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Isolation of Amylase Producing Bacteria from Solar Salterns of Nellore District, Andhra Pradesh, India.

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Article

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ABSTRACT

α-Amylases are a class of starch degrading enzymes catalyzing the hydrolysis of internal α-1,4-O-glycosidic bonds in polysaccharides. The following investigation were carried out to isolate haloalkaliphilic bacteria, a group of organisms with twin extremities of pH and salinity, capable of producing α-amylases from an artificial solar saltern. A total of 25 discrete colonies were isolated, 21 isolates showed amylase production. Among these 7 isolates produced amylase at extreme conditions such as salt, alkalinity and temperature. The isolates were characterized biochemically and also for other enzymes. From the results it is imperative that these isolates can be further studied to exploit them up to industrial scale.

INTRODUCTION

α-Amylases (E.C. 3.2.1.1.) are starch-degrading enzymes that catalyze the hydrolysis of internal α-1,4-*O*-glycosidic bonds in polysaccharides with the retention of α-ano meric configuration in the products. Most of the α-amylases are metallo enzymes, which require calcium ions (Ca^{2+}) for their activity, structural integrity and stability. They belong to family 13 (GH-13) of the glycoside hydrolase group of enzymes [1]. Amylases are one of the most important industrial enzymes that have a wide variety of applications ranging from conversion of starch to sugar syrups, to the production of cyclodextrins for the pharmaceutical industry. These enzymes account for about 30 % of the world's enzyme production [2]. The α-amylase family can roughly be divided into two groups: the starch hydrolyzing enzymes and the starch modifying, or transglycosylating enzymes [3]. The enzymatic hydrolysis is preferred to acid hydrolysis in starch processing industry due to a number of advantages such as specificity of the reaction, stability of the generated products, lower energy requirements and elimination of neutralization steps [4]. Due to the increasing demand for these enzymes in various industries, there is enormous interest in developing enzymes with better properties such as raw starch degrading amylases suitable for industrial applications and their cost effective production techniques [5]. Owing to increase in demand for these versatile enzymes the focus has turned to halophilic bacteria, microorganisms that live under extreme saline concentrations [6].

In one such attempt the haloalkaline salterns of Nellore district which harbor a Extremophilic group of microbes termed 'halophiles' due to their resistance to wide range of salt concentrations, were chosen [7,8]. The salterns natural conditions of moderately hot temperatures of 40-45^{0}C around the year and an alkaline pH of 8 – 9.5, and salt concentrations nearing saturation, selectively allow the survival of halophiles, classified into salt tolerant (2-5%), moderately halophilic (5-20%), extremely halophilic (20-30%) based on their resistance towards NaCl concentration. As these halophiles have been reported to produce highly thermo-resistant amylases, [9] applied in a variety of industrial processes like in fabric treatment and saccharification reactions etc [10].

MATERIALS AND METHODS

Sample Collection

The sediment samples were collected from the saltern zone from a depth of 15 cm in salt brines near Muthukur of Nellore district of Andhra Pradesh. Having temperatures ranging around 40-45^{0}C, pH in excess of 8 salt concentrations nearing saturation.

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The samples were collected using a sterile Polythene bags and were transferred to laboratory, processed within 24 hours of collection. All the samples were stored in the refrigerator at 4^{0}C. Serial Sample dilution was carried out using sterile saline. Dilutions up to 10^{-7} were made to perform Spread plate technique. All the samples were placed on Modified Nutrient Agar, amended to mimic the natural sample site conditions.

Salt, pH and Temperature Tolerance

All isolates were screened for their salt tolerance efficacy level by growing them in nutrient broth amended with concentrations of salt (NaCl) ranging from 0% to 35% (saturation), growth was recorded, Absorbance against salt concentration. Modified Nutrient broth was prepared at different pH range to detect the tolerance level of the isolated strains. The flasks were incubated in a rotatory shaker for 24 hrs at 37^{0}C and the O.D value for each pH flask was noted. The temperature tolerance was determined by using modified nutrient medium at increasing temperatures and absorbance was recorded. The isolates growing optimally under "haloalkaline" conditions were selected, further purified and subjected to Amylase production at "haloalkaline" conditions and biochemical characterization.

Screening for Enzyme Production

Initial screening was done using modified Starch Agar medium amended for the detection of starch hydrolysis by the test isolates. Starch Agar plates were prepared by modifying NAM medium to 7% NaCl and pH of 8.5 and inoculated with the isolates for 24 hours at 40^{0}C. Iodine was poured in drops on to the plate and clear zones around the colonies were expected. The positive isolates in regard to zone of degradation were selected for further analysis [11].

Amylase Production under NaCl, Temperature And pH Influence

The positive isolates from the initial screening were further tested for the enzyme activity under different concentrations and degree's of NaCl, temperature and pH after 24 hrs incubation at optimal temperature and for temperature influence varying temperatures was employed. The enzyme activity was scored as degrees of positiveness (+, ++, +++, etc ;.)

Biochemical Characterization

Gram staining was performed to study the morphological characteristics of the selected isolates. Various biochemical tests were performed *viz.,* citrate utilization, production of Catalase, Oxidase, Indole, Urease, acid production and starch hydrolysis, gelatin liquefaction. Various carbon sources utilisation was screened [12].

RESULTS AND DISCUSSION

In the present study the Sediment samples from Muthukur solar salterns were collected and were serially diluted in saline and plated onto Modified Nutrient Agar medium. Dilutions from 10^{-4} to 10^{-7} were used for plating. The media was prepared using 7% (w/v) NaCl with other common ingredients of Nutrient Agar medium. The sub-cultured colonies showed interesting characteristics like fast growth rate and unique cultural characters and moderate salt tolerance up to 12%. Finally 14 strains producing α-amylase were isolated and maintained on Modified Nutrient Agar medium at 37^{0}C.The isolates ability to produce zone of degradation in starch agar medium were recorded as positeveness (+,++,+++,etc;.). The enzyme activity at different influencing factors and biochemical characterization results were tabulated in Table1 and Table.2

The selected strains were based on their unique amylase production over a range of limiting factors and were further studied for different biochemical characters. The selected isolates were analysed for different carbon source utilisation and other enzymes like lipase, protease, cellulase, chitinase and pectinase were screened. The results are tabulated in Table 3 and Table 4 respectively. The isolates showed growth and Amylase production at moderately limiting conditions of pH, Salt and Temperature which can be exploited for their industrial applications

CONCLUSION

α-amylases are one of the most widely used enzymes required for the preparation of fermented foods [13]. Apart from food and starch industries [14], in which demand for them is increasing continuously, they are also used in various other industries such as paper and pulp, textile, *etc* [15]. With increase in its application spectrum, the demand is for the enzyme with specificity [16]. Research is focused on developing thermo tolerant and pH tolerant a-amylase from microbes, modifying them genetically or applying site-directed mutagenesis to acquire desired properties in the enzyme. Commercially most of the production of a-amylase is carried out in submerged fermentation, but solid-state fermentation is being looked at as a potential tool for its production, especially applying agro-industrial residues as substrate.

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Table 1: Amylase production screening for haloalkaliphilic isolates

Starch hydrolysis	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14
pH 6	+	+	+	+	+++	+	+	+++	+	+	+	+	+++	++
7	+	+	+	+	+++	+	+	+++	+	+	+	+	+++	+
8	++	++	++	+++	++	++	++	++	+++	+++	++	++	+	++
9	++	++	+	++	++	+	++	+	+	++	++	+	+	++
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Temperature 10^{0}C	-	-	-	-	-	-	-	+	-	-	+	-	-	+
20^{0}C	+	+	+	+	+	+	+	+	+	+	+	++	+	+
30^{0}C	++	++	++	++	+	+	+	+	+	+	++	++	++	++
35^{0}C	++	++	++	++	++	++	++	+	++	++	+	++	+	++
40^{0}C	++	+	+	++	++	+	+	++	+	++	+	++	+	+
50^{0}C	+	+	+	+	+	+	+	-	+	+	-	-	+	-
NaCl % 5%	++	++	++	++	++	++	+++	+++	+++	+++	++	++	+++	++
7%	+	+	++	++	++	++	+	+	++	++	++	++	++	+
10%	-	-	+	+	+	+	-	-	++	+	-	+	+	-
15%	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-- = negative, + = low, ++ = medium, +++ = fastidious.

Table 2: Biochemical characters of the selected isolates

S.NO	AA5	AA8	AA9	AA10	AA11	AA13	AA14
Citrate	+	-	+	+	-	+	-
Urease	+	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+
Oxidase	+	+	-	-	-	+	-
Methyl red	+	+	+	-	+	+	+
Vogues proskauer	+	-	-	+	-	+	+
Indole	-	-	-	-	-	-	-
H2S production	-	-	-	-	-	-	-

+positive, - = negative.

Table: 3 Carbohydrate Utilization of selected isolates

S NO	Lactose	Sucrose	Dextrose	Fructose	Maltose	Galactose	Ribose	Mannitol
A5	-	+	+	+/-	-	-	-	-
A8	-	-	-	-	+/-	-	-	-
A9	-	+	+	+/-	-	-	-	-
A10	-	+	+	+	-	-	-	-
A11	-	-	-	-	-	-	-	-
A13	-	+/-	+	+/-	-	-	-	-
A14	-	-	+	+/-	-	-	-	-

-- = Negative; + = positive

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Table: 4. Different enzyme production ability of the selected isolates.

S. No/ Enzyme	Lipase	Protease	Cellulase	Chitinase	Pectinas
A5	-	+	+	-	-
A8	-	+	+	-	-
A9	-	-	+	-	-
A10	-	+	+	-	-
A11	-	-	+	-	-
A13	-	+	+	-	-
A14	-	+	+	-	-

-- = Negative; + = Positive.

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