ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 08, pp.36723-36726, August, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ISOLATION, CHARACTERIZATION AND PRODUCTION OF LIPASE PRODUCING BACTERIAL STRAINS FROM PETROL SPILLED SOIL

[1]Bharathi D., [2,*]Rajalakshmi G., [3]Komathi S., [1]Mythili S., [1]Queen J and [1]Sithara N. V.

[1]Research Scholars, PG and Research Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore 641028

[2]Head of the Department, PG and Research Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore -641028

[3]Assistant Professor, PG and Research Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore -641028

ARTICLE INFO

Article History:
Received 03rd May, 2016
Received in revised form
24th June, 2016
Accepted 17th July, 2016
Published online 31st August, 2016

Key words:

Lipase, Petrol Spilled Soil, Tributyrin Agar, Carbon Source, Nitrogen Source.

ABSTRACT

Lipase is an industrially important enzyme and has a wide range of applications in dairy, food, detergent and pharmaceutical industries. Lipases are highly produced from microbes and specifically, bacterial lipases play a vital role in commercial importance. The present investigation deals with the production of lipase from petrol spilled soil sample. The sample was collected from petrol bunk situated in Coimbatore, followed by serial dilution and subsequently spread plated on nutrient agar plates. 8 colonies were selected and characterized based on morphological and biochemical test. The selected colonies were streaked on Tributyrin agar plates. From the 8 isolates, 5 bacterial isolates that showed clear zone after incubation were selected as lipase producing strain. Selected bacterial isolates were then optimized for maximum yield of enzyme under various parameters like carbon, nitrogen sources, pH and temperature on production media. Lipase production was increased with sucrose and lactose as a carbon source and yeast extract as a nitrogen source. Effect of pH and temperature indicated that, the lipase production was maximum in pH 6.0 and at 37°C.

Citation: Bharathi, D., Rajalakshmi, G., Komathi, S., Mythili P.S., Queen, J. and Sithara, N.V., 2016. "Isolation, characterization and production of lipase producing bacterial strains from petrol spilled soil", *International Journal of Current Research*, 8, (08), 36723-36726.

INTRODUCTION

Lipases are glycerol ester hydrolyses that act on acylglycerols to liberate fatty acids and glycerol. Lipases can hydrolyze long chain water soluble triglycerides into diglycerides, monoglycerides, glycerol and fatty acids (Veerapagu *et al*., 2013). Lipases are ubiquitous enzymes which are widely distributed in plants, animals and microbes (Larbi *et al*., 2014). Microbial production of lipase gained more importance, compared to all other production via, plants and animals. Microbial enzymes are also more stable than their corresponding plant and animal enzyme and their production is more convenient and safer. Many microorganisms are known as potential producer of extracellular lipase, including bacteria, yeast, and fungi (Sirisha *et al*., 2010, Udgire *et al*., 2015 and Sasmita *et al*., 2012).

Corresponding author: Rajalakshmi G.
Head of the Department, PG and Research Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore -641028.

Microbial lipases are mostly extracellular and their production is greatly influenced by media composition such as carbon and nitrogen source besides physicochemical factors like pH, temperature (Vijay *et al*., 2005). Lipase enzymes are generally produced in the presence of lipids such as olive oil, coconut oil or any other inducer such as tween 20/80, glycerol, fatty acids (Ashokkumar *et al*., 2011 and Patcha *et al*., 2013). The present study was on, isolation of lipase producing microorganisms from petrol spilled soil sample. Isolates were screened by Tributyrin agar plate assay and optimization of the pH, temperature, carbon and nitrogen source done for obtaining higher lipase production.

MATERIALS AND METHODS

Sample collection

Sample was collected from petrol spilled soil from petrol bunk situated in Coimbatore, Tamilnadu, India.

The soil was collected in sterile plastic sampling container and labelled properly.

Isolation of bacterial strains

Serial dilution techniques were used for the isolation of bacteria. In this technique, sample suspension was prepared by adding 10 g of soil with 100 ml of double distilled water (the stock) and shaken vigorously for at least 2-3 minutes. The dilute was then sedimented for a short period. Sterile dilution blanks were marked sequentially, starting from stock and10^{-2} to 10^{-4}. 1 ml from the stock was transferred to the 10^{-2} dilution blank using a fresh sterile pipette.

One ml from the 10^{-2} dilution was transferred to the 10^{-3} tube for each succeeding steps then from 10^{-3} to the 10^{-4}, then from 10^{-4} to the 10^{-5}. From each dilution tube 0.1 ml of dilution fluid was transferred into Nutrient Agar culture plate and incubated at 37°C for 24 hours. Nutrient Agar (NA) culture media contained 0.5% peptone, 0.3% yeast extract, 0.5% NaCl, 0.25% beef extract, 1.5% agar, 100 ml distilled water and pH was adjusted to 6 at room temperature. After successful growth, microorganisms were identified on the basis of colony morphology and named as SP1, SP2, SP3, SP4, SP5, SP6, SP7, SP8 and the pure cultures of isolates were sub-cultured in nutrient agar slants, incubated at 37°C to achieve vigorous growth.

Morphological characterization

Gram staining was performed to observe the cellular morphology and gram nature of the isolated 8 bacterial strains. Motility test was performed to check if the organisms were motile or not (Jahir *et al*., 2011and Gupta *et al.,* 2004).

Biochemical characterization

The 8 isolates were characterized by biochemical tests viz. IMViC reactions i.e. Indole production test, Methyl Red test, Voges Proskauer test, Citrate utilization test, Nitrate reduction test, Carbohydrate fermentation test, Urease test, Oxidase test, Catalase test, Hydrogen sulphide test and triple sugar ion test by standard method (Sherman, 2005 and Holt *et al*., 1994).

Characterization of lipase producing strains

From the above 8 isolates, lipase producing bacterial strains were characterized on the basis of tributyrin agar plate assay method. The composition of the tributyrin medium was yeast extract 3%, sucrose 3%, KH_2PO_4 0.5%, $CaSO_4$ 0.1%, $MgSO_4$.7H2O 0.1%, olive oil 1.0(%v/v), pH of the medium adjusted to 6-7. Isolated 8 cultures were grown on the tributyrin agar plate at 37°C for 24 hours to observe zone.

Optimization of lipase producing media

Effect of Carbon supplements on lipase production

Production media is supplemented with different carbon supplements like sucrose, glucose, lactose at different concentration of 1%, 2%, 3%, 4%, 5% (w/w). Lipase activity was measured by using photoelectric colorimeter at 610 nm.

Effect of Nitrogen supplements on lipase production

Production media is supplemented with different nitrogen supplements such as peptone, yeast extract, and ammonium sulphate at different concentration of 1%, 2%, 3%, 4%, 5% (w/w). Effect on nitrogen on lipase activity was measured by using photoelectric colorimeter at 610 nm.

Effect of pH on lipase production

To study the effect of pH, the lipase activity was measured at various pH ranging from 6 to 9. The pH was varied using different buffers. Lipase activity was measured by using photoelectric colorimeter at 610 nm.

Effect of Incubation temperature on lipase production

For selection of optimum temperature for the production of lipase the temperatures varying from 25°C, 37°C, 40°C. Lipase activity was measured by using photoelectric colorimeter at 610 nm.

RESULT AND DISSCUSSION

Isolation and Screening

The results of physiological and biochemical activities of all the 8 cultures isolated from petrol spilled soil are tabulated (Table 1) It was observed that 5 isolates were gram positive and 3 isolates were gram negative. It was reported that, 3 gram positive and 1 gram negative bacterial strains were isolated from oil contamination sites (Pallavi *et al*., 2015). Among the 8 bacterial isolates, 5 potential strains produced clear zone in tributyrin agar medium. This zone indicates these 5 bacterial isolates (SP1, SP3, SP5, SP7 and SP8) are able to produce lipase. These 5 bacterial strains were used for further optimization studies.

Optimization of Physiological and Cultural Conditions for Maximum Enzyme Production

Effect of carbon source on nutrient medium

Of the different carbon sources used, SP1, SP7, and SP8 showed maximum lipase production in the medium supplemented with sucrose followed by SP3 and SP5 showing maximum production in the medium supplemented with lactose. Less enzyme production was recorded in the medium supplemented with glucose. It has been reported that maximum lipase production can be obtained when lactose and maltose are used carbon source (Veerapagu *et al*., 2013)

Effect of nitrogen source on nutrient medium

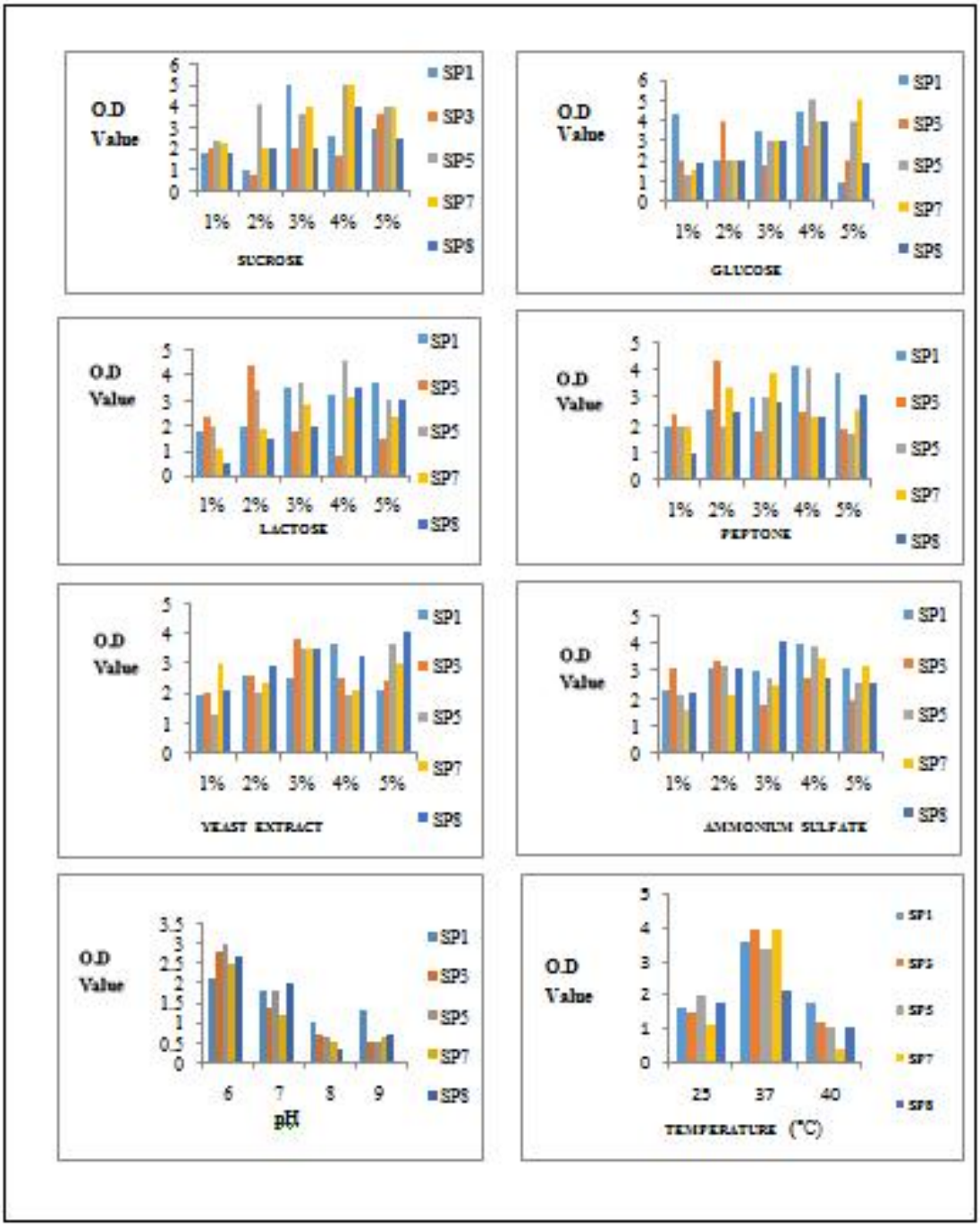
Different sources of nitrogen were tested in order to determine their influence on the production of lipase.

All 5 bacterial isolates showed maximum production in yeast extract (3%) in culture medium comparing to peptone and ammonium sulphate.

Table 1. Morphological and Biochemical characterization of bacterial isolates from petrol spilled soil

Parameters	Observation							
	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8
Gram staining	+	+	+	+	-	-	+	-
Morphology	rod	cocci	cocci	cocci	rod	rod	rod	rod
Motility test	-	+	+	-	-	+	+	+
Indole production test	-	+	+	+	+	-	-	+
MR test	-	+	+	+	+	-	-	+
VP test	-	+	+	-	-	-	+	+
Citrate utilization test	+	+	-	+	-	+	+	-
Carbohydrate fermentation test	+	-	-	+	-	-	-	+
TSI test	-	-	-	-	+	+	+	-
Oxidase test	+	-	+	-	-	-	+	+
Urease test	-	+	-	-	-	+	-	-
Catalase test	+	-	+	-	+	-	+	+
Nitrate reduction test	-	+	-	-	-	-	-	+
Hydrogen sulphide test	-	+	+	+	+	+	-	+

MR= Methyl Red, VP= Voges Proskauer, TSI= Triple Sugar Iron. + = Positive, - = negative.



Graph 1. Optimization techniques

Similar results were obtained for yeast extract (1%) as a nitrogen source for bacterial strains isolated from industrial rejection of gas station (Larbi *et al*., 2014)

Effect of pH on nutrient medium

Lipase production of 5 bacterial strains was observed between pH 6.0-9.0. The results suggested that there was a stimulation of enzyme production at a pH range of 6.0. So, considering this result lipase has high production at acidic pH in culture medium. It was reported that maximum lipase production was achieved at pH 7.2 and 8 (Sumathy *et al*., 2012 and Bhavani *et al*., 2012)

Effect of temperature on nutrient medium

Studies conducted for temperature shows that the bacteria produces lipase in wide range of temperature from 25°C, 37°C, and 40°C. Bacterial isolates S8 showed maximum production at 25°C, other isolates have limited enzyme production at 25°C. Isolates SP1, SP3, SP5, SP7 showed high production in optimum temperature at 37°C, and the enzyme production was affected and decreased after increase of temperature at 40°C. It was obtained maximum enzyme production was at 36°C for bacterial strains isolated from oil contaminated soils (Lakshminarasu *et al*., 2010)

Conclusion

The present study indicates that the various factors influenced commercially important lipase enzyme production. The temperature 37°C, pH 6, sucrose and lactose as carbon supplement and yeast extract as nitrogen supplement were found to maximize lipase production. Further techniques like molecular characterization of bacterial strains, purification and characterization of lipase enzyme are under study.

REFERENCES

Ashok Kumar, Varun Bhushan, Shikha Verma, Gaurav Srivastav and Sushil Kumar, 2011. Isolation and characterization of microorganisms responsible for different types of food spoilages, *International Journal of Research in Pure and Applied Microbiology*, 1(2): 22-31.

Bhavani, M., Chowdary, G.V., David, M. and Archana, G. 2012. Screening, isolation and biochemical characterization of novel lipase producing bacteria from soil samples, *International Journal of Biological Engineering*, 2012, 2(2), 18-22.

Gupta, R., Gupta, N. and Rathi, P. 2004. Bacterial lipases: an overview of production, purification and biochemical properties, *Application in Microbiology and Biotechnology*, 64: 763–781.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and Willians, S.T. 1994. Bergeys manual of determinative bacteriology, Williams and Wilkins, Baltimore. pp.787.

Jahir Alam Khan and Syed Hasan Abbas Rizvi, Isolation and characterization of micro-organism from oil contaminated site, Advances in Applied Science Research, 2011, 2(3):455-460.

Lakshminarasu, M., Sirisha, E., Rajasekar, N. 2010. Isolation and optimization of lipase producing bacteria from oil contaminated soils, *Advance in Biological Research*, 4(5), 249-252.

Larbi Daouadji, K., Benattouche, Z. and Abbouni, B. 2014. screening selection identification and optimization of bacterial lipase isolated from industrial rejection of gas station, *Journal of Chemical and Pharmaceutical Research*, 6(6): 455-459.

Pallavi, P., Ravikumar, T. and Ram Reddy, S. 2015. Isolation and characterization of lipase producing bacteria for biodegradation of oil contamination, *International Journal of Current Research and Academic Review,* 3(8), 56-66.

Patcha Boonmahome, Lipase-Producing Bacterium and its Enzyme Characterization, Journal of Life Sciences and Technologies, 2013, 1(4), 196-200.

Sasmita Sabat, V., Krishna Murthy, Pavithra M., Pala Mayur and Anitha Chandava, 2012. Production and characterisation of extracellular lipase from *Bacillus stearothermophilus* MTCC 37 under different fermentation conditions, *Journal of Engineering Research and Applications,* 2(3), 1775-1781.

Sherman, N., Cappuccino, J.G. 2005 Microbiology: a laboratory manual. Sixth edition, 81(3):265-267.

Sirisha, E., Rajasekar, N. and Lakshmi narasu, M. 2010. Isolation and optimization of lipase producing bacteria from oil contaminated soil, *Advance Biological Research*, 4(5); 249-252.

Sumathy, R., Vijayalakshmi, M., Deecaraman, M. 2012. Studies on lipase production from fungal strains by different inducers at varied concentration- A comparative study, *International Journal of Environmental Science*, 3(3), 1072-1078.

Udgire, M., Vivek, S. and Sinha, S. 2015. Isolation of thermo stable extracellular alkaline protease and lipase producing bacteria from tannery effluents, *International journal of Biotech Trends and Technology,* 10(2),9-13.

Veerapagu, M., Sankaranarayanan, A., Ponmurugan, K., Jeya, K.R. 2013. Screening selection identification production and optimization of bacterial lipase from spilled soil, Asian *Journal of Pharmaceutical and Clinical Research,* 6(3), 62-66.

Vijay Gunasekaran and debabtra Das, 2005. Lipase fermentation and progress, *Indian Journal of Biotechnology,* 4, 437-445.

****