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Isolation and Screening of Lipase Producing Microorganism from Soil and Comparative Study of Enzyme Activity with Different Substrates

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Abstract: Microbial enzymes are often more useful than enzymes derived from plants or animals because of the great variety of catalytic activity. Microbial enzymes are also more stable than corresponding plant and animal enzymes and their production is more convenient and safer. Lipases are the acyl hydrolases and water soluble enzymes that play a role in fat digestion by cleaving long chain triglycerides into polar lipids. Because of an opposite polarity between the enzyme (hydrophilic) and their substrate (lipophilic), lipase reaction occurs at the interface between the aqueous and oil phase. In this study the soil sample was collected from slaughter house, and screened for lipase by Rhodamine B plate method. The lipase producing organism was isolated and characterized as Bacillus sp. The isolated Bacillus.sp was grown in five different substrates. Among which rice bran substrate showed high enzyme activity (0.4675µmol/min/ml) and protein content (29.37mg/dl).The molecular weight of the lipase was 33KDa using SDS PAGE. The hydrolysis of milk was done using the partially purified sample.

Keywords: Lipase, Bacillus Sp. Rice Bran.

I. INTRODUCTION

Microbial enzymes are also more stable than corresponding plant and animal enzymes and their production is more convenient and safer (Wiseman, 1995). Bacterial strains are generally more used as they offer higher activities compared to yeast and tend to have neutral or alkaline pH optimum and are often thermostable (Frost,1987).Lipases are the acyl hydrolases and the water soluble enzymes that play a role in fat digestion by cleaving triglycerides into polar lipids. Because of the opposite polarity between the enzyme (hydrophilic) and their substrates (lipophilic), lipase reaction occurs at the interface between the aqueous and the oil phases (Reis et al., 2008). Bacteria produce different classes of lipolytic enzymes including car boxy lest erases(EC 3.1.1.1) and hydrolyzing water soluble esters and lipases (EC 3.1.1.3) which hydrolyse long chain triglycerol substrates (Rosenatu and Jaegar, 2000). Commercially useful lipases are usually obtained from microorganisms that produce a wide variety of extracellular lipases. Microbial lipases are special interest because of their stability in organic solvents and their lack of requirement s for cofactors, their broad substrate specification and high enantio selectivity. Lipases are extensively used in dairy industry for the hydrolysis of milk fat. The dairy industry uses lipases to modify the fatty acid chain lengths , to enhance the flavours of various cheese. Current applications also include the acceleration of cheese ripening of lipolysis of butter, fat and cream.

II. MATERIALS AND METHODS

A. Collection of Sample

The soil sample was collected from the slaughter house, Chennai. Stored at 4°C for further analysis.

B. Screening of Lipase Producing Microorganism And Enrichment

The sample was screened for lipases production in a selective media containing Rhodamine B and olive oil(Gisela and Karl Erich Jaegar, 1987).The screened samples were enriched in minimal media with five different substrates (Rice bran, coconut oil, palm oil, Palm oil ,rice bran + coconut oil, Ricebran + palm oil).

C. Characterisation of The Isolated Microorganism

The isolated organism was characterized by conventional biochemical test.

D. Partial Purification of Lipase By Cold Acetone and Determination of Molecular Weight by SDS

The enzyme was partially purified by cold acetone precipitation. (Stuers W. Jaegar and Winkler, 1986).The enzyme extract was added with two fold of cold acetone (-20°C) and vortexed and storefat (-20°C) for 20 mins. Then centrifuged at 15000rpm for 25 mins. The pellet was dissolved in 20mM Tris HCl buffer (pH 7.2) and stored at 4°C.The molecular weight was determined by SDS.(Gupta.et al., 2004).

E. Enzyme Activity

The activity of lipase was determined by titrametric method (Gabrien Alues Macedo and Titiana Fontes Pio, 2005).The lipase has the ability to hydrolyse triacylglycerols at an oil-water interface to release free fatty acids and glycerol. These fatty acids were titrated with relevant bases to estimate the enzyme activity. The acid value is calculated

from,56.1×N×V/M, where V is the amount of KOH solution (ml) used for titration ,N is Normality and M is the mass of the reaction mixture.

III. RESULTS AND DISCUSSIONS

The collected was screened for lipase production in a selective media containing Rodamine B and olive oil .The fluorescence emitting colonies indicated the hydrolysis of liquid (i.e., growth of lipase producing microorganism) (Figure.1). The screened samples were enriched in minimal media containing the five different substrates for five days at 35°C.The isolated organism was characterized as Bacillus sp by Biochemical tests (Table 1). The lipase activity for all the five substrates were calculated, among them the coconut oil had highest activity on third day, 0.3973 (µmol/min/ml). (Table-2 & Figure. 2).The enzyme activity after partial purification for all five substrates were calculated, among them the rice bran had highest lipase activity, 0.4675 (µmol/min/ml) (Table-3 & Figure. 3). The enzyme isolated used to reduce the fat content in milk. The SDS shows the molecular weight was 33KDa. (Figure4). The partially purified sample from rice bran showed higher activity ,which is a cheap source and can be effectively used for the large scale production of lipase.

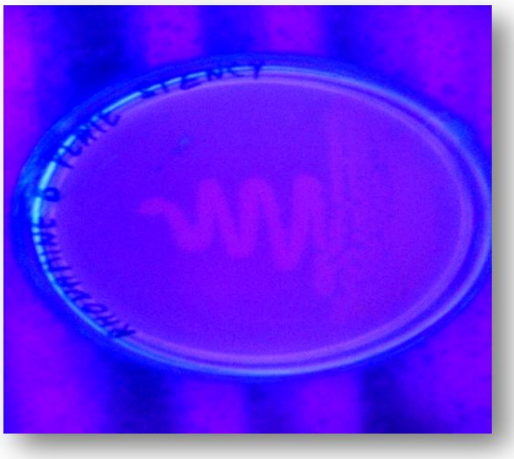

Fig.1. Appearance of Fluorescence emitting zone around the colonies.

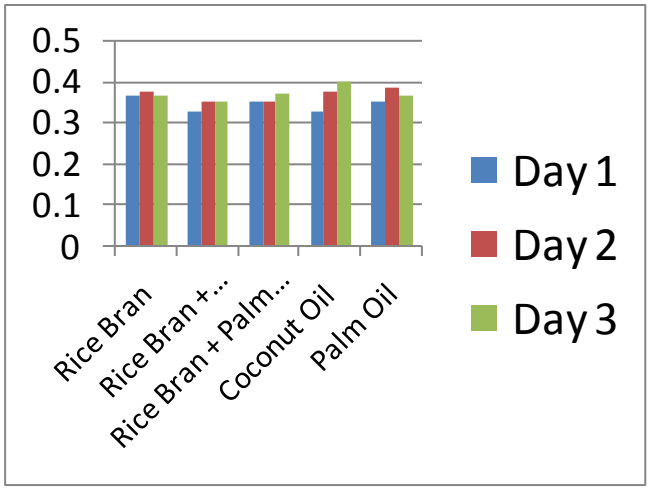

Fig.2. Lipase activity of the crude sample at 24 hours interval.

TABLE I: Characterization of tshe Isolated Microorganism

Biochemical test	Results
Gram's staining	*Gram positive rod*
Motility	*Motile*
Catalase	-
Oxidase	+
Spore staining	*Green colour spores*
Indole	-
MR	-
VP	-
Citrate	+
Urease	-
Nitrate	-
TSI	*AA G-*
Glucose	*Yellow colour*
Maltose	*Yellow colour*
Lactose	-
Mannitol	-

TABLE II: Enzyme Activity (Crude)

S.No	Substrates	Lipase Activity (µmol/min/ml)		
		Day 1	Day 2	Day 3
1.	Rice Bran	0.3623	0.374	0.3623
2.	Rice Bran + Coconut Oil	0.3272	0.3506	0.3506
3.	Rice Bran + Palm Oil	0.3506	0.3506	0.374
4.	Coconut Oil	0.3272	0.374	0.3973
5.	Palm Oil	0.3506	0.3856	0.3623

TABLE III: Enzyme Activity (Partially Purified Sample)

S.No	Substrates	Lipase Activity (µmol/min/ml)
1.	Rice Bran	0.4675
2.	Rice Bran + Coconut Oil	0.42075
3.	Rice Bran + Palm Oil	0.374
4.	Coconut Oil	0.42075
5.	Palm Oil	0.42075

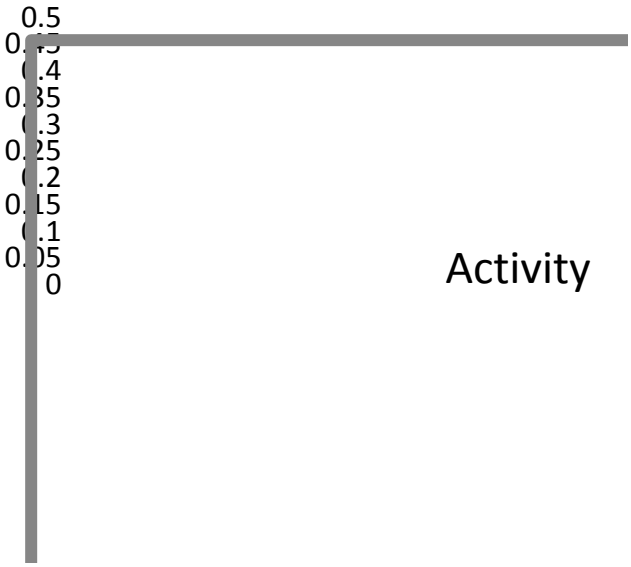

Fig.3. Enzyme Activity (Partially Purified Sample).

Fig.4.Sds Page - Determination of Molecular Weight.

IV. CONCLUSION

In this study, soil sample was collected from slaughter house for screening of lipase producing microorganism using Rhodamine B plate method. The isolated microorganism were characterized by Biochemical tests and it was identified as Bacillus sp .The isolated bacteria was grown on five different substrates. Among which rice bran showed high enzyme activity (0.4675 µ mol/min/ml).The molecular weight of lipase was approximately 33KDa using SDS PAGE. The hydrolysis of milk sample was done using the partially purified lipase extract. The amount of fat hydrolyzed was detected by calculating the amount of fatty acid released. This is an application of lipase used on milk industry to hydrolyse the fat contents.

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