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LIPOLYTIC BACTERIA: A SURVEY OF EDIBLE OIL FACTORY EFFLUENT

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ABSTRACT

Lipases are important enzymes used in various industries. Various bacteria produce lipases. Current study was undertaken to isolate bacteria which produce lipases. Effluent from edible oil factory was taken and seven different bacteria were isolated. These bacteria were characterized biochemically and physiologically. Oil degrading capacity of these bacteria was tested by inoculating these bacteria in different types of oil. The bacteria showed variation in degrading capacity as few were able to degrade only some oils and some many oils tested. Quantitative estimation of bacterial lipases was also carried out and it was found that stain PSl7 had the highest lipolytic activity.

KEYWORDS: Bacteria, Lipase, Effluent

Lipases are one of the most versatile enzymes as bring about hydrolysis of triglycerides, synthesis of esters from glycerol and long chain fatty acids, alcoholysis, acidolysis, esterification and aminolysis (Gunasekaran *et al.*, 2005). Lipases are produced by various different taxonomic groups of prokaryotes and eukartoytes like bacteria, fungi, and mammals. Lipase from soil have been isolated by Willerding *et al.* [8]. Various workers have discussed about lipases producing bacteria (Jaeger *et al.*, 1994, Gupta *et al.*, 2004, Lopes *et al.*, 2002). Arpigny and Jaeger, 2005, have classified different lipases and have discussed their properties.

MATERIALS AND METHODS

Effluent sample from an oil factory was brought in the laboratory. The sample was inoculated in nutrient agar medium and kept at 37˚ C for 48 hours. After incubation of 48 hours the NAM plates were observed. The bacteria colonies developed were pure cultured and then inoculated on to plate containing NAM and 1% coconut oil and methylene blue, modified from Reynolds, 2003. Bacteria that were able to degrade coconut oil were then chosen for further study and were characterized physiologically and biochemically. For physiological characterization growth of bacteria at different pH and NaCl concentration was studied. The tube of nutrient agar were made with different pH in the range of 2-10 using citrate and tris buffer for lower and higher pH respectively, and inoculated with bacteria and kept at their respective temperatures for growth. Similarly NAM plates with different NaCl concentrations were inoculated with bacteria and kept at respective temperatures for growth.

Biochemical characterizations of the bacteria were done by using the following tests. Gelatin hydrolysis test, starch hydrolyses test, MR-VP test, nitrate reduction test, indole production test, dnase test, catalase test, citrate utilization test, urease test, hydrogen sulphide production test, casein hydrolysis test, decarboxylase test, and tween hydrolysis test.

Qualitative estimation of lipase activity was carried out by the method given by Deeth, and Touch, 2000. Tributyrin, lipase preparation and phosphate buffer (0.2 M, pH 7.2) in ratio 1:1:2 were incubated for 24 hours at 37˚C. After incubation two ml of reaction mixture was taken out and mixed with 10 ml extraction mixture of isopropanol, petroleum ether and 4N sulphuric acid (40:10:1). The mixture was shaken and layers allowed to separate after which two ml of upper layer was titrated with 0.02 N methanolic KOH with methanolic phenolphthalein (50 µl) as indicator. Free fatty acid content was determined by formulae (TN/PV) $X10^3$. Lipase activity was expressed in U/mg. Bacteria selected for degrading coconut oil were subjected to degradation of 15 different types of oils and fats (Table-1). Bacteria were also tested for production of exo polysaccharide and PHA.

RESULTS AND DISCUSSION

Seven different types of bacteria were isolated from sample of oil factory. They were

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designated from PSL1-7. PSL 5 was gram negative while all the others were gram positive bacteria. All were endospore producing rod shaped bacteria and were catalase positive. PSL1 and PSL6 were alkalophilic bacteria while the rest were able to grow in acidic conditions also. PSL1 and PSL4 were halophilic bacteria and were able to grow up to 8% NaCl concentration while rests were not able to grow in NaCl concentrations more than 2%. PSL1, PSL3 and PSL6 were able to grow at temperature of 60˚ C while the other four were able to grow up to 55˚ C.

PSL7 was the only bacterium which was able to degrade all the types of oils and fats tested except wax which no bacterium was able to degrade. The results of oils and fats degrading activity of different bacteria isolated from the sample of oil factory is given in table – 1. The lipolytic activity ranged from 91.86 to 31.02 (U/mg). PSL 7 showed the highest activity while PSL4 the lowest. All the bacteria were able to produce EPS and PHA while there was no difference in amount of EPS produced by all bacteria, PSL6 was the highest and PSL4 was lowest producer of PHA while rest produced same amount (Data not shown).

All the bacteria isolated from the effluent showed different properties but in all it can be concluded that they have potential as biotechnologically useful microorganisms as they were all thermo tolerant as well as were able to produce lipases which degraded different types of oils and fats. Along with these properties they were also producing PHA and EPS which have potential uses as biodegradable plastics and packaging materials.

Table 1: Test in Different Oils

S. No.	Oil name	PSL1	PSL 2	PSL 3	PSL 4	PSL 5	PSL 6	PSL 7
1	Coconut oil	+ve	+ve	+ve	+ve	+ve	-ve	+ve
2	Mustard oil	+ve	+ve	-ve	-ve	+ve	-ve	+ve
3	Gulli oil	-ve	+ve	-ve	+ve	+ve	-ve	+ve
4	Olive oil	-ve	+ve	+ve	-ve	+ve	-ve	+ve
5	Soybean oil	-ve	+ve	+ve	+ve	+ve	+ve	+ve
6	Alsi oil	+ve	+ve	+ve	-ve	+ve	+ve	+ve
7	Tilli oil	-ve	-ve	-ve	+ve	+ve	+ve	+ve
8	Groundnut oil	+ve	+ve	-ve	-ve	+ve	+ve	+ve
9	Almond oil	+ve	+ve	+ve	+ve	+ve	+ve	+ve
10	Sunflower oil	+ve	+ve	-ve	-ve	-ve	+ve	+ve
11	Amla oil	-ve	-ve	-ve	-ve	+ve	-ve	+ve
12	Jasmine oil	-ve	-ve	-ve	-ve	-ve	+ve	+ve
13	Caster oil	+ve	+ve	-ve	+ve	+ve	+ve	+ve
14	Ghee	+ve	+ve	+ve	+ve	+ve	-ve	+ve
15	Vegetable fat	-ve	-ve	+ve	-ve	+ve	+ve	+ve
16	wax	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Table 2: PHA and EPS production

S.No.		PSL1	PSL 2	PSL 3	PSL 4	PSL 5	PSL 6	PSL 7
1	PHA production (mg/ml)	0.05	0.05	0.05	0.04	0.05	0.06	0.05
2	EPS production (mg/ml)	0.04	0.04	0.04	0.04	0.04	0.04	0.04

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