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ISOLATION AND SCREENING OF LIPASE PRODUCING BACTERIA FROM MUNICIPAL SOLID WASTES (MSW)

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ABSTRACT

Lipases (triacylglycerol acylhydrolases EC 3.1.1.3) are hydrolysis enzyme. They hydrolyze the carboxylic ester bond through various important reactions such as trans-esterification, alcoholysis, interesterification, esterification, aminolysis. Microbial enzymes are often more useful than enzymes derived from plants or animals because of the great variety of catalytic activity. In present study, several lipase producing bacteria were isolated, from which best lipase producing isolates were further identified by means of morphological and biochemical characterization. Based on the identification characteristics and Bergy's manual two potent isolates were screened and identified as the genus *Pseudomonas* (PS-4) and *Staphylococcus* (PS-7).

KEYWORD: Lipases (triacylglycerol acylhydrolases *Staphylococcus* (PS-7).

INTRODUCTION

Enzymes are the bio-catalysts playing an important role in all stages of metabolism and biochemical reactions. Certain enzymes are of special interest and are utilized as organic catalysts in numerous processes on an industrial scale. Microbial enzymes are known to be superior enzymes obtained from different microorganisms, particularly for applications in industries on commercial scales. Though the enzymes were discovered from microorganisms in the 20th century, studies on their isolation, characterization of properties, production on bench-scale to pilot-scale and their application in bio-industry have continuously progressed, and the knowledge has regularly been updated. Many enzymes from microbial sources are already being used in various commercial processes. The microorganisms including bacteria,

fungi and yeasts have been globally studied for the bio-synthesis of economically viable preparations of various enzymes for commercial applications.[1]

In conventional catalytic reactions using biocatalysts the use of enzymes, either in free or immobilized forms, is dependent on the specificity of enzyme. In recent advances of biotechnology, according to the requirements of a process, various enzymes have been and are being designed or purposely engineered. A large number of new enzymes have been designed with the input of protein-engineering, biochemical-reaction engineering and metagenomics. Various molecular techniques have also been applied to improve the quality and performance of microbial enzymes for their wider applications in many industries.[2] As a result, many value added products are being synthesized for a global market with the use of established bioprocess-technology. The main industries that apply microbial enzymes are the food, textile, leather, pharmaceutical, cosmetics, fine chemicals, energy, biomaterials, paper, cellulose and detergent industries. Immobilization processes allow the reuse of these enzymes and increase stability. The enzymes and the microorganisms themselves have also been much used for bioremediation processes.

Many microorganisms such as bacteria, yeast and fungi are known to secrete lipases. Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, etc. Bacterial lipases are mostly extracellular and are greatly influenced by nutritional and physico- chemical factors, such as temperature, pH, nitrogen and carbon sources, inorganic salts, agitation and dissolved oxygen concentration.[3] Lipases from several sources have been purified, and some of their properties investigated. Generally, they are acidic glycoproteins of molecular weights ranging from 20000 to 60000. Their biological function is to catalyze the hydrolysis of triacylglycerol to give free fatty acids diacylglycerol and monoacylglycerol and glycerol. Microbial lipases attracted more attention due to its easy isolation, ease of genetic manipulation, high yield possible, systematic amount due to absence of seasonal variations and quick growth of micro-organisms or low-priced media.

MATERIALS AND METHODS

Sample collection

Municipal solid waste samples were collected randomly from the Vijayawada municipal corporation dump yard, at a depth of 4-5 cm with the help of a sterile spatula in sterile plastic bags to an amount of 500g without any chaff or grass roots and brought to laboratory,

Department of Environmental science, Acharya Nagarjuna University, Guntur within 48 hrs. These collected samples from were sealed in sterile containers respectively and stored in the refrigerator at 4^{o}C until use.

Screening of lipase producing bacteria

Dissolved 1 gm of soil from each sample in 100 ml of 0.8% saline water separately by agitating the soil solutions for 30 minutes in an orbital shaker incubator at 30^{o}C and 100 rpm. Thus prepared soil suspension was used to make the dilutions of 10^{-1} to 10^{-6} by ten-fold serial dilution method. These diluted soil suspensions of 1ml from each soil sample were used as inoculum in Tween-80 agar medium (40 ml) for the screening of lipase producing bacteria by pour plate method. Thus prepared Tween-80 agar plates in duplicates from each dilution of the respective soil samples were incubated at 30^{o}C for 48 hrs. Appeared bacterial colonies were counted along with suspected lipase producing bacteria (colonies with clear zone formation). The total number of colonies for each soil sample was calculated. The pH of the medium was adjusted to 7.2. For preparation of nutrient broth agar medium, agar was added to the final concentration of 1.5% (w/v).

Screening of best lipase producing bacteria

The preliminary bacterial isolates capable of lipase production were further screened to isolate the best possible lipase producing bacteria based on agar well diffusion or cup well method. Nutrient broth media emulsified with Tween-80 was prepared using homogenizer and sterilized by autoclaving at 121^{o}C and 15 psig for 15 min. All the lipase producing isolates were inoculated separately in to the prepared Tween-80 broth media aseptically. These culture flasks were incubated in incubator separately at 37^{o}C for five days.

Identification of lipase producing bacteria

After successful growth of microorganisms, each colony morphology e.g., size, shape, margin elevation, consistency, color, transparency was determined. Gram stain was performed to observe the cellular morphology and gram nature of the bacteria and biochemical characterization of the strains were also carried out. The biochemical tests of sugar utilization, amino acid decarboxylation, nitrate reduction, hydrogen sulfide production, starch, casein, and urea hydrolysis and IMVIC tests were performed.

RESULTS AND DISCUSSION

Identification of lipase producing bacteria

Morphological and biochemical identification of the lipase producing bacteria

A total of 12 morphologically distinct bacterial strains were isolated from municipal solid wastes l of Vijayawada corporation dump yard, Andhra Pradesh, India.

Table 1: Morphological and biochemical characterization of Isolates.

Test	Isolate-1 (PS-4)	Isolate-2 (PS-7)
Colony morphology		
Configuration	Circular	Circular
Margin elevation	Undulate	Entire
Surface	Smooth	Smooth
Density	Opaque	Opaque
Pigments	Green	White
Shape	Rods	Cocci
Size	Small	Medium
Arrangement	Single	Single or in pairs
Gram reaction	Negative	Positive
Biochemical tests		
Citrate utilization	+	+
Catalase	+	+
Gelatin liquefaction	+	-
Nitrate reduction	-	+
Oxidase	+	-
Indole	+	-
Methyl red	-	-
H_2S production	-	-
Voges-proskauer test	+	-
Urease	+	-
Tween-80 hydrolysis	+	+
Starch hydrolysis	-	-
Casein hydrolysis	+	-
Sugar fermentation		
Glucose	+	+
Sucrose	-	+
Lactose	-	+

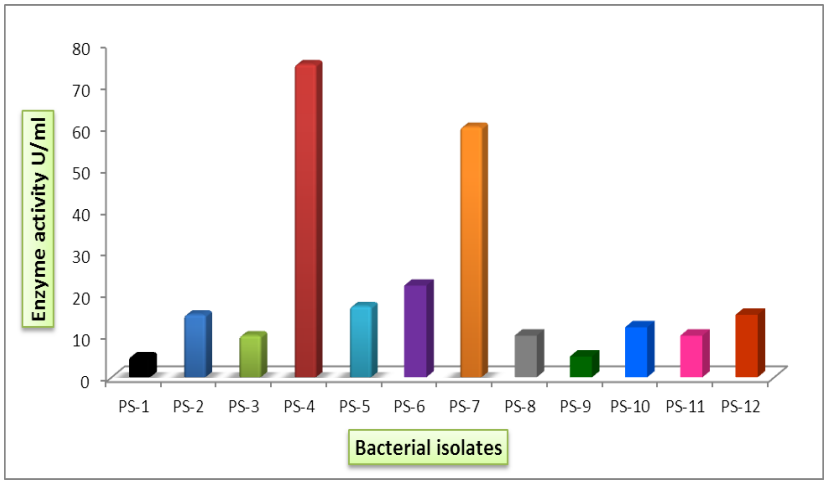

Figure 1: Enzyme activity of lipase positive bacteria selected for preliminary screening.

Waste contaminated soil in this study basically refers to soil from kitchen waste dumping sites. Municipal solid waste contains organic materials such as paper, food and yard waste and plastics. To be specific, MSW can be divided into six major chemical compound classes: non-cellulosic carbohydrates (hemicellulose, starch and monoand oligosaccharides), cellulose, proteins, lipids, lignin, and plastics (Pichler and Kögel-Knabner, 2000). The isolated strains were screened for extracellular lipase using Tween-80 agar medium. Two of the isolates (PS-4 and PS-7) produced larger clear zone than the others, indicating higher lipase production. These two isolates were identified based on morphological and biochemical characterization. In the two bacterial strains one is Gram positive and coccoid and second one is Gram negative rod in shape. In accordance with the Bergey's manual of systematic bacteriology, the isolates were genus *Staphylococcus* and *pseudomonas*. Results obtained for the second phase of screening, which was based on the maximum lipase production using different methods as Agar well diffusion method. From the results it is inferred that the bacterial strains PS-4 and PS-7 produces maximal lipase compared to other strains (Figure-1). Hence, these strains were selected as potential strains for lipase production. Out of these two isolates PS-4 (*Pseudomonas* spp.) was very effective in production of lipase enzyme. Table-1 shows the two isolates morphological and biochemical characteristics. The major factor for the expression of lipase activity has always been carbon, since lipases are inducible enzymes[4] and are thus generally produced in the presence of a lipid source such as oil or any other inducer, such as triacylglycerols, fatty acids, hydrolysable esters, tweens, bile salts and glycerol. However, their production is significantly influenced by other carbon sources such as sugars, polysaccharides, whey and other complex sources. The industrial demand for new sources of lipases with different catalytic

characteristics was stimulated the isolation and selection of new lipase-producing strains, lipases producing bacteria are distributed in diverse habitats in soils, water and plants in the fields, these organisms are occupy different locations as vegetable oil processing factories[5], industrial wastes[6], dairies, soil contaminated with oil decaying food, oilseeds, compost heaps, coal tips, and hot springs.[7],[8],[9] Reported that waste contaminated sites such as dumped with kitchen wastes, which are usually comprised of numerous lipid leftovers from processes of cooking and non-cooking, can be serving as excellent breeding grounds for the isolation of lipolytic bacteria of industrial significance.

CONCLUSION

The results obtained in this study show that municipal solid waste could be potential source for the isolation of lipase producing bacteria. Further optimization of potential isolates for the lipase production to be carried out.

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