Characterization of thermophilic bacterium: a compost pile isolate.

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Abstract

Starch degrading Gram positive rod shaped bacterium was isolated from compost pile. A Morphological and biochemical characters of strain indicates that it belongs to the genus *Bacillus*. Amylolytic activity of the strain recorded was 2.4 U/ml. Optimum catalytic activity of the strain was recorded at pH 8 and temperature at 40^{o}C.

1. Introduction

Temperature is one of the most important variables in cultivation and applications of microorganisms. Classification of microorganisms is therefore based on their relation to temperature and that has therefore always been considered as one of the most basic elements of biological system and divided into three classes: Psychrophiles, Mesophiles and Thermophiles. Thermophiles are organisms which are capable to thrive at relatively high temperature i.e. from 41 to 122^{o}C. On the basis of temperature optima thermophiles are classified into three major classes; Moderate thermophiles can grow at temperature range of above 50^{o}C where as Hyperthermophiles are organisms which are able to grow at very high

temperature ranges i.e. 80 to 105^{o}C. Thermophiles can be isolated from various natural and manmade habitats like composts, sun-heated soils, terrestrial hot springs, submarine hydrothermal vents and geothermally heated oil reserves and oil wells. (Satyanarayna et al 2005).

Thermophiles are the source of industrially important thermostable enzymes due to inherent flexibility, stability, high activity, specificity. Thermophilic amylases can be used in several biotechnological industries such as detergent, agricultural, starch liquefaction, saccharification, textile, paper, food, baking, novel food applications and analysis in medical and clinical chemistry (Pathak AP and Rathod MG 2016)

Thus isolating and manipulating thermophilic amylase producing bacteria from compost pile has manifold importance for various biotechnology industries.

Keywords: Compost pile, Thermophiles, amylolytic activity, *Bacillus carboniphilus.*

2. Materials and Methods

2.1.Isolation and screening of thermophilic amylolytic bacteria

Compost sample was collected from compost pile of Vishnupuri area, Nanded, Maharashtra, India. One gram of compost was weighted and diluted in 100 ml of sterile distilled water and allowed to decant for 2 hours. 100 µl of the suspension was spread on nutrient agar plates and incubated at 50^{o}C for 24 h. Incubated plates were observed and isolated colony was selected for further experiments. Selected isolate was inoculated on starch agar plates to confirm amylolytic activity of bacterium. (Pathak et al 2015; Pathak AP and Rathod MG 2015; Rathod MG and Pathak AP 2014; Hingole SS and Pathak AP 2013; Pathak AP and Sardar AG 2012).

2.2.Identification of isolate

The selected isolate was subjected for gram staining and motility. Carbohydrate utilization profile of isolate was studied by inoculating pure culture in basal nutrient medium in which additional carbohydrates such as sucrose, fructose, maltose and lactose were used. IMViC test and Catalase test were also carried out (Gavali JT and Pathak AP 2016; Polkade et al 2015; Sharma et al 2015; Sonalkar et al 2015; Sharma et al 2009).

2.3.Production and Extraction of enzyme

1 ml of 24 h active culture of isolate was inoculated into 100 ml production medium containing Yeast Extract 5 g, Tryptone 5 g, Sodium Bicarbonate 5 g and dried potato powder 50 g. The production was carried out at 40^{o}C for 72h at 120 rpm in orbital shaking incubator. The whole fermented broth was filtered through Whatman filter paper 41µm.The filtrate was then used to determine enzyme assay (Pathak AP and Rathod MG 2016; Pathak et al 2014; Khairnar et al 2012).

2.4.Enzyme Assay

Amylolytic activity of thermophilic bacterium was estimated by the analysis of reducing sugar released during hydrolysis of 1% (w/v) starch in 0.1 M sodium citrate buffer by the Dinitrosalicylic acid (DNS) method. One unit of amylolytic activity was defined as the amount of enzyme that releases 1mMol of reducing sugar as glucose per minute under assay condition (Pathak AP and Rathod MG 2016; Pathak et al 2014; Khairnar et al 2012)

2.5.Characterization of starch degrading enzyme

2.5.1. Effect of pH and temperature on catalytic activity of enzyme

In order to determine optimum assay pH and temperature for catalytic activity, the assay was carried out at various pH ranging from 3 to 9 and temperature from 10 to 60^{o}C respectively. (Pathak et al 2014; Gavali and Pathak 2015; Pathak AP and Rathod MG 2014).

3. Results and Discussion

3.1.Isolation, screening and identification

The selected isolate TS1 was gram positive, motile, rod shaped, spore forming bacterium having white, circular, opaque colony with flat elevation and sticky consistency. Based on colony morphology, microscopic and biochemical characters TS 1 was identified as *Bacillus carboniphilus.*

Results of morphological characters, staining features and biochemical characters are given in table 1.

Table 1: Morphological, Microscopic, Biochemical and Physiological Characterization of isolate TS 1

Colony characteristics	**Isolate**	**IMViC Test**	**Isolate**
Shape	Circular	Indole test	-
Size (mm)	0.3	Methyl red test	+
Color	White	VP test	-
Elevation	Flat	Citrate utilization test	-
Margin	Regular	**Sugar utilization pattern**	
Surface	Smooth	Maltose	-
Consistency	Sticky	Fructose	-
Opacity	Opaque	Sucrose	-
Microscopic Features		Lactose	-
Cell Shape	Rod	**Enzyme profile**	
Cell size (Micron)	0.5	Catalase	+
Cell Motility	Motile	Amylase	+

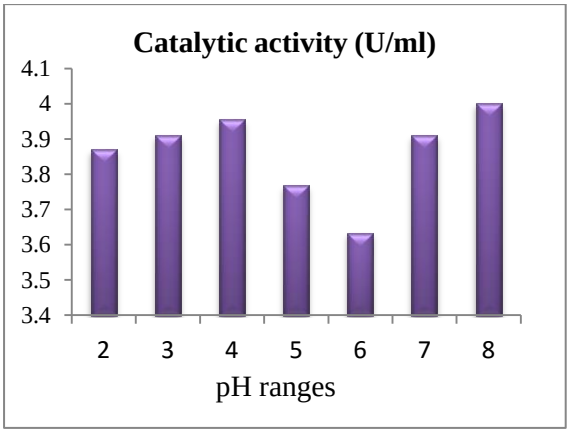
Grams nature	+ve	Protease	+
Sporulation	Spore former	Lipase	+

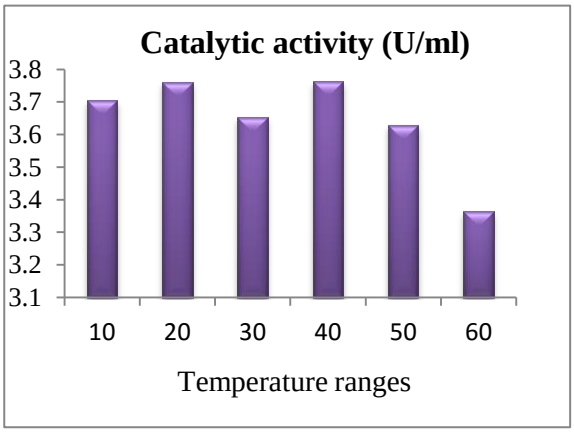
3.2.Production of thermostable enzyme

Starch degrading amylolytic activity of *Bacillus carboniphilus* was recorded was 2.4 U/ml of after 72 h of production period.

3.3.Characterization of crude thermostable enzyme

Bacillus carboniphilus showed optimum catalytic activity of its crude thermostable starch degrading enzyme at pH 8 and temperature 40^{o}C.





4. Conclusion

Potent thermophilic amylolytic bacterium was isolated from compost pile and identified as *Bacillus carboniphilus*. Thermozymes are one of the most widely used enzymes required for the preparation of fermented foods. Apart from food and starch industries, these organisms can be used in various other industries such as paper and pulp, textile, etc.

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