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SCREENING OF LIPOLYTIC ALKALIPHILIC BACTERIAL ISOLATES FROM LONAR LAKE

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Abstract:-Alkaliphililic bacteria were isolated from the soil and water samples collected from lonar lake area which is a natural alkaline environment. Different media were used for isolation pH of the media was adjusted at 10.5. Isolates were screened for lipolytic activity on butter fat agar. Lipolytic activity of five alkaliphilic isolates was confirmed by using tributyrin agar medium . Amongst the isolates C1ps showed maximum lipolytic activity after 36 hours of incubation. Diameter of the zone of lipolysis noted was 26mm.

Keywords: alkaliphilic, isolates, screened, lipolytic activity.

INTRODUCTION :-

Growth and survival of micro-organisms is greatly influenced by pH of the environment and all bacteria & other microbes differ as to their requirements. Each species has the ability to grow within a specific pH range that maybe broad or limited with the most rapid growth occurring within a narrow optimum range. Alkaliphiles grow at pH value above and often between 10 & 12. These micro-organisms also called as extremophiles can be a source of novel enzymes (Bertin Vanderberg, 2003). Lipases (try-acyl glycerol acyl-hydrolase e.c.3.1.1.3) are ubiquitous enzymes of considerable physiological significance and industrial potential, and remain a subject of intensive study (Alberghina etal 1991; Bornschever 2000).
Enzymes from micro-organisms that can survive under extreme pH could be particularly useful for application under highly acidic or alkaline conditions for e.g. in the production of detergents (Gaur etal 2012). Many lipases are active in organic solvents where they catalyze a number of useful reactions including esterification (Choudhary etal 2001). These lipolytic enzymes are also used as research tools for hydrolysis, synthesis, analysis, and bio-transformation and affinity separation. They have cosmetic applications as denture cleanser and several other applications. Considering the several applications of lipases, in this study screening and isolation of lipase producers from water and soil samples collected from Lonar Lake was carried out.

MATERIALS AND METHODS:

Collection of Samples

We collected a total of twelve samples (three water and nine soil samples) were collected from Lonar lake area (Impact Crater salt lake with coordinates of 190 582 363N, 760 362 303E) in Maharashtra. Sampling was done from surface and sub-surface at a depth of 3 cm. All samples were kept in Ice Box and immediately transported to laboratory and stored at 40 C aseptically for further study. Plant material and other large particle were removed from the soil. PH of water samples was measured at the sight and also in the laboratory. PH of soil was determined by shaking 10g of soil sample in 20 mL sterile distilled water for 20 minutes followed by measurement with PH meter (McLein, 1982).

Isolation of Bacteria

Each of the collected samples of water and soil was subjected first to enrichment in general purpose media (Nutrient broth). Flasks were incubated at 280 C for 48 hours on incubator shaker at 120 rpm. Hundred milliliter of Different selective media (pseudomonas broth, actinamycetes broth, cetrimide broth and kingsbee broth) of PH 10.5 where inoculated with 5%

V/V inoculum from the first enrichment flask. All flasks were incubated at 280 C for 48 hours. After further 2 enrichments, a loop full of samples (calibrated for 0.05 mL) from each well grown culture flask was streaked on agar plates of respective selective medium. These plates were incubated at 200 C for 48 hours to develop colonies. Pure Cultures were maintained on slants and preserved at 40 C for further studies.

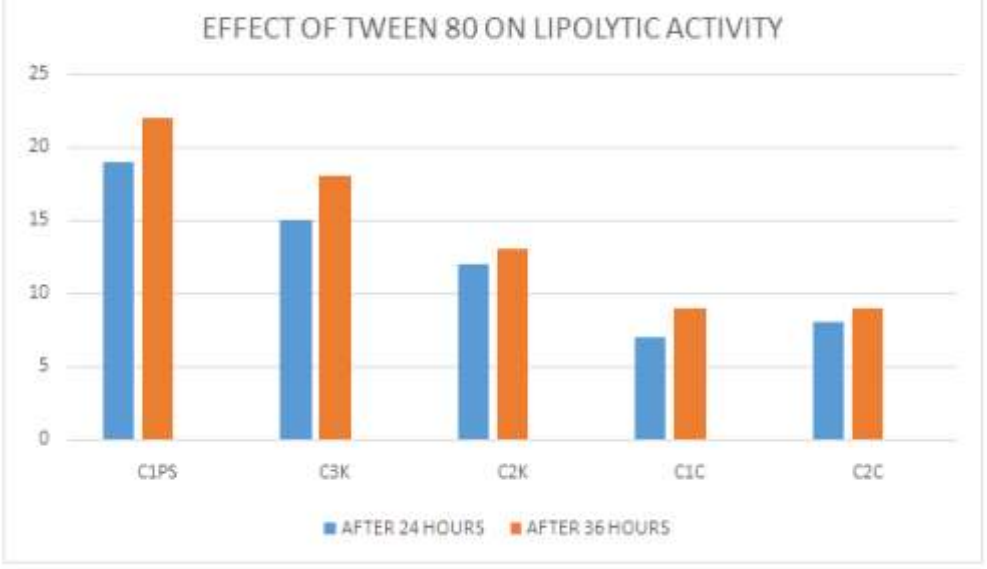
Sr.No.	Source	Medium	Isolate No.
1.	Soil	Cetrimide Agar	C_1C
2.	Water	Cetrimide Agar	C_3C
3.	Soil	Pseudomonas agar	C_1PS
4.	Soil	Pseudomonas agar	C_2PS
5.	Water	King's B agar	C_2K
6.	Soil	King's B agar	C_3K
7.	Soil	King's B agar	$CRYEC_2$
8.	Water	Pseudomonas Agar	C_1GM
9.	Soil	Cetrimide Agar	$CRYEMAC_2$
10.	Soil	Cetrimide Agar	$CRYMAC_3$
11.	Soil	Cetrimide Agar	C_2C
12.	Soil	Cetrimide Agar	RC_3K
13.	Soil	Cetrimide Agar	NC_2K
14.	Soil	Pseudomonas Agar	$N_2FGA\text{-}C_1$
15.	Water	Pseudomonas Agar	C_2GG
16.	Water	Pseudomonas Agar	C_3PS
17.	Soil	Pseudomonas Agar	C_4PS
18.	Soil	Actinamycetes agar	$ACT\text{-}C_1$
19.	Soil	Potato Dextrose Agar	$PDA\text{-}F_1$
20.	Water	Potato Dextrose Agar	$PDA\text{-}F_2$

	Alkaliphilic isolation	Zone of Lipolysis in mm.
1.	C_2K	17
2.	C_3K	20
3.	C_1C	15
4.	$C_1C./25/6$	10
5.	C_2C	17
6.	C_1PS	22

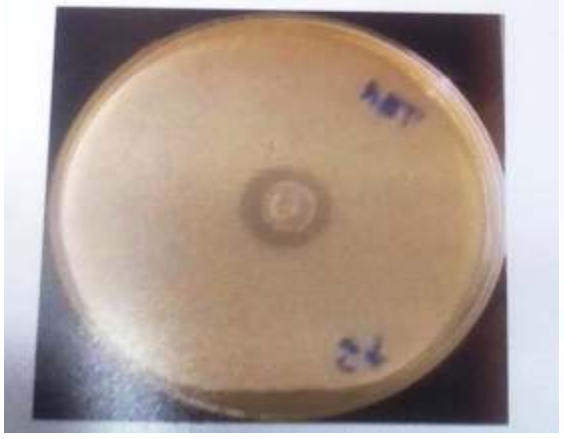
Screening for Lipolytic bacteria

For primary screening of lipolytic bacteria butter fat agar PH 10.5 was used. Culture (0.05 mL) was spot inoculated with calibrated loop of high media. Zone of lipolysis was noted by using CuSO4 solution. For confirmation of lipolytic activity tributyrin agar medium was used (Collins, 1964; Collins and Lyne, 1980; Limpon et al. 2006). The bacterial colonies which form clear zone around them on the plates were recorded and the strains showing more lipolytic potential (depending upon the zone diameter) were selected and screened further for efficient lipase production. Effect of tween 80 was on the lipolytic activity of microorganisms was also studied.

Sr.no.	Alkaliphilic isolates	Diameter of zone(mm) of Tributyrin agar with 0.5%Tween 80 After 24 Hours	After 36 Hours
1.	C_1PS	19	22
2.	C_3K	15	18
3.	C_2K	12	13
4.	C_1C	07	09
5.	C_2C	08	09

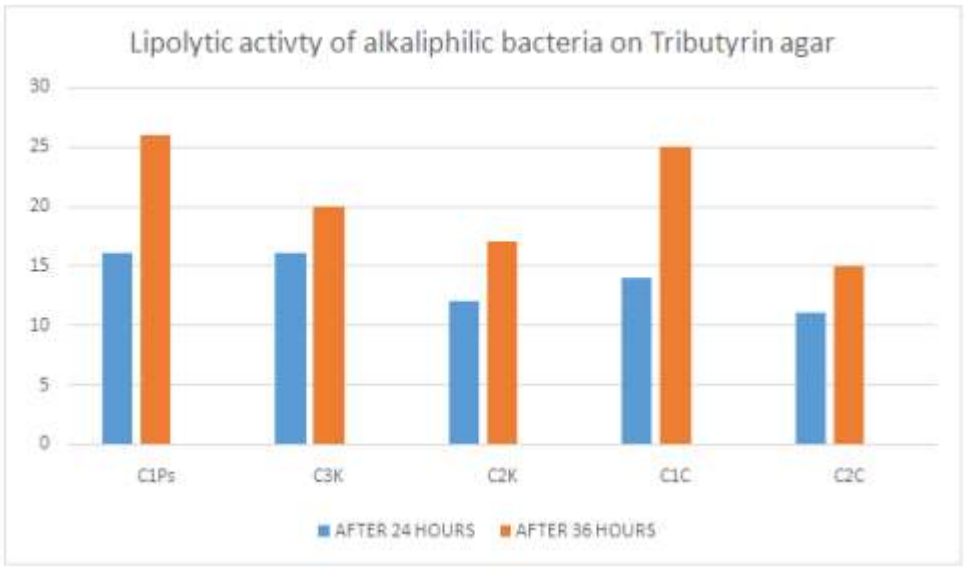


Sr.no.	Alkaliphilic isolates	Diameter of zone (mm) of Lipolysis After 24 Hours	After 36 Hours
1.	C_1Ps	16	26
2.	C_3K	16	20
3.	C_2K	12	17
4.	C_1C	14	25
5.	C_2C	11	15



Zone of lipolysis by C3K alkaliphilic bacterial isolate

Zone of lipolysis by C1Ps alkaliphilic bacterial isolate



RESULTS AND DISCUSSIONS

In the present investigations, a total of twenty bacteria and fungi were isolated from Lonar Lake area (which has extreme alkaline environment) and were tested for lipolytic activity. Out of these six strains showed notable zone of lipolysis. Secondary screening of these alkaliphilic bacteria was carried out on tributyrin agar. Out of the five strains, C1Ps showed largest zone of lipolysis thereby indicating more lipase production.

Effect of tween 80 (a natural surfactant) was also studied by incorporating 0.5% of the solution in tributyrin agar and incubating for 24 hours and 36 hours respectively.

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