International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 10, Issue 4 -2023

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2023.10.04.008

Isolation and identification of lipase producing bacterial strain from grey wastewater for grey wastewater treatment

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Abstract

Microbial treatment of wastewater is thus an effective way to degrade organic compounds. Microbial lipases have already established their vast potential regarding usage in numerous applications. Specifically, they are employed in waste water treatment, pharmaceutical, dairy, leather, detergent of and medical industries. The objective of this study was to isolate and characterizing lipase producing bacterial strain from grey wastewater and developing high potential lipase producing bacterial strain for industrial as well as for sewerage system. In the present study bacterial cultures were isolated from Cafeteria, Restaurants, Schools and Garage settling pits and were screened for Lipase production and activity. These bacterial species were grown in production Tween 80 media and the lipase enzyme produced was estimated. After optimization of factors like pH and salt concentration a maximum lipase enzyme activity was recorded. Twenties bacterial species were found to have lipolytic ability. Out of the 20 isolates 12 were Gram-negative bacteria and 8 were Gram-positive and genus *Pseudomonas Acinetobacter, Enterobacter, Aeromonasus* and *Burkholderia* were identified by biochemical assy. Of the isolates the *pseudomonas spp* were highly effective for lipase enzyme production in 8& 10% saline concentration and *Bacillus spp*, are highly effective for lipase enzyme production at alkaline condition. The value of oil contents of untreated sample collected initially from the kitchen wastewater was cafeterias, restaurants, schools and garage settling pit 10,12,9, and 20 mg L-1 which decline to 0,1,1,5 mg L-1 respectively after 48 hours incubation of bacteria. The oil is partially and completely degraded by bacteria isolates mixture. That means the bacteria isolates are effective for wastewater treatments.

Keywords: wastewater, lipase producing bacteria, lipase assay, oil contents

1. Introduction

1.1. Background of the Study

World population is at the verge of water shortage which demands proper management of water resources to meet requirements of rapidly growing population. Collection and reuse of wastewater has never been a priority in past but the concept is much more popularized in current scenario (Volkman, 2003; Abdel-Halim *et al*., 2008).

Grey wastewater is defined as wastewater without any input from toilets, which means that it corresponds to wastewater produced in bathtubs, showers, hand basins, laundry machines and kitchen sinks, in households, office buildings, schools, etc. (Jefferson *et al*., 2004; Wididana, 1995). Grey wastewater is less polluted than municipal wastewater in the absence of faces, urine and toilet paper

There is an increasing interest in the reuse of wastewater in many parts of the world, including both industrial and developing countries. One reason is water shortage, caused by too low amounts of rainfall in combination with high evaporation or too large demands of freshwater from the population. On the other side in some countries, the driving force for reuse of wastewater is environmental and economic considerations.

A low cost treatment system to reuse wastewater in agriculture sector needs pre-treatment in Health aspects, mainly micro-organisms, and environmental perspectives. Use of grey wastewater for urinal and toilet flushing is one of the possibilities since the water that is used for toilet flushing required to be developed and evaluated (Abdel-Halim *et al*., 2008), because proper recycling and use of wastewater may aid in meeting water shortages.

Microbial treatment of wastewater is thus an effective way to degrade organic compounds (Ronald and Richard, 1981). Microbial lipases have already established their vast potential regarding usage in numerous applications. Specifically, they are employed in waste water treatment (degreasing of lipid clogged drains), pharmaceutical (resolution of racemic mixtures), dairy (hydrolysis of milk, fat), leather (removal of lipids from hides and skin), detergent (removal of oil/fat stains) and medical (diagnostic tool in blood triglyceride assay) industries.

The large part of the earth's biomass is represented by lipids. Lipids are essential to all living systems. They are the most important source of energy, play structural roles in membranes and are involved in signaling events. To be able to carry out these functions, lipids require lipolytic enzymes during their metabolism. Lipolytic enzymes catalyze the turnover of this water-insoluble compounds (Gilham and Lehner 2005). They also breakdown lipids and make them mobile within the cells of individual organisms (Beisson, *et al..,* 2000).

Lipases can be produced from various sources such as animals, plants and microorganisms. However, for industrial applications, lipases from microorganisms are more interesting because (Yasuo S *etal..*, 2002), they can be produced in the high yields (Pogori N, *etal..*, 2007). There are many varieties of catalytic activities that can be used in many applications. (Sharma DB, etal.., 2011). The genetic manipulation is easily available (Hasan F, *etal*.,2006).

Lipolytic enzymes are grouped into 3 main categories, which are esterase, phospholipases and lipases (Arpigny and Jaeger 1999). Lipases (triacylglycerol acylhydrolases) are enzymes having a biological function of catalyzing the hydrolysis of triacylglycerols (Yasuo *et al*, 2002). Lipases can play an important role in the processing of g-linolenic acid, a polyunsaturated fatty acid; astaxanthin, a food colorant; methyl ketones, flavor molecules characteristic of blue cheese ; 4-hydroxydecanoic acid used as a precursor of g-decalactone, a fruit flavor; dicarboxylic acids for use as prepolymers; interesterification of cheaper glycerides to more

valuable forms (e.g., cocoa butter replacements for use in chocolate manufacture) (Undurraga D *et al*., 2001); modification of vegetable oils at position 2 of the triglyceride, to obtain fats similar to human milk fat for use in baby feeds; lipid esters, including isopropylmyristate, for use in cosmetics; and monoglycerides for use as emulsifiers in food and pharmaceutical applications.

Lipase producing bacterial strains is generally widespread in nature (Veerapagu M. *etal*., 2013).

Lipase producers have been isolated mainly from soil, or spoiled food material that contains vegetable oil. The present studies were carrying out with an objective to isolate and characterizing lipase producing bacterial strain from grey wastewater and developing high potential lipase producing bacterial strain for industrial as well as for sewerage system.

2. Materials and Methods

2.1. Description of the study area

The study was conducted at Debremarkose district, East Gojjam Zone, Amhara National Regional State, Ethiopia. It is situated 300 km west of Addis Ababa, 265km of east Bahir Dar. Geographically; it is located between 10°20′N latitude and 37°43’ E longitudes and at an altitude of 2446 meters above sea level (masl).

2.2. Samples techniques

45 samples of grey wastewater /kitchen wastewater were taken from different cafeterias, Restaurants, schools and Garages settling pit (untreated). From each level approximately 5 liters volume was collected with sterilized jars. Then the samples were transferred to the laboratory for parameters analyze and for isolation and characterization of lipase producing bacterial strain.

2.3. Lipolytic bacteria isolation

Samples were serially diluted with sterile distilled water and inoculated on Tributyrin Agar Plates (TBA) containing per litre of peptone, 5g; beef extract, 3g; tributyrin, 10ml and agar-agar, 20g and Penicillium G followed by incubation for 24-48 hours at 37 °C.

2.4. Screening of lipolytic isolates

Isolated bacterial strains were screened for their lipolytic activity on the basis of Tributyrin Agar Plates(TBA). Tributyrin agar media along with 1.0%(v/v) olive oil were prepared and sterilized at 121^0C for 15 min and then sterilized media were poured into petri plate. Isolated strains were streaked on the tributyrin agar plate and it was incubated at 37^0C for 24 hr to observe clear zone.

2.5. Biochemical characterization of the isolates

Identification of isolated lipolytic bacteria up to genus level will be performed with the help of morphological and different biochemical characteristics. Gram’s nature, bacterial colony characters, and motility of the organism be studied for morphological analysis while different tests like Indole test, Nitrate reduction test, Catalase test, Oxidase test along with carbohydrate / sugar fermentation were performed for analysis of biochemical characters of isolated lipolytic bacteria with the help of Bergey’s manual of Systematic Bacteriology.

2.6. Lipase assay

Extracellular Lipase activity was measured using polyoxyethylenesorbitan ester (Tween 80) as substrate by the method described by Tirunarayanan and Lundbeck with slight modifications. The reaction mixture contains 0.1ml of 10% Tween 80 in 50mM Tris hydrochloride buffer (pH 7.6), 0.5ml of concentrated culture supernatant as a source of enzyme, 0.1ml of 1M CaCl2 in oTris buffer, and 2.3ml of Tris buffer (pH 7.6) with optical density 400nm against enzyme blank .

2.7. Optimization of lipolytic bacteria at different saline solution concentration

The fermentation media were prepared with different saline (NaCl) concentration such as 2%, 4%, 8% and 10% inoculated by the bacterial cultures then incubate at 37° C for 24- 48 hours. Enzyme assay for each isolate was performed in triplicates and the optical density was measured against enzymes blank.

2.8. Effect of lipolytic bacteria against different pH values

In this methods, the reaction mixture contains different amount of pH values (4, 5, 6, 7, 8, 9 and 10) inoculated by the bacterial cultures. Then the culture and the media were incubated at temperatures i.e. 37° C for 24- 48 hours. Reaction mixture with 0.5ml of deionized water instead of supernatant was considered as a blank. Enzyme assay for each isolate was performed in triplicates. In this spectrophotometric assay, Tween was cleaved to produce fatty acid and alcohol. Presence of calcium in the reaction mixture leads to the formation of an insoluble fatty acid salt, giving a precipitate which can be measured spectrophotometrically at 400nm. One unit of Lipase activity was defined as the amount of enzyme resulted in an increase of optical density at 400nm (OD 400) of 0.01 after 2 hours under the assay conditions.

2.9. Analysis of oil contents

The quality of treated kitchen wastewater was analyzed by oil contents. Oil contents was determined by standard methods (5210, 5220 and 5520 respectively) as adopted from APHA (2005).

3. Results and Discussion

3.1. Selection of lipolytic microorganisms

Of the total of 45 samples bacterial isolates, 43 bacterial isolates were positive for lipase and showed lipolytic activity in the culture medium.

3.1.1. Screening of lipolytic bacteria

To get highly potential Lipase enzyme producing bacteria screening 43 isolated bacteria was done by streaking the isolates on Tributyrin Agar Plates (TBA with selected antibiotics (Penicillin G). Of the 43 bacterial isolates 20 bacterial isolates shows high clear zone of hydrolysis when they grow on the Tributyrin agar.

S/No	Sample ID	List of purified isolates	Total no of purified isolates
1	W3	W3-2	1
2	W4	W4-1, W4-3, W4-7	3
3	W5	W5-1-W5-4, W5-6	3
4	W6	W6-2, W6-3, W6-5, W6-7	4
5	W7	W7-1, W7-2, W7-3, W7-4	4
6	W8	W8-2, W8-3, W8-5, W8-9, W8-10,	5
Total no of purified isolates			20

Table:- 1. Screened bacterial isolates Codes.

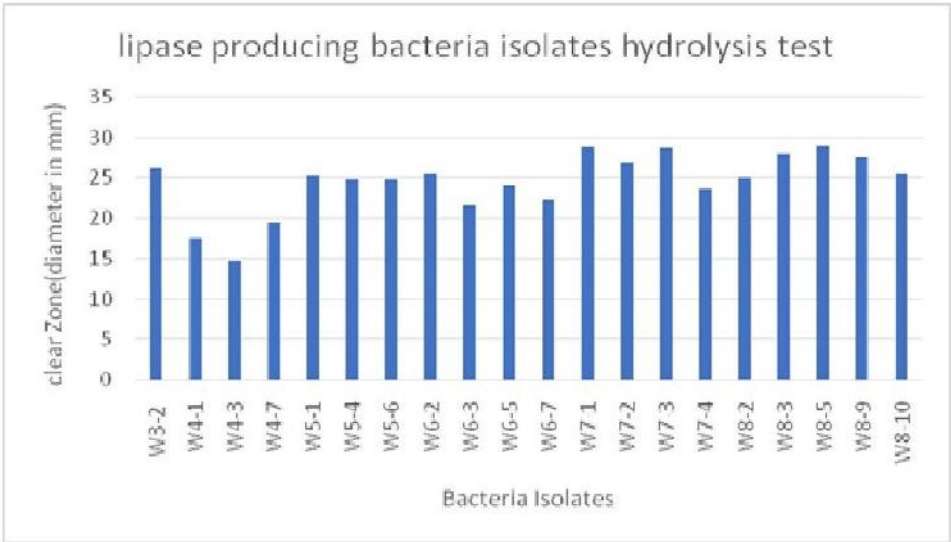

Figure 1:- Bacterial isolates of clear zone of hydrolysis on Tributyrin agar plates (TBA) with selected antibiotics (penicillin G).

The twenty selected isolates were subjected to rapid screening by using Tributyrin Agar Plates (TBA). The results were showed that all the twenty isolates exhibited high clear zones (14.8-28.8) mm with Tributyrin Agar Plates (TBA) in the figure (1), the clearly apparition of the zone of hydrolysis around colony were considered as important parameters for selection of lipase producer strains. The evaluation of the lipase efficiency based on the clear zones around colonies was showed that all of them could produce lipase.

3.1.2. Biochemical identification

Out of the 20 isolates 12 were Gram-negative bacteria and 8 were Gram-positive, being Twelve of them were identified as, three of the genus *Pseudomonas*,(W7-4,W4-7and W8-2) three of the genus *Acinetobacter (W4-3,W5-6,W7-1)*, four of the genus *Enterobacter (W6-2,W7-2,W7-3,W8-3)*, one of the genus *Aeromonas (W8-5)* and one of the genus *Burkholderia* (W8-9) which is in agreement with the research of several authors, which affirm that the majority of lipolytic bacteria found in nature are Gram-negative (Ramnath *et al.*, 2017 and Dharmsthiti and kuhansuntissak,1998). Out of eight Gram-positive, being three of them were identified as genius *Bacillus (W4-1, W5-4, W6-7)* and five of the genus *Staphylococci (W3-2, W5-1, W6-5, W6-3, W8-10)* as using biochemical assays. As (Ankit *etal.*, 2011) investigating lipolytic microorganisms for industrial use, isolated species of lipolytic bacteria from the genera *Pseudomonas* sp. and *Bacillus* sp. from highly contaminated water samples of several rivers in the Bhopal region of India. (Odeyemi etal., 2013) examining microorganisms capable of use palm oil as substrate, found 32 lipolytic bacteria grouped in *Enterococcus* sp., *Escherichia* sp., *Klebsiella* sp., *Pseudomonas* sp., *Serratia* sp. and *Staphylococcus* sp., isolated from a restaurant wastewater trap tank.

3.2. Lipase optimization result

The extracellular lipase enzyme was measured by spectroscopy by using tween 80 as a media with salt (2%,4%,6% and 8% concentration)

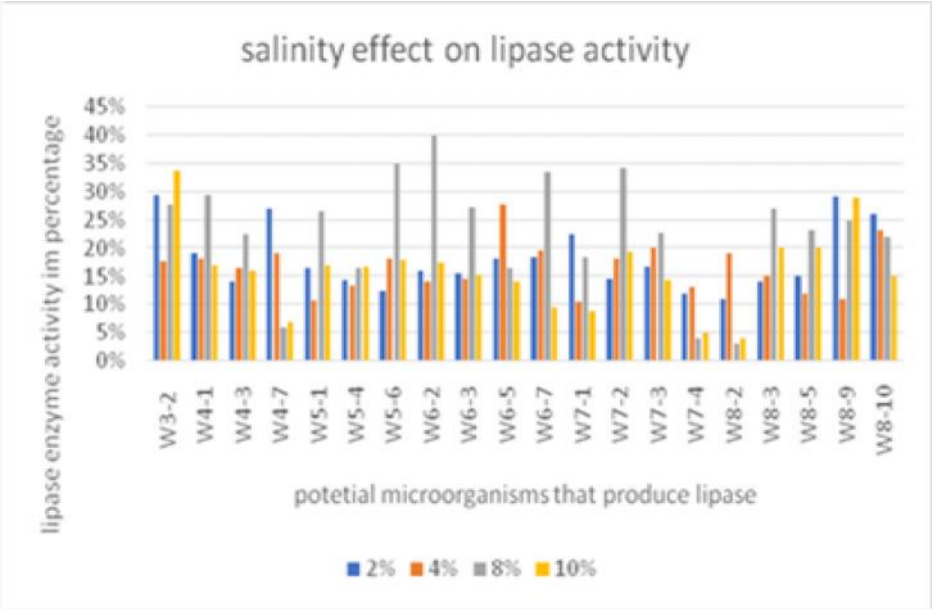

Figure 2:- Lipase enzyme activity at 2%, 4%, 8% and 10% NaCl salt concentration.

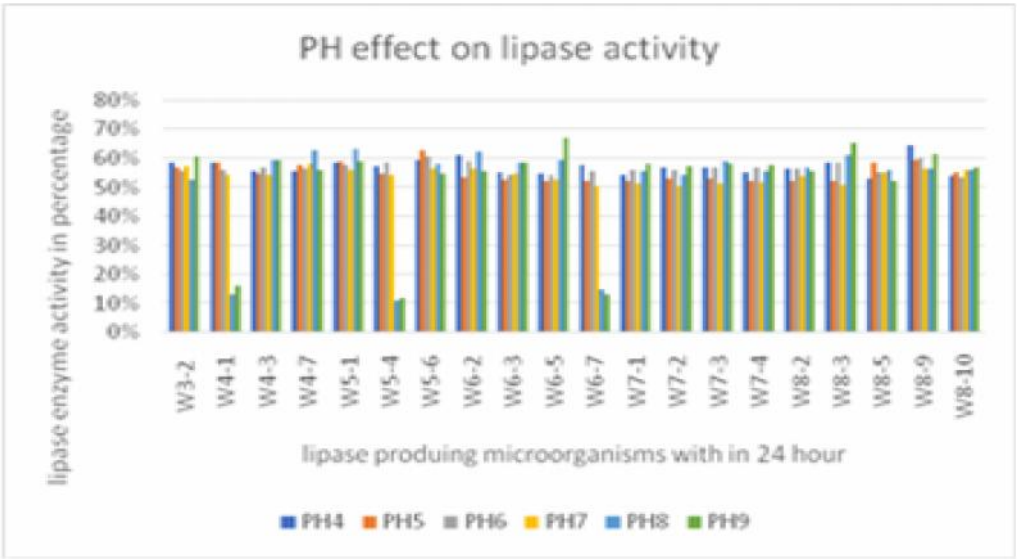

Figure 3:- Lipase enzyme activity at pH 4, 5, 6, 7, 8 and 9.

Environmental pressures are important factors for the expression of enzymes such as lipases by microorganisms. Highly contaminated environments, such as effluents with high fat and oil loads, have been shown to be suitable for the screening and isolation of bacterial species that produce these enzymes.

The optimization of lipase production was carried on the variation of saline and pH of fermentation medium. As figure 2 shows that the *pseudomonas spp* are highly effective for lipase enzyme production in at 8& 10% saline concentration. As figure 3 shows that *Bacillus spp*, has high lipase enzyme production in alkaline condition (pH 9 value). This result indicates the activity of lipase

at two condition is important for the wastewater treatment because the kitchen waste water is a full of salt and soap. So, the microorganism can multiply and produce high amount of lipase with in short period of time. This result is agreed with *Kumar S, etal.,* 2005 , Lipase activity 1.16 U of culture medium was obtained in 48 h at 55°C and pH 8.5 with refined mustard oil as carbon source and a combination of peptone and yeast extract (1:1) as nitrogen sources.

3.3. Oil contents analysis of kitchen wastewater

Oil contents Mean values for four different samples collected were arranged in descending order. Comparison of means significance decline in the kitchen wastewater and garage settling pits are by aerobic treatment of the bacteria. Fig. 4 shows that the value of oil contents of untreated sample collected initially from the kitchen wastewater was cafeterias, restaurants, schools and garage settling pit 10, 12, 9, and 20 mg L-1 which decline to 0, 1, 1,5 mg L-1 respectively after 48 hours incubation of bacteria. Oil includes the accumulation of oils, fats, cellulose, starch, proteins, and wax. The results showed that the bacterial activity completely and partially removed oil contents from the kitchen wastewater and made it perfect for the irrigation purpose.

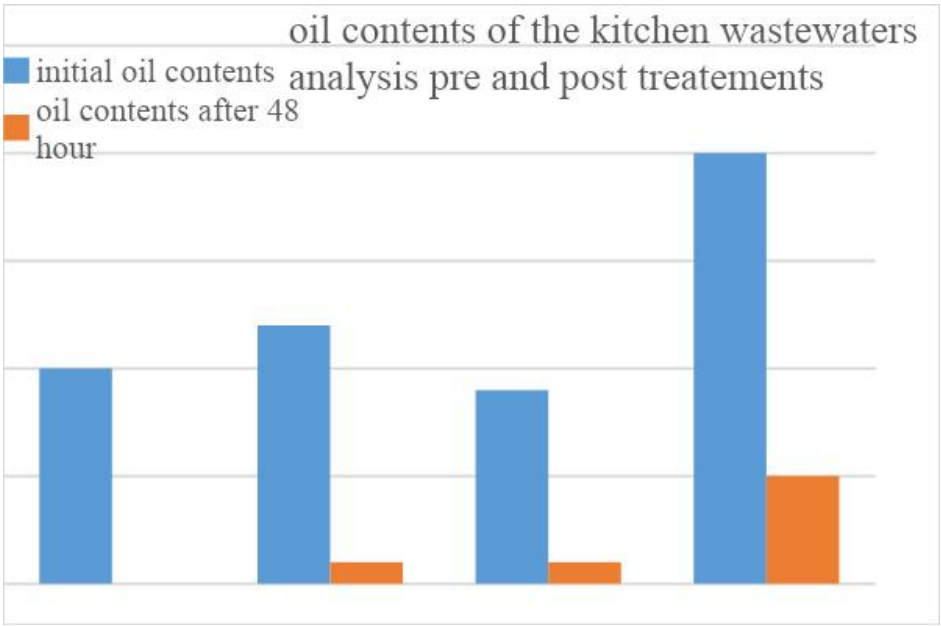

Figure 5 oil concentration of the waste water before and after treatment.

4. Conclusion

The waste water is a reservoir of a huge and diverse microbial population, which is considered a rich source of many types of microbial strains which can afford a particular group of microbial strains necessary for the degradation of different contaminants thrown into the waste water. Hence the waste water samples may be used to isolate the novel strains that can be used as a part of the microbial collection for the production of lipase at research labs and industries. All the bacterial isolates as *pseudomonas spp, Bacillus spp, Aeromonas spp Acinetobacter spp, Staphylococcus spp, Enterobacter spp, and Burkholderia spp,* were isolated by biochemical assay. To study lipase producing activity Tween 80 agars is used as media. The oil content of the waste water is decrease after treating by lipase producing microorganisms. Our present study provides useful information for the optimization of culture conditions such as pH and saline concentration to provide the best lipase production.

The stability of the enzymes at basic pH and at high saline concentration makes them excellent candidates for application in the treatment of wastewater. These results show that lipase producing bacteria are widespread in oil contaminated wastewaters.

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DOI:10.22192/ijarbs.2023.10.04.008	

How to cite this article:
Rahel Gebeyehu, Adebabay Tazeb, Miraf Mekonnen. (2023). Isolation and identification of lipase producing bacterial strain from grey wastewater for grey wastewater treatment. Int. J. Adv. Res. Biol. Sci. 10(4): 103-111.
DOI: http://dx.doi.org/10.22192/ijarbs.2023.10.04.008