SCREENING OF ENZYME PRODUCING BACTERIAL STRAINS ISOLATED FROM THE SOLAR SALTPAN

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Abstract : In this study, soil sample was collected from the saltpan in Kanyakumari District, Tamilnadu State, India. The samples were utilized for microbiological analysis. The soil samples were serially diluted aseptically, plated on Nutrient Agar, and incubated at 37 °C for 72 hours. After incubation, 15 dominant bacterial colonies were isolated and tested for enzyme activity. A total of fifteen bacteria (SA1, SA2, SA3, SA4, SA5, SA6, SA7, SA8, SA9, SA10, SA11, SA12, SA13, SA14, and SA15) were isolated from the saltpan. Among them, ten bacterial strains produced >15 mm zone of hydrolysis. The most potent bacterial strain was SA5, which produced 22 mm zone around the bacterial colony. Among the bacterial strains, the strain SA5 exhibited maximum amylase producing potential than other strains. A total of three bacteria showed >15 mm zone around the colony. The isolated bacterial strains were subjected for the production of lipase on Tributyrin agar medium and the zone of hydrolysis was measured. Among the screened bacterial isolates, the isolated strain SA5 produced maximum zone of hydrolysis (17 mm zone) around the bacterial colonies, followed by SA8 (16 mm zone). The other screened bacterial isolates showed less lipase activity. Among the 15 bacterial isolates, only six exhibited tannase producing potential. Among the strains, SA5 showed maximum tannase activity and the zone of hydrolysis was 14 mm.

IndexTerms **– Saltpan; Bacteria; Enzymes**

1. Introduction

Enzymes break down a variety of biological interactions utilizing naturally occurring fundamental organic substrates, such as carbohydrates, proteins, lipids, and organic acids, into simpler parts. They are preferable than artificial or synthetic catalysts since they are essential to all metabolic reactions. Since their discovery, enzymes have drawn the attention of scientists all over the world, resulting in the understanding of their structure and its applications in both academic and industrial sectors. From the agricultural to the health industries, enzymes have demonstrated their influence on a variety of industries (Kelly et al., 2017). Microorganisms are the most frequent source of enzymes despite the fact that enzymes have been isolated, purified, and investigated from microbial, animal, and plant sources as well. This is because of their extensive biochemical variety and the possibility of producing them on a large scale using resources that are both affordable and abundant (Jiang et al., 2012). The capacity of microorganisms to be cultivated in large quantities quickly using recognized forms of fermentation is another benefit. Due to the enormous diversity of microorganisms, there is always an opportunity to identify the right microorganisms for creating novel enzymes with improved features that is acceptable for commercial use. For a long time, isolated bacterial enzymes have been applied to use in a variety of industrial processes. Along with the traditional industrial enzymes such amylases, lipases, proteases, and cellulases, more recently, new enzymes have been used because of their unique activity and potential for industrial and environmental applications. Pectinase, chitinase, laccase, tannase, xylanase, pullulanase, mannanase, aspartase, and other such enzymes are among those that have gained attention (Setati, 2010).

The hypersaline natural habitats that cover certain area of India are found in the interior and coastal regions of South India. Man made ponds, naturally salted logoons, artificial ponds supplied with brine, or combinations of artificial ponds and natural logoons all contribute to these ecosystems. There have been reports of microorganisms from this habitat belonging to the three major domains (Bacteria, Eukarya, and Archaea). Halophilic and halotolerant species thrived in these settings because they are equipped to survive harsh environmental conditions including high temperatures and low and high pH levels. The average salt concentration in the water was 2.5%, but in certain hyper-saline conditions, the concentration of salt can approach saturation (>40%). In addition to being adapted to high salt concentrations, microorganisms that are evolved to living in extreme settings, such as severe saline ecosystems, are typically also adapted to a broad range of other conditions, including intense irradiation, high temperatures, and poor oxygen diffusion. Specialized cellular and enzymatic adaptations are necessary for survival in such settings in order to maintain the osmotic equilibrium. This equilibrium is crucial because bacteria can dehydrate, which results in shrivelling and catastrophic loss of cellular structure and function. Proteins may become dehydrated as a result of cells having less water, which might lead to undesirable interactions that can be avoided by adjusting their net charge. Due to the high concentration of glutamate and aspartate on the surface of halophilic proteins, this is the primary distinction between halophilic and non-halophilic proteins (Margesin and Schinner, 2001). The severe osmotic pressure produced by the saline habitat may be tolerated by bacteria isolated from the halotolerant environment. The use of several enzymes from halophilic and halotolerant organisms, including lipases, hydrolases, tannases, proteases, esterases, and nucleases, has been enhanced. The primary benefit of these biocatalysts comes from their structure, since more acidic amino acids are present on the protein surface, increasing the hydrophobicity of the protein (Niño de Guzmán et al., 2008). Some of the microbial enzymes that halotolerant bacteria employ are capable of functioning in a variety of physicochemical situations, including those with severe pH, temperature, and salt concentration. The main aim of the study is to screen bacteria for the production of enzyme from the hypersaline environment.

2. Materials and methods

2.1.Samples

The saltpan in Kanyakumari District, Tamilnadu State, India, served as the source of the samples for this investigation. The temperature ranged from 30 °C to 41 °C and was warm and humid. From the reservoir pond, a sample was taken. In an uncontaminated vial, the sediment sample was stored. The samples were utilized for microbiological analysis after being brought to the lab in an ice box. From a depth of 20 cm in the saltpan, a sample of soil sediment was taken.

2.2.Isolation of bacteria

The soil samples were serially diluted aseptically, plated on Nutrient Agar (NA) containing 5% NaCl, and incubated at 37 °C for 24, 48, and 72 hours and 10 to 50 CFU/ml per plate was the acceptable plate count for bacteria. After incubation, 15 dominant bacterial colonies were isolatedand tested for enzyme activity.

2.3. Protease screening

Protease screening was performed using nutrient agar plates. To the nutrient agar plates, 1% casein was added and bacteria were inoculated. The plates were incubated at 37°C for overnight. Based on the zone of clearance, the strains that produce proteases were chosen.

2.4. Screening of amylase producing bacterial isolates

The thermotolerant bacterial strains were grown on LB agar plates containing 1% (w/v) soluble starch to examine their capacity to produce amylase. Plates were flooded with iodine solution after one day at 30 °C of incubation to analyze for the appearance of distinct halos surrounding bacterial colonies]. For further research, bacteria having amylase secretions were chosen.

2.5. Isolation and screening of lipase producing bacteria

The isolated bacteria were plated using the spread plate technique onto Tributyrin agar bases comprising 0.5%(w/v) peptone, 0.3% (w/v) yeast extract, 1.0% (v/v) Tributyrin, and 2% agar with (pH of 7.0). Tributyrin was hydrolyzed by lipase during the 48-hour incubation period at 37 ºC and a zone of clearance was visualized.

2.6.Tannase screening

Tannic acid agar plates were used for the initial screening to identify the potent tannase producers. The isolate was spread over the plates and incubated at 30° C. After 96 hours of incubation, the diameter of the clear zones—which included the colony diameters—formed surrounding the microbial colonies as a result of the hydrolysis of tannic acid was determined, and the highest tannase producers were selected based on comparison.

3. Results

3.1. Protease screening

A total of fifteen bacteria (SA1, SA2, SA3, SA4, SA5, SA6, SA7, SA8, SA9, SA10, SA11, SA12, SA13, SA14, and SA15) were isolated from the saltpan. Among them, ten bacterial strains produced >15 mm zone of hydrolysis. The most potent bacterial strain was SA5, which produced 22 mm zone around the bacterial colony. The scheme of protease production was depicted in Fig. 1.

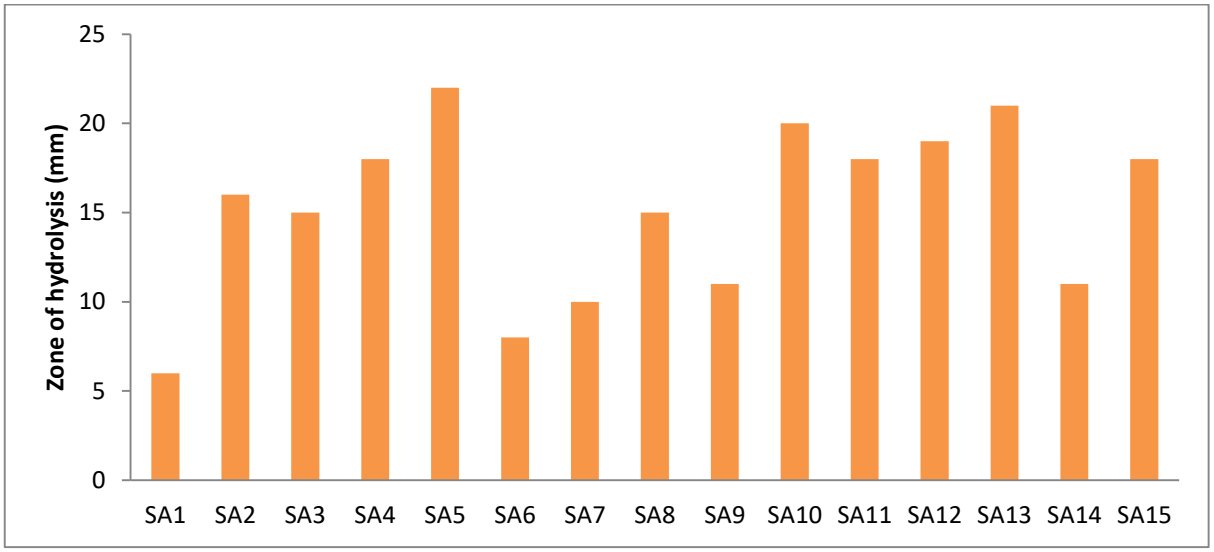

Fig. 1. Production of protease by bacteria isolated from the halotolerant environment.

3.2.Amylase screening

The isolated bacterial strains were screened for amylase production using 1% soluble starch. Among the bacterial strains, SA5 exhibited maximum amylase producing potential than other strains. A total of three bacteria showed >15 mm zone around the colony (Fig. 2).

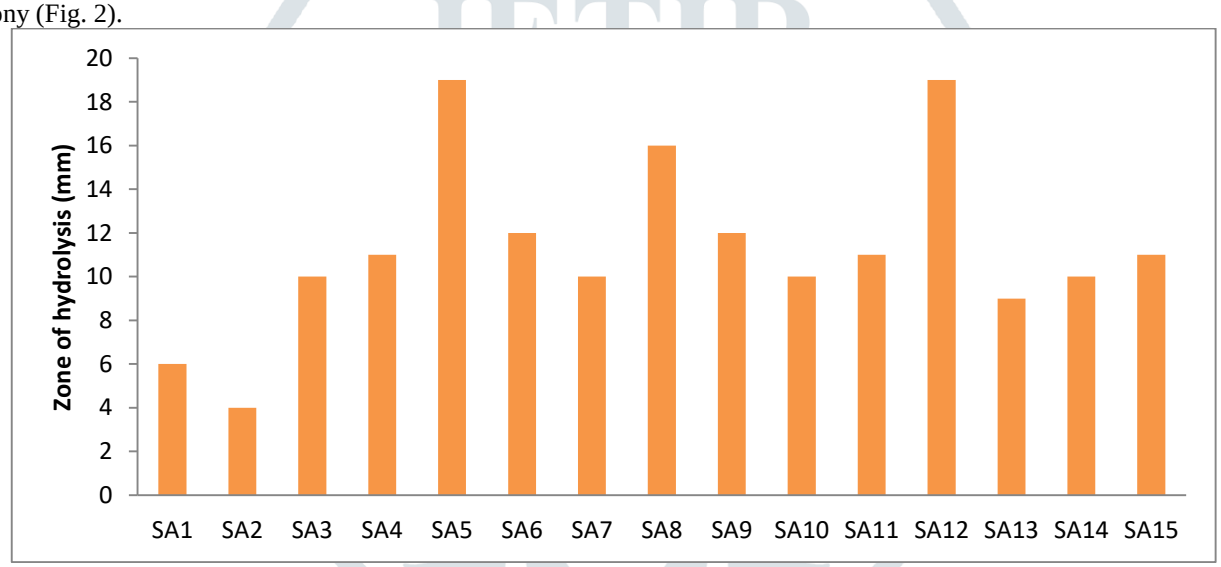

Fig. 2. Production of amylase by bacteria isolated from the halotolerant environment.

3.3. Lipase screening

The isolated bacterial strains were subjected for the production of lipase on Tributyrin agar medium and the zone of hydrolysis was measured after 72 h incubation at 37 ºC. Among the screened bacterial isolates, the isolated strain SA5 produced maximum zone of hydrolysis (17 mm zone) around the bacterial colonies, followed by SA8 (16 mm zone). The other screened bacterial isolates showed less lipase activity (Fig. 3).

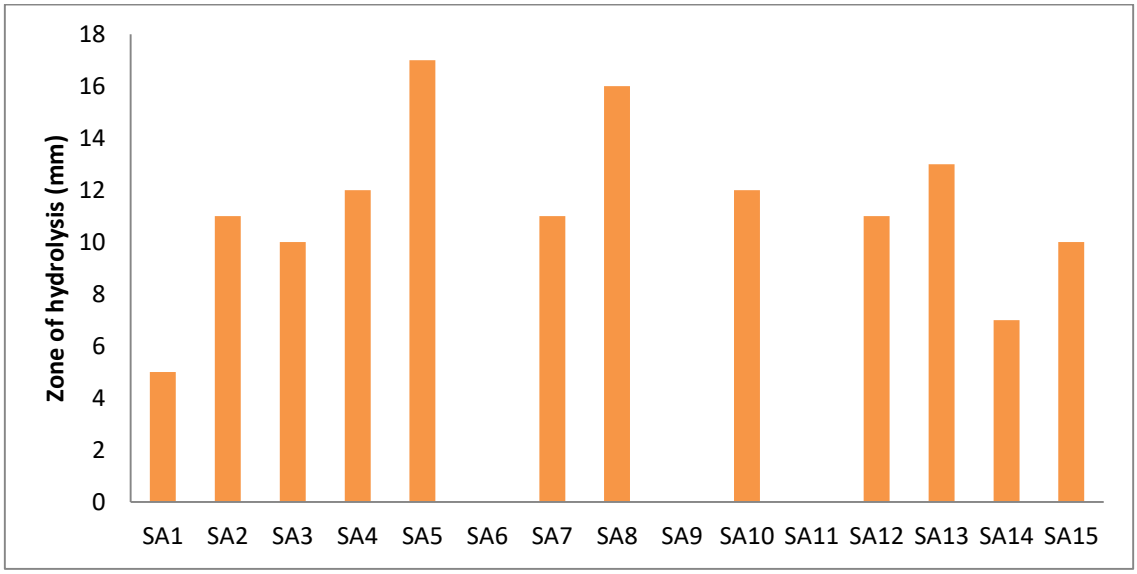

Fig. 3. Production of lipase by bacteria isolated from the halotolerant environment.

3.4. Tannase screening

Bacteria were screened for the production of tannase using tannic acid agar plates. Among the 15 bacterial isolates, only six exhibited tannase producing potential. Among the strains, SA5 showed maximum tannase activity and the zone of hydrolysis was 14 mm (Fig. 4).

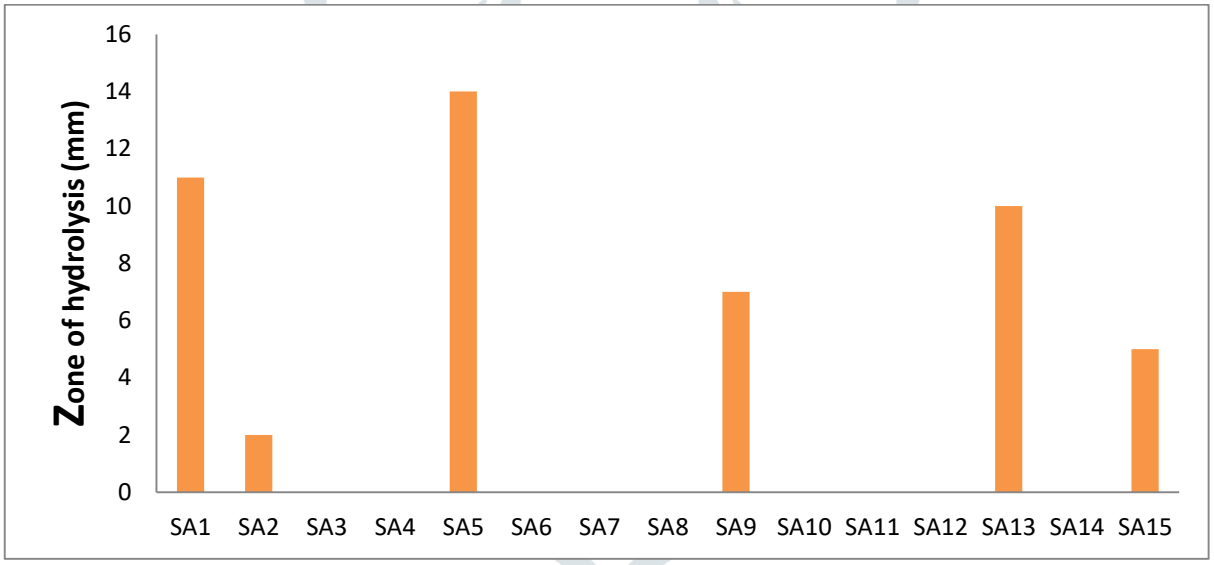

Fig. 4. Production of tannase by bacteria isolated from the halotolerant environment.

4. Discussion

Extremozymes can catalyse enzymatic reactions in harsh industrial processes. In this study, a total of 15 bacteria were isolated for the screening of enzymes (protease, amylase, lipase and phytase). Proteases are one of the enzymes which find wide applications in industries. Among the microorganisms of different origin, saltern microbes are competitive candidates with novel metabolites. Fifteen different bacterial strains producing protease enzyme were isolated from marine sediment. Among the 15 strains, the strain SA5 exhibited maximum protease production. The production of protease is chiefly affected by the factors such as pH and temperature in most of the organisms. Bajaj et al. (2013) stated that the secretion of protease varies in microorganisms and it is dependent on environmental factors. because of these properties (Lakshmi et al., 2014). The amount of enzyme production varied based on the types of microorganisms. Because habitats that contain substrates (e.g., protein, tannins, sugars) are likely to harbour enzyme-producing bacteria, those environments were chosen as the isolation sources. Similar isolation sources were utilized by other publications to separate hydrolytic enzyme-producing bacteria. Moreover, tannase producing ability of the bacterial population from this saltern was not elucidated. It was exceedingly challenging to observe the clean zones when screening for tannase. The pH of the soil sediment in the saltpan varied widely and was alkaline (Thys et al., 2006). Like other salterns, the pH of the soil sediment was alkaline and the soil pH may vary based on the ionic concentration of brine in the condenser. Enzymes-producing bacterial strains have been reported from various hypersaline environments. The saltpan is one of the most diversified microbial sources and various enzymes producing bacterial strains have been reported from the saltpan. Even though colony forming bacteria in saline samples often represent a limited fraction of the total count. In addition, plate screening

was carried out using specific inducing media able to reveal (under the tested conditions) both constitutive and inducible activities (Kandasamy et al., 2016). Other researchers, mainly aimed to obtain ecological and/or nutritional information, determined the microbial enzyme activities on non inducing media thus revealing constitutive activities. This work was essentially aimed to discover new strains for potential applications but it could also partially contribute to the knowledge of the nutritional versatility of the isolates and in some measure to their ecological role. No significant differences were found among number and typology of the enzyme activities expressed by the isolates obtained from the soil sample from the halotolerant environment. The isolates investigated in this study showed diversified enzymatic patterns thus expressing different degrees of nutritional and ecological versatility. The strains showing various extracellular enzymes could be considered having high specificity and limited versatility. On the contrary, those having a more diversified enzymatic competence (9-10 activities) revealed a wide nutritional versatility (Fenice *et al*., 1997). As expected, the various enzymes related to the degradation of bio macromolecules (polysaccharides, lipid, proteins, nucleic acids) were very common. Lipolytic activity appeared to be the most widespread and, as already reported, it could be related to the degradation of some lipid components; in fact, after proteins, lipids are the most important fraction (Pogaku et al., 2010). In our samples 100% of the isolates revealed protease activity. Although many lipolytic microorganisms are already known, due to the huge interest on lipases at biotechnological level, some of the isolates studied here that apparently produced high level of activity merit further investigations. Microbial enzymes from the extreme environment have potential applications in industrial sectors. Bacteria from the clades of Gammaproteobacteria, Alphaproteobacteria and Firmicutes have been described previously. In this study, the isolated bacteria were the major producer of the hydrolytic enzyme. Halophilic enzymes are widely used in various fields, including the production of fermented food, leather industries, pharmaceutical, textile, and environmental bioremediation. These enzymes showed stability in solvents such as chloroform, toluene and benzene, which are used in various industries. Halophilic enzymes such as proteases, amylases, cellulases, nuceleases, xylanases, chitinases, lipases, tannases and alcohol dehydrogenases are useful for various industrial processes (Kai and Peisheng, 2016).

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