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ISOLATION, CHARACTERIZATION OF CELLULASE PRODUCING *LYSINIBACILLUS SPHAERICUS* MTCC NO. 9468 FROM GUT OF *EISENIA FOETIDA*

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ABSTRACT
Twenty bacteria were isolated from the gut of earthworm (*Eisenia foetida*)**.** Cellulolytic enzyme activity of the cultures from the gut was screened on CMC (Carboxy Methyl Cellulose) medium. All the isolates were found positive for cellulase production. Among the twenty isolates, EGB1 isolate which showed maximum activity was categorized as *Lysinibacillus sphaericus* with MTCC No. 9468 (formerly *Bacillus sphaericus*) based on cultural, morphological, physiological and biochemical characteristics.

INTRODUCTION

Cellulose is the major polysaccharide constituent of plant cell wall and one of the most abundant available organic compounds in the biosphere. Cellulose serves as a vast reservoir of glucose residues linked by β - 1,4 glycosidic bonds. The conversion of cellulosic mass to fermentable sugars from cellulolytic organisms has been suggested as a feasible process and offers potential to reduce use of fossil fuels and reduce environmental pollution (Lynd *et al.,* 2002).

A great deal of research on the enzymatic degradation of cellulose and hemicellulose from different substrates has been developed in the last two decades. Actinomycetes degradation of cellulosic biomass in agricultural wastes plays a vital role in carbon recycling. Treatment of cellulose by cellulolytic enzymes for practical purposes has attracted the continuing interest of biotechnologists. A number of biomass conversion methods have been proposed and employed ranging from direct chemical methods like acid hydrolysis and pyrolysis to biological methods such as application of cellulase enzymes. For a long-range solution to our resource problems of natural fertilizers, cellulose is available in large quantities in the form of agricultural residues like straws, hulls, stems, stalks, forestry and timber residues. Currently, bioconversion of these cellulosic residues is highly competitive. The undervalued agro-residues can be converted to value-added products. Plant residues contain 15-60 percent cellulose, 10-30 percent hemicellulose, 5-30 percent lignin, 2-15 percent protein and 10 percent sugars, amino acids and organic acids. Cellulose occurs in a semi crystalline form and has glucose units with β-1,4-linkages. The individual chains of glucose are held together by hydrogen bonds. Cellulase enzyme complex decomposes cellulose to disaccharide cellobiose which is hydrolyzed by the enzyme cellobiase to glucose.

Earthworms are farmer's best friends and eco-friendly by playing variety of roles in agro ecosystem. The gut of earthworm is the factory to manufacture the beneficial microbial density and their products to excrete thousand times more to enrich the surrounding soil. Experiments have proved that crops grown in earthworm inhabitant soils had increased the yields from 25% to over 300% than in earthworm free soil (Barley, 1961). Researchers had reported that bacteria living in the gut of worms breakdown many hazardous chemicals such as hexachloro cyclohexane (HCH) into detoxified forms maintaining the biological buffering of the soil. Due to lack of favorable conditions influencing the growth of earthworms, most of the agricultural lands likely account for lower abundance of earthworms (or) leading them to abandon the croplands.

Investigation of the interaction of actinomycetes with soil invertebrates is one of the ways to study the development in biogeocenoses. At present the literature contains sufficient evidence for the presence of actinomycetes in the gut of soil invertebrates (in particular earthworms) (Szabo, 1974; Chu *et al*., 1987). Microbial community indigenous to the earthworm *Eisenia foetida* was studied (Toyota and Kimura, 2000). The earthworm gut is favorable for the development of actinomycetes due to neutral pH, optimal humidity and temperature (Polyanskaya *et al.,* 1996; Striganova*,* 1989). In addition, the increased organic carbon and nitrogen content in earthworm gut may also stimulate microbial activity (Karsten

and Drake, 1995).

Hence to better understand the role of earthworms in nature and their potential usage, the metabolic functions of earthworm intestinal microorganisms must be rigorously defined. However, little attention has been paid on this topic to learn the contributions of earthworm gut flora and its products to soil health. In the present study owing to the importance of earthworms and their effective role in degradation, bacteria were isolated from earthworm gut and screened for cellulase production.

MATERIALS AND METHODS

Collection of Earthworms and sampling procedure

Earthworms (*Eisenia foetida)* were collected from vermicompost pits of S. V. Agricultural University, Tirupati, A.P., India. Earthworms were washed with sterile tap water and then placed on a sterile petriplate moistened with filter paper and subjected to starvation for 24 hr. Further they were disinfected with 70% ethanol; gut was dissected out, weighed and homogenized (for 5 min with a vortex mixture) in sterile 0.85% NaCl solution and bacterial isolates were obtained by dilution plate method. (Cappucino and Sherman, 2008).

Screening for Cellulase Activity

The isolated bacteria were inoculated on Carboxy Methyl Cellulose (CMC) agar to screen for cellulase activity. Efficient cellulose degrader was selected based on the formation of larger clearing zones on CMC agar.

Identification and characterization of selected isolate

Based on morphological and biochemical characteristics the isolate EGB1 was identified as a strain of *Bacillus* in our laboratory. Subsequently the isolate was submitted to Institute of Microbial Technology (Chandigarh).

Cellulase Production by *Liscinibacillus sphaericus*

Basic liquid mineral medium (100mL in 500 mL Erlenmeyer flasks) was inoculated with 1mL of overnight bacterial culture and incubated at 37ºC with vigorous aeration in a shaker at 150 rpm for 2 days. Culture filtrate was separated by centrifugation at 8,000 x g for 20 minutes at 4ºC and used as crude enzyme source. Filter paper activity is a measure of total cellulolytic activity (Mandels and Weber, 1969). In this method, filter paper as cellulosic substrate was incubated with *Lysinibacillus sphaericus* as a source of enzyme. The liberation of reducing sugar was measured by dinitro salicylic acid (DNS) method (Miller, 1959). One unit of cellulase was defined as the amount of enzyme releasing 1μmole of reducing sugar per mL per minute. *Lysinibacillus sphaericus* MTCC No.9468 exhibited highest cellulolytic activity in terms of filter paper units.

Table 1: Morphological tests

Tests	Isolate EGB1
Colony Morphology	
Margin	regular
Elevation	convex
Surface	dull
Pigment	—-
Opacity	opaque
Gram's reaction	+ ve
Cell shape	long rods
Endospore	+
Position	C
Shape	—-
Sporangia bulging	+
Motility	+
Fluorescence (UV)	—-

RESULTS AND DISCUSSION

A total of twenty bacterial cultures were obtained from the earthworm (*Eisenia foetida*) gut. All the isolates were found positive for cellulase production. Among the twenty isolates, EGB1 isolate which showed maximum activity was categorized as *Lysinibacillus sphaericus* with MTCC No. 9468 (formerly *Bacillus sphaericus*) based on cultural, morphological, physiological and biochemical characteristics.

Cellulolytic bacteria were identified by morphological and biochemical tests using Bergey's Manual of Systemic Bacteriology. Identified and characterized by IMTECH, Chandigarh (Table 1 to 4) and identified as *Liscinibacillus sphaericus* MTCC 9468 (Formerly *Bacillus sphaericus)*.

Table 2: Physiological tests

Tests	EGB1
Growth at 4ºC	—
10ºC	+
15ºC	+
25ºC	+
30ºC	+
37ºC	+
42ºC	+
45ºC	—
55ºC	—
65ºC	—
Growth at pH 4.0	—
pH 5.0	+
pH 6.8	+
pH 8.0	+
pH 9.0	+
pH 11.0	+
Growth with NaCl (%)	
2.0	+
4.0	+
6.0	+
8.0	—
10.0	—

Table 3: Biochemical tests

Tests	EGB1
Growth on MacConkey agar	—
Indole test	—
Methyl red test	—
Voges Proskauer test	—
Citrate Utilization	—
Casein hydrolysis	—
Gelatin hydrolysis	+
Starch hydrolysis	—
Urea hydrolysis	+
Nitrate reduction	—
H_2S production	—
Catalase test	+
Oxidase test	+

Table 4: Acid production from carbohydrates

Tests	EGB1
Salicin	—
Arabinose	—
Galactose	—
Dextrose	—
Meso-inositol	—
Raffinose	—
Rhamnose	—
Fructose	—
Mannitol	—
Sorbitol	—
Sucrose	—
Lactose	—
Xylose	—

After identification of bacterial culture, the efficiency of the organism for cellulase production was determined using the mineral salts medium. Cellulolytic activity of EGB1 isolate was determined according to filter paper assay method (Mandels and Weber, 1969). The amount of soluble reducing sugars that was (glucose) released in the production medium was determined. The cellulase activity was expressed in terms of Filter Paper Units (FPU). The volume of EGB1 isolate filtrate responsible for the release of 1μ mole of glucose per min was considered to be one filter paper unit. Since *Liscinibacillus sphaericus* MTCC 9468 had been detected to exhibit highest cellulolytic activity in terms of filter paper units of 1.92 FPU/ mL in subsequent experiments this organism was further exploited to assess the cellulolytic potential.

The *Liscinibacillus sphaericus* MTCC 9468 isolate of the present investigation need to be further studied in depth of its cellulolytic potential for actual application in the conversion of waste products into value-added and useful products.

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