Production and Preliminary Optimization of Lipase Produced by *Pseudomonas Sp* AA1 Strain Isolated from Mangrove Sediment

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ABSTRACT ***Background and objective:*** *Production of bacterial based enzymes is found to be the more significant area of research due to their potential applications in broad range of industries. The objective of the present study was to identify a potent bacterial strain from the mangrove environment and the optimization of lipase enzyme.* ***Materials and Methods:*** *Bacterial samples were screened for potential lipase production. The potent isolate was biochemically characterized and the enzyme was optimised for salinity and pH.* ***Results:*** *Based on the biochemical test, the potential bacterial stain was Pseudomonas sp AA1. Lipase enzyme production was more at pH7 and salinity 5% condition.* ***Conclusion:*** *The lipase production was more at pH7 and salinity 5% condition. The bacterial species should be further characterised by 16S rRNA sequence. Further optimization of this enzyme production is needed.*

Keywords: *mangrove sediment, Bacterial stain, Pseudomonas, Lipase, optimization*

INTRODUCTION

Mangrove ecosystem shows diversity of microbes such as bacteria, fungi, actinomycetes *etc.* Bacteria include various types like nitrogen fixing bacteria, phosphate solubalizing bacteria, sulphate reducing bacteria, photosynthetic bacteria, methanogenic bacteria and enzyme producing bacteria[1]. Microbes have served and continue to serve as one of the largest and useful source of many enzymes[2]. Many enzymes from microbial source are already being used in various commercial processes[3]. Most of industrial microbial lipases are derived from fungi and bacteria. Lipases isolated from different sources have wide range of properties depending on their sources with respect to positional specificity, fatty acid specificity, thermostability, pH optimum, *etc*.,[4]. Veerapagu *et al.*[5] studied the isolation and identification of novel lipase producing bacteria (*Psuedomonas*) from oil spilled soil. Several studies were carriedout in isolation and identification of lipase producing bacteria from diverse samples[6,7,8]. Here we have screened some isolate of bacteria from mangrove sediment and test the lipase production as a preliminary study.

MATERIALS AND METHODS

Bacterial stain isolation

The soil sample was collected from mangrove forest of Vellar estuary, South East Coast of India. The sample were taken from surface sediment to a depth of 3-5 cm using sterile spatula to collect sample and transferred into sterile polythene bag and transported to the laboratory.

One gram of sediment sample was dissolved in 100 ml of nutrient broth, soil was enriched in nutrient broth after 1 day of incubation at 37ºC. Dilution was made by taking 1 ml of the enriched sample transferred into 5 ml of NaCl (physiological saline). Serially tenfold dilutions of mangrove soil sample was spread in to nutrient agar plate and incubated for 24 hrs. Morphologically dominant of total five isolates were selected for further study. All the isolates were assigned for specific number *i.e,* Mangrove sediment (MS-1, 2, 3, 4, 5). The selected colonies was maintained by sub-culturing on nutrient agar slants and stored at 4ºC for further use.

Gram staining

The smear was prepared and air dried then flooded with Gram's crystal violet for 1 minute. After washing with water Grams iodine was added for 1 minute. Followed by washed with water and decolourise with Grams decolouriser until no further violet colour comes off. Finally the counter stain, 0.5% Saffranine was added for about 1 minute. After washing with distilled water, the slide was observed under oil immersion. The lipase- producing bacterial strain was identified by standard procedures described in Bergy's manual of determinative bacteriology[9].

Citrate utilization test

This test was used to determine the ability of an organism to utilize sodium citrate as its only carbon source and inorganic ammonium salts as its only nitrogen source. Using a sterile loop the organism was inoculated into the Simmon's citrate agar slant tubes by means of the stab and streak method and incubated 37ºC for 24 hours. The colour change and growth indicates that the organism has utilize citrate as the sole source of carbon. No growth and no colour change indicates that the organism has not utilized citrate.

Indole production test

Using a sterile loop the organism was inoculated in peptone water in a test tube and incubated for 37ºC for 24 hours. After incubation, few drops of Kovac's reagent was added to the test tube. On adding the Kovac's reagent to the test tube there is red colour formation on the surface of medium indole positive. In the test tube there is no red colour formation indole negative.

Methyl red test

The test organism was inoculated into the MR broth in a test tube. The tubes were incubated at 37ºC for 48 hours. After incubation 5 drops of methyl red reagent was added to the test tube. The inoculated test tube developed a stable red colour, the organism is methyl red positive, remained in yellow in colour, the organism methyl red negative.

Voges proskauer test

The test organism was inoculated into the VP broth in a test tube. The tube was incubated at 35°C for 24 hours. After incubation 5% α-napthol and 40% potassium hydroxide was added. The tubes were gently shaken to expose the medium to atmospheric oxygen and tubes were left undisturbed for 10-15 minutes. The tube inoculated with organism developed red colour at the surface of the broth (within 15 minutes), the organism is VP positive. The tube inoculated with organism remained yellow in colour, the organism is VP negative.

Primary screening for lipase production

All the isolates were done by spirit blue agar. The plates were incubated for 2 days at 32ºC. The positive lipase activity was confirmed by clear zone around the bacterial colonies.

Optimization of lipase production in solid medium

To optimize the salinity for lipase production, the spirit blue agar plates were prepared at 1, 2, 3, 4, 5% salinity and incubated at 30°C for 24 hrs. After the incubation is over, the clear zone was formed on spirit blue agar plates and clear zone were measured. To optimize the pH of for lipase production, the spirit blue agar plates were prepared at the pH level of 4, 5, 6, 7 and 8 then incubated at 30°C for 24 hrs. After the incubation is over, the clear zones were formed on spirit blue agar plates and clear zone were measured.

RESULTS AND DISCUSSION

Numerous colonies of yellow, red and white were observed from mangrove sediment sample. From all the bacterial strains, the potent lipase producing was found as round white gram negative and designated as *Pseudomonas* sp AA1 strain (Plate 1) based on the other biochemical characterization.

Plate 1. *Pseudomonas* sp AA1 strain

Among the five various salinity concentrations, 5% salinity was showed higher zone (6mm) of clearance. The result is given in Fig.1. Among the five various pH conditions, pH7 showed higher zone (5.6mm) of clearance and the results are shown in Fig.2.

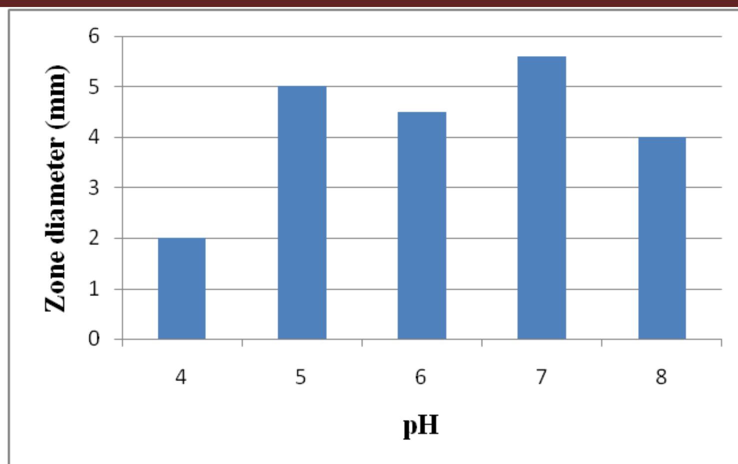

Fig. 2. The zone of enzyme activity at different pH at solid medium

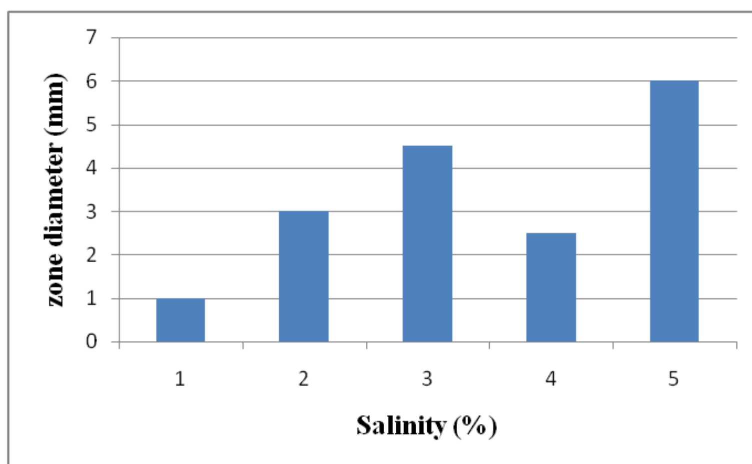

Fig.1 The zone of enzyme activity at different salinity at solid medium

As per the previous studies the bacterial lipase production was considerably more at pH 7 [5,9,10]. Some research have proved that the production also more in pH 8-9 [11,12,13]. In this study using *Pseudomonas* sp AA1 we have observed more liposae production in pH 7. Earlier, Sharma and Rathore *et al.*[9,14] confirmed the more protease production in 9% ,7.5% and 4.5% respectively. The effect of salinity on lipase production was investigated here and 5% salinity showed better result.

CONCLUSION

In this investigation, using *Pseudomonas* sp AA1, the lipase production was more at pH7 and salinity 5% condition. This is a preliminary study and in the next stage the bacterial species should be further characterised by 16S rRNA sequence. Further optimization of lipase production with carbon souse, temperature and other physical parameters are warranted.

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