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ORIGINAL ARTICLE

Optimization and molecular identification of novel cellulose degrading bacteria isolated from Egyptian environment

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Abstract Cellulase producing bacteria were isolated from both soil and ward poultry, using CMC (carboxymethylcellulose) agar medium and screened by iodine method. Cellulase activity of the isolated bacteria was determined by DNS (dinitrosalicylic) acid method. The highly cellulolytic isolates (BTN7A, BTN7B, BMS4 and SA5) were identified on the basis of Gram staining, morphological cultural characteristics, and biochemical tests. They were also identified with 16S rDNA analysis. The phylogenetic analysis of their 16S rDNA sequence data showed that BTN7B has 99% similarity with *Anoxybacillus flavithermus*, BMS4 has 99% similarity with *Bacillus megaterium*, SA5 has 99% homology with *Bacillus amyloliquefaciens* and BTN7A was 99% similar with *Bacillus subtilis*. Cellulase production by these strains was optimized by controlling different environmental and nutritional factors such as pH, temperature, incubation period, different volumes of media, aeration rate and carbon source. The cellulase specific activity was calculated in each case. In conclusion four highly cellulolytic bacterial strains were isolated and identified and the optimum conditions for each one for cellulase production were determined. These strains could be used for converting plant waste to more useful compounds.

1. Introduction

Cellulose is the most abundant biomass and most dominating agricultural waste on earth [36]. It is a polymer chain of glucose units connected by β-1, 4 linkages. Cellulose waste is a huge renewable bioresource produced by the photosynthetic process [15,39]. It has a high potential for bioconversion to important bioproducts such as ethanol. The ability to obtain cheap ethanol will depend on the successful identification of novel cellulase producing strains [21]. More research activities are needed to obtain novel cellulases with hyper activity on pretreated biomass substrates by screening and sequencing novel classes of microorganisms and engineering cellulases

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Table 1 Biochemical characteristics for the identification of selected isolates.

Biochemical test	Isolate code			
	BTN7A	SA5	BMS4	BTN7B
Gram stain	+	+	+	+
Cell shape	B	B	B	B
Endospore stain	+	+	+	+
Oxygen requirements	F	F	F	F
Motility test	–	–	–	+
Catalase test	+	+	+	+
Starch hydrolysis	+	+	+	+
Citrate utilization	–	–	–	–
Methyl red	–	+	+	+
Vogas Proskauer	+	+	–	–
Nitrate reduction				
NH_3	–	–	–	–
NO_2	–	–	–	–
NO_3	+	+	+	+
Carbohydrates fermentation				
Glucose	–	–	A	A
Lactose	–	–	–	–
Mannitol	–	–	–	A
Arabinose	–	–	–	–
Growth at 55 °C	–	–	–	+
6.5% NaCl growth	+	+	+	–

F = Facultative anaerobes, A = Acid, B = Bacilli.

Table 2 Isolates accession number and their sequence similarities based on 16SrDNA.

Isolates	Source	Accession number	99% similarity strains
SA5	Ward poultry	KC438369	*Bacillus amyloliquefaciens subsp. plantarum*
BMS4	Soil	KC429572	*Bacillus megaterium*
BTN7A	Soil	KC438368	*Bacillus subtilis subsp. subtilis*
BTN7B	Soil	KC429571	*Anoxybacillus flavithermus WK1*

with improved industrial qualities and by identifying proteins that can stimulate cellulases [38].

One of the requirements of the biological conversion of lignocellulosic wastes into industrial products is the use of cellulolytic and hemicellulolytic enzymes [18]. Microorganisms that can produce cellulase enzymes (cellulolytic microorganisms) can degrade cellulose. Fungi and bacteria isolated from soil secrete several enzymes which degrade lignocellulosic biomass [7]. These enzymes are commonly produced by some bacterial genera such as *Cellulomonas, Pseudomonas* [26] *Bacillus*, and *Micrococcus* [14] and fungi [32] that are widely used now in industrial applications. Cellulosic biomass hydrolysis requires successive action of three types of enzymes, which are cellobiohydrolase, endoglucanase carboxymethyl cellulase (CMCase), and β-glucosidases [5].

Scientific research efforts try to improve the hydrolysis process in an economical way. There are different factors which affect bacterial growth and enzyme production such as pH, temperature, aeration, incubation period, inoculum size and carbon source. The air pollution in Cairo is a matter of serious concern. One of the most notable sources of pollution is open-air waste-burning. A black cloud over Cairo has been noticed each year for many decades during harvest time where farmers burn leftover rice husks at the end of the growing season. The overall aim of this research was to produce more healthy air by converting plant wastes to economical products. Any bacterial strain has its own identity and has to be investigated for its optimum culture conditions for its best activity for the application purpose. On the other hand, economical high-efficient bacterial strains are not available unless paying their Know-How. As an initial step this article aimed to isolate indigenous bacterial strains efficient in cellulose degradation and maximize their activity using different nutrients and culture condition.

2. Materials and methods

2.1. Sample collection

Soil and ward poultry samples were collected from Beni-Suef and El-Sharkia Governorates, Egypt, and stored under sterile conditions at 4 °C until bacterial isolation.

2.2. Media

Luria-Bertani agar medium (LB) [4] was used for different physiological and molecular biology procedures. Bushnell Haas medium (BHM) [8] was used for isolation of cellulose hydrolytic bacteria after amendment with carboxymethylcellulose (CMC) as the sole carbon source.