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Research Article

ISOLATION AND SCREENING OF AMYLASE PRODUCING BACTERIA FROM DIFFERENT MARKET SOILS

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ABSTRACT

The objective of present study was to isolate and screen amylase producing bacteria from soil samples collected from local markets. Total sixteen bacterial colonies were isolated and streaked on starch agar plate and incubated at 37ºC for 48 hours, after which four isolates showed clear zone around the colony indicating amylase activity. This was followed by morphological, biochemical test and MALDI-TOF-MS analysis. Among the four isolates (T1, T2, T3, T4), T2 isolate produced highest amylase activity, identified as *Pseudomonas lundensis* DSM 625. The optimization parameters like pH, temperature, incubation time was observed and noted a maximum activity of 28.58 U/ml at pH 7 and temperature for maximum activity (24.25 U/ml) was found to be 30°C in 24 hrs. The unknown concentration of crude amylase was determined using maltose standard curve. These parameters were carried out for rest of the 3 isolates and values recorded which showed similar trend as that of T2 isolate. Hence, these bacterial isolate can be used as biotechnological tool for industrial purpose.

Key words: Amylase, Bacteria, pH, temperature, incubation time.

INTRODUCTION

Microbes are important in human culture and health in many ways, serving to ferment foods, treat sewage, produces fuel, enzymes and other bioactive compounds (Tyrell and Kelly, 2017). Microbes have become increasingly important as producer of industrial enzymes. However, about less than 50 species are actually used to produce the entire list of microbial enzymes of commercial importance (Sundarapandiyan and Jayalakshmi, 2017).

Several microorganisms have been studied for amylolytic enzymes production, with species of Aspergillus, Rhizopus, and Bacillus genera being the most used in

industrial processes (Silva *et al*., 2009). Enzymes that catalyze the hydrolysis of starch are used in the production of maltodextrins, modified starches, glucose, and maltose syrups and can be applied in several industrial processes, such as the production of biofuels, food, pharmaceuticals, detergents, beer, bread, and animal food (Oliveira *et al*., 2016). Factors such as pH, temperature, and carbon and nitrogen sources also play vital roles in the rate of amylase production, particularly in fermentation processes. Because microorganisms are amenable to genetic engineering, strains can be improved for obtaining higher amylase yields (Sundarram and Krishnamurthy, 2014).

Amylases are significant enzymes for their specific use in the industrial starch conversion process. Amylolytic enzymes act on starch and related oligo- and polysaccharides (Pandey *et al*., 2000). The enzymes have been produced by a wide range of microorganisms and substrates and categorized as exo-, endo-, de-branching and cyclodextrin producing enzyme (Singh *et al*., 1995).

Microbes can also be exploited for the production of various enzymes, especially antioxidant enzymes. This enzyme can be used therapeutically for the treatment of various diseases related to oxidative stress (Radha and Chandra, 2017). Microbial amylase has almost surpassed the synthetic sources in different industries (Pandey *et al*., 1999). Amylase are hydrolyzing enzyme which causes hydrolysis of molecules.

Amylases are proven essentially vital in the field of Biotechnology due to its uses (Burhan *et al*., 2003). In this study, we aimed to isolate newer source of extracellular amylase from the local market soil samples.

MATERIALS AND METHODS

Source of sample collection

Four different markets, namely, local fruit market, local vegetable market, local fish market and local market selling corn were visited in Puliampatti, Erode district, Tamil Nadu to collect soil samples. Hand full of soil was collected and packed in polythene bags and brought to the lab for further studies.

Isolation of Amylase producing bacteria

One gram of soil sample was weighed and serial dilution (10^{-2} to 10^{-6}) of each soil samples were carried out and spread on nutrient agar plate (37˚C, 24 hr). After incubation the colonies were streaked on starch agar plate and incubated for 37˚C for 48 hr and flooded with 1% iodine to observe the zone of hydrolysis for each sample. The colony showing highest zone of hydrolysis was selected for further study.

Identification of Bacteria

Morphological Test

The identification of bacteria was carried out by morphological studies.

Biochemical Test

Indole test, Methyl red, Voges-Proskauer, Citrate utilization, H_2S production, starch hydrolysis, urease production and nitrate reduction was done.

MALDI-TOF-MS analysis

The organisms were further confirmed using MALDI-TOF-MS analysis (Singhal *et al*., 2015).

Qualitative test for protein

To identify the crude sample is protein in nature, Ninhydrin test was done.

Quantitative assay of protein

The total protein content of the samples was determined by Lowry's method, using Bovine Serum Albumin (BSA) as a standard (1mg/ml).

Crude enzyme preparation

The protease producing bacterial colony was inoculated in casein broth medium, incubated at 37˚C for 48 hrs. After filtration, the filtrate was subjected to centrifugation (10,000rpm for 10 minutes). The supernatant was used as crude enzyme preparation for further studies.

Amylase activity assay

To study the amylolytic activity, the supernatant was used as the enzyme source and the assay was carried out using DNS method.

Calculation:

$$\text{Amount of Maltose} = \frac{\text{concentration of standard maltose}}{\text{OD value of standard}} \text{ X unknown OD}$$

$$\text{Enzyme Activity (U/ml)} = \frac{\text{micromole maltose equivalent releases X total assay volume (ml)}}{\text{vol.of enzyme assay (ml)X time of assay (min)X vol. in calorimeter(ml)}}$$

$$\text{Specific activity} = \frac{\text{Enzyme activity in U/ml}}{\text{Total protein content in mg/ml}}$$

Specific activity is the activity of an enzyme per milligram of total protein (expressed in µmol/min/mg). It is the amount of product formed by an enzyme in a given amount of time under given conditions per milligram of total proteins. The specific activity U/mg was calculated using formula given in the calculation.

Effect of temperature and incubation time on enzyme activity

The following temperatures were chosen for optimization 30˚C, 40˚C and 50˚C and incubated for 24, 48 and 72 hrs. The contents were then centrifuged at 10,000 rpm at 4˚C for 10 min. Enzyme activities were determined by standard enzyme assay.

Effect of pH on enzyme activity

This was observed using different pH ranging from 7 to 9. It was incubated at 37˚C for 24hr. The contents were centrifuged at 10,000 rpm at 4˚C for 10 min. Enzyme activities was determined by standard enzyme assay.

RESULTS AND DISCUSSION

Isolation and screening of bacteria

Among the 16 isolates, 4 isolates showed maximum zone of hydrolysis around the streaked lines on starch agar plate. Those 4 bacterial isolates were selected for amylase production in fermentation broth. Zone of hydrolysis was obtained on starch agar plate.

Bacterial identification and characterization

Morphological and Biochemical test

The following morphological and biochemical tests were carried out to confirm the species which is elaborately shown in the Table 1.

Table 1. Morphological test and Biochemical Test

S. No	Indole	Methyl Red	Voges-Proskauer	Citrate utilization	H_2S	Motility	Gram staining	Shape	Organism
T1	N	N	N	P	N	P	N	Round	*Acinetobacter johnsonii*
T2	N	N	N	P	N	P	N	Rod	*Pseudomonas lundensis*
T3	N	N	N	N	N	P	P	Rod	*Arthrobacter polychromogens*
T4	N	N	N	P	N	P	P	Rod	*Pseudomonas fragi*

N= Negative P=Positive

Further these isolates were confirmed using MALDI–TOF–MS, which confirmed the bacterial genus and species with their identification numbers.

Qualitative test for protein

The present study showed that all the 4 samples were protein due to the presence of purple colored product obtained in Ninhydrin test. This test gives color reactions on the basis of the composition of different amino acids present in the sample.

Quantitative assay for protein

The amount of protein present in the crude samples was estimated by Lowry's method using BSA as a standard. Rupali, 2015 used this method to prove the quantity of protein in the samples.

Amylase production by bacterial isolates

The activity of amylase was studied using 3, 5-dinitrosalicylic acid. The specific activity of any enzyme can be defined as the activity of an enzyme per milligram of total protein (expressed in µmol/min/mg), a standard curve using maltose was plotted was shown in Figure 1.

Effects of temperature and incubation time on isolates

The temperature range from 30˚C to 50˚C was chosen. Incubation was done for 24, 48 and 72 hours of duration. From four different isolates, *Pseudomonas lundensis* showed maximum activity at 30˚C. The maximum activity of enzyme was found at 30˚C in 24 hrs as shown in the figures 2 & 3.

The similar trend was observed in *Pseudomonas fluorescens, Bacillus subtilis and E.coli.* The activity of the enzyme was reduced to half at the third day of incubation (72 hr). It was proved by Alariya *et al*., in 2013 that as the incubation time increases, the enzyme activity gets reduced.

Similarily, incubation at 30ºC for 24hrs in *Bacillus subtilis,* the enzyme yield was 370 U/mg (Nanganuru *et al.*, 2012).

Effects of pH

To optimize the pH, range from 6 to 8 were chosen in this study. The activity is shown in figure 4. Among the four isolates, all have shown maximum activity at pH 7 which gradually reduced in the alkaline pH

at 8. However the activity was reduced by half in the acidic pH of 6 by all the four species namely *Acinetobacter johnsonii, Pseudomonas ludensis, Arthrobacter polychromogens* and *Pseudomonas fragi.*

For many enzymes it is clearly proved that the activity was maximum at the pH of 7 (Viswanathan *et al.,* 2014). Similar result was observed in *Bacillus subtilis* at a pH of 7 as it produced 20 U/ml/min of enzyme (Sundarapandiyan and Jayalakshmi, 2017). It was also proved in another similar study that amylase production by *Bacillus sp* was found to be maximum at pH 7 and produced 26.5U/ml/min of enzyme (Vasantha and Hema, 2012).

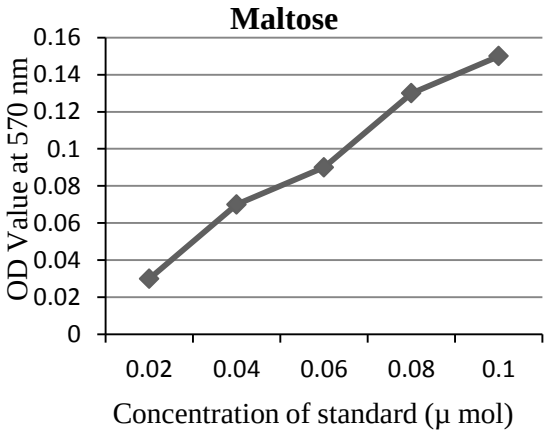

Figure 1. Maltose standard curve

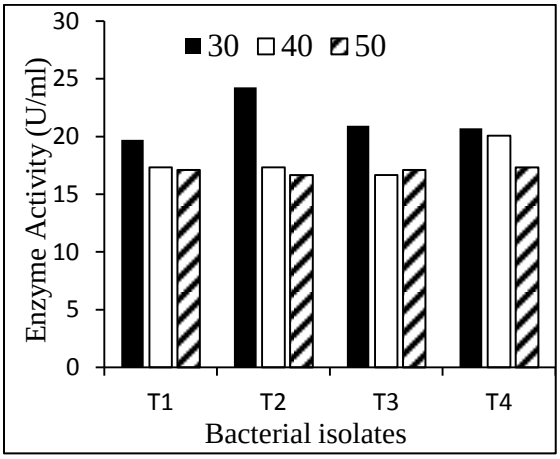

Figure 2. Effects of temperature

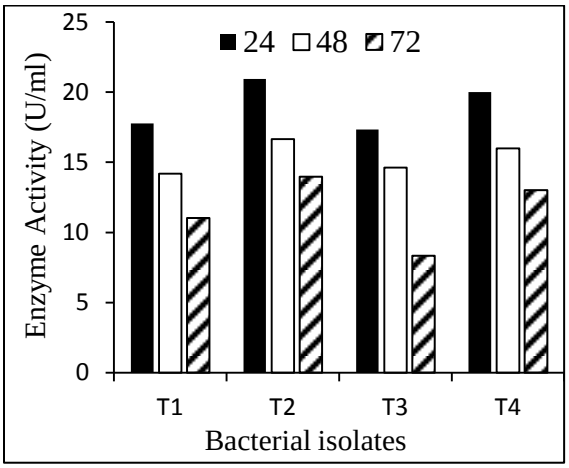

Figure 3. Effects of incubation time

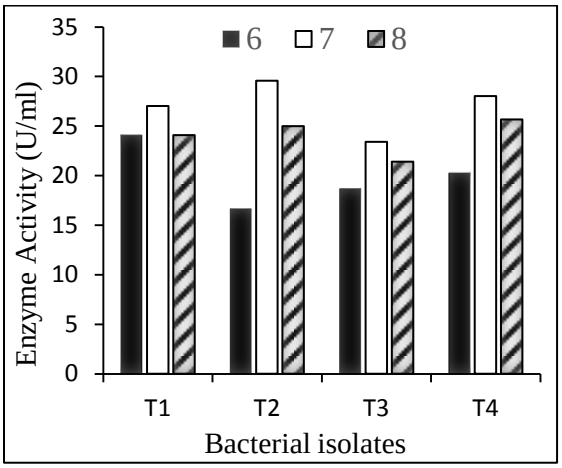

Figure 4. Effects of pH

CONCLUSION

The microorganism isolated from different local market soils showed the potential for the production of enzyme amylase and optimized with varying condition of pH, temperature and incubation time. The amylase producing bacteria was optimized, the maximum activity was found in *Pseudomonas lundensis* at 30ºC in 24 hours at pH 7. The rest of the isolates

showed optimal results. These bacterial isolates with considerable amylase activity can be further purified for their use in various industrial purposes. Therefore these isolates can be used as an alternative source in the production of amylases in Biotechnology based industries.

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