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**RESEARCH ARTICLE**

**SCREENING AND IDENTIFICATION OF *BACILLUS SPP.* LIPASE PRODUCING BACTERIA FROM
 LIPID CONTENT OF KITCHEN WASTES.**

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Abstract

Lipases are enzymes which have several applications in many industries like pharmaceuticals, leather, detergents, and soaps, textile, food, biofuel, etc. Many organisms like bacteria, fungi, and plants are eligible to produce lipase enzyme. The present study was aimed for isolating lipase producing bacteria from lipid containing kitchen waste as samples. As lipid samples are rich in lipid and fatty acid content it makes a very good source to find microbes capable of degrading lipids by producing lipase enzymes. These isolates were identified by morphological, and biochemical characterization. The maximum lipase activity by the *Bacillus cereus* and *Bacillus Megaterium* isolates were at pH 7 at 35° C for 24 hours.

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Introduction:-

An Indian city produces about 0.8 to 1 kg solid wastes per capita per day (waste management at military station, 2009). These wastes are collected and dumped into the landfills, causing major pollution (**Bouallagui, R. et al., 2003; Bouallagui, H., et al., 2005**). The biological treatment of these wastes appears to be most cost effective and carry a less negative environmental impact (**Coker, C., 2006**).

Lipase (EC 3.1.1.3) is an ester hydrolase, which catalyze the hydrolysis of triacylglycerol and production of glycerol and fatty acids (**Sharma, R., et al., 2001**). Lipases are ubiquitously enzymes which are found in animals, plants, fungi and bacteria. Microbial lipase had played a very crucial role than animal and plant lipases in enzymatic theoretical research as well as practical application, including hydrolysis, esterification, trans esterification, and ester chiral synthesis (**Alfonso, I and Gotor, V., (2004); Joseph, B., et al., 2008**). Bacterial enzymes are more preferred over fungal enzymes because of their higher activities and neutral or alkaline pH optima. In order to increase the cell yields and the enzymatic activities of the cells, genetic and environmental manipulations can be performed more readily on bacterial cells due to their short generation times, their simple nutritional needs and easy screening procedures for desired properties (**Hasan, F., et al., (2006)**).

Growth conditions affect the synthesis of lipase by microorganisms. Carbon and nitrogen sources, the presence of activators and inhibitors, incubation temperature, pH, inoculum amount and oxygen tension can influence lipase production (Gupta, R., et al., 2004). The aim of present study is to isolate and characterize bacterial strains from lipids containing kitchen waste by determining their ability to produce lipase enzymes and their efficacy of effective bacteria use in the degradation of kitchen waste.

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Materials and Methods:-

Sample collection:-

Lipids and oil content of kitchen waste were collected in plastic bags from Cafeteria house at near College of Life sciences, CHRI hill campus, Gwalior in Madhya Pradesh, India. The isolation of bacterial isolate was carried out by serially diluting the kitchen waste as samples in sterile water and subsequently plating on the Nutrient agar medium.

Isolation and screening of lipase producing bacterial isolates:-

In the present study, lipids contain kitchen waste as sample was collected from a Cafeteria nearby College of Life Sciences, CHRI, Hill campus, Gwalior in a sterile container for the isolation of lipase producing bacteria under laboratory condition. For the isolation of lipolytic bacteria, 1.0 gm of sample was dissolved in 100 ml of distilled water. It was then serially diluted (10^{-1} to 10^{-6}) and the diluted samples were inoculated on tributyrin agar for screening of lipase producing bacteria. And inoculated petriplates were incubated at 37°C for 24 to 48 hours and the formation of clear zone around the colonies on the plate was considered as lipolytic microbes.

Medium composition for lipase activities:-

Qualitative lipase activity assay was evaluated on Tributyrin agar medium which contained (per liter): as given below. The formation of a white precipitate around the colonies resulting from the deposition of crystals of the calcium salt formed by the fatty acid liberated by the enzyme, indicates positive lipolytic activity (7 mm or more) (Gopinath, S.C.B. *et al.*, 2005).

MM Composition	Quantity (Gm/Ltr)
Tributyrin	10 mL
Peptone	10 gm
CaCl ₂	0.1 gm
NaCl	5 gm
Agar powder	15 gm
pH	7-7.4
D/W	1000mL

The qualitative lipase assay was determined by Tributyrin agar medium (Harrigan, W.F., 1998).

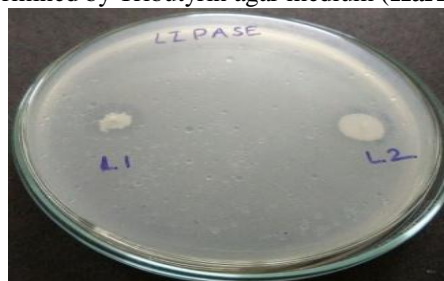


Fig 01:- Screening of Lipase producing bacterial isolates.

Identification of bacterial isolates by Morphological, physiological and biochemical characteristics

Bacterial identification was carried out using microbiological techniques like morphological and biochemical characterization as per Bergey's manual of determinative bacteriology ((Holt, J.G., *et al.*, 1994).

S. N.	Morphological, biochemical and physiological analyses of bacteria isolated from Lipids contain kitchen waste.		
	Characteristic	(L1)	(L2)
1	Gram's staining test	+ve	+ve
2	Morphology	Rods	Rods
3	Endospore	Central	Central
4	Oxidase	-	-
5	Catalase	+	+
6	Motility	+	+
7	Indole production	-	-
8	H ₂ S production	-	-

10	Citrate utilization	-	-
11	Gelatin hydrolysis	-	-
12	Lecithinase	+	-
13	Casein hydrolysis	+	+
14	Phenylalanine	-	-
15	Urease	+	-
16	Starch hydrolysis	+	+
17	Methyl red test	+	-
18	Voges Proskauer test	+	-
19	Nitrate reduction	+	_d
	Acid production from Glucose	+	+
21	Mannitol	-	+
24	Inositol	-	-
25	Lactose	-	+
27	Growth at 50 °C	-	-
28	Growth 7.5% (w/v) NaCl	-	-
29	Anaerobic growth	Facultative	-

+, positive reaction; -, negative reactions; d, dubious.

Fig-Table-01- Identification of lipase producing bacterial isolates by Morphological, physiological and biochemical characteristics.

Lipase assay:-

To determine the lipase activity on the basis of olive oil hydrolysis (Macedo, G.A., et al., 1997). The sample were mixed with 1.0ml of substrate containing 10.0 ml of 10% homogenized olive oil in 10% gum Arabic (Neem), 2ml of 0.6% of CaCl₂ solution and 5ml of 0.2mol (Alberghina, L., et al., 1991) citrate buffer, pH7.0. The enzyme substrate mixture was incubated on an orbital shaker at 100rpm at 37°C for 1hr. To stop the reaction mixture, liberated fatty acids were titrated with 0.1mol l⁻¹NaOH. The extracellular lipase enzyme activity was expressed as units (U) per ml of broth.

Results And Discussion:-

Isolation and Screening of lipase producing bacterial isolates:-

The isolation of bacterial isolate were carried out by serially diluting the volatile samples in sterile water and subsequently plating on the 1.5% Tributyrin agar base medium by using pour plate method. The plates were incubated at 37°C for 24-48hrs. And observe for zone formation (**Fig.-01**). A clear zone around the colonies indicates the production of lipase (**Cardenas, J., et al, 2001**). The lipase positive bacterial strains were further purified, grown in nutrient broth at 37°C for 24 hrs and screened for the ability to produce lipase with tributyrin, the bacterial isolates L1, and L2. During lipase activities, more lipase production by L1 bacterial strains than the L2 bacterial strain (**Fig.-02**). These bacteria was identified and further studies were carried out by morphological, physiological and biochemical characteristics (**Table-01**) of the potent lipolytic bacterial strains were determined according to the method described in 'Bergey's Manual of Determinative Bacteriology' (Holt, J.G., et al., 1994).

Table 02:- Bacterial Isolates Showing Zone of hydrolysis (Diameter in mm)

S.N.	Bacterial Isolates	Diameter of zone of hydrolysis (mm)
1.	L1 Bacterial Isolates	12
2.	L2 Bacterial Isolates	11

Enzyme assay of lipase producing bacterial isolates-

According to lipase activity of L1 and L2 bacterial strains are given at 24hrs of incubation at 37°C, the *Bacillus cereus* (L1) showed a maximum produced amount recorded as 55.0 U/ml, whereas the minimum production observed at 37°C, at 24hrs and the amount was recorded as 15.0 U/ml by *Bacillus megaterium* (Fig-02).

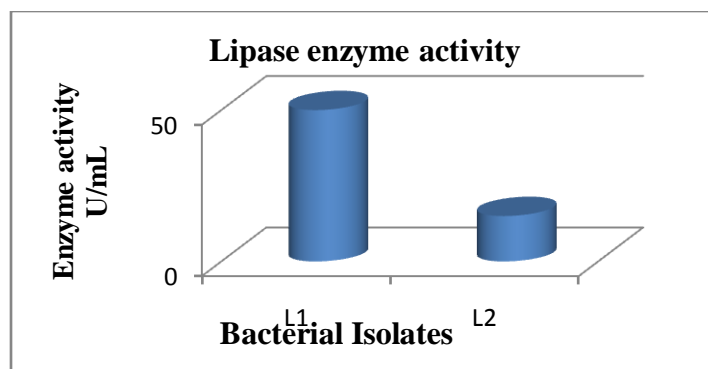


Fig 02:- Lipase enzyme activity assay of L1 and L2 strains by spectrophotometric method.

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