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Comparative Analysis of α-Amylase Activities of Different *Bacillus species* Isolated from Various Compost Materials and Process Optimization

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This work has been carried out for the isolation of different *Bacillus* species isolated from various compost materials and their α-amylase activites were optimized and compared. Different compost materials such as agriculture compost, garbage waste and vegetable waste were selected for isolation of *Bacillus* species on the basis of their morphological and biochemical characteristics. Out of ten *Bacillus* species, four species were selected on the basis of amylase test. Among these four species, *Bacillus coagulans* showed the highest amount of amylase activity of 8.4 U/ml at 35°C and pH 7.0 after 24 h incubation period.

Keywords: *Bacillus,* α-amylases, compost, waste.

α-amylases (EC:3.2.1.1) are extracellular enzymes which hydrolyze starch into wide range of products such as glucose and maltose or specific malto-oligosaccharide or mixed maltooligosaccharides. The enzyme hydrolyzes the internal α -1,4 linkages in starch in a random fashion leading to the formation of soluble maltodextrins, maltose, and glucose. These enzymes account for about 30% of the world's enzyme production and have a great significance with extensive biotechnological applications in bread and baking, food, textile, and paper industries (Sivaramakrishnan *et al.*,, 2006 and Gutpa *et al.*, 2003).

Although amylases can be derived from several sources, including plants, animals and microorganisms, microbial enzymes, generally meet industrial demands (Pandey *et al.*, 2000). Today a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry. In spite of the wide distribution of amylases, microbial sources, namely fungal and bacterial amylases, are used for the industrial production due to advantages such as cost effectiveness, consistency, less time and space required for production and ease of process modification and optimization (Burhan *et al.*, 2003).

Sources of amylases in bacteria, yeast and other fungi have been reported and their properties described by (Chi *et al.*, 2007; Gupta *et al.*, 2008; Liu and Xu, 2008). Now a days the new potential of using microorganism as biotechnological sources of industrially relevant enzymes has stimulated interest in exploration of extra cellular enzymatic activities in several microorganisms (Akpan *et al.*, 1999; Bilinski and Stewart, 1995; Buzzini and Martini, 2002). These enzymes are found in animals (saliva, pancreas), plants (malt), bacteria and molds (Abu *et al.*, 2005). Sources of amylases in yeast, bacteria and molds have been reported and their properties have been described..Compost is the

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decomposition of organic matter such as kitchen scraps, wood shavings, yard trimmings, paper and cardboard. Composting is the natural process of 'rotting' or decomposition of organic matter by microorganisms under controlled conditions. Compost supplies nutrients and develops better soil structure, and helps prevent overflow that can contaminate water streams such as rivers and lakes. Compost helps the soil soak up and preserve nutrients and provides plants protection from pests and diseases. Compost introduces and feeds diverse life in the soil, including bacteria, insects, worms, and more, which support vigorous, plant growth. In this paper different *Bacillus* species were isolated from various compost materials and their α-amylase activites were optimized and compared.

MATERIALS AND METHODS

Collection of compost samples

For the present study, four different compost samples collected from various places of Raipur. The compost samples collected are agriculture compost, garbage waste and vegetable waste.

Isolation and characterization of microbes

The bacterial species were isolated from each compost samples using serial dilution method. 1 mg of the compost sample was added to 9 ml sterilized water in test tube 1 to make a 1/10 fold dilution. Then 1ml of the diluted sample was transferred from the tube 1 to tube 2 to make it $1/10^2$ dilution. Subsequent dilutions were made up to $1/10^{10}$ dilution (tube 10). 0.1 ml of the diluted sample from tube 10 was inoculated in to tryptone soya agar plate and incubated overnight for single colony isolation. Plates containing well separated colonies were streaked on another plate to obtain pure culture of isolated colonies. Gram's staining, microscopic observation, growth characteristics and biochemical tests of the isolated bacteria were studied (results not shown). All the biochemical tests were performed and the organisms were identified as *Bacillus sp.* according to the Bergey's Manual of systematic Bacteriology (Williams *et al.*, 1989).

Preparation of inoculum

The bacterial isolates were transformed from stock culture to100 ml nutrient broth. The inoculated flasks were incubated overnight at 37°C. The broths were then centrifuged at 10,000Xg at 4°C for 10 min. After centrifugation, the pellets were suspended in 10 ml sterile water and the absorbance was recorded at 660 nm to have a suspension of 4.5 x 100000 cells ml^{-1}.

Media composition for fermentation

The media for submerged fermentation composed of 1.5% peptone, 0.5% soluble starch, 0.3% di-potassium hydrogen phosphate and 0.1% $MgSO_4.7H_2O$. 1 ml of the bacterial cell suspension was inoculated into 100 ml of the medium in a conical flask and allowed to ferment for 96 h. The broth was centrifuged at 10,000xg for 15min. The supernatant obtained was used as the source of enzyme.

Assay of amylase

Estimation of amylase activity was carried out according to the 3, 5-Dinitro salicylic acid (DNSA) method of Miller (1959). The assay mixture consisted of 1ml of phosphate buffer (pH 6.5), 100µl 1% starch solution and 1ml of enzyme extract. The reaction mixture was incubated for 20min. The reaction was stopped by addition of 0.5 ml DNSA reagent and cooled in a water bath. 2.5ml distilled water was added to the mixture and the absorbance was read at 540 nm using an UV-Visible spectrophotometer (SL-218, ELICO) against glucose as the standard. One unit of amylase activity was defined as the number of ì moles of glucose liberated by 1 ml of enzyme solution per minute under assay condition.

Optimization of enzyme activity

To determine effect of pH, temperature and incubation temperature on amylase activity were studied. The pH of the medium was varied between 4 to 10 to find the effect of pH on amylase production in bacteria. The incubation temperature and time were also varied. The incubation temperature of the culture flasks varied in between 20-50°C. The culture flasks were incubated for 6, 12, 24, 36 and 48 h. All the experiments were performed with three replications and the mean values are presented with ±SD.

RESULTS

Physico-chemical parameters of the sample

No drastic variation was observed in the physico-chemical parameters of the compost

samples used for isolation of amylase producing microorganisms. The pH of the compost samples varied between 5.5 -8.0 where as the temperature varied between 40-44 °C (Fig.1)). The aerobic plate count (APC) measured in term of colony formic units (CFUs/ml) varied for different compost samples (Fig.2)

Isolation and identification of the isolates

A total of ten isolates of bacteria were obtained from the three different compost samples. All the bacterial isolates were identified on the basis of their Gram reaction, and Biochemical tests. The studies of morphological, physiological and biochemical characteristics of *Bacillus* species showed yellow spore chain, smooth spore surface, production of divisible pigment and melanin on tyrosine agar, reduction of nitrate to nitrite, utilization of phenylalanine and no growth at 45 °C indicated it to belong to *Bacillus sp*. Based on the amylase test four different *Bacillus* species were identified out of the ten isolates (Table.1).

Amylase activity

The OD value of glucose taken by the spectrophotometer plotted against the concentration of glucose gives straight line indicating the standard curve for the test. This

Table 1. Nomenclature of bacterial isolates

S No.	Name of bacterial isolates	Symbol used
1	*Bacillus pantothenicus*	Bb1
2	*Bacillus coagulans*	Bb2
3	*Bacillus stearothermophilus*	Bb3
4	*Bacillus licheniformis*	Bb4

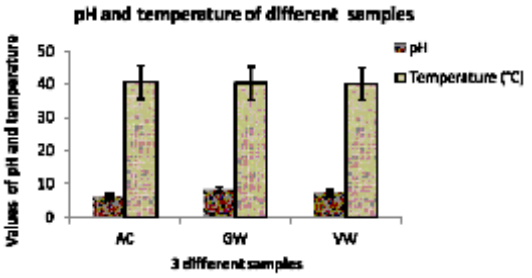

Fig.1. Variation of pH and temperature among compost samples

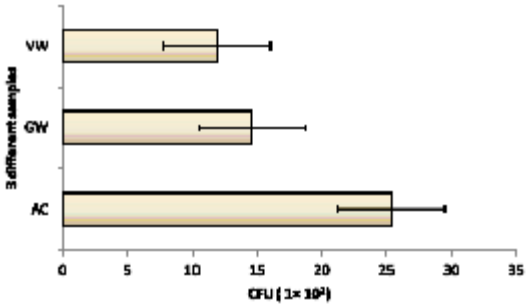

Fig. 2. Bacterial enumeration among various compost samples

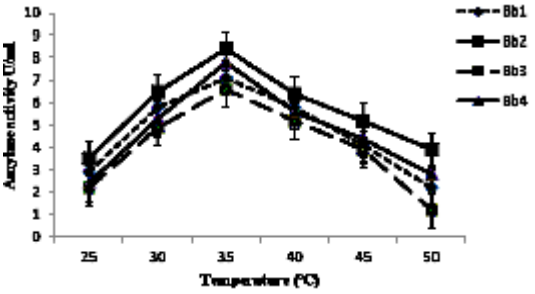

Fig. 3. Amylase production under different incubation temperatures

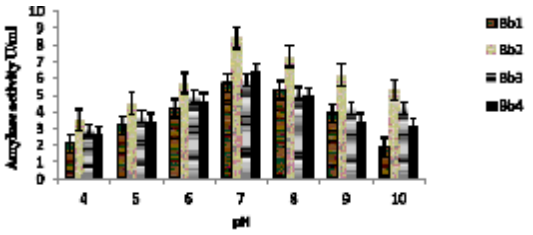

Fig. 4. Effect of pH on amylase activity

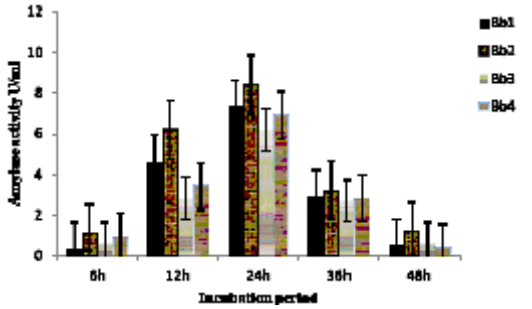

Fig. 5. Amylase production under different incubation periods

standard curve was used to find out the amylase activity unit throughout the study. The amylase activity of these four bacterial species were evaluated at various pH, temperature and incubation period (Fig.3,4,&5 Among 4 species, *Bacillus coagulans* showed the highest amount of amylase activity of 8.4 U/ml at 35°C and pH 7.0 after 24 h incubation period.

DISCUSSION

Our present study reflects the isolation and application of *Bacillus* species in various industrial sector as far as the amylase enzyme point is concerned. *Bacillus coagulans* showed the highest amount of amylase activity of 8.4 U/ml at 35°C and pH 7.0 after 24 h incubation period. Other species were also showing amylase activites having lesser amount. Several authors have been also reported the presence of α- amylase enzyme in *Bacillus spp*.(Bernfeld, 1955; Aiyer, 2004; Ajayi *et al*., 2006)..

These studies will definitely help towards the exploration of more enzymes needed for food and pharmaceutical sectors beneficial to the mankind in a sustainable manner. These organisms can be further exploited for the presence of other industrial important compounds which would be a great benefit to biotechnological industry.

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