Screening and Identification of Amylase Producing Bacteria from Marakkanam Saltpan Environment, Tamil Nadu, India.

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ABSTRACT :

Saltpans are an extreme environments, that inhabits organisms are survive in terribly high level of salinities, high temperatures and endure severe solar radiations. The present study was carried out to isolate, screen and optimize amylase enzyme producing bacteria from saltpan environment at Marakkanam. The results of present study was revealed that a total 25 bacterial strains were isolated of which 12 strains were produced significant amount of starch hydrolysis. Given value of zone of inhibitions are in the range of 1.2 mm to 13.1 mm. The isolated SUS3, strain was identified as *Bacillus subtilis* using Bergy's manual 1994. The present study disclosed that this thermo stable amylase producing strain could also be helpful industrial application particularly in food and beverages, animal feed, brewing textiles, detergents and health care.

Keywords: Amylase, *Bacillus subtilis*, pH, Saltpan, Temperature.

INTRODUCTION:

Saltpans are an extreme surroundings, that inhabit organisms that survive terribly high salinities, high temperatures and endure severe solar radiations. Therefore, these organisms might serve sources of novel secondary metabolites. Numerous halophilic and halo tolerant microbes inhabiting the salt pans are yet to be fully explored as potential producers of pharmaceutically important molecules. Few reports are available on their antimicrobial potential in India [1, 2].

Enzymes are thought of as natural bio catalysts that promotes specific chemical reactions. Most enzymes are produced by the fermentation of bio based materials [3]. Microbial enzymes are most popular to those from each plants and animal sources because they are inexpensive to produce and their enzyme contents are more expected, convenient to handle and reliable [4]. Enzymes are mainly performing in the conversion of macro molecules to body energy and new materials, also for growth, repair and cell maintenance. The source of enzymes is animal, plant and microorganisms; however, the industrial applications of commercial enzymes, microorganisms are the foremost vital source of assorted enzymes [5].

Amylase is one among such enzymes that are vital in the field of biotechnology. Apparently the primary enzyme produced industrially was amylase from a fungal basis in 1894. It was used as a pharmaceutical acid for the treatment of digestive disorders [6,7]. Amylases are derived from several microorganisms like fungi, yeast, bacteria and actinomycetes but affiliate of the genus *Bacillus* are heterogeneous species and that organisms are awfully versatile and their ability to the surroundings. Amylases are purified earlier from various bacillus species such as *Bacillus megaterium* [8] from *Bacillus subtilis* [9] from *Bacillus licheniformis* SPT 27. In view of above, the present investigation was made on isolation, screening, identification of amylase enzyme producing bacteria from saltpan environment at Marakkanam.

MATERIAL AND METHODS

Collection of sample:

The sediment samples were collected from Marakkanam saltpan environments (Latitude 12^0 14'.12" N, Longitude 079^0 56'.28" E). The collected sample were transferred in sterilized polyethylene bag and kept in portable ice box and transported to the laboratory for the further investigation.

Isolation of saltpan microbes

Isolation of microbe was done by using the halophilic agar in (Hi-Media, Mumbai) suspend 32.5 grams in halophilic agar in 100ml distilled water. The media aws boiled and dissolved the medium completely and then sterilized by autoclaving at 15Ibs pressure (121°C) for 15 minutes. Mix well and pour the sterile petri plates.

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Isolation of saltpan microbes are result expressed as CFU/ml or g [11]. Totally, 25 bacterial strains were isolated from saltpans environments. Isolated bacterial strains were stocked in nutrient agar sland.

Screening of Amylase producing strains

All isolated bacteria were tested for amylase production by starch hydrolysis. The 24hrs old isolated bacteria culture was transferred in to starch agar medium (peptone – 0.5 g, beef extract – 0.15, yeast extract – 0.15, NaCl – 0.5g, starch – 1g, Agar – 2g, and distilled water- 100 ml) and the plates were incubated at 37^{0}C for 48hrs. After 48hrs the animal pates flooded with iodine solution (iodine – 0.2%, KI- 0.4%, distilled water - 100ml), the highest zone of inhibition was recorded and selected bacterium species were identified by according to the Bergey's Manual of systematic Bacteriology [12].

Assay of amylase:

Assay of amylase was evaluated by using 3, 5 – dinitrosalicylic acid (DNS) method as described by [13]. By reacting 0.5 ml of enzyme to 0.5 ml of soluble starch and incubating the enzyme mixture at 37°C for 15 minutes. The reaction was stopped by addition of 1ml of DNS reagent and boiled for 15 minutes. The Absorbance was read at 540 nm and obtained results were compared with standard of amylase One unit of enzyme was defined as one micromoles of amylase released per ml of enzyme.

Effect of pH for Amylase:

One ml of the sample from the enzyme production medium was taken and centrifuged at 2000 rpm for 20 minutes. Then the 0.5 ml of supernatant was transferred to different test tubes. The supernatant containing enzyme was mixed with 0.5ml of 0.1M –Phosphate buffer was used at different pH 5, 5.5, 6, 6.5, 7, 7.5 and 8 incubated at 37 °C for 10 minutes. Then the enzyme activity was measured by DNS method.

Effect of temperature on Amylase activity

One ml of the sample from the enzyme production medium was taken and centrifuged. Then the 0.5 ml of supernatant was transferred to different test tubes at different temperatures (40, 45, 50, 55, 60, 65, 70 °C). The supernatant containing enzyme was mixed with then the enzyme activity was measured by DNS method.

RESULTS:

The present investigation, totally 25 bacterial strains were isolated from Marakkanam saltpan environment. Among these, 12 strains were acceptable for amylase production by starch hydrolysis (Table 1). Name of SUS1 to SUS25 maximum activity was observed on SUS3 (13.1mm) and minimum activity was recorded on SUS21 (1.2mm). The isolated SUS3, strain was identified as *Bacillus subtilis* Table 4.

The biochemical characterization of *B. subtilis* has been observed on the analyzed sediment sample. Morphologically it has rod in shape and brown color pigment. Positive results were showed on Gram stain followed by Starch production; Catalase, Indole production, Glucose and negative result were found on Citrate, Hydrogen sulphite, MR reaction, VP reaction, Lactose and Sucrose.

The maximum activity of amylase (6.23 U/ml) was observed in the fermentation medium adjusted at pH 6. These results are suggested that there is a stimulation of enzyme synthesis at neutral pH and that the higher enzyme production at this pH was a result of increased cell growth.

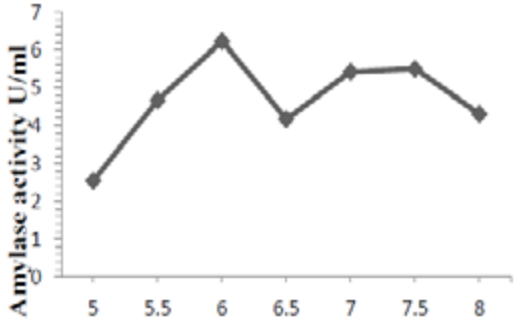
Enzyme production was gradually increased with increasing temperature and maximum enzyme production of 7.0 U/ml was observed at 45°C. Results show on Table 3.

Phenotypic name	Zone of inhibition (mm) enzyme activities
SUS1	2.3mm
SUS3	13.1mm
SUS6	4 mm
SUS10	6 mm
SUS16	3mm
SUS 17	10.5mm
SUS18	5.1mm
SUS20	5.8mm
SUS21	1.2mm
SUS22	1.3mm
SUS23	3.9mm
SUS25	3.5mm

Table 1: Starch hydrolysis test.

Different pH	Amylase activity U/ ml
5	2.54
5.5	4.67
6	6.23
6.5	4.17
7	5.42
7.5	5.50
8	4.31

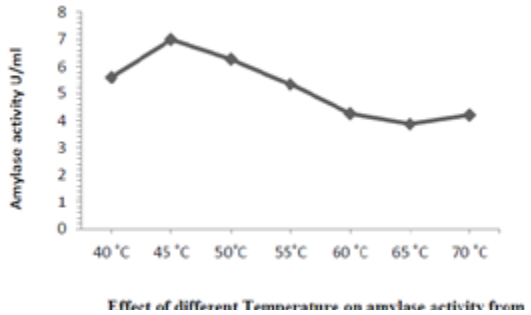
Table 2: Effect of different pH on amylase activity from *Bacillus*



Effect of different pH on amylase activity from *B. subtilis*

Different temperature ^{0}C	Amylase activity U/ml
40 ˚C	5.61
45 ˚C	7.0
50˚C	6.28
55˚C	5.34
60 ˚C	4.25
65 ˚C	3.87
70 ˚C	4.20

Table 3: Effect of different temperature on amylase activity from *Bacillus subtillus*



Effect of different Temperature on amylase activity from

S.No	Biochemical reactions	*Bacillus subtilis* SUS3
1	Morphology	Rod
2	Gram staining	+
3	Pigmentation	Brown
4	Starch production	+
5	Citrate (simmons)	-
6	Hydrogen sulphite (TSI agar)	-
7	Catalase	+
8	Indole production	+
9	MR reaction	-
10	VP reaction	-
11	Glucose	+
12	Lactose	-
13	Sucrose	-

(+) Positive (-) Negative , (TSI- Triple Sugar Ione), (MR- Methyl Red), (VP- Voges-proskauer)

Table 4: Biochemical characterization of amylase producing strains

Discussion:

Amylolytic enzymes were produced by many microorganisms. Industrial demand for the thermo stable enzymes was energy us to screen microorganism capable of producing significant thermo stable enzymes. The results of present investigation revealed that the significant amount of amylase production was occurred at 45 ^{0}C and pH-6. Compared to other ranges of pH and temperature investigators the earlier report have state that maximum growth and enzyme production was occurred between the ranges of temperature 50- 55 and pH 8-9 [14,15,16],. The present study optimum temperature and pH required for enzymes production was comparatively low. It might be due to the environment where the organisms was isolated or it may due to the mutation of the strains *B. subtilis* and it indicates that optimum temperature and pH for enzyme production was strain specific. Najafi *et al.* (2004), have recorded 590U/ml at 36h from marine *Vibrio* sp and the maximum enzyme activity found at 36h with this strain.

Conclusion:

The present study disclosed that this thermo stable amylase producing strain could also be helpful industrial application particularly in food and beverages, animal feed, brewing textiles, detergents and health care.

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