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Production of lipase from *Micrococcus flavus* and influence of bioparameters

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Abstract: *Lipase enzymes from microbial sources are currently enormous attention by potential industrial uses. The aim of the study is to investigate some bioparameters affecting lipase production by spectrophotometric method. Extracellular lipase producing organisms isolated from different places of 'ERNAKULAM DIST'(KERALA) and identified as Micrococcus flavus (NYT) by morphological and biochemical characters and 16 rRNA sequencing .Optimization of lipase production and enzyme activity was measured with varying pH (4-9) , incubation temperature (27^0C & 37^0C) , growth media incubation time (24hr,48hr,72hr) , reaction mixture time intervals (10, 20 and 30 min) and substrates as sunflower oil and palm oil. The maximum lipase production by using sunflower oil as substrate was recorded at pH 8, 27^0C during 48h of culture period and 10 min incubation time and in palm oil higher production was at pH 7, 27^0C during 48h of culture period and 10 min incubation time.*

Keywords: Enzyme *activity,Lipase , ,Micrococcus , Tributryn ,Optimization.*

I. Introduction

Lipases extensively present in nature, catalyze the hydrolysis and the synthesis of ester formed from glycerol and long chain fatty acids [1]. Plants, animals and microorganisms are capable to producelipase enzymes [2 – 4]. Lipases havingadvantageous properties related to their stability as organic solvent-tolerant [5] and thermo stable enzymes [6].

Microbial lipases are more considerable and greatly demanded sources due to several industrial potentials [7]. Lipases are usually used in large number of fields such as food, dairy, textile, detergent, pharmaceutical and cosmetic industries . These enzymes are also used in the processing of fats and oils, detergents and decreasing formulation, food processing, the synthesis of fine chemicals, pharmaceuticals,[8 , 9] and paper manufacture, .

Aim of this work is to isolate and characterize the lipolytic bacteria from polluted water from different places of ERNAKULAM DIST(KERALA) ,optimize the growth parameters for higher lipase production.Organism used for this research work was*Micrococcus flavus*(NYT) ,is foundin everywhere water, dust, air and soil, and as a part of the normal flora of mammalian skin. It is agram positive, yellow pigmented cocci,obligate aerobeand arranged in tetrads. Commercially easily available sunflower oil and palm oil were used as the substrate for the determination of the lipase activity of *M. flavus*.

II. Metrials And Methods

11.1 SAMPLE COLLECTION

In this study, water samples were collected from different places of ERNAKULAM DIST and aseptically transfer to the laboratory immediately. The isolation of organisms was done by using serial dilution method.

11.2 .ISOLATION OF LIPOLYTIC BACTERIA

Lipase producing organisms were screened by qualitative plate assay. 9 Isolates were separately inoculated on tributyrin agar base plates [containing 0.5 % (w/v) peptone, 0.3% (w/v) yeast, 1% (v/v) tributyrin and 2% agar, pH7.0] and incubated at 30^0C for 2 days. Zone of clearance due to the hydrolysis of tributyrin was observed. Colony showing clear zone around was taken for the further studies.

11.3. IDENTIFICATION

The more potent lipase producer , was identified based on morphological, biochemical and physiological characters and 16SrRNA sequencing.

11.4. OPTIMIZATION OF PARAMETERS

Varying the following parameters one at a time with fermentation media were checked. The parameters were i. incubation period, ii. incubation temperature iii. pH and iv. lipid substrate. The influence of incubation period and incubation temperature were assessed by culturing NYT in tributryn broth at different time duration (24, 48, 72 hours) andwith temperatures of 27^0C& 37^0Crespectively . Growth media was adjusted to pH ranged from 4 to 9 before the inoculation of NYT,to determine the effect of pH on lipase production.Enzyme activity of all parameters were assessed with sunflower oil as a substrate and varying incubation time such as 10, 20 and 30mins.

11.5. ENZYME PRODUCTION

Composition of productionmedium used (tributryin broth) in this work was: peptone (5g); yeast (3g); tributryin (10 ml); distilled water (1000ml). NYT was inoculated in to the production media. After the incubation time, culture broth was centrifuge at 4^0C, 4000rpm for 15minutes, for the separation of an enzyme and the supernatant was used as an enzyme source for further studies. Determination of lipase activity of an organism is done by colorimetric assay of lipase activity using copper soap method [10].

11.6. LIPASE ACTIVITY ASSAY

0.3 ml of substrate taken as reagent blank , vortex and centrifuge 5 minutes at 1000 x g , room temperature. 0.5ml of enzyme added with 25ml of substrate to initiate lipolysis on the emulsion substrate, start timer, and continue stirring. Remove duplicate 0.3ml subsample of the reaction mixture at pre determined time intervals (e.g.: 10, 20, 30 minutes) and place 5ml of benzene and 1ml of cupric acetate/pyridine reagent. Immediately vortex to stop the reaction and form colored fatty acid cupric soaps. Centrifuge 5 minutes at 1000 x g , room temperature , to obtain the clear benzene upper phase. Measure at the A715 nm for the benzene layer of each sample using glass cuvettes.

III. Results And Discussion

In this study, the lipase producing bacterial strain was isolated from ERNAKULAM DIST and identified as *Micrococcus flavus*(Table-1). Optimization of an enzyme production and yields were assessed at different incubation period (24hr, 48hr, 72hr) ,temperature (27^0C & 37^0C)and at pH (4-9) by using substrate as sunflower oil (Fig - 1) and palm oil (Fig - 2) . The higher production of lipase enzyme was 18.4935 IU/L in sunflower oil and 12.1832 IU/L was in palm oil as substrate.

From the values of lipase assay, the maximum lipase production recorded at pH 8 when sunflower oil used as substrate and in palm oil the higher production at pH 7. Related work on *Bacillus mycoides*[11], has the maximum lipase activity on pH 7 the lipase activity was less in low and high medium pH tested, In both substrates , NYT has an optimum incubation temperature at 27^0C while comparatively less activity was obtained at 37^0C . Similar study was reported [12] isolated 5 *Bacillus* strain from oil mill waste and analyze their lipase activity and optimization ,the maximum lipase production was at pH -7, substrate concentration (coconut oil) at 0.5% and at 24 hr. NYT shows higher lipase activity during 48hr of culture period and at incubation time 10 mins than other two incubation times in sunflower oil and also in palm oil. *Streptomyces griseus*has achievedthe maximum activity at 24 & 48 h of incubation period using sunflower oil and palm oil as a substrate [13].

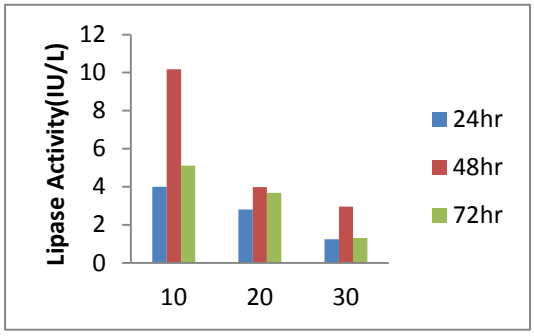
IV. Figures And Tables

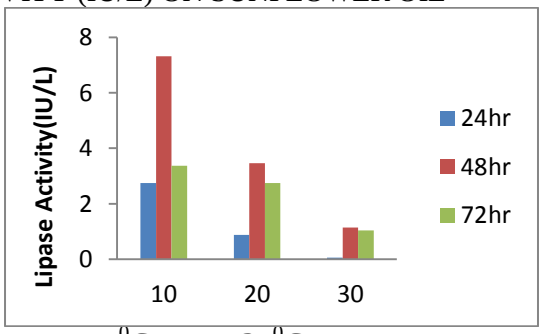
TABLE 1- CHARACTERS OF NYT

Sl No	Biochemical test	Result
1	Grams reaction	+
2	Shape	Cocci,Tetrads.
3.	Aerobic growth	+
4.	Growth above 40^0C	-
5.	Mac conkey growth	NLF colonies.
6.	Motility	-
7.	Endospore	-
8.	Capsule	+
9.	Starch hydrolysis	+

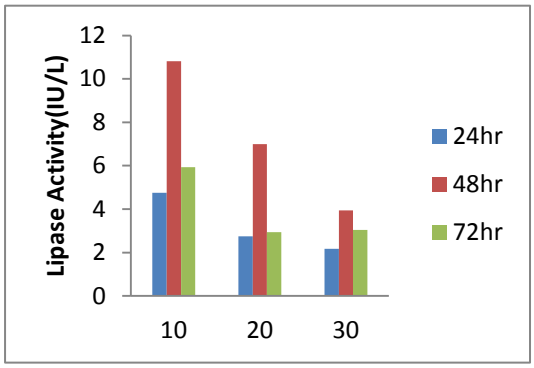
10.	Gelatin hydrolysis	-
11.	Glucose fermentation	-
12.	Lactose fermentation	-
13.	Sucrose fermentation	-
14.	Manitol fermentation	-
15.	H_2S production	+
16.	Indole test	-
17.	Methyl red	-
18.	VP	+
19.	Citrate	+
20.	Catalase	+
21	Oxidase	+
22.	Urease	+
23.	6.5% NaCl	+
24.	Pigmentation	Yellow
	Identify(genus)	*Micrococcus sp*

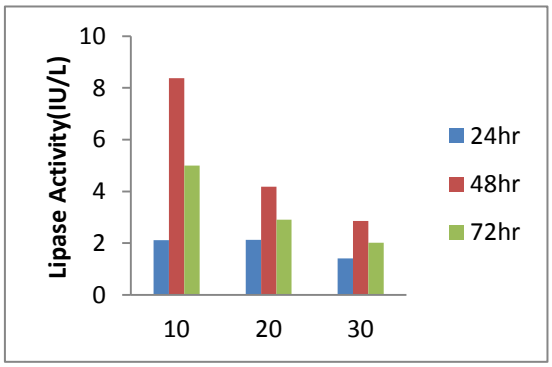
FIGURE- 1- LIPASE ACTIVITY (IU/L) ON SUNFLOWER OIL





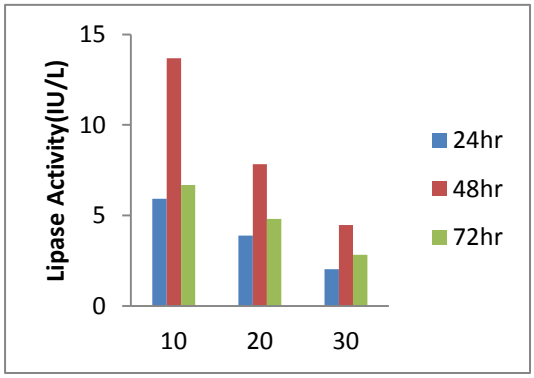
pH-4 ,27^{0}CpH-4 , 37^{0}C

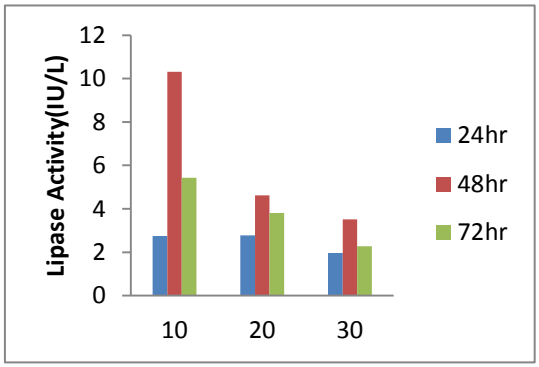




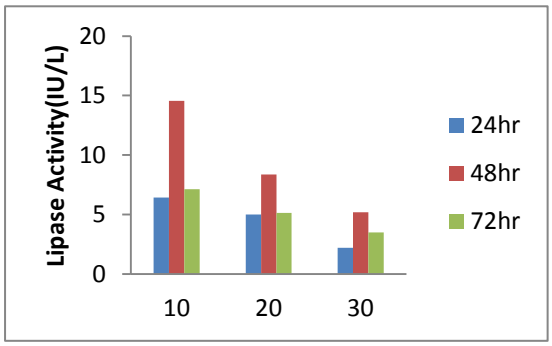
pH-5, 27^{0}C

pH-5, 37^{0}C

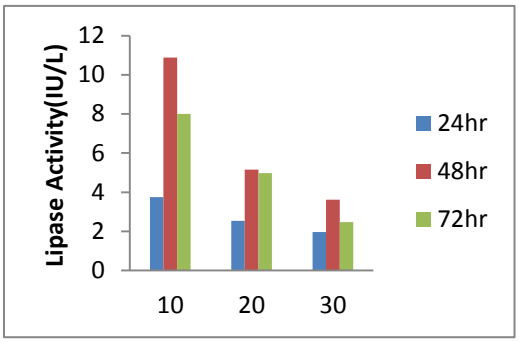




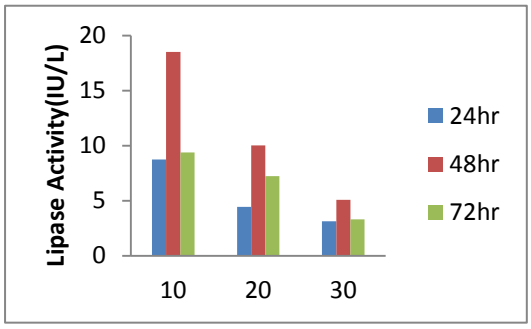
pH-6, 27^{0}C



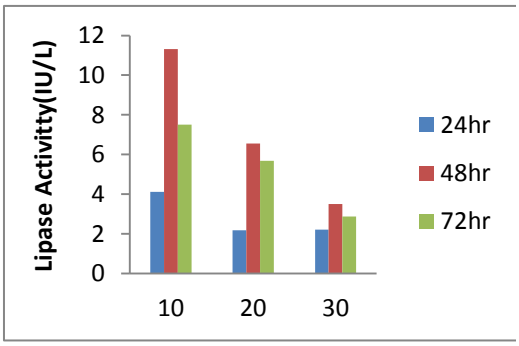
pH-6, 37^{0}C



pH-7 , 27^{0}C

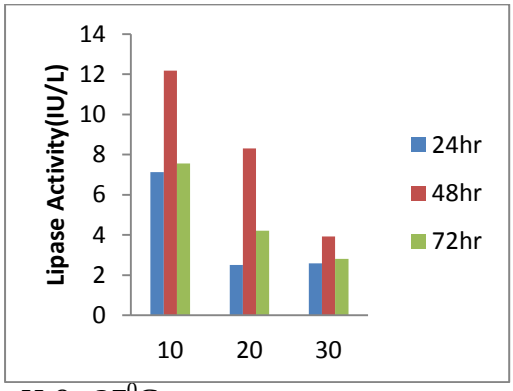


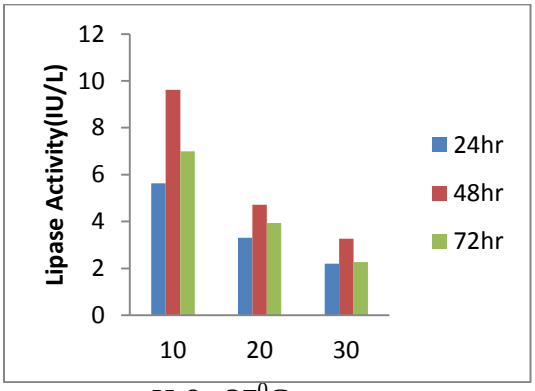
pH-7 , 37^{0}C



PH-8 , 27^{0}C

PH-8 , 37^{0}C

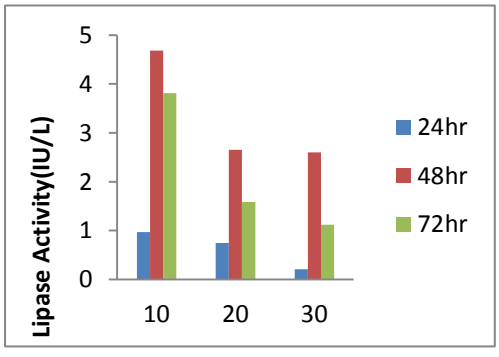


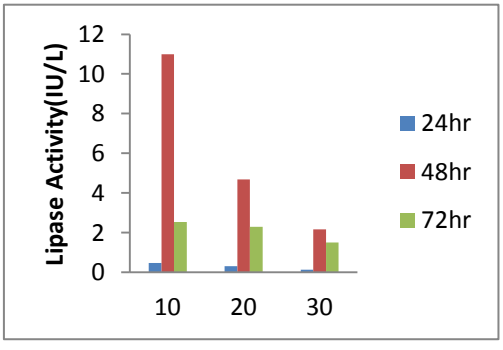


pH-9 , 27^{0}C

pH-9 , 37^{0}C

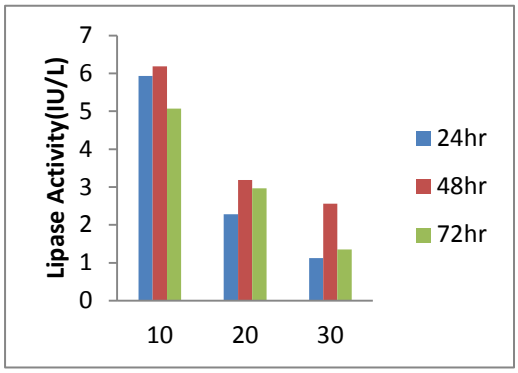
FIGURE- 1- LIPASE ACTIVITY(IU/L) ON PALM OIL



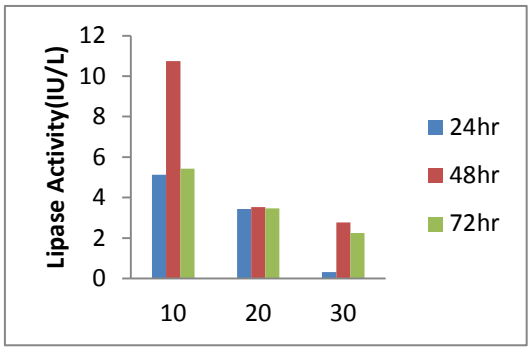


pH-4, 27^{0}C

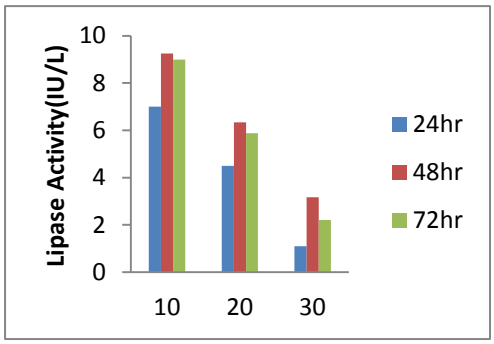
37^{0}C



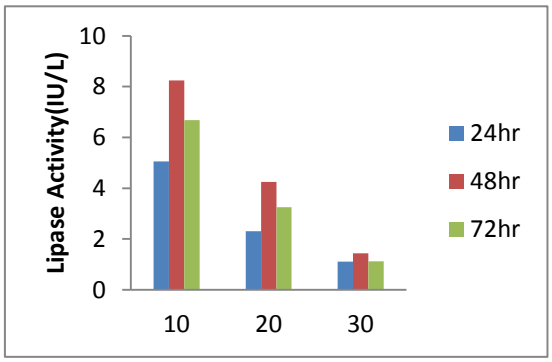
PH-5 , 27^{0}C



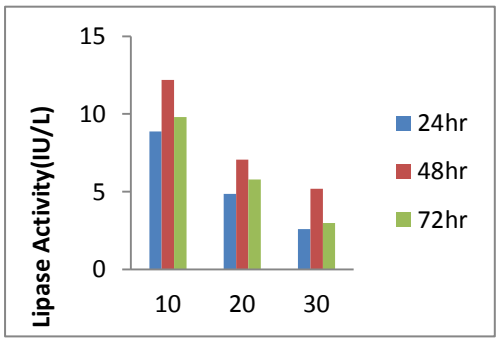
37^{0}C



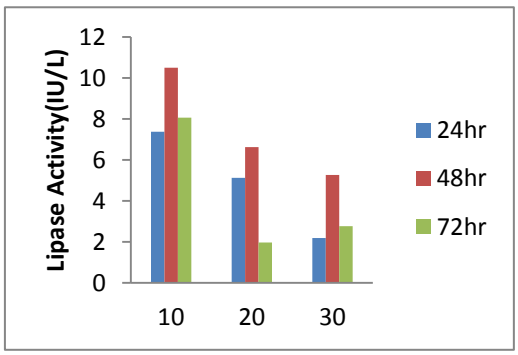
pH-6 , 27^{0}C



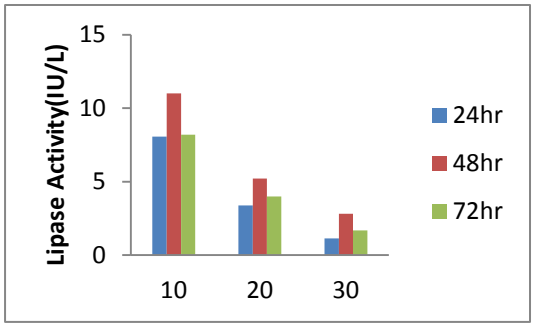
37^{0}C



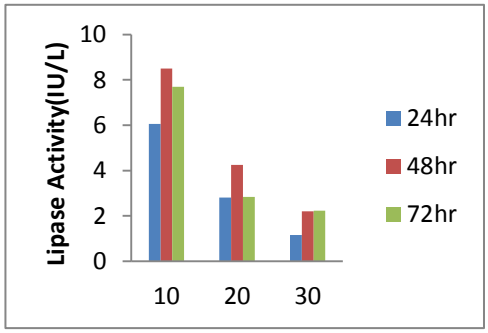
pH-7 , 27^{0}C



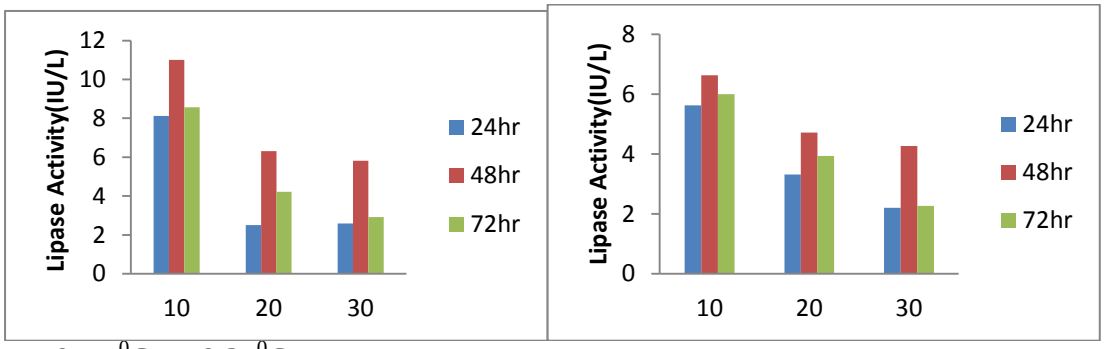
37^{0}C



pH-8 , 27^{0}C



pH-8 , 27^{0}C



pH-9 ,27^{0}CpH-9 37^{0}C

V. Conclusion

The bacteria isolated from different places of ERNAKULAM DIST was screened extracellular lipase production by qualitative plate assay (tributyrin agar base plate method) and identified as *Micrococcus flavus* by morphological and biochemical characters and also by sequencing. . Lipase activity(colorimetric assay by copper soap method) was measured with varying pH (4-9),incubation temperature (27^0 C& 37^{0}C,) varying incubation time such 24hr,48hr and 72hr with sunflower oil and palm oil as substrates.

The optimal growth conditions for the maximum lipase production by *Micrococcus flavus*was recorded at pH 8, 27^{0}Cof incubation temperature, during 48hr of culture period and reaction mixture incubation time 10 mins in both substrates, comparatively higher lipase production was in sunflower oil as a substrate.

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