produced by <i>Pediococcus lolii</i> KU-E1	455
P-FA-07	Evaluation of prebiotic property in edible mushrooms	463
P-FA-08	Rapid detection of fungi and aflatoxigenic fungi in brown rice using near infrared spectroscopy	470
P-FA-09	Biocontrol activity by soil bacteria and plant growth promoting ability by PGPR for fungal disease management in soybean	479
P-FA-10	Characterization of <i>Azospirillum</i> spp. with plant promoting abilities, and biocontrol of rice disease caused by <i>Rhizoctonia solani</i> using marine bacteria	487
P-FA-11	Removing dextran contamination in the syrup evaporator by using dextranase	495
P-FA-12	Determination of fungal contamination and moisture content in brown rice using near infrared spectroscopy	502
P-FA-13	Control of fungal growth and mycotoxin production of <i>Aspergillus flavus</i> and <i>Aspergillus carbonarius</i> by using lactic acid bacteria: an in vitro study	511
P-FA-14	Isolation of plant-growth-promoting epiphytic bacteria from organic rice (<i>Oryza sativa</i> L.)	517
P-FA-15	Evaluation of antioxidant activity of Khem-ngen rice bran oil By ABTS ^{•+} Assay Measurement	524
P-FA-17	Isolation and functional characterization of caleosin gene from the green microalga <i>Chlorella vulgaris</i>	531
P-FA-18	Development of the actinomycete inoculum for biological control of soft rot disease of Dendrobium, commercial orchid caused by <i>Erwinia carotovora</i>	538
P-FA-19	Investigation of nisin and cetylpyridinium chloride treated <i>Escherichia coli</i> using FE-SEM and Raman spectroscopy	546
P-FA-20	Mobilization and tolerance of molybdenum trioxide by wood-rotting basidiomycetes	551

P-FA-22	Monitoring the iodine levels in the 14 selected small-scale iodized salt processing plants in the Bangkok metropolitan area	555
P-FA-23	Rate constant of thermal denaturation of protein in chicken breast	561
P-FA-24	Phytochemical screening, antioxidant and anticancer activities of <i>Phlogacanthus pulcherrimus</i> leaves	568
P-FA-25	The antimicrobial and shelf-life study of vegetable sanitizer from herbal extracts and <i>Acacia concinna</i> (Willd.) D.C.'s pod water	575
P-FA-26	Screening for ligninolytic enzymes from novel isolates of fungi and their potential to degrade paraquat	580
P-FA-27	Antioxidant activity of <i>Allium tuberosum</i> Rottl. ex Spreng under different extraction methods	586
P-FA-28	<i>In vitro</i> multiplication of <i>Pseuderatherum palatiferum</i> and its antibacterial activity	592
P-FA-29	Effects of microwave treatments on selected bacterial and fungal strains and applications	597
P-FA-30	Ultrasound-assisted extraction of phenolic compound from southern Thai indigenous vegetables	604
P-FA-31	Effect of furanone C-30 compound on luminescence and white leg shrimp protection from luminescent vibriosis by <i>Vibrio harveyi</i>	612
P-FA-33	Effects of cultivar and harvesting time of cassava roots on physicochemical properties of cassava starch	617
P-FA-34	Production of flavor compounds by co-culturing of <i>Zygosaccharomyces rouxii</i> , <i>Meyerozyma guilliermondii</i> and <i>Tetragenococcus halophilus</i> in Thai soy sauce fermentation	622
P-FA-36	Development of paper-based devices for detection of <i>Vibrio parahaemolyticus</i> DNA from loop-mediated isothermal amplification	628
P-FA-37	Comparative study on phenolic antioxidant extraction from <i>Camellia sinensis</i> var. <i>siensis</i> and <i>assamica</i>	633
P-FA-39	Effect of ohmic pretreatment on tissue integrity and extraction yield of Chinese chives (<i>Allium tuberosum</i> Rottl.) leaf oil	640
P-FA-40	Total phenolic content, antioxidant and antibacterial activities of two varieties of mango fruit extracts	648
P-FA-41	Chloroplast transformation of <i>Chlamydomonas reinhardtii</i> for production of antiviral double-stranded RNA in shrimp aquaculture	659
P-FA-44	Total flavonoid content and antioxidant activities of edible flower tea products from Thailand	663
P-FA-46	Development of enzyme-linked immunosorbent assay for detecting progesterone in milk	670
P-FA-47	Comparative studies of antioxidant content in black sesame (<i>Sesamum indicum</i>) seed from different extraction methods.	676
P-FA-49	Effects of Ohmic Heating on Thawing Time, Energy Consumption and Water Holding capacity of Frozen Chicken Breast	683
P-FA-51	Antioxidant activity and anti-inflammatory effects of defatted rice bran (<i>Oryza sativa</i> L.) protein hydrolysates on Raw 264.7 Macrophage cells	691
P-Me-01	Comparative repellency effect of three essential oils against <i>Aedes aegypti</i> (Linn.) and <i>Anopheles dirus</i> (Payton and Harrison)	699
P-Me-02	Comparative study of enterovirus 71 subgenotype B5 and C4 and harvesting of viruses by freeze-thaw method	704
P-Me-05	Antibacterial activity of Thai traditional medicinal plants used as traditional drugs in Roi Et Province, Thailand	711
P-Me-06	Screening for bioactive compounds from Thai medicinal plants with suppressing activity against inflammasomes for treatment of Gout	718

P-Me-07	Partial purified protein hydrolysate from rhizome of <i>Gloriosa superba</i> Linn possess anti-breast cancer activity	723
P-MB-01	Genetic modification of <i>Escherichia coli</i> for L-lactic acid production	729
P-MB-02	Development of loop-mediated isothermal amplification method for detecting enterotoxin A gene of <i>Staphylococcus aureus</i>	734
P-MB-04	Role of superoxide dismutase in tolerance to cell wall stress in <i>Saccharomyces cerevisiae</i>	741
P-MB-05	Gadd45 β silencing reduced Hsp70, Hsp60 and Thioredoxin-1 protein expression in cholangiocarcinoma cell line, HuCCA-1	745
P-MB-07	Identification of low differentially expressed responsive genes in microarray data of LPS-stimulated macrophage RAW264.7	752
P-MB-08	Genetic engineering of plasmid vector expressing biotin tag fused recombinant allergen	758
P-MB-09	Magnetic nanobead-based immunoassay for rapid detection of CEA tumor biomarker	764
P-MB-16	The Effect of <i>ubi4</i> Disruption on High Temperature Growth in <i>Saccharomyces cerevisiae</i>	773
P-MB-17	Rapid detection of the rs16879552 and rs7835688 of the <i>neuregulin 1</i> in hirschsprung disease using real-time PCR with taqman minor groove binder probes	780
P-MB-18	Construction of recombinant <i>Hansenula polymorpha</i> for expression of the codon-optimized L1 major capsid protein of Human papillomavirus genotype 52	786
P-MB-19	18S rRNA sequences to reveal microbial eukaryotes in the Central Gulf of Thailand	792
P-MB-20	Morphological and PCR based identification of <i>Phytophthora</i> spp. isolated from the Para Rubber Tree (<i>Hevea brasiliensis</i>)	800

Characterization of a novel alkaline lipase from *Pseudomonas* sp. HCU2-9 for detergent formulation

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Abstract

A novel alkaline lipase was purified and characterized from *Pseudomonas* sp. HCU2-9. The lipase was purified to homogeneity using ammonium sulphate precipitation and gel filtration chromatography. The molecular weight of lipase was approximately 45 kDa by SDS-PAGE. The lipase showed maximal activity at pH 9 and 60°C, respectively. The enzyme was stable in the pH range 5-9 for 30 min and at 45-55°C for 60 min. Higher activity was observed in the presence of Ca^{2+} , Mg^{2+} , Mn^{2+} ions, Tween 80 and Triton X-100 while Co^{2+} , Cu^{2+} , Zn^{2+} ions, DTT and PMSF showed inhibitory effect. The enzyme hydrolyzed both synthetic and natural triglycerides with maximum activity for tripalmitin and palm oil, respectively. It also hydrolyzed esters of *p*-nitrophenol with highest activity for *p*-nitrophenyl palmitate. Organic solvents were found to have little effect on the activity of the lipase. The enzyme showed stability towards selected commercial detergents, oxidizing agents and protease. The remarkable resistance capability of the lipase makes it a potential additive for better detergent formulation.

Keywords: characterization, detergent, lipase, oxidizing agent, purification

Introduction

Lipases (acylglycerol acylhydrolase, EC 3.1.1.3) are an important group of biotechnologically valuable enzymes. Although lipases have been found in many species of animals, plants, bacteria, yeast and fungi. The enzymes from microorganisms are the most interesting because of their potential applications in various industries such as dairy, food, pharmaceutical, paper, biodiesel, textile, cosmetic industries and chemical industries (Saxena et al., 1999; Jaeger and Eggert, 2002). As for industrial enzymology, lipases are commonly included in detergents (Liu et al., 2009). As a detergent additive, the increasing usage of alkaline lipase is mainly due to its affiliation with the nonphosphate detergents. At low temperature (especially below 40°C), capability of removing fat-containing dirt is very poor if the alkaline lipase become nonfunctional. Ideally, alkaline lipase in a detergent should have high activity and stability over a broad range of temperature and pH. Moreover, alkaline lipases should be also compatible with different components in a detergent including surfactants, metal ions, oxidants and proteases (Wang et al., 1995).

A considerable number of bacterial lipases have been commercially produced. Of these, the important ones are *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Chromobacterium* and *Pseudomonas* (Jaeger et al., 1994; Palekar and Vasudevan, 2000). As for industrial enzymology, lipases are commonly included in detergents. For this reason, increasing numbers of lipases have been exploited by researchers in the detergent industry for example, *B. subtilis* DR8806 and *P. stutzeri* PS59 (Shirin et al., 2013; Li et al., 2014). However, very few information were available on the compatibility of the lipases with detergent ingredients (Mohamed et al., 2000; Liu et al., 2009)

In present study, a number of lipase-producing bacteria in soil were isolated and optimized the production of lipase by using the waste of industrial vegetable oil as a carbon source. After optimization of culture conditions, the enzyme was purified by using ammonium sulphate precipitation and gel filtration chromatography. The lipase from the novel isolated bacterium was also characterized and evaluated as an additive for detergent formulation.

Materials and methods

Microorganism

A bacterial strain was isolated from an oil-contaminated soil that showed the highest lipolytic activity. The initial assay to detect lipolytic activity was done on lipase test medium (Tween 80 agar) containing, per litre of distilled water, 15 g agar, 10 g peptone, 5 g NaCl, 0.10 g CaCl₂·2H₂O and 10 ml Tween 80. The lipolytic activity was indicated by the zone of white sediment after it had been incubated for 24 h at 37°C. Then, agar well diffusion was done on lipase test medium (Tween 80 agar) and tributyrin agar plates containing, per litre of distilled water, 15 g agar and 15 ml tributyrin. The lipolytic activity was indicated by the clear zone after it had been incubated for 24 h at 37°C. Bacterial isolate was completely identified and characterized based on morphological and biochemical characteristics.

Enzyme production

The strain was inoculated into 25 ml of basal medium containing, per litre of distilled water, 0.2% (w/v) glucose, 0.5% (w/v) tryptone, 0.01% (w/v) MgSO₄·7H₂O, 0.1% (w/v) K₂HPO₄ supplemented with 2% (v/v) olive oil [partially modified basal medium of Silva et al. (2005)] in 125-ml Erlenmeyer flasks incubated on rotary shaker at 200 rpm for 18-24 h at room temperature (27-30°C). At which time it was approximately with 0.08-0.1 of OD₆₀₀, 0.5 ml of culture was inoculated into 250 ml of basal medium supplemented with 2% (v/v) wastewater from palm oil refining process in 500-ml Erlenmeyer flasks incubated on rotary shaker at 200 rpm for 24 h at room temperature (27-30°C).

Purification of lipase

In the first step of purification, ammonium sulphate precipitation was carried out overnight at 4°C at final concentration of 80% saturation. Following centrifugation at 13,200g for 40 min at 4°C, the pellet was dissolved in 50 mM sodium phosphate with 0.15 M NaCl buffer pH 7.0 and dialyzed against distilled water containing 1 mM EDTA. The dialyzed enzyme was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) to evaluate its extent of purity. Gel filtration was used for a subsequently purification step. The dialyzed enzyme was applied onto a pre-equilibrated HiPrep 16/60 Sephacryl S-100 column (Pharmacia, Sweden). The purification was operated at the flow rate of 0.1 ml/min. Fractions (0.5 ml) were collected automatically by AKTA FPLC collector (Amersham Biosciences, Germany). Fractions with lipase activity were dialyzed extensively and run on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) to evaluate lipase purity. Protein concentration and lipase activity were also assayed.

Lipase assay

Lipase activity was measured spectrophotometrically using *p*-nitrophenyl palmitate (*p*NPP) (Sigma) as the substrate. The assay mixture consisted of 0.1 ml of sample mixed with 0.9 ml of substrate solution containing 10 mg of *p*NPP dissolved in 1 ml propan-2-ol diluted in 9 ml of 50 mM Tris-HCl pH 8.0 containing 40 mg of Triton X-100 and 10 mg of gum arabic. The assay mixture was incubated at 37°C for 30 min and the *p*-nitrophenol released was measured at 410 nm. One unit of activity was defined as the amount of enzyme that liberated 1 nanomole of *p*-nitrophenol per min under the assay conditions (Maia et al., 2001).

Protein measurement

The protein concentration of enzyme preparations was measured according to the method described by Bradford (1976) with bovine serum albumin as the standard.

Gel electrophoresis and activity detection

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was done according to Laemli (1970). After the electrophoresis, the method of Lima et al. (2004) was used to detect lipolytic activity.

Characterization of the purified lipase

Effect of pH on activity and stability

Activity assays were done using the *p*NPP method at 37°C in assay mixtures buffered to various pH values. The following buffers were used to study the effect of pH and type of buffer on activity and stability of purified lipase: citrate-phosphate pH 5-6; phosphate pH 7; Tris-HCl pH 8; glycine-NaOH pH 9-10, all at 50 mM. Stability assays were done by incubating the purified lipase at 37°C for 30 min in buffers of different pH values (citrate-phosphate pH 5-6; phosphate pH 7; Tris-HCl pH 8; glycine-NaOH pH 9-10, all at 50 mM). The residual lipase activity was measured at 37°C using 50 mM Tris-HCl buffer pH 8.0 by the *p*NPP method.

Effect of temperature on activity and stability

Activity assays were done using the *p*NPP method with 50 mM Tris-HCl buffer pH 8.0, at 40, 45, 50, 55 and 60°C. Stability assays were done by incubating purified lipase at 40, 45, 50, 55 and 60°C for 60 min prior to the activity assay. Residual lipase activity was measured at 37°C using 50 mM Tris-HCl buffer pH 8.0 by the *p*NPP method.

Effect of various substances on purified lipase activity

The purified lipase was incubated in the presence of various substances including metal ions (1 mM), surfactants (0.1%, w/v), emulsifier (0.1%, w/v), inhibitor (1 mM) and oxidizing agents (0.1%, w/v) at 37°C for 30 min. Residual lipase activity was then measured using the *p*NPP method.

Evaluation of purified lipase as an additive for detergent formulation

The purified lipase was biochemically characterized for its potential usage in the detergent industry and all experiments were done in triplicate. The effects of various commercial detergents (1%, w/v) on lipase activity were examined by incubating the purified lipase at pH 9 at 50°C for 60 min. Residual lipase activity was measured at 37°C using 50 mM Tris-HCl buffer pH 8.0 by the *p*NPP method.

Effect of various organic solvents on purified lipase stability

The stability of purified lipase in organic solvents was investigated (Ogino et al., 2000) by appropriately mixing 3 ml of purified lipase and 1 ml of solvent in screw cap vials to obtain a final solvent concentration of 25% (v/v). The solution was incubated in shaker (150 rpm) at 37°C for 0-4 h and residual lipase activity was measured in the aqueous phase.

Substrate specificity of purified lipase

The purified lipase was used to study its ability on hydrolyze *p*NP esters and triacylglycerides. In case of *p*NP esters (*p*-nitrophenyl caprate, laurate and palmitate), the experiments were determined colourimetrically by the *p*NPP method. For triacylglycerides (tributyrin, trilaurin, tripalmitin and triolein) and oils (coconut oil, corn oil, olive oil, palm oil, soybean oil and sunflower oil), the experiments were determined by colorimetric method for determination of free fatty acids.

Statistical analysis

An analysis of variance (ANOVA) was used to compare the results. A post-hoc test was conducted to determined differences among treatment means using Turkey HSD following a check for homogeneity of variance by the Levene test. Means with $P < 0.05$ were considered to be significantly different

Results and discussion

Lipolytic activity

Sixty-five strains of bacteria were screened with respect to production of lipase. Detection of lipolytic activity on lipase test medium was carried in a preliminary study, followed in order by agar well diffusion by using tributyrin agar and lipase activity was then measured using the *p*NPP method.

Consequently, a strain HCU2-9 was isolated as the best producer and finally identified as *Pseudomonas* sp. HCU2-9 by conventional biochemical methods.

Purification of lipase

The purification scheme is summarized in Table 1. The enzyme was purified 36.3-fold with 38.9% recovery. The purity was confirmed by the presence of a single band on SDS-PAGE suggesting that *Pseudomonas* sp. HCU2-9 secretes only one extracellular lipase. The molecular weight of the purified lipase was estimated to be 45 kDa by SDS-PAGE and zymogram assay.

The purification of an extracellular lipase from *Pseudomonas* sp. HCU2-9 using a two-step procedure was similar to previous reports on lipase purification from other by conventional precipitation and chromatography. Examples include purification of lipase from *Pseudomonas* sp. DMVR46 (Patel et al., 2014) and *P. aeruginosa* AAU2 (Bose and Keharia, 2013).

Table 1: Summary of lipase purification from *Pseudomonas* sp. HCU2-9

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification (fold)
Crude lipase	2540	252	10.1	100	1
Ammonium sulfate precipitation	1623	59.6	27.2	63.9	2.7
Sephacryl S-100 HR column	989	2.7	366.3	38.9	36.3

Characterization of purified lipase

Effect of pH on the activity and stability of purified lipase

Lipase activity of *Pseudomonas* sp. HCU2-9 was maximal at pH 9 (Table 2). Even at more acidic pH values, lipase activity was observed with value at pH 5 being 89.16% of the maximum activity. The lipase was stable between pH 5 and pH 9 after incubation for 30 min at 37°C (Table 2). Nevertheless, this is similar to other bacterial lipases. Results were reported by Sarkar et al. (2012) for lipase from *Staphylococcus aureus* with maximum activity and stability at pH 8 and pH 9, respectively. Li et al. (2014) also reported the lipase from *P. stutzeri* PS59 with maximum activity at pH 8.5. Since the lipase from *Pseudomonas* sp. HCU2-9 was found to be active and stable over a wide pH range of 5 to 9 which indicates its potential applicability in the laundry industry.

Table 2: Effect of pH on the activity and stability of purified lipase from *Pseudomonas* sp. HCU2-9

pH	Relative activity (%)	
	Lipase activity	Lipase stability
5	89.16 ± 0.24	90.12 ± 0.24
6	94.08 ± 0.11	92.56 ± 0.11
7	97.45 ± 0.06	95.40 ± 0.15
8	98.05 ± 0.18	97.19 ± 0.04
9	100.00 ± 0.16	100.00 ± 0.09
10	87.30 ± 0.02	88.22 ± 0.12

Effect of temperature on the activity and stability of purified lipase

The optimal temperature for lipase activity was 60°C (Table 3). Results show that the enzyme could hydrolyzed (*p*NPP) at 40°C (approximately 85% relative activity) and the activity gradually increased with increasing temperature until it reached the optimum at 60°C. The optimal temperature of 60°C was similar to that of lipase from *Aeromonas caviae* AU04 (Velu et al., 2012). The purified lipase from *Pseudomonas* sp. HCU2-9 was stable from 45 to 55°C (Table 3) and it retained 94.25% of its activity for 60 min at 60°C.

Table 3: Effect of temperature on the activity and stability of purified lipase from *Pseudomonas* sp. HCU2-9

Temperature (°C)	Relative activity (%)	
	Lipase activity	Lipase stability
40	85.12 ± 0.35	90.48 ± 0.10
45	89.08 ± 0.15	100.01 ± 0.06
50	94.16 ± 0.24	98.09 ± 0.13
55	96.25 ± 0.19	97.05 ± 0.04
60	100.00 ± 0.05	94.25 ± 0.11

Effect of various substances on purified lipase stability

It was observed that DTT and PMSF strongly inhibited lipase activity (Table 4). On the other hand, lipase activity was stimulated by Ca²⁺, Mg²⁺, Mn²⁺ ions, surfactants and emulsifier. Moreover, the enzyme exhibited significant stability in the presence of commercial detergents and oxidizing agents. Stimulation of *Pseudomonas* sp. HCU2-9 lipase by Ca²⁺, Mg²⁺, Mn²⁺ ions had some similarity to the results of Sarkar et al. (2012) and Li et al. (2014). For inhibition of lipase of *Pseudomonas* sp. HCU2-9 by DTT indicated that the presence of a sulphur containing amino acid(s) at its active site (Lima et al., 2004) and inactivation of the lipase by PMSF may be caused by modification of an essential serine residue that plays a key role in the catalytic mechanism (van Oort., 1989). The enzyme exhibited significant stability in the presence of commercial detergents and oxidizing agents. Results suggest that purified lipase of *Pseudomonas* sp. HCU2-9 can be considered as a potential candidate to be used as in biotechnology and essentially for further commercial utilization as a additive in detergent formulations.

Table 4: Effect of various substances on the activity of *Pseudomonas* sp. HCU2-9 lipase

Compounds	Relative activity (%) ^a
Control ^b	100.00 ± 0.04
Metal ions (1 mM)	
AlCl ₃	98.36 ± 0.32
BaCl ₂	95.11 ± 0.22
CaCl ₂	150.08 ± 0.12
CuSO ₄	35.13 ± 0.29
CoCl ₂	44.47 ± 0.18
MgCl ₂	158.25 ± 0.25
MnCl ₂	142.16 ± 0.65
ZnCl ₂	67.30 ± 0.02
Surfactants (0.1%, w/v)	
Tween 20	125.25 ± 0.23
Tween 80	132.13 ± 0.09
Triton X-100	135.11 ± 0.18
SDS	120.05 ± 0.06
Sodium cholate	113.10 ± 0.17
Emulsifier (0.1%, w/v)	
Gum arabic	123.12 ± 0.49
Gelatin	128.16 ± 0.14
Inhibitor (1 mM)	
β-mercaptoethanol	58.13 ± 0.16
EDTA	98.02 ± 0.18
DTT	20.14 ± 0.15
PMSF	18.06 ± 0.11

Compounds	Relative activity (%) ^a
Oxidizing agents (0.1%, w/v)	
Hydrogen peroxide	99.26 ± 0.28
Sodium hypochlorite	98.11 ± 0.26
Detergents (1%, w/v)	
Breeze power	95.78 ± 0.32
Pao silver nano	91.22 ± 0.89
SA8 premium	69.45 ± 0.13
Breeze excel liquid	89.15 ± 0.75
Hygiene Laundry Detergent	92.23 ± 0.26
Fineline machine wash detergent	83.12 ± 0.33
Enzyme (0.05 mg/ml)	
Protease	89.12 ± 0.22

^a The relative activity was calculated by using the control as a reference (100%).

^b Control was represented the assay mixture without the addition of any substance.

Effect of various organic solvents on purified lipase stability

Effect of various organic solvents on the stability of *Pseudomonas* sp. HCU2-9 lipase are shown in Table 5. The enzyme was found to be quite stable and active in most of the organic solvents. The highest stability was achieved in hexane and isopropanol with the relative lipase activity of 124.29% and 114.08%, respectively after 4 h. The activation of lipase could be explained by the interaction of organic solvents with hydrophobic amino residues present in the lid that covers the catalytic site of the purified lipase, thereby maintaining the lipase in its open conformation (Singh and Banerjee, 2007)

Table 5: Effect of various organic solvents on the stability of purified lipase from *Pseudomonas* sp. HCU2-9

Organic solvents (25%, v/v)	Relative activity (%) ^a
Control ^b	100.00 ± 0.04
Acetone	84.28 ± 0.50
Chloroform	89.18 ± 0.36
Ethanol	87.15 ± 0.45
Hexane	124.29 ± 0.35
Isopropanol	114.08 ± 0.29
Methanol	91.13 ± 0.19
Toluene	92.17 ± 0.08

^a The relative activity was calculated by using the control as a reference (100%).

^b Control was represented the assay mixture without the addition of any organic solvent.

Substrate specificity of purified lipase

The hydrolytic rate of the lipase towards various synthetic triglycerides and *p*-nitrophenyl esters was examined. As shown in Table 6, the enzyme hydrolyzed all synthetic triglycerides and maximum activity was obtained with tripalmitin. Activity on *p*-nitrophenyl palmitate (*p*NPP) was 10-fold higher than that on *p*-nitrophenyl caprate (*p*NPCA). Similar results were proposed by Saxena et al. (2003). It could be confirmed that this enzyme has greater specificity for long-chained triacylglycerols. The enzyme also hydrolyzed all vegetable oils, with the highest activity obtained using palm oil.

Table 6: Specificity of purified lipase from *Pseudomonas* sp. HCU2-9 in respect of various lipids

Substrate	Relative activity (%) ^a
Triacylglycerols ^b	100.00 ± 0.04
Tributyrin	75.19 ± 0.32
Trilaurin	78.15 ± 0.04
Tripalmitin	100.00 ± 0.08
Triolein	95.33 ± 0.27
<i>p</i> -nitrophenylesters ^c	
<i>p</i> -nitrophenyl caprate (<i>p</i> NPCA)	12.11 ± 0.32
<i>p</i> -nitrophenyl laurate (<i>p</i> NPL)	45.03 ± 0.21
<i>p</i> -nitrophenyl palmitate (<i>p</i> NPP)	100.00 ± 0.04
Oils ^d	
Coconut oil	85.42 ± 0.19
Corn oil	79.23 ± 0.05
Olive oil	95.63 ± 0.16
Palm oil	100.00 ± 0.03
Soybean oil	95.04 ± 0.09
Sunflower oil	75.15 ± 0.11

^a The relative activity was calculated by using the tripalmitin, *p*-nitrophenyl palmitate and palm oil as the reference (100%).

^b Activity toward tripalmitin was accepted as 100%

^c Activity toward *p*NPP was accepted as 100%

^d Activity toward palm oil was accepted as 100%

Conclusions

In conclusion, a novel alkaline lipase from *Pseudomonas* sp. HCU2-9 was purified and characterized. The lipase has an optimal pH and temperature of pH 9 and 60°C, respectively. Moreover, the analysis of the stability of the enzyme in the presence of various metal ions, surfactants, emulsifier, oxidizing agents, commercial detergents, organic solvents and protease, as well as its broad substrate specificity, demonstrates that the enzyme exhibits potential as a commercial additive in detergents. Further research is needed to study the mechanism of the high-washing performance of the *Pseudomonas* sp. HCU2-9 lipase.

References

- Bose, A., and Keharia, H. 2013. Production, characterization and applications of organic solvent tolerant lipase by *Pseudomonas aeruginosa* AAU2. *Biocatalysis and Agricultural Biotechnology*, 2: 255-266.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-252.
- Jaeger, K.E., and Eggert, T. 2002. Lipases for biotechnology. *Current Opinion in Biotechnology*, 13: 390-397.
- Jaeger, K.E., Ransa, S., Dijkstra, B.W., Colson, C., van Heuvel, M., and Misset, O. 1994. Bacterial lipases. *FEMS Microbiology Review*, 15: 29-63.
- Laemmli, U.K. 1970. Cleavage of structure proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.
- Li, X.L., Zhang, W.H., Wang, Y.D., Dai, Y.J., Zhang, H.T., Wang, Y., et al. 2014. A high-detergent-performance, cold-adapted lipase from *Pseudomonas stutzeri* PS59 suitable for detergent formulation. *Journal of Molecular Catalysis B: Enzymatic*, 102: 16-24.
- Lima, V.G.M., Krieger, N., Mitchell, D.A., Baratti, J.C., de Filippis, I., and Fontana, J.D. 2004. Evaluation of the potential for use in biocatalysis of a lipase from a wild strain of *Bacillus megaterium*. *Journal of Molecular Catalysis B: Enzymatic*, 31: 53-64.

- Liu, R., Jiang, X., Mou, H., Guan, H., Hwang, H.M., and Li, X. 2009. A novel low-temperature resistance alkaline lipase from a soda lake fungus strain *Fusarium solani* N4-2 for detergent formulation. *Biochemical Engineering Journal*, 46: 265-270.
- Maia, M.M.D., Heasley, A., Camargo de Moraes, M.M., Melo, E.H.M., Moraes Jr, M.A., and Ledingham, W.M. 2001. Effect of culture conditions on lipase production by *Fusarium solani* in batch fermentation. *Bioresource Technology*, 76: 23-27.
- Mohamed, A.M., Mohamed, T.M., Mohamed, S.A., and Fahmy, A.S. 2000. Distribution of lipases in the Gramineae: partial purification and characterization of esterase from *Avena fatua*. *Bioresource Technology*, 73: 227-234.
- Ogino, H., Nakagawa, S., Shinya, K., Muto, T., Fujimura, N., Yasuda, M., et al. 2000. Purification and characterization of organic solvent-stable lipase from organic solvent tolerant *Pseudomonas aeruginosa* LST-03. *Journal of Bioscience and Bioengineering*, 89: 451-457.
- Palekar, A.A., Vasudevan, P.T., and Yan, S. 2000. Purification of lipase: a review. *Biocatalysis and Biotransformation*, 18: 177-200.
- Patel, V., Nambiar, S., and Madamwar, D. 2014. An extracellular solvent stable alkaline lipase from *Pseudomonas* sp. DMVR46: partial purification, characterization and application in non-aqueous environment. *Process Biochemistry*, 49: 1673-1681.
- Sarkar, P., Yamasaki, S., Basak, S., Bera, A., and Bag, P.K. 2012. Purification and characterization of a new alkali-thermostable lipase from *Staphylococcus aureus* isolated from *Arachis hypogaea* rhizosphere. *Process Biochemistry*, 47: 858-866.
- Saxena, R.K., Davidson, W.S., Sheoran, A., and Giri, B. 2003. Purification and characterization of an alkaline thermostable lipase from *Aspergillus carneus*. *Process Biochemistry*, 39: 239-247.
- Saxena, R.K., Ghosh, P.K., Gupta, W., Sheba, D.W., Bradoo, S., and Gulati, R. 1999. Microbial lipases, potential biocatalysts for the future industry. *Current Science*, 77:101-115.
- Shirin, E., Ahmad, A., and Shamsi, E. 2013. Molecular cloning of a thermo-alkaliphilic lipase from *Bacillus subtilis* DR8806: expression and biochemical characterization. *Process Biochemistry*, 48: 1679-1685.
- Singh, M. and Banerjee, U.C. 2007. Enantioselective transesterification of (RS)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol using *Pseudomonas aeruginosa* lipase. *Tetrahedron: Asymmetry*, 18: 2079-2085.
- van Oort, M.G., Debeer, A.M.T.J., Dijkman, R., Tjeenk, M.L., Verheij, H.M., De Haas, G.H., et al. 1989. Purification of substrate specificity of *Staphylococcus hyicus* lipase. *Biochemistry*, 28: 9278-9285.
- Velu, N., Divakar, K., Nandhinidevi, G., and Gautam, P. 2012. Lipase from *Aeromonas caviae* AU04: isolation, purification and protein aggregation. *Biocatalysis and Agricultural Biotechnology*, 1: 45-50.
- Wang, Y.X., Srivastava, K.C., Shen, G.J., and Wang, H.Y. 1995. Thermostable alkaline lipase from a newly isolated thermophilic *Bacillus*, strain A30-1 (ATCC 53841). *Journal of Fermentation and Bioengineering*, 79: 433-438.



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