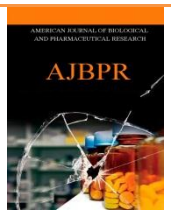
e-ISSN - 2348-2184
Print ISSN - 2348-2176

AMERICAN JOURNAL OF BIOLOGICAL AND PHARMACEUTICAL RESEARCH

Journal homepage: www.mcmed.us/journal/ajbpr



ISOLATION OF CELLULASE ENZYME PRODUCING MICRO ORGANISM FROM SOIL AND SELECTION OF MEDIUM FOR GROWTH AND ENZYME PRODUCTION

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Article Info

Received 29/01/2015
Revised 24/02/2015
Accepted 20/03/2015

Key words: -
Cellulose, polysaccharide, Degrading microorganisms,

ABSTRACT

To isolate a good strain of cellulose degrading microorganism from soil, samples of compost are collected from different parts of Burdwan District (West Bengal, India). Among 102 isolates one is found good for degrading cellulosic materials and it was identified as *Aspergillus flavipes*, seems to constitute a new report of cellulose degrading microorganism and the identification was confirmed by the Commonwealth Mycological Institute, Kew, Surrey, England(Accession No- 355051). The selected isolate is grown in different medium and found to grow and produce enzyme well in Mandels and Reese (1985) medium.

INTRODUCTION

Cellulose is one of the major structural compounds of plant cell wall. As it is an indigestible polysaccharide the utilization of this complex compound takes place after chemical or enzymatic hydrolysis into simple sugar glucose [1]. The use of cellulolytic enzyme is more economic than chemical hydrolysis [2]. Much attention has been made by several authors [3, 4] to hydrolyze cellulose material by using cellulose hydrolyzing enzymes from different microbial sources. They have attempted to develop an enzymatic saccharification process for various kinds of cellulosic materials. The value of cellulose as renewable source of energy by its enzymatic hydrolysis has made the subject of intense research and industrial interest [5]. The product of cellulose hydrolysis has made commercial Interest in production of ethanol [6], organic acids [7] and others like protein.

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For further enhancement of productivity of cellulolytic enzymes production by the microorganisms for perfect saccharification of cellulose a continuous work is going on till now as the hydrolyzing product has great importance in various fields [8]. *Trichoderma* and *Aspergillus* are thought to be the major cellulose producers [9-11] and the crude enzymes produced by these microorganisms are commercially available in the market. Today 20% of the world markets of these enzymes are the product of *Trichoderma* and *Aspergillus* [1,9-11].

The objectives of the present investigation are to isolate a good strain of cellulose degrading microorganism from soil and to select a suitable medium for its growth and production of cellulose degrading enzymes.

MATERIALS AND METHODS

Soil and compost are collected from different parts of Burdwan district of West Bengal (India), air dried, powdered and sieved. The soil samples are kept in properly labeled sterile glass phials. For isolation of microorganisms, soil samples are diluted with distilled water serially up to 10^{-3} time and plated on Doelger and

Prescott agar medium. The agar plates are incubated at 30^0C. After three days of incubation, the colonies are picked up on Czapek and Dox slant (for fungal colony) or nutrient agar slant (for bacterial colony). The slants are incubated till good growth occurs. They are kept in cold (10^0C) and sub-cultured at regular interval.

From slant cultures fungal spores are collected and spore suspension is prepared by wetting the spores with 5 ml of 0.05% aqueous solution of Manoxal O. T. (dioctyl ester of sodium sulphosuccinic acid) and then washed with 5 ml sterile distilled water. The spores are collected in 100 ml sterilized flask with glass beads. The flasks are shaken in a shaker to break the spore clumps. Spore suspension thus prepared is diluted suitably with normal saline solution. The spore suspension is measured in Thomas counting chamber keeping the count at 10^7 spores/ ml. This spore suspension is plated on Czapek and Dox medium containing carboxymethyl cellulose (CMC) as only the carbon source instead of glucose. After inoculation the plates are incubated at 30^0C for three days. The cellulose decomposing fungi grow only and they are picked up into agar slants containing Czapek and Dox medium.

The slants are incubated till good growth occurs. From these slants spores are collected and inoculated in each culture tube containing 10 ml of liquid Czapek-Dox medium with filter paper as carbon source instead of glucose. After inoculation the culture tube are incubated at 30^0C. The cellulose decomposing fungi grow and they are picked up in pure culture. The cellulose decomposing microorganisms are separately grown in 25 ml of different liquid medium with 1% CMC instead of glucose as carbon source in 100 ml flask.

After incubation at 30^0C for 7 days, the mycelium is harvested by centrifugation (5000Xg) for 10 minute and the culture filtrates are analyzed for reducing sugar. Protein is estimated by Lowry method [12] with the Folin phenol reagent. Reducing sugar is estimated with Dinitrosalicylic acid reagent [13].

RESULTS

In course of survey of 20 soil samples involving 103 microorganisms 18 strains are isolated capable of degrading cellulose as evident from their growth in CMC medium. The isolates are numbered according to the following method; soil number (numerically 1, 2, 3, 4,……) followed by isolates number (alphabetically a, b, c, d…..). The 18 isolates are grown again separately in Czapek-Dox agar medium for sporulation. The result is represented in table No-1. Selected strains are separately grown in 25 ml of liquid Czapek-Dox medium in 100 ml flask at 30^0C for 7 days. After incubation period the culture filtrates are analyzed for reducing sugar. The results obtained are represented in the table No-2. From the results obtained six strains (4a, 4d, 6b, 7f, 10a, 15b) are selected on the basis of production of reducing sugar in the culture filtrate. For final selection these six selected strains are grown in 100 ml flask containing 25 ml of liquid Czapek-Dox medium with 1% CMC at 30^0 C for 5, 8, 10 and 12days. After incubation period the culture filtrates are analyzed for reducing sugar. The results are represented in table No-3. From this result, the strain 6b is finally selected as a potent producer of cellulose hydrolyzing enzymes. To find out a suitable liquid medium for growth and enzyme production, the selected strain is grown in five different types of medium such as, Saunders medium . Haynes, Wickarham & Hesselitine medium . Mandels & Weber medium modified by Tangnu et al (1981), Nystrom & Allen medium. The results obtained are presented in table No- 4 and it has been found that the medium of Mandles & Reese is the best for production of cellulytic enzymes though the medium of Saunders as well as of Nystrom & Allen are good for the growth of the strain.

Table 1. Primary screening of microorganisms for cellulose degrading activity

Isolatioin no.	Growth in CMC medium	Isolation no.	Growth in CMC medium	Isolation no.	Growth in CMC medium	Isolation no.	Growth in CMC medium
1a	-	5a	-	9b	-	14e	-
1b	-	5b	-	9c	-	15a	-
1c	-	5c	-	9d	+	15b	+
1d	+	5d	-	9e	-	15c	-
2a	-	5e	-	9f	-	16a	-
2b	-	6a	-	10a	+	16b	-
2c	-	6b	+	10b	-	16c	-
2d	-	6c	+	10c	-	16d	-
2e	-	6d	-	10d	-	17a	+
2f	+	6e	-	11a	-	18a	-
2g	-	7a	-	11b	-	18b	-
3a	-	7b	+	11c	-	18c	-

3b	-	7c	-	12a	-	19a	-
3c	+	7d	-	12b	-	19b	-
3d	-	7e	-	12c	-	19c	-
3f	+	7f	+	12d	-	19d	-
3g	-	7g	-	12e	-	20a	+
3h	-	7h	-	12f	-	20b	-
4a	+	7i	-	13a	+	20c	+
4b	-	7j	-	13b	-	20d	-
4c	-	7k	-	13c	-	20e	-
4d	+	8a	-	13d	-	20f	-
4e	-	8b	-	14a	-	20g	-
4f	-	8c	-	14b	-	20h	-
4g	-	8d	-	14c	-		
4h	+	9a	-	14d	-		

Table 2. Growth and cellulase production by selected isolates

Isolate No.	Reducing sugar(mg/ml)	Mycelial dry wt.(mg/25ml)
1d	0.22	62
2f	0.26	43
3c	0.20	36
3f	0.18	60
4a	0.35	76
4d	0.42	66
4h	0.26	40
6b	0.46	56
6c	0.15	37
7b	0.22	42
7f	0.42	78
9d	0.25	32
10a	0.36	56
13a	0.28	58
15b	0.40	62
17a	0.30	36
20a	0.16	55
20c	0.26	42

Table 3. Cellulose degradation by selected strains

Isolates No.	Reducing sugar in c.f.(mg/ml)			
	Days of cultivation			
	5	8	10	12
4a	0.30	0.38	0.32	0.25
4d	0.32	0.44	0.40	0.32
6b	0.38	0.52	0.46	0.40
7f	0.26	0.42	0.40	0.38
10a	0.38	0.40	0.38	0.34
15b	0.22	0.40	0.39	0.34

Table 4. Growth and Cellulase production in different liquid medium

Medium	Reducing sugar in c.f.(mg/ml)	Mycelial dry wt.(mg/25ml)	Protein in c.f.(mg/ml)	Final pH of c.f.
A	0.54	68	-	6.3
B	0.55	56	0.132	6.0
C	0.52	68	-	7.1

D	0.60	60	-	7.0
E	0.56	62	-	7.1

a = liquid Saunders (1948) medium,
b = Haynes, Wickerham and Hesseltine (1955) medium,
c = Mandels Reese (1985) medium,
d = Mandels and Reese (1985) medium,
e = Nystrom and Allen (1976)

DISCUSSION

Cellulose is a highly crystallized polymer of glucose molecules. In nature it undergoes decomposition by several microorganisms. The hydrolysis of cellulose by cellulolytic enzymes is reported by several authors in several microorganisms *Trichoderma viridae* and *T.koningii* [1], *Chaetomium thermophile* [14], *Myrothecium verrucaria* [15], *Fusarium solani* [16] *Cellulomonas fimi* [17] Ogawa and *Aspergillus niger*) [11]. These organisms can be grown in medium containing cellulosic material only as carbon source that helps to isolate them. They produce cellulolytic enzymes in the medium that causes the hydrolysis of cellulosic materials into glucose molecules.

This reducing sugar is used to estimate the potentiality of cellulose hydrolysis by microorganisms. Based on this principle a cellulose decomposing strain is isolated from the soil. The selected strain is identified as *Aspergillus flavipes*, seems to constitute a new report of cellulose degrading microorganism and the identification was confirmed by the Commonwealth Mycological Institute, Kew, Surrey, England(Accession No- 355051). Conidiophores are 300-500 µ in length and 8-10µ in breadth. Conidia are 2-3µ in diameter. Phialides are in two series, primary (4.-7 µ x 2 µ) and secondary (5-8 µ x 1.5-2 µ). The isolate, however, differs from standard description (A manual of soil fungi; Gilman 1969) in that hulle cells are absent. A number of species of the genus *Aspergillus* have been reported to be producer of cellulolytic enzymes. These are *A. niger*[11], *A.terreus*[9], *A.nidulans* [10], *A. phoenices*[2]. The present report of cellulolytic enzyme production by *A. flavipes* , therefore, seems to constitute a new report in this regard.

The growth and production of cellulolytic enzymes by the selected strain in culture depends on various factors. The nutrient composition of the culture medium is one of them that provide support the results of experiment on effect of various culture medium used for it [13].

ACKNOWLEDGEMENT

The author is grateful to the Director, Identification Services Commonwealth Mycological Institute, Kew, Surrey, England, for confirming the identity of the selected organism.

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