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ORIGINAL RESEARCH ARTICLE

Comparative Study of the Lipase Enzyme Extracted From the Bacterial Isolates in Lipid Enriched Soil of Kathmandu Valley in Nepal

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ABSTRACT

The lipid and fatty acids containing waste being mixed into the soil is a major problem worldwide .The main aim of this research work is to isolate the microorganisms which survive and grow in the lipid rich soil of slaughter house, garage and oil mills. Characterization and identification of isolates was done according to Bergey's manual of systemic bacteriology 1984 Vol 1 and 1986 Vol 2 and the lipase enzyme production and lipolytic activity was studied..The isolated organism was grown on spirit blue agar media supplemented with Tween 80 .Lipase production media supplemented with Tween 80 was used for lipase production which was indicated by increase in turbidity of the media. The crude extract was obtained and effect of different pH on lipase activity was also studied .A growth curve in relation to lipase activity was established .A crude extract of lipase enzyme showed zone of hydrolysis(measured in mm).

Key words: Lipase enzyme ,Microorganisms**(** *Acinetobacterium calcoaceticum* **,** *Pseudomonas aeruginosa* ,*Bacillus subtilis* , *Staphylococcus aureus),* Spirit Blue Agar Media, Tween 80.

INTRODUCTION

Triacyl glycerol hydrolases also known as lipase are the enzyme that catalyses the hydrolysis of long chain acyl glycerols in aqueous emulsions (Lee *et al* 2003). Lipase enzyme also catalyzes transesterification reaction, inter esterification and esterification between a fatty acid and alcohol which is the reverse reaction of hydrolysis (Macrae 1983) [1]. They are generally extracellular enzymes which are secreted into the medium in massive amounts by many microbial species including bacteria, fungi, actinomycetes and yeasts.

Over two billion tons of petroleum is produced annually worldwide. Environmental contamination with petroleum introduces a myriad of hydrocarbons, causing a variety of problems (Atlas and Philip, 2005) [2].

Slaughterhouse lipid waste, tallow, is a fat-rich material and has been used as raw material for the production of low value products like soaps and detergents [3].

Uncontrolled releases of petroleum compounds that are carcinogenic, mutagenic and are potent immunotoxicants into soil and groundwater poses a serious threat to human and animal health. Biodegradation of hydrocarbon-contaminated soils has been established as an efficient, economic, versatile and environmentally sound treatment [4].

Microorganisms or their enzymes are used in a wide range of biotechnological activities such as polymer hydrolysis, synthesis of value-added compounds, decontamination of soils etc.. Microbial enzymes is one of the most important fields of research because enzyme-catalyzed reactions are highly efficient and selective, are less polluting, and usually require mild conditions and less energy, which leads to the lowering of costs. Thus, there is an increasing interest for isolating new enzymes and new enzyme-producing strains for their use in industrial conversions. Among these enzymes, lipases play an important role in many biotechnological processes [6].

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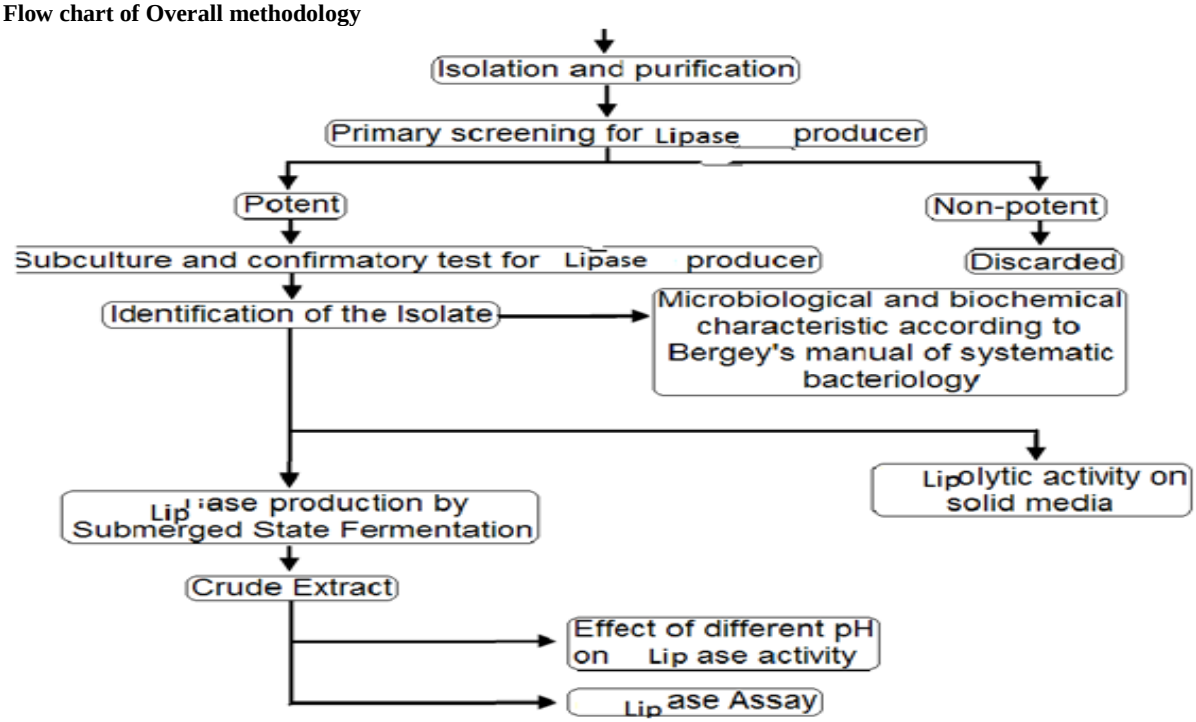
METHODOLOGY

Fig 1: Flow chart of Overall methodology



RESULTS

5 samples were preceded for the isolation of lipolytic bacteria. Among 5 samples, each sample were taken from Garrage (Maitidevi), Bhaktpur Oil Mill (Bhaktpur), Kalimati Oil Mill (Kalimati), Fish supplier (New Road) and Slaughter house, Chabhil. Among these 5 bacterial samples 4 bacterial species were isolated as different species and taken for further tests and production and were given the code number. Different characteristics and cultural conditions of isolated lipolytic bacteria were compared with those bacteria outlined in Bergey's Manual of Systematic Bacteriology (1984) Vol. 1 and Vol. 2(1986).

Table 1: Characterization and identification of isolates

S.No	Characteristics	RKS1	RKS2	RkS3	RKS4
1	Gram stain	-ve	-ve	+ve	+ve
2	Spore	-ve	-ve	+ve	+ve
3	Motility	-ve	+(unipolar motility)	+ve	+ve
	Biochemical test				
4	Catalase	+ve	+ve	+ve	+ve
5	Oxidase	-ve	+ve	+ve	-ve
6	VP test	-ve	-ve	+ve	-ve
7	Citrate utilization	-ve	+ve	+ve	-ve
8	Indole	-ve	-ve	-ve	-ve
9	Tween 80 hydrolysis	+ve	+ve	+ve	+ve
10	Growth at pH				
	5.7	+	+	+++	+
	6.8	++	++	+++	+++
	8.0	+++	+++	+++	+++
	9.0	++	+++	+++	++
11	Growth at NaCl				
	5%	++	+	++	++
	7%	+++	+	++	++
	9%	-	++	++	+++

Table 2: List of identified lipolytic bacteria are shown in table below

S. No	Code Name	Identification
1	RKS1	*Acinetobacterium calcoaceticum*
2	RKS2	*Pseudomonas aeruginosa*
3	RKS3	*Bacillus subtilis*
4	RKS4	*Staphylococcus aureus*

Growth curve in relation to lipase activity:

Various carbon sources are required for the growth and production of alkaline, acid and thermostable lipase. The best carbon source is olive oil, tween 80, tween 20 and sometimes glucose [5]. The best nitrogen source is yeast extract, trypon etc.

The media taken for the production of lipase was lipase production media that was supplemented with tween 80 and it was produced as 30°C. The lipase production was associated with bacterial growth. The growth curve of the organism is shown by the density of the media by the increase in turbidity.

From the result it was found that *Acinetobacterium calcoaceticum* produced maximum lipase among all isolated lipolytic bacteria. So this bacterial species was selected for detail study of their enzyme activities. The effects of pH were studied on their lipase activity.

Optimum pH for lipase activity:

pH affects enzymes strongly and the activity of an enzyme is determined in a large degree by the pH of the system in which it operates. The lipase activity of *Bacillus subtilis* was very good at low

pH i.e. at 5 which gradually decreased with increase in pH and was very less towards neutrality and again the activity greatly increased with the rise in pH and was maximum at pH 9. But *Acinetobacterium calcoaceticum* and *Staphylococcus aureus* showed low activity at low pH and the activity increased with increase in pH and the maximum activity was at pH 8 and 9 respectively.

Fig 2: Zone of hydrolysis at 30ºC at 3% tween-80

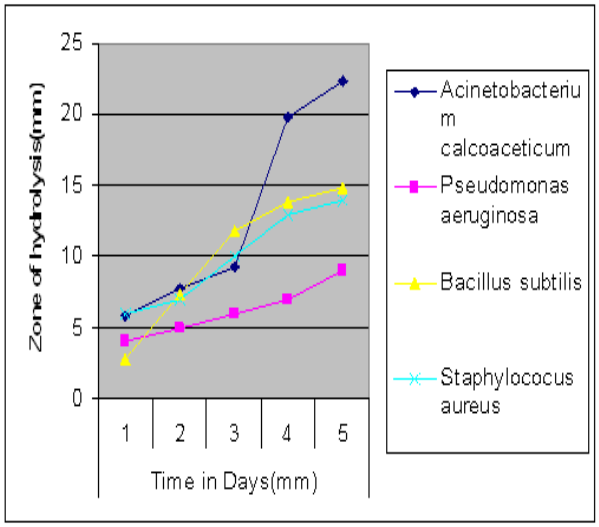


Fig 3: Effect of pH on lipase activity using 100µl crude lipase

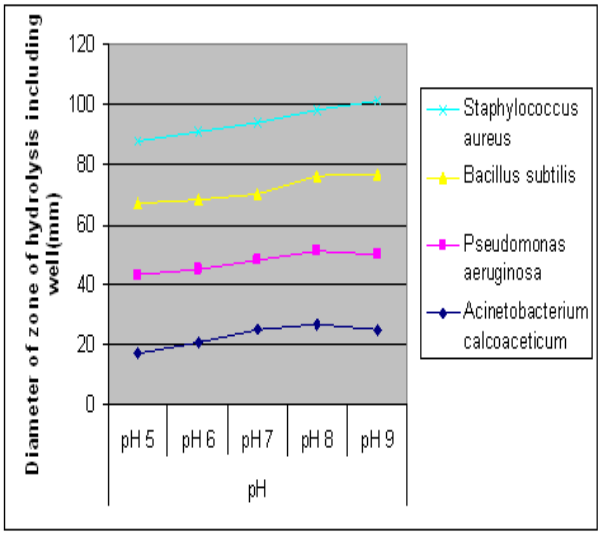
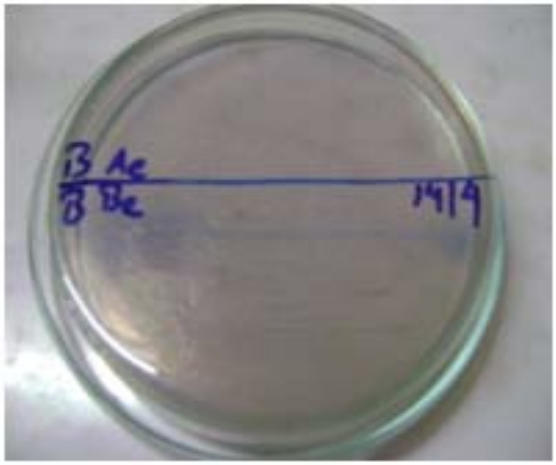


Fig 4: Screening of lipolytic bacteria



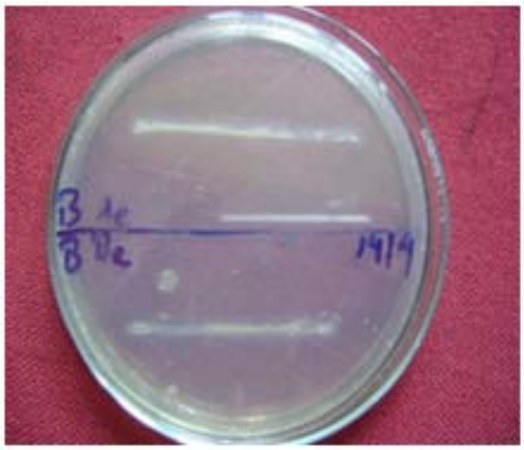


Fig 5: Zone of hydrolysis given by lipase extracted from *A. calcoaceticum*

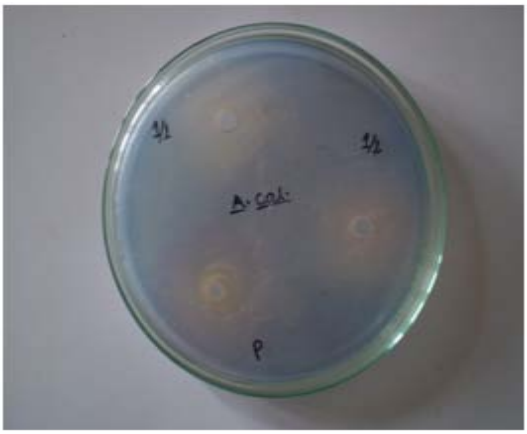
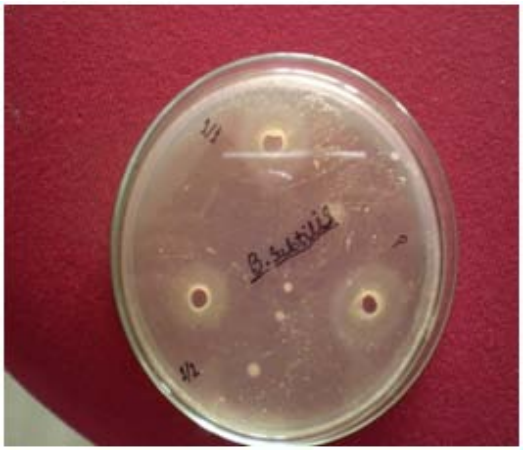


Fig 6: Zone of hydrolysis given by lipase extracted from *P. aeruginosa*

Fig 7: Zone of hydrolysis given by lipase extracted from *S.aureus*

Fig 8: Zone of hydrolysis given by lipase extracted from *B. subtilis*



DISCUSSION

Firstly the soil samples were collected from those areas where the availability of lipolytic bacteria was higher. Screening of the samples came with 16 isolates which were likely to be different species according to the morphological studies but the further biochemical test came with only 4 different species. The soil sample from Fish supplier was more likely to be the house of best lipolytic bacteria throughout our studies as 3 isolates out of 4 found there. So it was considered to be the best sample and the best lipolytic bacteria were *Acinetobacterium calcoaceticum* which came from all the sites except oil mill of Kalimati.

All the isolated lipolytic bacteria were grown on spirit blue agar medium supplemented with tween-80 for their ability to produce lipase at maximum. It was found that the isolated bacterial species hydrolysed tween-80 more rapidly. Spirit blue with tween-80 was found to be the suitable substrate for all bacteria to produce lipase. A special type of medium, lipase production medium was selected for lipase production. The optimum pH for the lipolytic bacteria was at pH 8-9. The lipolytic activity of bacteria was not directly proportional to its enzyme.The substrate has been found to affect the production of lipase. Nitrogen source plays an important role in growth of microorganisms. Different sources of nitrogen were added to the media to stimulate the lipase production as well as bacterial growth. Hydrolyzed proteins such as Bacto Tryptone enhance lipase production. Yeast extract was a good nitrogen source, because the additions of Yeast extract enhanced the production of lipase.

CONCLUSION

From soil samples of five different places with different geographical and climatic make up, four very good lipolytic bacteria were isolated showing tremendous production of lipase enzyme effective at different salt concentration and pH of different values from 5 to 9, which covers the condition of mostly all of the waste from the natural sources in Nepal which are probably used for all the washing and cleaning purposes in here. So, the lipase enzyme produced from the bacteria from our local resource is very beneficial at all the physiological and geographical conditions.

Recombinant lipase enzymes is used in applications such as baking, laundry detergents and even as biocatalysts in alternative energy strategies to convert vegetable oil into fuel. In addition to their role in synthetic organic chemistry, these also find extensive applications in chemical, pharmaceutical, and food industries. Microbial lipases have been widely used for biotechnological applications in fat, food ingredients, detergents, dairy and textile industries, production of surfactants, and oil processing. Promising fields for the application of lipases also include the biodegradation of plastics.

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