Original Research Article

# Isolation and characterization of thermostable lipases from themophilic bacteria isolated from hot water spring

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## Abstract

Twelve isolates were screened for lipases production from hot water spring, Vajreshwari, Thane, Maharashtra on a Nutrient agar and nutrient broth medium consisting of olive oil. The isolate showed highest lipase activity was VIII which later was identified as *Sphingomonas paucimobilis*. The effect of pH, temperature and substrate concentration was studied on partially purified lipases by ammonium sulphate precipitation from *Sphingomonas paucimobilis*. The partially purified lipase had maximal activity at pH of 7.The optimum temperature for the hydrolysis of olive oil at 60°C.The lipases has ability to hydrolyse used fried oil. The transesterification activity of lipases was used to synthesis biodiesel. The analysis of synthesized biodiesel was performed by FTIR( Fourier transform infrared spectroscopy) and free fatty acid content.

**Key Words:** Vajreshwari hot water spring, Lipases, Biodiesel, *Sphingomonas paucimobilis.*

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Received Date: 25/10/2017 Revised Date: 12/11/2017 Accepted Date: 21/12/2017
DOI: https://doi.org/10.26611/2026210

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Volume 8
Issue 1

## INTRODUCTION

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are serine hydrolases that catalyze the hydrolysis, esterification and transesterification of long-chain triacylglycerols at an oil-water interface. The lipases are able to reverse the reaction in absence of water to form triacylglycerides from fatty acids and glycerols by esterification reaction. The another catalytic activity exhibited by lipases is transesterification utilizing fats, oils and fatty acids to produce alkylesters[2,9,20]. Lipases are carboxylic ester hydrolases attracting an enormous attention due to they are the most versatile and widely used enzyme in biotechnological applications viz. processing of fats and oils, detergents, food processing, the synthesis of fine chemicals and pharmaceuticals, paper manufacture and production of cosmetics and pharmaceuticals, bakery, brewing, biofuels, leather processing and general cleaning. Lipases are produced by microorganisms (bacteria and fungi), plants and animals from that microbial lipases are more useful since they have great variety of catalytic activities and microorganisms are easy to manipulate genetically continuous supply due to absence of seasonal fluctuations and capable of rapid growth on inexpensive media[4,12,16]. Thermostable lipases are in need due to many processes use temperatures around 50°C by having high melting points of the lipids that are used as substrate. The industrial demand for thermostable lipases stimulates the search for microorganisms that produce such thermostable enzymes. The advantage to have elevated temperatures for reaction is favorable changes in most physical properties of fats at high temperature and increased stability of thermostable lipases to organic solvents determine the increased interest in e new thermostable[2,16] The source of thermostable enzyme is thermophilic bacteria which can survive at elevated

How to cite this article: R J Pungle *et al.* Isolation and characterization of thermostable lipases from themophilic bacteria isolated from hot water spring. *International Journal of Recent Trends in Science and Technology.* January to March 2018; 8(1): 67-73.

temperature 45°C–80°C[9]. It has been revealed that 99% of bacteria are unexplored in laboratory cultivation from various unexploited regions of the earth[4,12]. Thermophilic lipases show higher thermostability, higher activity at elevated temperature and often show more resistance to chemical denaturation. This makes them ideal tools in industrial and chemical processes where relatively high reaction temperatures and/ or organic solvents are used. The industrial demand search for the thermostable continues to stimulate the microorganism's produces of thermostable enzymes[20]. It has been found out that energy consumption for development made more dependency on fossil fuel which is causing green house effect. The alternative source may the biodiesel (fatty acid alkyl monoester) derived from feedstocks such as vegetable oils and animal fats. Biodiesel is biodegradable, non-toxic, and can be used directly or blended with conventional petrodiesel in unmodified diesel engines. For production of biodiesel alkali treatment used having drawback of saponification in presence of water contamination. To have a adventitious method the lipases are getting interest due to their versatility and stability[5,18]. Thus the present study isolation and screening of thermophilic bacteria for lipases was demonstrated. It was first time *Sphingomonas paucimobilis* was isolated from hot water spring from Vajreshwari, Thane, Maharashtra. Lipases from *Sphingomonas paucimobilis* was studied for production and optimization for physical parameters with exploitation for biodiesel production.

## MATERIALS AND METHODS

All the chemicals used in the study were procured from Himedia Pvt. Ltd. And SD Fine India Pvt. Ltd.pH meter, weighing balance (Shimazdu), water bath, Autoclave (Kumar lab product), Incubator (Meta-lab), centrifuge (Remi).

**Collection of sample:** Hot water spring sample was collected in sterile tubes from Vajreshwari, Thane, Maharashtra.

**Isolation of Thermophilic Micoorganisms and Screening for Lipase producers:** The water samples were streaked over nutrient agar plate for isolation and purification of bacterial culture. The isolates were spot inoculated on lipase medium consisting of nutrient agar with oil and incubated at $60^0$C for 24hr, 48hr and 72hr. The Bacterial colonies showing zone of clearance were selected as lipase producer[3,15]. The isolates capable of lipase production were further screened to isolate the best possible lipase producing bacteria based titrimetric assays. The selected isolates were inoculated in Nutrient broth medium consisting olive oil as substrate with Tween 80 as emulsifier and incubated at $60^0$C for 24hr, 48hr and 72hr. The enzyme activity of supernatant (crude enzyme) was evaluated. The culture having highest enzyme activity was selected for production of lipases, partial purification of enzyme and optimization[15]. All the experiments were performed in duplicate.

**Lipase Production:** The composition of lipase production media was: peptone (0.2gm), glucose (0.2gm), $(NH_4)_2SO_4$ (0.1gm), $K_2HPO_4$(0.2gm), olive oil (1ml), tween80 (1-2 drops) and distilled water (100ml).The 1ml of overnight culture was inoculated in production media and incubated at $60^0$C with constant shaking at 120rpm for 48hr. After 48 hours of incubation, the culture was centrifuged at 10,000 rpm for 20 min at 4ºC and the cell free culture supernatant fluid was used as the sources of extracellular enzyme. The lipase activity in the supernatant was determined by the titrimetric method. The bacterial isolate that produced maximum lipase was selected for further work[21].

**Enzyme assay:** The inoculums was centrifuged at 10,000rpm at $4^0$C to pellet out the cells. The titrimetric method was applied for determination of enzyme activity. From collected supernatant 1ml was added in 10% of olive oil substrate with tween 80 as emulsifier. To the reaction mixture 500µl of phosphate buffer was added. The reaction mixture incubated at $60^0$C for 1hr with constant shaking at 120rpm. The 500 µl of ethanol was added to stop the reaction. The released fatty acid was titrated with sodium hydroxide (0.05N) in buret with phenolphthalein. Titration was carried out until persistant light pink colour appears. Control experiment was done by mixing phosphate-buffered olive oil with tween 80 emulsion substrate mixture and titrating with sodium hydroxide (0.05 N). The quantity of fatty acid liberated is equivalent to the volume of NaOH used and it was calculated using Eq. (1), where N is the normality of NaOH titrant used (0.05 in this case).

Eq. (1):

µlmole fatty acid per ml sample = (ml NaOH of sample – ml NaOH of control) * N*1000/

Volumn of reaction mixture

One unit (U) of lipase activity is defined as the amount of enzyme that releases from the emulsion substrate 1 µmole of fatty acid per ml per minute under specific assay condition.[9]

**Protein Estimation:** Protein estimation was performed by Foline lowry method[4].

**Partial Purification of Lipase:** Lipase purification was carried out at 4°C. The culture medium was centrifuged at 10000 rpm for 20 min to obtain crude enzyme, the supernatant fluid was subjected to precipitation with ammonium sulphate to 30 % saturation and allow to precipitate for 24 hours. The precipitate was collected by centrifugation at 10000rpm at $4^0$C for 20min.Lipase

activity both in the precipitate. The pellet was dissolved in TriCl buffer of pH 7.4 and dialyzed for overnight at $4^0$C.The dialyzed enzyme was used for characterization of enzyme[21].

**Characterization of Lipase:** The enzyme was incubated at $60^0$C with olive oil emulsified with tween 80 substrate in buffers viz. citrate buffer (for pH 5.0), phosphate buffer (for pH 6.0,7.0,8.0 ) and Tris-HCl buffer ( for pH 9.0,10.0,11.0) under assay conditions and enzyme activity was determined. The optimum temperature for the enzyme activity was determined by incubating the assay mixture at different temperatures ($20^0$C, $37^0$C, $50^0$C, $60^0$C and $70^0$C, $80^0$C) and enzyme activity was detected[15]. The effect of substrate concentration for enzyme activity was determined by incubating the assay mixture at different substrate concentration (1%,2%, 4%, 6%, 8%,10% and 12%) and enzyme activity was detected.

**Lipases on used Fried Oil:** The ability of lipases to act on high acid value oil was investigated.10% 0f used fried oil was mixed with 0.5ml phosphate buffer and 50µl of enzyme was added and incubated at $60^0$C with a control and enzyme activity and free fatty acid content was determined by enzyme assay.

**Lipases in biodiesel production:** The partially purified lipase was mixed with fried oil and methanol in the ratio of oil, methanol was 1:1 and 0.5ml of lipase was added. The transesterification was operated at $55^0$C temperature for 24 hours. The free fatty acid content of the was measured after free fatty acid conversion to biodiesel[10].

**The analytical method:** Biodiesel from lipase was characterized for free fatty acid content and FTIR analysis. Fatty acid content were determined by titration method[8,10]. FTIR (Bruker) was performed at RUSSA center, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.[14]

## RESULT AND DISCUSSION

**Isolation of Thermophilic Microorganisms and Screening for Lipase production:** From the hot water spring Vajreshwari, Thane, Maharashtra the bacteria isolated were 12 in number as reported by Pimpliskar, 2014.The isolated microorganisms were plated on lipase medium consisting of nutrient agar with Olive oil and incubated at $60^0$C for 24hr and 48hr.The bacterial colonies having clear zone ariond them were considered as lipase producer. Colony no. I,II,III,IV,V,VI,VII,VIII,XIII and XVIII had cleare zone around it. The isolates having clear zone was assayed for maximum enzyme activity by inoculating in Nutrient broth with olive oil and detected for enzyme activity by titrimetric method. From Graph 1 Colony I and VIII has maximum enzyme activity, thus selected for production of lipases. Colony no. I and VIII had maximum enzyme activity in 48hr. The enzyme activity of all isolates was declined in 72hr. Of incubation.

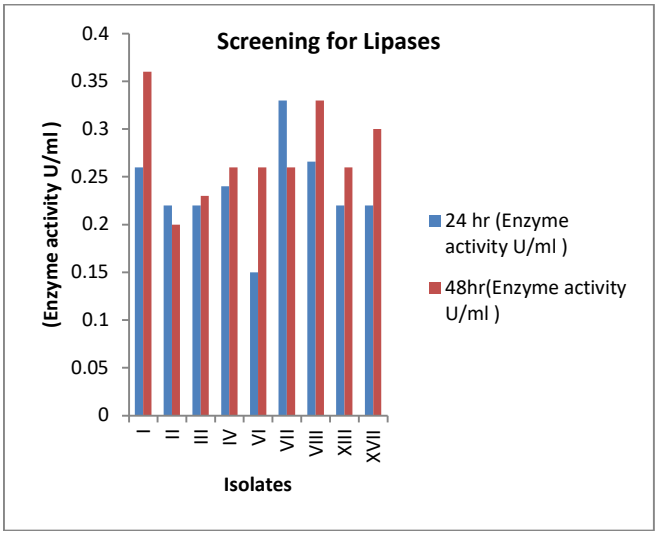


**Figure 1:** Secondary screening

**Identification of Bacteria:** Bacteria was identified which had given highest enzyme activity after partial purification of lipases. The bacteria was identified as *Sphingomonas paucimobilis* by VITEK-2 at Dr, Jagatap-Patil Speciality Laboratory, Barashi. The Studies found out that *Sphingomonas paucimobilis* has lipase activity[17].

**Table 1:** Biochemical Identification by VITEK-2

| Well | Test | Mnemonic | Isolate 8 | Well | Test | Mnemonic | Isolate 8 |
|---|---|---|---|---|---|---|---|
| 2 | Ala-Phe-Pro Arylamidase | APPA | – | 34 | D-Tagatose | dTAG | + |
| 3 | Adonitol | ADO | – | 35 | D-Trehalose | dTRE | + |
| 4 | L-Pyrrolydonyl-Arylamidase | PyrA | – | 36 | Citrate (Sodium) | CIT | – |
| 5 | L-Arabitol | IARL | – | 37 | Malonate | MNT | – |
| 7 | D-Cellobiose | dCEL | + | 39 | 5-Keto-D-Gluconate | 5KG | – |
| 9 | Beta-Galactosidase | BGAL | – | 40 | L-Lactate alkalinization | ILATk | – |
| 10 | H2S Production | H2S | – | 41 | Alpha-Glucosidase | AGLU | – |
| 11 | Beta-N-Acetyl-Glucosaminidase | BNAG | – | 42 | Succinate alkalinization | SUCT | – |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 12 | Glutamyl Arylamidase pNA | AGLTp | – | 43 | Beta-N-Acetyl-Galactosaminidase | NAGA | – |
| 13 | D-Glucose | dGLU | + | 44 | Alpha-Galactosidase | AGAL | – |
| 14 | Gamma-Glutamyl-Transferase | GGT | – | 45 | Phosphatase | PHOS | – |
| 15 | Fermentation Glucose | OFF | – | 46 | Glycine Arylamidase | GlyA | – |
| 17 | Beta-Glucosidase | BGLU | – | 47 | Ornithine Decarboxilase | ODC | – |
| 18 | D-Maltose | dMAL | + | 48 | Lysine Decarboxylase | LDC | – |
| 19 | D-Mannitol | dMAN | + | 52 | Decarboxylase Base | 0DEC | – |
| 20 | D-Mannose | dMNE | + | 53 | L-Histidine assimilation | lHISa | – |
| 21 | Beta-Xylosidase | BXYL | – | 56 | Courmarate | CMT | + |
| 22 | Beta-Alanine arylamidase pNA | BAlap | – | 57 | Beta-Glucuronidase | BGUR | – |
| 23 | L-Proline Arylamidase | ProA | – | 58 | O/129 Resistance (comp. vibrio.) | O129R | – |
| 27 | Palatinose | PLE | – | 59 | Glu-Gly-Arg-Arylamidase | GGAA | – |
| 29 | Tyrosine Arylamidase | TyrA | – | 61 | L-Malate assimilation | IMLTa | – |
| 31 | Urease | URE | – | 62 | Ellman | ELLM | – |
| 32 | D-Sorbitol | dSOR | – | 64 | L-Lactate assimilation | ILATa | – |
| 33 | Saccharose / Sucrose | SAC | – | | Organisms identified by VITEK-2 | | Sphingomonas pausimobilis |

**Partial Purification of Lipase:** Partial purification of lipases was carried out by 30% ammonium sulphate precipitation. It was noted down that enzyme was precipitated at 30% saturation only as precipitate has enzyme activity. The specific enzyme activity was increased after precipitation of lipase. The specific enzyme activity in crud lipases from colony VIII was more than colony I. Thus for further studies colony VIII was taken.

**Table 2:** Partial purification of lipases

| No. | Name | Protein concentration (mg/ml) | Enzyme activity (U/ml) | Specific activity (U/ml/mg) |
|---|---|---|---|---|
| 1 | Crude extract I | 46.77 | 0.11 | 0.002 |
| | VIII | 3.5 | 0.27 | 0.077 |
| 2 | Ammonium sulphate precipitation (30%) VIII | 1.58 | 8.1 | 5.12 |

**Characterization of Lipases: Optimization for pH:** The enzymes have effect over activity due to hydrogen ion concentration. Thus evaluation of effect of pH at various pH buffers from pH 5 to pH 11 was performed by using olive oil as substrate and incubating for 1 hr. at $60^0$C. It was found that The enzyme activity of Lipases was maximum at pH 7.0 (Graph 2). The enzyme is active at pH 5-pH 6.

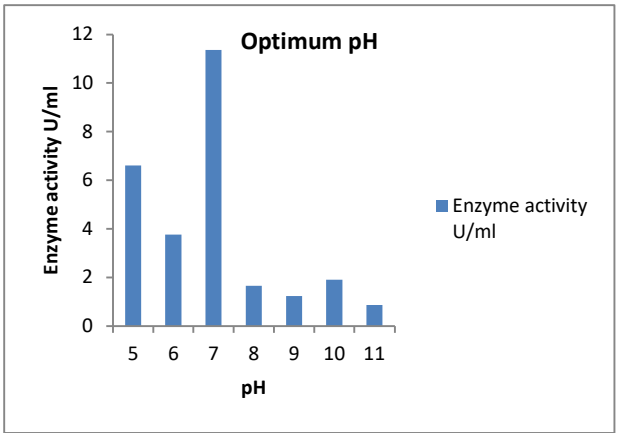


**Figure 2:** Effect of pH

**Optimization for Temperature:** Temperature is the critical factor for enzyme activity. The enzyme activity was determined by incubating reaction mixture at various temperatures ranging from $20^0$C to $70^0$C. It is evident from Graph 3 that gradual increase in enzyme activity of

Lipases upto $60^0$ C and then decrease. The enzyme activity was maximum at $60^0$C.

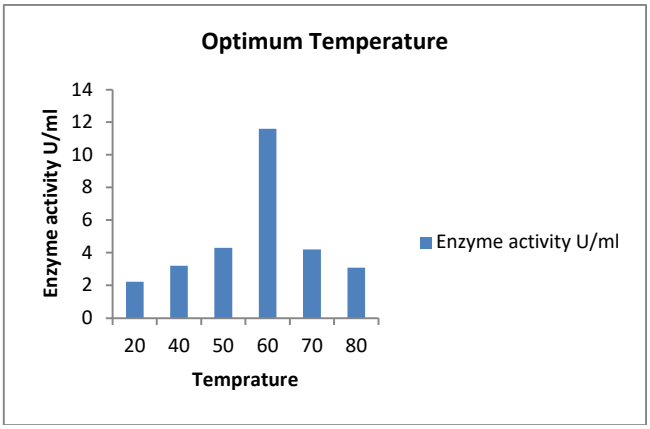


**Figure 3:** Effect of Temperature

**Effect of Substrate concentration:** The effect of substrate concentration for enzyme activity was determined by incubating the assay mixture at different substrate concentration (1%, 2%,4%,6%,10% and 12%) and enzyme activity was detected. Optimum Enzyme activity was detected 14% at substrate concentration.The substrate concentrations has to be determined to obtain the saturation point of enzyme.

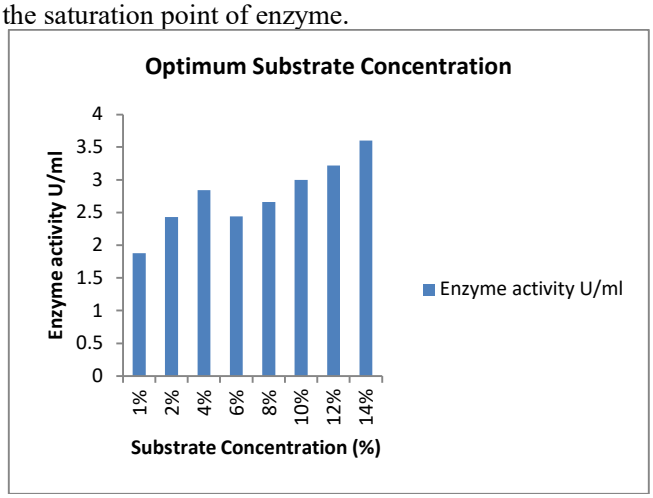


**Figure 4:** Effect of Substrate Concentration

**Lipases on used Fried Oil:** The lipases were applied on fried oil obtained from the sweet shop. It was observed from Graph 5 the content of fatty acid was increased after treating lipases with fried oil suggesting lipases effective over fried oil.

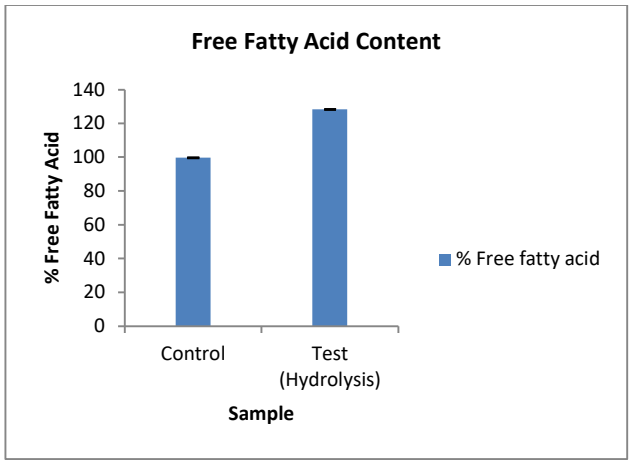


**Figure 5:** Free fatty acid content

**Lipases in biodiesel production: Analysis of Free Fatty acid:** The free fatty analysis after transesterification reaction carried out in presence of lipases with used fried oil and methanol was reported according to Graph 6 as fatty acid content was reduced from 99.5% to 4.23% suggesting the conversion of free fatty acid to FAME.

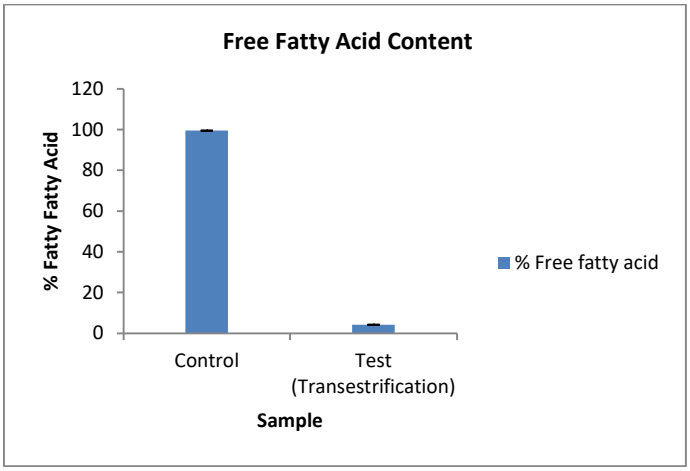


**Figure 6:** Free Fatty Acid content

**FTIR Analysis:** For the confirmation of trasesterification reaction and identification of Fatty acid methyl ester the FTIR analysis was carried out. The FTIR analysis of aqueous phase (Graph 7) was with bands at 3328.84 $cm^{-1}$ corresponding to –OH group. According to the peaks 2945.20$cm^{-1}$ and 2833.70$cm^{-1}$ fall between characteristic peaks of lipids at 3100$cm^{-1}$-2800$cm^{-1}$[16]. The amid III and amid II bands were represented by 1652.86$cm^{-1}$ and 1410.15$cm^{-1}$ respecrively. Carbohydrate specific peaks were 1111.90$cm^{-1}$ and 1017.64$cm^{-1}$. FTIR analysis confers the presence of protein which was congugated with carbohydrate group suggesting enzyme may be glycoprotein[11]. The lipid moity observed was may be due to emulsion of lipids or bound lipid to lipases.

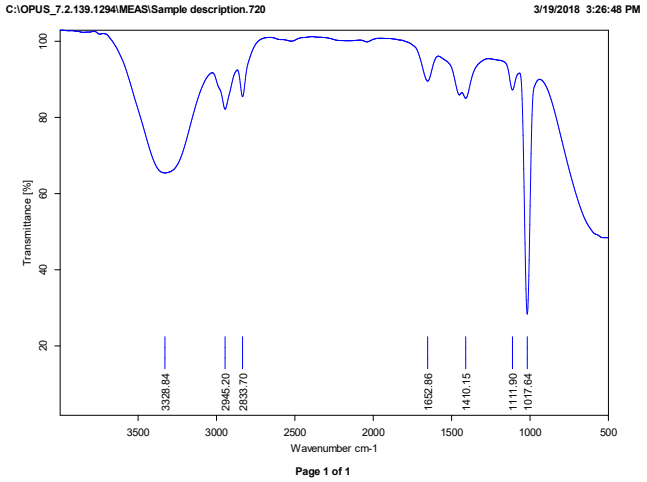


**Figure 7:** FTIR of Aqueous phase

The FTIR results of oil-methanol phase (Graph 8) obtained demonstrates that peak at 2922.04cm$^{-1}$ and 2854.10 cm$^{-1}$ were typical of lipids of C-H stretching. peak at 1457.62 cm$^{-1}$ was due to asymmetric stretching of –$CH_3$ in FAME and 1158.60 cm$^{-1}$ was characteristic of O-$CH_3$ typical of biodiesel. In the region from 1800-1700 cm$^{-1}$, it can be observed peaks that can be attributed to the stretching of C=O, typical of esters. The peak 1370.13 cm$^{-1}$ can be attributed to the glycerol group O-$CH_2$ (mono-, di- and triglycerides) 1075–1100 cm$^{-1}$, covering the asymmetric axial stretching of O-$CH_2$-Ce-$CH_2$-OH[16].The reaction was in progress as it was observed for peaks 1370.13 cm$^{-1}$ and 1075–1100 cm$^{-1}$

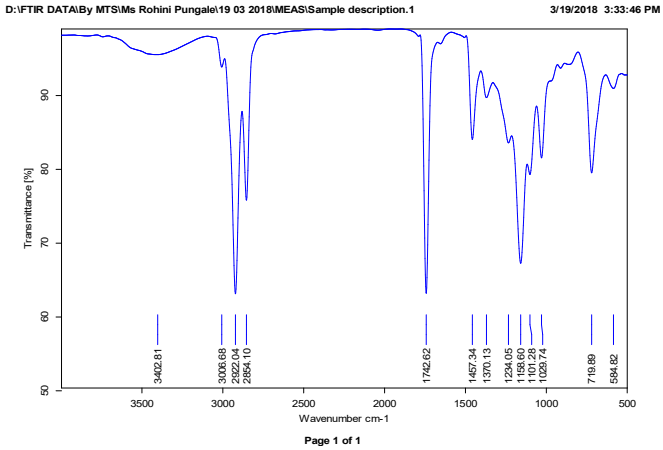


**Figure 8:** FTIR of Oil and Methanol mixture treated with Lipases

## CONCLUSION

From Vajreshwari Hot water spring 12 bacteria were isolated. From that Sphingomonas paucimobilis was identified a culture having maximum lipase activity. *Sphingomonas paucimobilis* was first time evaluated for production, partial purification of of lipase as well as characterization for optimum pH, temperature and substrate concentration as per cited references. Partially purified lipase can hydrolyse used fried oil and its trasesterification reaction exploited for biodiesel synthesis by methanol and used fried oil as substrate. Isolated lipases will be beneficial to utilize waste in the form of used fried oil for a eco-friendly energy generation source i.e. biodiesel.

## ACKNOWLEDGMENTS

The authors wish to acknowledge our gratitude towards Dr. P.V. Ashtekar, Principal and S. A. Kate, Head, Department of Biotechnology Shivchhatrapati College, Aurangabad for the facilities extended to complete this research work.

## REFERENCES

1. Ayuob M., Alhamdani Hanaa Jaffer Jabbar Alkabbih:Isolation And Identification Of Lipase Producing Bacteria From Oil-Contaminant Soi:Journal Of Biology, Agriculture And Health care. 2016; Vol.6, No.20
2. Bakir B., Metin K.:Production And Characterization Of An Alkaline Lipase from Thermophilic Anoxybacillus Sp. Hbb16z. :Chem. Biochem. Eng. Q..2017;31 (3) 303–312
3. Bhavani M,Chowdary G, David M, Archana G:Screening, Isolation And Biochemical Characterization Of Novel Lipase Producing Bacteria From Soil Samples: International Journal Of Biological Engineering 2012
4. Bora L.,Bora M.:Optimization Of Extracellular Thermophilic Highly Alkaline Lipase Fromthermophilic Bacillus Sp Isolated From Hotspring Of Arunachal Pradesh, India:Brazilian Journal Of Microbiology.2012; 30-42
5. Chi-Yang Yu, Liang-Yu Huang, I-Ching Kuan,Shiow-Ling Lee:Optimized Production Of Biodiesel From Waste Cooking Oil by Lipase Immobilized On Magnetic Nanoparticles: Int. J. Mol. Sci. 2013; 14, 24074-24086
6. Indriati G., Ruth Rize Paas Megahati:Isolation Of Thermophilic Bacteria And Optimizing The Medium Growth:Conditions :https://Doi.Org/10.20546/Ijcmas.2018;701.177
7. Jardine J.,Akebe Luther King Abia, Mavumengwana V., Eunice Ubomba-Jaswa:Phylogenetic Analysis and Antimicrobial Profiles of Cultured Emerging Opportunistic Pathogens (Phyla Actinobacteria and Proteobacteria) Identified in Hot Springs: Int. J. Environ. Res. Public Health 2017; 14, 1070
8. Kimtuna P, Opas Choonuta,Yunua T.,Paichida N.,Klomkloab S., Sangkharaka K: Biodiesel Production Using Lipase From Oil Palm Fruit As A Catalyst: Energy Procedia.2015; 822 – 826
9. Kotogán A.,Németh B., Vágvölgyi C., PappT., Miklós Takó M.:Screening For Extracellular Lipase Enzymes With Transesterification Capacity In Mucoromycotina Strains.: Food Technol. Biotechno.2014; 52 (1) 73–82.
10. Muhannad I. Massadeh, Fatima M. Sabra:Production And Characterization Of Lipase From Bacillus

Stearothermophilus: African Journal Of Biotechnology Vol.2011; 10(61), Pp. 13139-13146
11. N. Saifuddin, A Z. Raziah, H. Nor Farah: Production Of Biodiesel From High Acid Valuewaste Cooking Oil Using An Optimized Lipaseenzyme/Acid-Catalyzed Hybrid Processe; Journal Of Chemistry. 2009;6(S1), S485-S495
12. Natalello A., Ami D,Brocca C., Lotti M., Silvia M. Doglia S:Secondary Structure, Conformational Stability And Glycosylation Of A Recombinant Candida Rugosa Lipase Studied By Fourier-Transform Infrared Spectroscopy: Biochem. J.2005; 385, 511–517
13. Panda1 M.,Sahul M., Kumanandatayung :Isolation And Characterization Of A Thermophilic Bacillus Sp. With Protease Activity Isolated From Hot Spring Of Tarabalo, Odisha:Iranian Journal of Microbiology. 2013; Volume 5 Number 2, 159-165
14. Pimpliskar M.,Vanmali H.,Jadhav R., Menon S: Detection of Industrially Important Thermostable Enzyme of thermophiles Isolated from Hot Water Spring at Vajreshwari,T…: Asian Journalof Microbiology. 2015;Vol. 17,No.(1),205-207
15. Rabelo S, Ferraz V., S. Oliveira L. Franca A.: FTIR Analysis for Quantification of Fatty Acid Methyl Esters in Biodiesel Produced by Microwave-Assisted Transesterification: International Journal of Environmental Science and Development.2015; Vol. 6, No. 12
16. Shahinyan G,Margaryan A.,Panosyanm A.,Trchounian A :Isolation And Characterization Of Lipase-Producing Thermophilic Bacilli From Geothermal Springs In Armenia And Nagorno-Karabakhbiolog: Journal Of Armenia 2015; 2 (67)
17. Shapaval1 V., Afseth N., Vogt G., Kohler A.: Fourier Transform Infrared Spectroscopy For The Prediction Of Fatty Acid Profiles In Mucor Fungi Grown In Media With Different Carbon Sources: Microbial Cell Factories. 2014; 13:86
18. Shih-Yi Sheu, Yi-Ling Chen, Wen-Ming Chen: Sphingomonas Fonticola Sp. Nov., Isolated From Spring Wate: International Journal Of Systematic And Evolutionary Microbiology.2015; 65, 4495–4502
19. Tripathia R.,Singha J.,Bhartia R.,Thakura S.:Isolation, Purification And Characterization Of Lipase From Microbacterium Sp. And Its Application In Biodiesel Production :Energy Procedia.2014; 54,518 – 529
20. Willsey G., Wargov M.: Extracellular Lipase And Protease Production From A Model Drinking Water Bacterial community Is Functionally Robust To Absence Of Individual Members: Bacterial Community Exoenzyme Production.November23,2015
21. Youssef M, Mohammed A. Al-Omair, Hassan E. Abd-Elsalam: Isolation And Identification Two Thermophilic And Lipolytic Bacterial Strains From Saudi Arabia Environmentinternational: Journal Of Advanced Research.2014; Volume 2, Issue 9, 217-228.
22. Zouaoui B.,Bouziane B.: Isolation, Optimisation And Purification Of Lipase Production By Pseudomonas aeruginosa isolation, Optimisation And Purification Of Lipase Production By Pseudomonas aeruginosa: Zouaoui and Bouziane. J Biotechnol Biomaterial.2011

Source of Support: None Declared
Conflict of Interest: None Declared