Bioprospecting Hot Spring Metagenome: Lipase for the Production of Biodiesel.

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RESEARCH ARTICLE

Bioprospecting hot spring metagenome: lipase for the production of biodiesel

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Abstract Screening of metagenomic library from Taptapani Hot Spring (Odisha) yielded a positive lipase clone (pUC-lip479). Sequence analysis showed an ORF (RK-lip479) of 416 amino acid residues which was overexpressed in *Escherichia coli* BL21 (DE3). Optimum pH and temperature of purified lipase RK-lip479 were 8.0 and 65 °C, respectively, and found to be stable over a pH range of 7.0–9.0 and temperatures 55–75 °C. RK-lip479 could hydrolyse a wide range of 4-nitrophenyl esters (4-nitrophenyoctanoate, 4-nitrophenyldodecanoate, 4-nitrophenylpalmitate, 4-nitrophenylmyristate and 4-nitrophenylstearate), and maximum activity was observed with 4-nitrophenyldodecanoate. RK-lip479 was resistant to many organic solvents, especially isopropanol, DMSO, methanol, DMF, ethanol, dichloromethane, acetone, glycerol and ethyl acetate. RK-lip479 also showed activity in the presence of monovalent (Na^+ and K^+), divalent (Mg^{2+}, Mn^{2+}, Ca^{2+}, Hg^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+} and Ag^{2+}) and trivalent cations (Fe^{3+} and Al^{3+}). Yield of biodiesel production was in the range of 40–76% using various waste oils with RK-Lip479 under optimized conditions.

Keywords Hot spring · Metagenomic · Lipase · Solvent tolerance · Metal tolerance · Biodiesel

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Introduction

Biodiesel is a mixture of monoalkyl esters or fatty acid methyl esters produced from vegetable oils, animal fats and waste cooking oils by using transesterification process in the presence or absence of catalysts. It has a similar property of petroleum-based fuels like petrol, diesel and gasoline. In recent decades, biodiesel has drawn extensive attention for its ability to substitute fossil fuels, which are probable to become eaten up in the anticipated future (Yan et al. 2014). Emissions of various toxic gases produced from burning of fossil fuels have a serious effect on the environment and human health. Biodiesel is considered to be a carbon neutral fuel because its carbon content is originally geared up from the atmosphere which makes this fuel ecofriendly and promotes its commercial production (Ranganathan et al. 2008). The production of biodiesel can be conducted using different catalysts, such as alkali catalyst, acid catalyst and biocatalyst but in many of the biodiesel-producing industries, only alkali catalysts has been used for biodiesel production (Maceiras et al. 2009). The disadvantages of using a chemical catalyst are manifold like separation of catalyst and unreacted methanol, formation of soap and the hydrolysis of triacylglycerol during the reaction (Baron et al. 2014; Bonet-Ragel et al. 2015). On the other hand, an enzymatic transesterification method has been proposed as a cleaner and greener alternative conversion method (Narwal and Gupta 2013). Biodiesel production is currently facing many technological challenges such as high cost of

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lipase, low lipase activity or enzyme inhibition by organic solvents (Maceiras et al. 2009). Metagenomics is an innovative methodology of extracting all microbial genomic DNAs from a certain environmental habitat, constructing metagenomic libraries, and its screening to seek novel functional genes (Ferrer et al. 2005). Metagenomics overcomes the disadvantages of isolation and cultivation procedures of the traditional microbial methods and thus greatly broadens the scope of microbial resource utilization.

Hot springs are attractive sources for the retrieval of biocatalysts having tolerance to harsh conditions like solvent, pH, temperature and metal ions. These environmental resources are highly explored by researchers all over the world. As earlier reported, the biodiversity and abundance of lipase-producing bacteria from Taptapani Hot Spring, Odisha, India (Sahoo et al. 2014; Sahoo et al. 2015). However, there is no report available regarding the functional metagenomics from the hot springs of Odisha. In the present study, using functional metagenomics, solvent tolerant lipase having a potential to produce biodiesel using waste vegetable oils from Taptapani Hot Springs of Odisha is retrieved and characterized.

Materials and methods

Materials and strains

All chemicals used in the present study were of analytical grade and were purchased from Himedia, Sigma and Thermo Scientific, India. *Escherichia coli* 10G and *E. coli* BL21 (DE3) for lipase gene expression were purchased from Lucigen, USA.

Construction of metagenomic library

Sediment sample was collected from Taptapani Hot Springs (latitude 19°30'16.8″N, longitude 84°24'4.6″E) in triplicate. On-site measurement of the temperature and pH were 50 °C and 8.5, respectively. Metagenomic DNA of sediment samples (5 g) was extracted as described by Kumar and Khanna (2014). Metagenomic DNA (5 μg) was partially digested with *Sau*3A1 as per manufacturer's instructions and DNA size ranging from 3 to 8 kb was gel purified using GeneJET Gel Extraction Kit (Thermo Scientific, India). The digested DNA was ligated into the pUC19/BamH1 vector which was then transformed into electrocompetent *E.coli* DH5α by using Micropulser (Bio-Rad, USA). Transformants were grown on Luria-Bertani (LB) agar plate containing olive oil (1% (*v*/*v*)), Rhodamine B (0.01% (*w*/*v*)) and ampicillin (50 μg/ml) for screening their lipolytic activities. After 48-h incubation at 37 °C, there would be halo orange around the colony when exposed to UV light if the colony contained lipolytic activity. The positive lipolytic clone was primer walked using M13 forward and reverses primers followed by different internal primers for obtaining a full sequence of the insert (Xcelris Pvt. Ltd. Ahmedabad, India). Recognition of the open reading frames (ORFs) was done by using the NCBI's open reading frame finder tool (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), and sequence homology searches were executed by the BLAST program. A signal sequence search was done by the SignalP 4.1 program (http://www.cbs.dtu.dk/services/SignalP/).

Nucleotide sequence accession number

The nucleotide sequence for lipase gene was submitted to the NCBI database under accession number KR232658.

Expression and purification RK-Lip

Lipase gene was expressed using the Expresso T7 Cloning and Expression System (Lucigen Corporation, Middleton, WI). The full-length lipase gene was moved out from pUC19 vector by PCR using the following lipase gene specific primers: LipexpF (GAAGGAGATATACATATGAGCC TGCGCGCTAATGAT) and LipexpR (GTGA TGGTGGTGATGATGCGGGCGCAGAGACGCCAG). Underlined sequences in the forward facilitate the ligation in pETite C-His Kan vector (Lucigen). Underlined sequence in the reverse primer adds His-tag to the lipase gene. Primers were designed to amplify the full-length coding regions of lipase gene, excluding the signal peptide. The lipase gene was amplified by HOT start PCR (T100 Thermal Cycler, Bio-Rad, USA) under the defined PCR conditions (initial denaturation 10 min at 95 °C followed by 40 cycles of 30 s at 95 °C with annealing/extension step at 60 °C for 1 min). PCR amplified product was then ligated in pETite vector which was then transformed into *E. coli* 10G (Lucigen, USA). After confirming the gene sequence, the recombinant plasmid was then transformed into HI-Control *E. coli* BL21 (DE3) competent cells. Transformants were grown in 50 ml of LB broth with kanamycin (50 μg/ml) at 37 °C in an incubator shaker (Excella E24, New Brunswick, Germany) till 0.5 to 0.7 absorbance (600 nm) was reached. Afterwards, the expression was induced by adding 1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) and 2 ml of samples was picked up periodically for determining the lipase activity.

Purification of recombinant protein

Induced cells were harvested by centrifugation (Z 326K, Hermle, Germany) at 12,000 × g, 4 °C for 10 min and cell lysis was done by sonication (Sonics Vibracell, USA). After centrifugation, crude cell-free enzyme extract was purified using cobalt nitrilotriacetic acid (Co^{2+} − NTA) HisPur

Cobalt Spin Column Kit system (Thermo Scientific, India) according to the manufacturer's recommended protocol.

SDS-PAGE and zymogram analysis

Purified lipase was loaded at 12% SDS-PAGE (11). Protein bands were visualized by Coomassie Brilliant Blue R-250 Staining. For zymogram analysis, the lipase was electrophoresed on native gel which was then soaked in 25 mM methylumbelliferyl (MUF) butyrate and 0.1 M Tris-HCl (pH 8.0) and incubated for 30 min at 37 °C (Prim et al. 2003). Activity bands resulting from MUF liberation would be visible under UV illumination.

Lipase assay

Lipase was assayed by spectrophotometrically (Evolution 201, Thermo Scientific, USA) at 405 nm using p-nitrophenyl laureate as a substrate described by Sahoo et al. (2014). One unit (IU) of lipase activity was defined as the amount of enzyme that catalyses the release of 1 μM of 4-nitrophenol per minute under the specified assay conditions. Lipase activity was also determined by using various waste oils as described by Pinsirodom and Parkin (2001). Enzyme assay was performed in triplicates.

Biochemical characterization

Optimum pH was determined by incubating the lipase in various buffers ranging from pH 4.0 to 10 as described by Kumar et al. (2011). Similarly for optimum temperature determination, the lipase was assayed over a range of temperatures from 30 to 90 °C at optimum pH. For thermostability and pH stability, the residual lipase activity was measured after incubating the lipase at different temperatures 55 to 95 °C and pH 7 to 11, respectively, up to 6 h. Effect of various organic solvents such as methanol, n-hexane, DMSO, DMF, ethanol, dichloromethane, chloroform, xylene, acetone, glycerol, butanol, ethyl acetate, benzene, isopropanol, isoamyl alcohol and ethyl ether (each at 5, 10 and 25% (*v*/*v*)), metal ions such as NaCl, KCl, $CaCl_2$, $CoCl_2$, $HgCl_2$, $CuSO_4$, $ZnCl_2$, $MgCl_2$, $FeCl_2$, $MnCl_2$, $AgNO_3$ and $Al_2(SO_4)_3$ (each at 1, 5 and 10 mM), detergents, SDS, CTAB and Tween20, (each at 0.1, 0.5 and 1% (*w*/*v*)) and TritonX-100 (at 0.1, 0.5 and 1% (*v*/v)) and enzyme inhibitors EDTA (ethylenediaminetetraacetic acid) and PMSF (phenylmethylsulfonyl fluoride) (each at 1, 5 and 10 mM) were determined by incorporating these into lipase reaction at optimum pH and temperature. The substrate specificity was determined by assaying the lipase with 4-nitrophenyl esters with different aliphatic side chains: C12 (laurate), C14 (myristate), C16 (palmitate) and C18 (stearate). Michaelis–Menten constant (K_m) and the maximum velocity of the reaction (V_{max}) were calculated by Lineweaver–Burk plot.

Transesterification of waste oils to biodiesel

For the biodiesel synthesis reaction, 0.625 ml of 50% methanol (1:1 ratio of methanol and 0.1 M phosphate buffer pH 7.0) was added to 1.5 ml waste vegetable oil. Reaction was initiated by adding the different enzyme concentrations as described below. The solution was gently mixed with a vortex and then placed on a shaker at 200 rpm under standard enzyme assay conditions. After a regular interval of time, 10 μl aliquots of the oil layer were taken for analysis by gas chromatography (Korman et al. 2013). The amount of FAME in the sample was determined from the standard curves. Concentrations of the four fatty acid methyl esters (methyl palmitate, methyl stearate, methyl oleate and methyl linoleate) were quantified using calibration curves prepared by analysing standard solutions of mixed methyl esters. The biodiesel yield was determined as a ratio of the total concentration of these four methyl esters to the total concentration of corresponding fatty acids in the initial reaction mixture. The effect of oil to methanol ratio (1:1 to 1:5), lipase concentration (0.5 to 25 mg protein) and glycerol concentration (0 to 15%) on biodiesel production was also studied. After optimization of biodiesel production with waste olive oil, the biodiesel production was performed with various waste vegetable oil such as sesame oil, sunflower oil, palm oil and peanut oil under standardized conditions.

Results and discussion

A metagenomic library of 13,298 clones was constructed using metagenomic DNA isolated from the sediment of Taptapani Hot Spring, Odisha, India. The insert size was in the range of 3–8 kb with an average insert size of 5 kb. Seven clones forming a fluorescent halo on the plates were selected as lipase positive transformants. Further screening of these seven clones showed that these clones are able to grow in BHB medium with olive oil. Among the seven clones, pUC-lip-479 showed maximum activity (58 IU/ml) as compared to other following clones: pUC-lip-823 (0.5 IU/ml), pUC-lip-1981 (1.2 IU/ml), pUC-lip-2781 (0.7 IU/ml), pUC-lip-3432 (1.9 IU/ml), pUC-lip-6727 (2.1 IU/ml) and pUC-lip-6734 (0.24 IU/ml).

Nucleotide sequence analysis of the pUC-lip-479 revealed 1251 bp ORF encoding a lipolytic protein of 416 amino acid residues. A signal peptide sequence of 28 amino acids was found at the N-terminal end by using SignalP 4.1 server, which indicated that lipase is a secretory protein. The deduced protein without signal peptide consisted of 387 amino acids with theoretical calculated molecular size of 42.57 kDa. Sequence analysis at the protein level revealed the conserved Ala-His-Ser-Gln-Gly motif consistent with the consensus

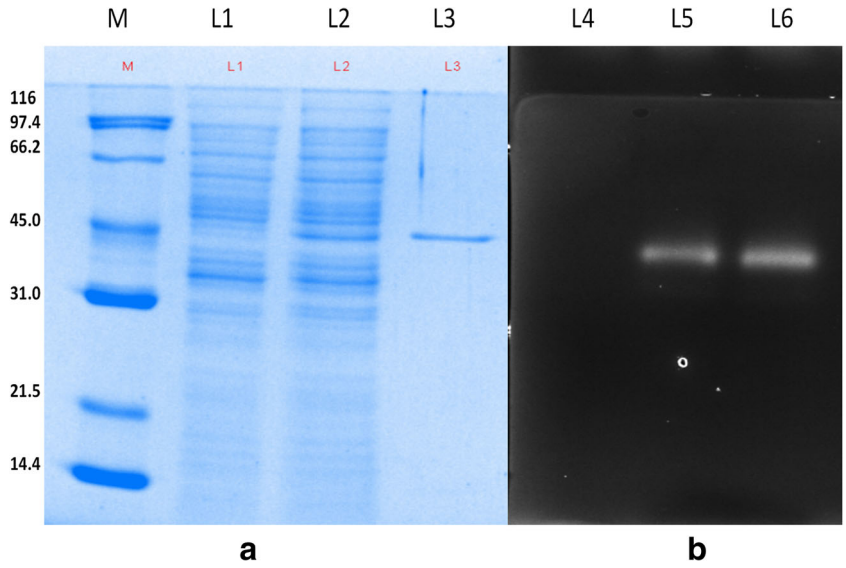

Fig. 1 Electrophoresis analysis of purified lipase RK-lip479. **a** SDS-PAGE (12% polyacrylamide) analysis of purified lipase from cell lysate, *M* protein molecular weight marker, *L1* uninduced cell lysate, *L2* induced cell lysate and *L3* purified lipase. **b** Zymogram analysis of lipase using methylumbelliferyl (MUF) butyrate as substrate, *L4* uninduced cell lysate, *L5* induced cell lysate and *L6* purified lipase

motif Ala-X-Ser-X-Gly (Arpigny and Jaeger 1999). This ORF also contained a Zn^{2+} binding consensus sequence (GAAHAAKH) which confirms that the present lipase is matching with the members of family 1.5 (Chakravorty et al. 2011).

Heterologous expression and purification

Lipase gene without signal sequence was successfully cloned into pETite C-His as a fusion protein with His-tag which was further confirmed by sequencing. Maximum enzyme titre was observed at the 16th hour after induction. The recombinant lipase (RK-lip479) was purified by Co^{2+}-NTA resin affinity chromatography and the purified recombinant protein was eluted using 200 mM imidazole (data not shown). The purified protein showed a single band of the ~42 kDa against the protein marker on 12% SDS-PAGE, and the recombinant lipase revealed a well-defined band of methylumbelliferyl (MUF) butyrate hydrolysis by zymogram analysis (Fig. 1).

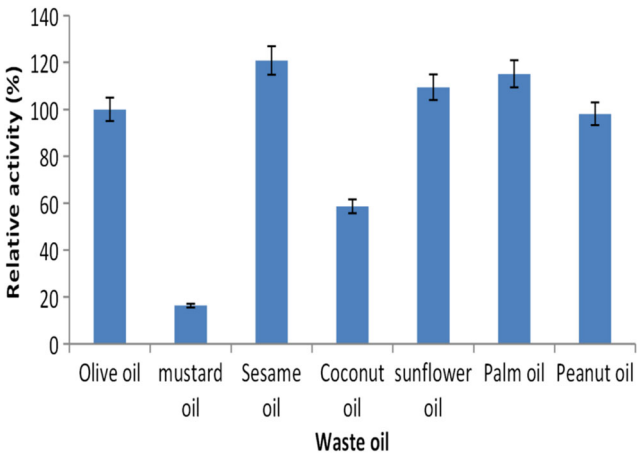

Fig. 2 Substrate specificity of lipase RK-lip479 towards various waste oils. The activity towards olive oil was defined as the 100% level and all measurements were performed in triplicates

Effect of pH and temperature

Maximum lipase activity 598.7 ± 7.4 IU/mg was observed at pH 8.0 which is found to be higher than that of the previous reports. Khan and Mookambikay (2013) characterized two lipases SmlipB and SmlipD retrieved from soil metagenome with activity of 112 and 105 IU/mg, respectively. Similarly, Fan et al. (2011) reported the highest activity of 236 IU/mg from a metagenome-derived lipase clone. pH stability analysis showed that RK-lip479 retained 87, 94, 77 and 54% relative activity at pH 7, 8, 9 and 10, respectively (Fig. S1). This is in agreement with previous reports of Khan and Mookambikay (2013) and Yang et al. (2015). Optimum temperature for RK-lip479 activity was 65 °C (596.1 ± 7.6 IU/mg). The thermal stability assay showed that RK-lip479 retained 89, 92, 60 and 32% relative activity even after 6-h incubation at temperatures 55, 65, 75 and 85 °C, respectively (Fig. S2). Whereas, lipolytic enzymes derived from a Malaysian hot spring retained 80% activity at 70 °C only up to 120 min (Tirawongsaroj et al. 2008). This proves lipase production from RK-lip479 clone to be alkali and thermophilic stable. Ala-Ser-Leu-Arg-Ala (Ala-X-X-X-Ala) motif of RK-lip479 could be responsible for its thermal stability (Chakravorty et al. 2011; Fujii et al. 1996). Probably, the AXXXA motif lead to dimerization of protein causing better stability by strong van der Waals interaction in our thermostable lipase at elevated temperature as also stated by Kleiger et al. (2002).

Table 1 Effect of various solvents on the lipase activity

Solvent (*v*/*v*)	Relative activity (%) 5%	Relative activity (%) 10%	Relative activity (%) 25%
Control	100.0	100.0	100.0
DMSO	129.1 ± 4.3	118.8 ± 1.45	116.5 ± 3.88
DMF	123.3 ± 3.78	129.5 ± 0.95	129.3 ± 4.0
Methanol	124.05 ± 3.93	114.8 ± 3.53	102.1 ± 3.31
Ethanol	118.9 ± 1.3	100.2 ± 1.87	83.2 ± 1 .85
Dichloromethane	123.3 ± 2.19	115.9 ± 2.57	115.3 ± 4.86
Acetone	115.1 ± 1.91	115.7 ± 4.71	134.9 ± 3.0
Isopropanol	119.2 ± 3.22	126.3 ± 4.12	73.8 ± 1.53
Chloroform	42.1 ± 1.43	26.7 ± 3.25	21.2 ± 4.32
Glycerol	99.4 ± 0.99	87.7 ± 3.24	68.1 ± 1.72
n-Hexane	56.2 ± 1.73	10.06 ± 1.22	Nd
Xylene	4.16 ± 0.76	0.48 ± 0.33	Nd
Butanol	23.3 ± 1.97	0.46 ± 0.24	Nd
Ethyl acetate	76.5 ± 2.42	2.72 ± 1.95	Nd
Benzene	4.67 ± 0.64	0	Nd
Isoamyl alcohol	15.6 ± 3.94	0.36 ± 0.09	Nd
Ethyl ether	2.51 ± 1.32	0	Nd

Nd Not determined

Substrate specificity and activity

RK-lip479 hydrolysed a broad range of substrates with maximum activity of 597.3 ± 3.7 IU/mg was observed with 4-nitrophenyl laureate (C12) followed by 577.9 ± 3.5 IU/mg with 4-nitrophenylplamitate (C16). The lowest lipase activity was observed (195 ± 3.8 IU/mg) with 4-nitrophenylbutyrate (C4). Lipase activity of RK-lip479 (on C12) was 3.74 fold higher than lipase reported from *Bacillus* sp. strain 42 (Hamid et al. 2009), 2.75 fold higher than lipase clones from soil metagenomic (Fan et al. 2011) and 1.6 fold higher than clones obtained from bovine rumen metagenome (Privé et al. 2015). Based on Lineweaver–Burk analysis, K_m and V_{max} of RK-lip479 were 482 mg and 1111.11 μM/min with 4-nitrophenyl laureate as substrate. Furthermore, RK-lip479 was able to release fatty acid from various waste oils and maximum release was observed with sesame oil (120.7 ± 1.79%) followed by palm oil (115 ± 2.4%). The lowest fatty acid release was observed with mustard oil (16.3 ± 2.19%) as compared to that with olive oil (100%) (Fig. 2).

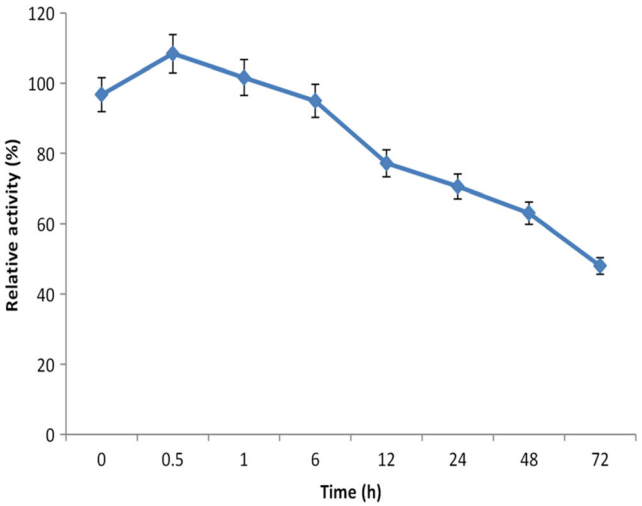

Fig. 3 Methanol stability of lipase RK-lip479. Methanol stability was investigated by incubating the purified lipase at 25% (*v*/*v*) methanol at various time intervals

Effect of metal ions, inhibitors and detergents

The effects of metal ions were recorded at three different concentrations: 1, 5 and 10 mM. At 1 and 5 mM, all the metals showed stimulatory effect on the lipase activity except Ag^{2+} and Al^{3+} (Table S1). At 10-mM concentration of metal ion, lipase was stable in the presence of Na^{+}, K^{+}, Ca^{2+}, Mn^{2+} and Zn^{2+} metals only but either lost the activity (Hg^{2+} and Ag^{2+}) or strongly inhibited by Co^{2+} (53%), Cu^{2+} (42%), Fe^{3+} (22%) and Al^{3+} (23%). Lipase is reported to be activated by $Zn^{2+}$and has a zinc-binding site which is involved in structural stabilization at high temperature as well as increased catalytic activity (Choi et al. 2005). Lipase activity was reduced to approximately 61% of the control in the presence of chelating agent EDTA. This phenomenon indicates that RK-lip479 is a metalloenzyme (Zheng et al. 2013). In the presence of 5

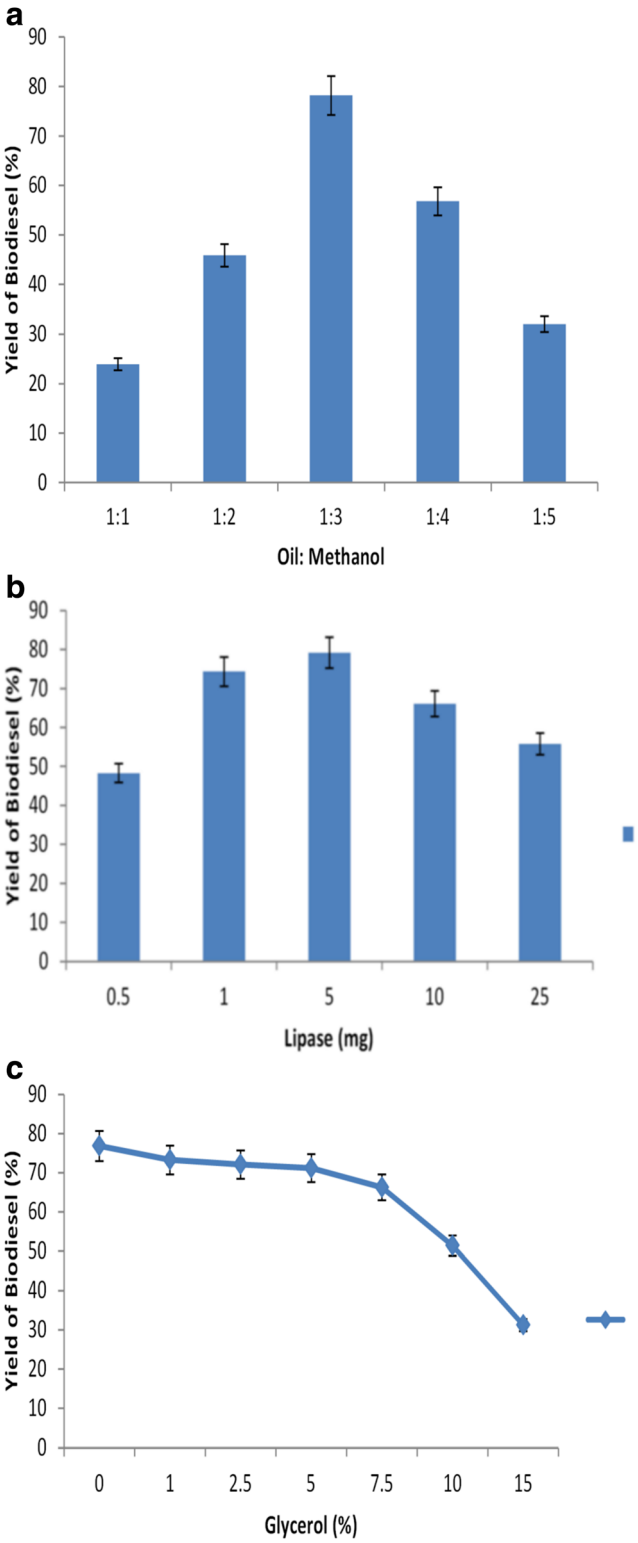

Fig. 4 **a** Effect of oil to methanol ratio on methanolysis of olive oil. **b** Effect of quantity of lipase on biodiesel production. **c** Effect of glycerol on biodiesel production using olive oil as substrate

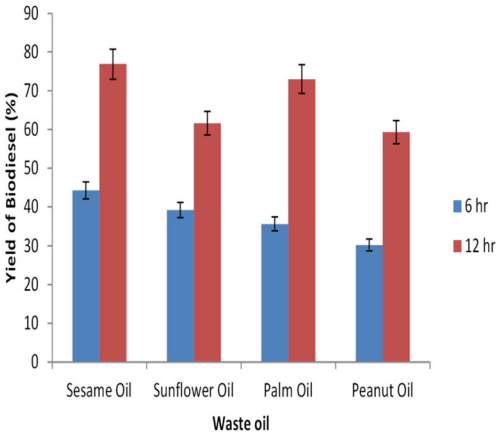

Fig. 5 Biodiesel production by using different waste oils

and 10 mM of PMSF, lipase activity was reduced to 84 and 39%, respectively (Table S1). It is very likely that active site serine is somehow accessible to PMSF, resulting to reduced lipase activity because of some conformational changes occurring at high temperature in the protein, and this makes the catalytic serine accessible to the inhibitor (Kumar et al. 2013). RK-lip479 displayed significant resistance to heavy metal ions compared to other published lipases that were tested (Hamid et al. 2009; Privé et al. 2015; Lee et al. 1999). Decrease in lipase activity was observed with the addition of CTAB (36%), SDS (1.9%), Triton X-100 (21%), tween20 (22%) and as compared with control (Table S1).

Effect of organic solvents

RK-lip479 showed varying stability in all the organic solvents tested. The higher relative activity of RK-lip479 was observed with DMSO (116%), DMF (129%), methanol (102%), dichloromethane (115%) and acetone (134%) (each at 25% *v*/*v*), whereas activity was 83, 73, 68 and 21% in the presence of ethanol, isopropanol, glycerol and chloroform, respectively (Table 1). The enzyme activity was highly suppressed in the presence of n-hexane, xylene, butanol, ethyl acetate, benzene, isoamyl alcohol and ethyl ether (each at 10% *v*/*v*), respectively. Methanol stability studies also showed that the lipase was stable at 25% methanol for 1 h and retained 102% enzyme activity (Fig. 3). On the other hand, enzyme showed 78 and 71% of enzyme activity after 12 and 24 h, respectively, and sharply decreased to 50% after 72 h. These results indicated higher stability of RK-lip479 than that of other previously reported lipases (Sahoo et al. 2014; Emtenani et al. 2013; Yoo et al. 2011). Methanol-tolerant lipase has been proved to be suitable for the production of biodiesel by many workers (Yoo et al. 2011; Mander et al. 2012; Bose and Keharia 2013; Li et al. 2014). Lipases were able to catalyse transesterification reactions for production of biodiesel in the presence of organic solvents like methanol (Sahoo et al. 2014).

Effect of oil and methanol molar ratio on biodiesel production

Molar ratio of oil to methanol is one of the most important factors as it affects the yield of biodiesel. The reaction was carried out using olive oil to methanol molar ratio of 1:1 to 1:5 (mole to mole). The results showed that the biodiesel yield was the highest (78.1 ± 2.1%) at 1:3 oil to methanol molar ratios and a further increase in molar ratio to 1:4 and 1:5 led to decrease in biodiesel yield to 56.7 ± 1.8 and 32 ± 1.7%, respectively (Fig. 4a). In a recent study, 1:4 M ratios were reported as an optimum ratio of oil to methanol for biodiesel production (Narwal et al. 2015).

Effect of quantity of lipase on biodiesel production

The effect of lipase dosage on the methanolysis of olive oil for biodiesel production was also determined (Fig. 4b). It was observed that with an increase of enzyme concentration from 0.5 to 5 mg, the initial rate of biodiesel conversion was increased from 48 to 79%, and it remained constant with further increase in lipase quantity to 10 and 25 mg. The highest yield was 79.1 ± 1.22% with 5 mg of purified lipase. Soumanou and Bornscheuer (2003) found 23.2 and 48% yield of biodiesel from sunflower oil after 24 h by using 0.5 g of commercially available lipases of *Pseudomonas cepacia* and *Pseudomonas fluroescens*.

Effect of glycerol on biodiesel production

Transesterification reaction yields two major products: biodiesel and glycerol. Glycerol is reported to be an inhibitory factor for production of biodiesel because it decreases both the rate and extent of the conversion of biodiesel and its insolubility in oil (Hong et al. 2011). During the biodiesel formation, about 8–10% (*v*/*v*) glycerol was synthesized as a byproduct (data not shown). Addition of glycerol (0, 1 and 2.5) over and above which was already formed during the bioconversion reaction showed no effect on the yield of biodiesel using olive oil (Fig. 4c). The yield of biodiesel was reduced when the total amount of glycerol reached 15%. Bélafi-Bakó et al. (2002) and Hong et al. (2011) reported the reduction in yield to 50 and 37% in the presence of 5 and 7%, respectively. Thus, the lipase reported in the present study exhibits tolerant to higher-amount glycerol addition into reaction environment as compared to that in the previous finding (Hong et al. 2011; Bélafi-Bakó et al. 2002).

Biodiesel production using different waste vegetable oils

Among the various waste oils used for the production of biofuels, the maximum yield of 44.2 ± 2.6 and 76.8 ± 1.2% was observed with sesame after 6- and 12-h incubations, respectively (Fig. 5). Similarly, after 12 h of incubation, yields of 73, 61 and 59% were observed with palm oil, sunflower oil and peanut oil, respectively. Biodiesel conversion yield is higher compared with that of other studies (Sanchez and Vasudevan 2006; Dos Santos Corrêa et al. 2011).

Conclusion

The multifunctional properties of lipase enzymes are only due to its diverse structure, therefore these enzymes are thermostable, alkaline stable, cold adaptable, tolerant to various organic solvents, metals and detergents and could be of great interest for various industrial applications. The novel lipase RK-lip479 had been isolated and characterized by metagenomic approach from the pristine hot spring of Odisha and had a wide range of substrate specificity, exhibited good stability over a wide range of temperatures and pH, high activity in the presence of solvent and metals, tolerance to detergents and specific activity towards long chain of triacylglycerols and a high conversion of biodiesel yield in transesterification using waste vegetable oils. This study can conclude that hot spring metagenome is a potential source of various thermostable enzymes and biomolecules.

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