Research Paper

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Isolation, Identification and Characterization of cellulase producing *Actinomycetes* bacterium FMR 3 (KF011478) from Slaughter House Waste

Microbiology

KEYWORDS : Actinomycetes, Slaughter house waste, Cellulase.

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ABSTRACT

In the present study, slaughter house waste was collected and bacterial colonies were isolated by serial dilution method. The colonies were screened for cellulase production by Congo Red Assay. The isolate which produced maximum zone of clearance was identified as Actinomycetes bacterium by biochemical test, sugar fermentation test and 16S rRNA sequence analysis. The Actinomycetes was found to be gram positive, motile, rod shaped, filamentous, spore forming bacteria. It has fermented glucose, maltose, sucrose and starch. The identified bacterial sequence was submitted in GenBank and obtained the accession number KF011478. The phylogenetic tree analysis by neighbor-joining method also confirmed that the isolate belongs to the family Actinomycetes.

Summary of the paper:

The cellulase producing Actinomycetes bacterium was isolated from slaughter house waste. The isolated Actinomycetes sp. was found to be gram positive, motile, rod shaped, filamentous, spore forming bacteria. The 16S rRNA analysis was carried out and the PCR product was sequenced. The sequence was submitted in GenBank and obtained the accession number KF 011478. The phylogenetic tree was constructed by neighbor-joining method.

1. Introduction:

Cellulose, the complex biopolymer, is the most abundant component of plant biomass. In nature, it is found almost exclusively in plant cell wall (Lynd *et al*. 2002). A number of biomass conversion methods have been proposed and employed ranging from direct chemical methods like acid hydrolysis and biological methods such as application of cellulase enzymes (Cooney *et al*. 1978). Any process which could efficiently and economically convert cellulosic material to glucose would be of high industrial significance (Walsh, 2002). Utilization and conversion of cellulose is done by enzymatic method using cellulase, a cellulase degrading enzyme produced by cellulolytic microbes.

The biggest obstacle in commercial success of cellulase enzyme production is the high cost of raw material used as substrate which could be overcome by resorting to microbial fermentation technology using low value biological substrates including agro-waste, viz., rice straw, wheat straw, rice bran, wheat bran and sugarcane baggasse, etc. (Nigam and Singh, 1996 ; Ikram *et al*. 2006).

Cellulases studied to date are mainly from *Mesophiles,* with the focus being on those from *Trichoderma* species (Eriksson, 1982; Goksoyr and Eriksen, 1980; Reese and Mandels, 1984). Cellulases from *Thermophilic fungi* (Coudray *et al*., 1982) *Thermophilic bacteria* (Avgerinos and Wang, 1980; Ljungdahl, 1983) and *Actinomycetes* (Fennnington *et al*. 1982; Montenecourt, 1983) are studied to a larger extent. Cellulase producing *Bacillus* was isolated from cow dung (Shanmugapriya et al. 2012).

Actinomycetes , one of the known cellulase producers has concerned considerable research interest due to its potential application in the recovery of fermentable sugars from cellulose that can be of benefit to human consumption (Jang and Chen, 2003; Arunachalam *et al*. 2010).

The present study is undertaken to isolate and characterize the microbes from slaughter house waste and to screen the ability of the organism to hydrolyze cellulose.

2. Materials and Methods:

Slaughter house waste sample was collected and filtered through double layer muslin cloth. From this, 1 ml of filtrate was used for serial dilution, 100µl of each dilution (10-6 to 10-9) was cultured by spread plates using Hungate's medium (Hungate, 1969). Five isolates were obtained and named as F1 to F5 and subcultured on 1% Carboxyl Methyl Cellulose (CMC) agar plates in duplicates and incubated at 37^0C for 48 hours. After 48 hours of incubation the colonies were screened for cellulase production by Congo red Assay (Apun *et al.* 2000). The formation of a clear zone of hydrolysis indicated cellulose degradation. The F1 isolate produced comparatively maximum zone of clearance than the other isolates. Hence, the F1 isolate was further studied and characterized for biochemical and sugar fermentation test (Buchanan and Gibbons, 1974). Identification of the isolate F1 was confirmed by 16S rRNA analysis (Altschul *et al.* 1990) and sequence was submitted to GenBank for Accession number.

3. Results:

2.1 Screening and isolation of cellulase producing bacteria:

The isolate F1 produced comparatively maximum zone of clearance than the other isolates by Congo Red Assay (Fig .1).

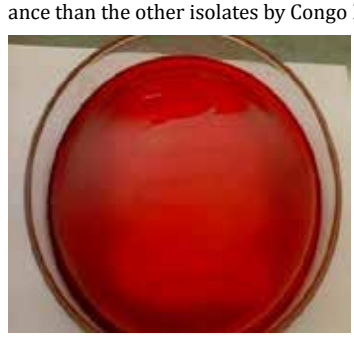

Figure 1: Screening of Cellulase production by Congo red Assay.

2.2 Identification of cellulase producing bacteria:

The isolate F1 was found to produce white, powdered, opaque colonies. It is Gram positive, Rod shaped, filamentous, motile and spore forming. The bacteria appeared single or in pairs (Fig2).

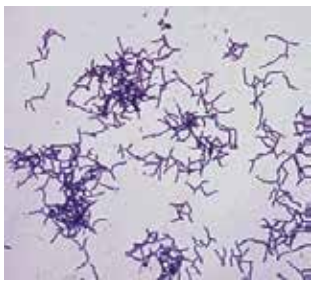

Figure2. Photomicrograph of *Actinomycetes* **bacterium FMR 3 KF011478** *(1000x)*

3.3 Biochemical Characterization and Sugar Fermentation Test:

The isolate F1 was found to be positive for Voges proskauer, Citrate and Urea hydrolysis and Negative for indole, methyl red, catalase, oxidase and hydrogen sulphide (H2S) production. Alkaline slant, Acid Butt and no gas production in triple sugar iron test. The isolate F1 fermented glucose, maltose, sucrose and starch and did not ferment fructose, lactose, xylose and arabinose. The results of the Biochemical characterization & Sugar fermentation was compared with Bergey's Manual (Bergey's manual of determinative bacteriology, 2000) and the isolate was identified as *Actinomycetes* Bacteria.

3.4 16S rRNA sequence analysis:

The PCR product of 16S rRNA was sequenced and the sequence result was submitted to BLAST-N to obtain the possible hits based on 97-99% similarity. The isolate belongs to the genus *Actinomycetes.* The 16S rRNA sequence of the isolate F1 (*Actinomycetes* bacterium FMR3) was submitted in GenBank and obtained the Accession number **KF011478**.

3.5 Phylogenetic Tree:

The phylogenetic tree analysis was performed using CLUSTAL-W program to validate the results obtained from BLAST-N. The results are correlated with the BLAST-N results confirming that 16S rRNA have a close relationship with *Actinomycetes*. Fig.3 shows the phylogenetic tree obtained by neighbor-joining method with 97-99% sequence similarity.

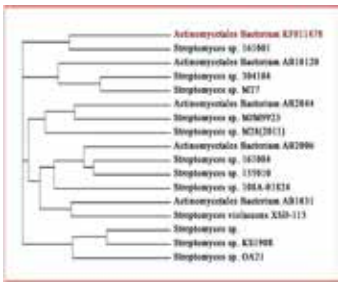

Figure 3.Comparitive sequence analysis of 16S rRNA from *Actinomycetes* **bacterium FMR3 and representative strains of the genus** *Actinomycetes* **from GenBank, using the neighbor-joining method.**

4. Discussion:

Bacillus sp., was isolated from cow dung, gives better cellulase production when combined with carboxyl methyl cellulose (CMC). Compare to coir waste and saw dust used as substrate for cellulase production, CMC was found to be the best cellulase producer (Shanmugapriya *et al*. 2012). *Pseudomonas aeuroginosa, Micrococcus*, *Streptococcus, Bacillus, Penicillium, Mucor* and *Fusarium* were isolated from rumen of ruminants and they were able to hydrolyze cellulose (Oyeleke *et al.* 2008). *Streptococcus* sp. was the predominant cellulolytic microorganisms that are associated with the possession of complex cellulase enzyme systems as reported (Schwartz, 2001).

Actinomycetes were isolated from high altitude Shola soil of tropical montane forest, the best carbon sources were found to be xylose followed by fructose and mannose (Rinoy Varghese *et al*. 2012). A cellulolytic *Actinomycete* species was isolated from Iraq soil and the strain grew well on a mineral medium containing CMC, produced cellulase enzyme at different temperature; 28^0, 37^0 and 48^0C and has an acido-alkalophilic growth ability (Amira *et al*. 1989). 282 isolates of *Actinomycetes* were isolated from soil samples collected from few selected areas in Peninsular Malaysia and were observed to have different colony colors and screened for their cellulase activity (Jeffrey *et al*. 2007).

In the present study, a gram positive, spore forming, motile *Actinomycete* bacterium FMR3 was isolated from slaughter house waste was found to be having cellulase activity. It is the first report of cellulase producing *Actinomycetes* from slaughter house waste.

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