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Annals of Biological Research, 2011, 2 (3) :453-460
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library

ISSN 0976-1233
CODEN (USA): ABRNBW

CMC-ase Activity of some soil fungi

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ABSTRACT

Cellulose as a degradable substance with hight abundant in nature constitutes moiety of gross weight product of green plant. The fungi are one of the major microorganisms to produce cellulase enzymes as β 1, 4 Glucanase, β-Glucosidase and Cellubiohydrolase. In this reaserch, the ability of CMC degradation of several soil fungi as Trichoderma, Aspergillus and Fusarium isolated from different region of Iran was investigated and characterization of cellulase from superior isolates. CMC (Carboxy Methyl Cellulose) is a basic liquid mineral medium as just carbon source were inoculated. Selected fungal inoculums in a randomised complete block design with three repetitions .Glucose and protein production assay were performed four days after inoculation and repeated each three days up to 30th day. The result of reaction of glucose with arsenat molibdat reagent was read with spectrophotometer at 525 nm and protein asay with Bradford metod was read with spectrophotometer at 595 nm. There were variations in measure glucose and protein, Glucose levels were increased to to 14th days and were decreased up to 25th days and were constant in the next days. Protein rate were increased to 9th day and were decresed to 25th day and it were fixed in next days. Our result showed that Trichoderma harzianum 15-6 isolate had highest and Fusarrium solani had least ability for CMC degradation and CMCase product in the experimental condition. The study clearly demonstrated that CMC is a good inducer for extracellular CMCase production by the fungus.

Key words: Carboxy Methyl Cellulose**,** Protein assay, sugar assay, *Trichoderma, Aspergillus and Fusarium.*

INTRODUCTION

Cellulose, as the most abundant digestible organic material, constitutes almost the half of gross production of vital material which are produced during photosynthesizes by plant. This substance

is a polysaccharide composed of,-D-glucopyranosylunits joined by 1, 4-glycosidic bonds [10]. inclusive the large proportion of cell wall in plants [14,16]. Moreover cellulose can be found in algae, fungi and some protozoa cysts. Hydrolysis of cellulose is usually performed by two methods, enzymatic and chemical which are done by cellulase enzymes and acids respectively. Because of some difficulties of acidic hydrolysis, enzymatic hydrolysis is more considered [2,6] Usually the outputs of cellulose hydrolysis are reduced glucose sugars. Preference of enzymatic hydrolysis is its low cost in comparison with the chemical method cost because enzymatic hydrolysis is usually done in a moderate condition which is in 45 to 50 ℃ and pH= 4.8 and there is no oxidation[7]. Cellulases are enzymes which work in a complex of three groups of enzymes within a synergistic manner that can hydrolyse β1-4 glycoside links[16]. These enzymes are contained 1-4- β-D glycanases or endogluconases (EG, EC 3.2.1.4) which can hydrolyse internal glycoside links, 1-4-β-D glucan cellobiohydrolase or exogluconases (CBH, EC.3.2.1.91) which are able to disjoin cellobiose units at the end of the cellules chain and 1-4- β-D glycosides (βGL,EC, 3.2.1.21) that can make glucose units separate from the linear cellules chain [3]. The history of enzymatic related process use is returned to ancient civilizations. From old times, mankind has known the role of some creature in production of nutrition food. Today it is shown that their released enzymes have played significant role in quality and manufacturing of new products. Increasing both human knowledge and technologies have prepared the possibility of purification and use of these enzymes in the absence of producer organisms and introduce some of them as expensive commerical goods. Today it is approved that microorganisms and specially fungi are the main origins of a considerable number of industrial enzymes [3,4]. Enzymatic hydrolysis of polysaccharides promises a new way to produce a renewable source of energy [19]. The most promising technologyfor the conversion of the lignocellulosic biomass to ethanol biofuel is based on the enzymatic breakdown ofcellulose using cellulase enzymes [12, 1]. Cellulose which is the polymer of D-glucose is the main component in all plants[8]. If we produce a large amount of this sugar with a low price in vitro, it can be easily transformed to ethanol or methane by fermentation. Saprophytic fungi can be one of the most beneficial sources of cellulase enzymes and it is verified that more than hundreds of different fungal species can grow on cellules and use it as only source of carbon while merely small number of them are able to secrete digestive enzymes of cellulose [18]. Fungi are one of the main microorganisms which can produce cellulase enzymes and use them in the secondary metabolisms pathways.the escreening done on the difrrent species fungi.alot of difrrent spaices fungi have ability cellolose degradation, Trichoderma, Aspergillus and Fusarium species had the highest activity. These enzymes are included endo β1, 4 gluconase, β- glycosidase and cellobiohydrolyses that can degrade and hydrolyse cellulose completely in a cooperative manner. Enzymes (as biological catalyzers) have various biotechnological applications such as agriculture, food industries, sewage purification and many other types of consumption. Furthermore cellulase enzyme is one of the most momentous enzymes in degradation of lingo-cellulose wastes in agriculture, reduction of cellules in raw materials in food industry and making wood soft in papermaking industry[11, 13]. Coughlan, 1990, suggested that fungi and bacteria are the most importand producers in nature. Although the bacterial cellulase activities are equal or even more than fungal, the rate of bacterial cellulase is less than fungi. Cellulase enzymes which are produced by infective fungi such as Fusarium, Trichoderma and Aspergillus play crucial role in smoothing the cell wall and also making easy to penetrate and progress of infectious ingredients through the hast issues. The main aim of this research is study on ability of selected fungal isolate to degrade cellulose in vitro to prepare the preface of selecting the superior species to both molecular capacities and gene expression level examinations and ultimately present those to industrial applications.

MATERIALS AND METHODS

Fungal isolates and sugar induction

Available fungi isolated from cultural soils in north of Iran were used in this experiments. To reassure the purity of species, fungi were cultured on water-agar medium and then the tips of these grown fungi hyphae were re-cultured grown on PDA (Potato dextrose agar) slants and stored at 4ºC (15). Induction of fungi to produce enzyme was performed on 100 cc liquid medium contain CMC as sole carbon resource. Experience was repeated three times in the mentioned inoculated medium condition in 25 Ċ.

Cellulase inducer medium:

Estimating of cellulose degradation was done by minimum liquid mineral medium included nitrogen, microelements and CMC as an only source of carbon. To prepare this medium 5 mgr of FeSO4 .H2o, 25% gram of MnSO4 .H2O, 25% gram of CoCl2, 25% gram of ZnSO4, 25% gram of (NH4)2SO4, 2 grams of KH2Po2, 25% gram of MgSO4 .7H2O, 0.4 gram CaCl2, 10 grams of CMC, 0.3 grams of Urea, 0.2 gram of Tween 80 and 1 gram peptone were used.used of pH 6 for liguid media]15]. Then prepared medium was divided into 20 glass bottles (each one contained 50 ml) and then autoclaved at 120 ºC for 20 min.

Sugar assay:

Sugar assay was started four days after inoculation and repeated every three days during one month [15]. Rate of glucose production from cellulose was determined by adding Arsenate-Molybdate reagent to collected samples and absorbance was read in 575 nm by spectrophotometer. To compare and score the samples, standard was made by preparing a serial dilution of glucose (0.1 to 1 gram per liter). Their absorbance were read by adding Arsenate-Molybdate reagent and compared with absorbance of glucose concentration in samples [14].

Protein assay

Five hundred µl of medium in each clean test tube were used to release proteins assays. Concentrations of released fungal extracellular proteins was determined using Bradford method [5]. BSA was used as a standard protein.

RESULTS AND DISCUSSION

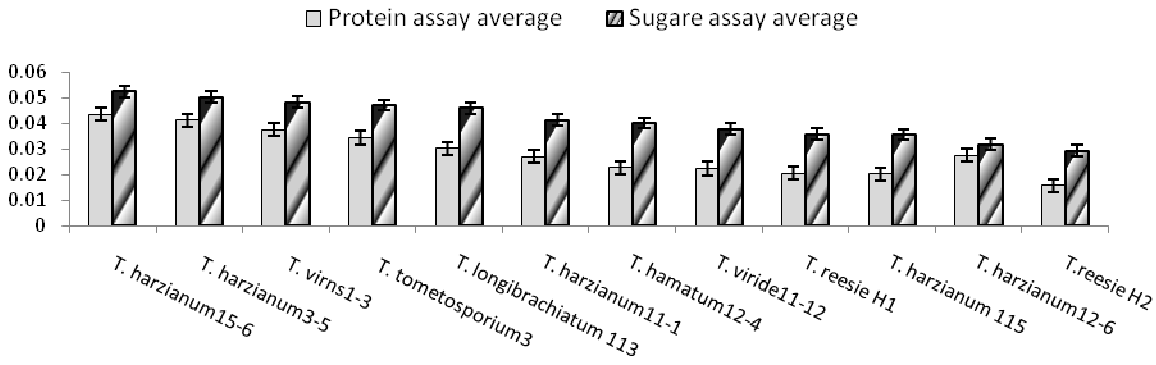

Fig. No. 1: Comparison between the mean protein and glucose released in CMC in Trichoderma isolates

In this study we screened different genus of fungi isolated from soil cultural to estimate cellulose degradation activity in vitro. A minimal medium contained nitrogen, microelements and CMC as

an sole source of carbon was used. The sampling process was started twelve hours after inoculation. Sugar assay indicated that there are some variations between tested species in inducer medium. All isolates studied which were cultured in CMC medium showed more or less a variation in amount of released glucose and protein. It means that the general trend of glucose and protein production were upward until 14th and 10th day, respectively in Trichoderma isolats (figure 1)

Our results showed that highest level of glucose and protein production was belonged to Trichoderma harzianum 15-16 isolate which was around 0/099341 g/L and 0/07354 mg/L, respectively (figure 2).

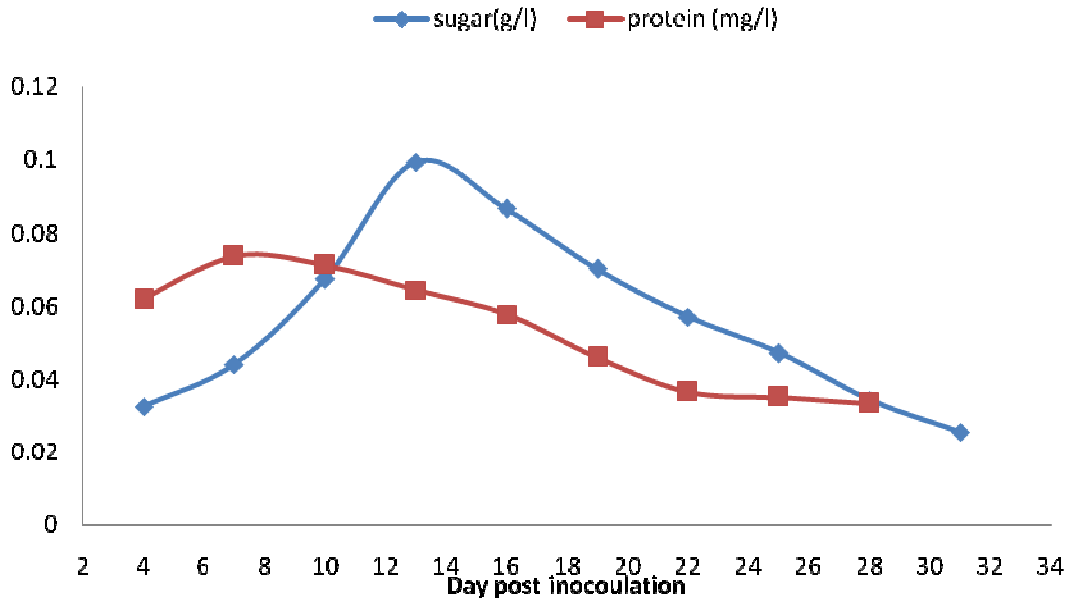

Fig. No. 2: Rate of Inducing sugar and protein produced by *Trichoderma harzianum* 15-6 isolate

For Aspergillus and Fusarium isolates the general trend of glucose and protein production were upward until 16th day in while after 16th mounts showed downward trend and in the next days they were stable (figure 3,4).

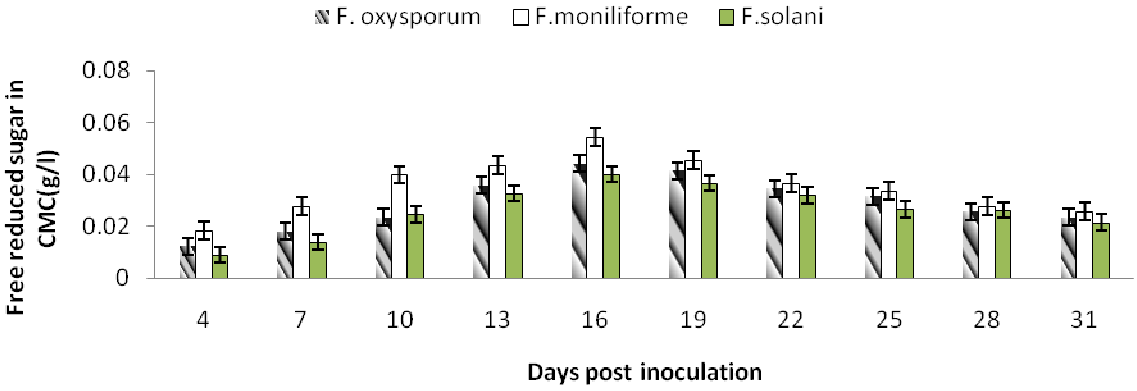

Fig. No. 3: Variations of glucose released by *Fusarium* isolates in CMC media

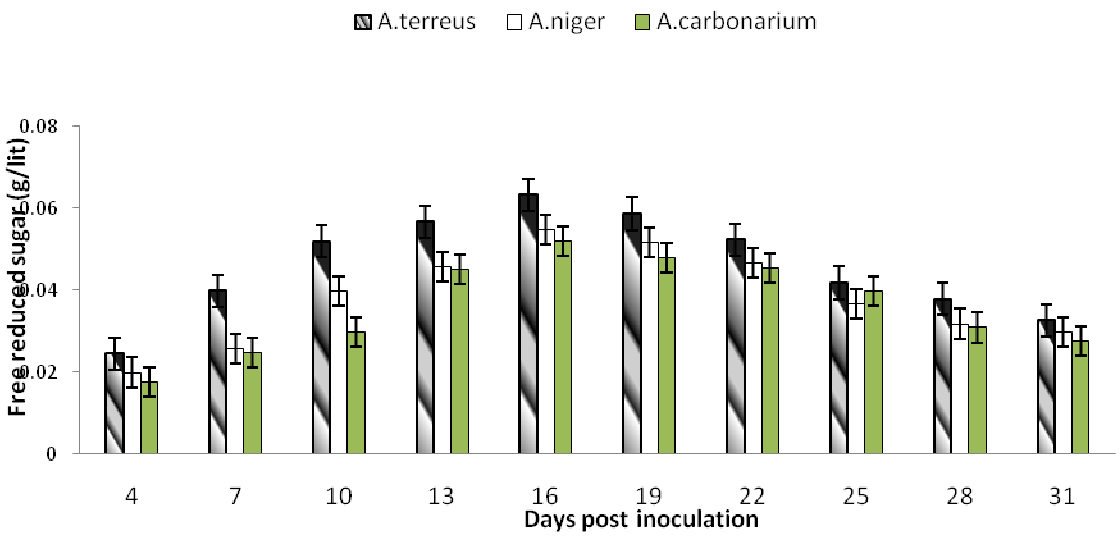

Fig. No. 4: Variations of glucose released by *Aspergilluse* isolates in CMC media

These variations and also increase in fungus biomass verified that not only these species were able to degrade cellulose crystals in CMC medium as an only source of carbon but also they were able to produce cellolase to transform cellulose to glucose.

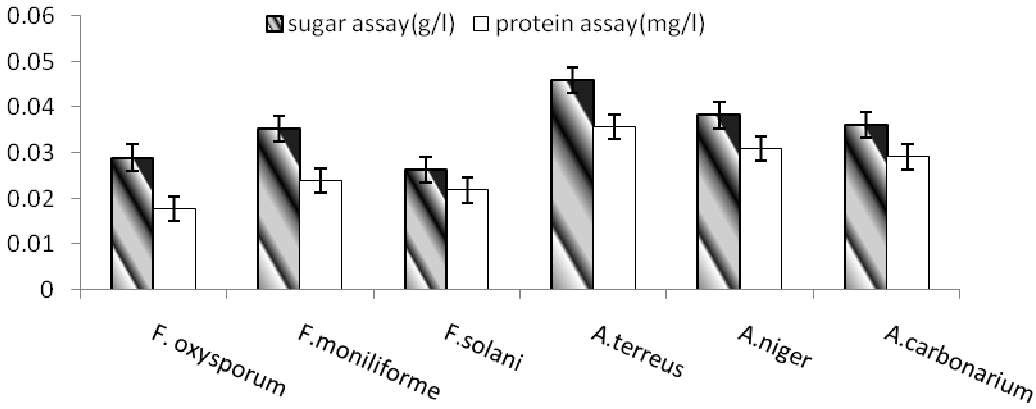

Fig. No. 5: Comparison between the mean protein and glucose released in CMC in Fusarium and Aspergillus isolates

Table No-1: Fungal isolates used are arranged base on cellulose degradation ability in CMC medium at g/L and Protein Extant In there cellulase pik (mg/L).

	Fungi species	Protein assay In there cellulase pik (mg/L)	Rate of sugar production In there glucose pik g/L		Fungi species	Protein assay In there cellulase pik (mg/L)	Rate of sugar production In there glucose pik g/L
10	*Trichoderma hamatum*12-4	0/04265	0.05100978	1	*Trichoderma harzianum*15-6	0/07354	0/099341
11	*Trichoderma viride* 11-12	0/04236	0.048754	2	*Trichoderma harzianum*3-5	0/07123	0/0812340
12	*Trichoderma reesie* H1	0/04048	0/046789	3	*Trichoderma virns* 1-3	0/06756	0/078234
13	*Trichoderma harzianum* 115	0/04021	0/046578	4	*Trichoderma tometosporium*3	0/05436	0/075806
14	*Trichoderma harzianum*12-6	0/03756	0/045879	5	*Trichoderma longibrachiatum* 113	0/06012	0/074898
15	H2 *Trichoderma reesie*	0/03561	0/045237	6	*Aspergillus terreus*	0/05461	0/063345
16	*Fusarium oxyspoium*	0/03124	0/0440664	*7*	*Trichoderma harzianum*11-1	0/04697	0/051234
17	*Fusarium solani*	0/02765	0/040120	*8*	*Aspergillus niger*	0/04878	0/054876
18	*Fusarium moniliforme*	0/04098	0/054340	*9*	*Aspergillus carbonarium*	0/04611	0/051982

In Fusarium species F.moniliform has the highest amount of sugar and protein production and F. solani showed lowest activity (Figure 3, 5). For Aspergillus species maximum and minimum activity were observed for A.trreus and A.niger, respectively (figure 4, 5)

Table 1 shows tested isolated in order of priority of maximum sugar production in the peak of existed cellulase rate and also variations in different days.

Based on the results, Trichoderma harzianum 15-6 and Fusarium solani species had the highest and the lowest ability to produce glucose in the CMC medium culture respectively.

Varition in released sugar protein in cmc media amoung three isolate of three diffrent species is obvioused. (Fig 6, 7). Trichoderma harzianum 15-16 has more CMC-ase activity than all isolates in our research cellulose.

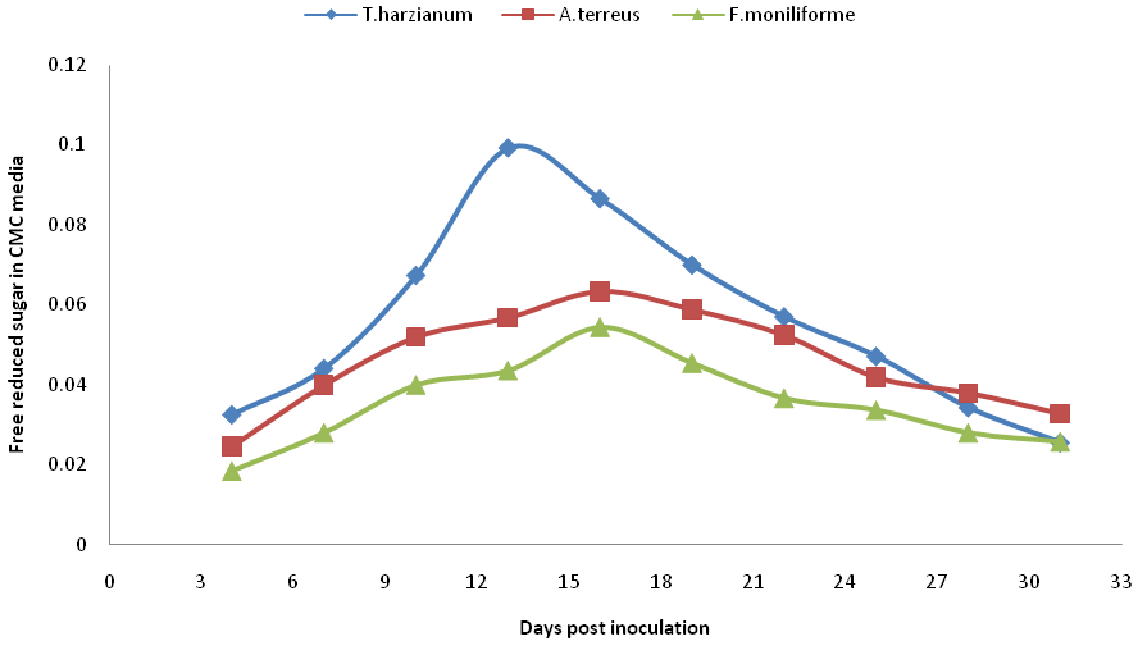

Fig. No. 6: Variations in released glucose from three high activity isolate in different species

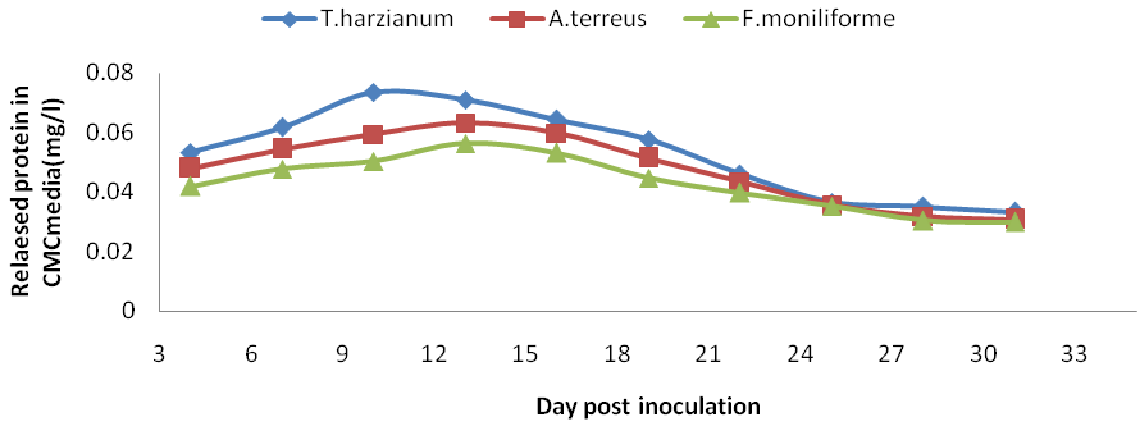

Fig. No. 7: Rate of Variations Inducing protein produced (mg/L) from three high activity isolate in different species

Complex enzymes of cellulase have a potential use in industrial applications that increase their commercial importance. This study verifies the result of Bergman and Peterson (1974) who found that T.koningii and T.viride are able to secret cellulase to degrade cellulose in a filtered medium. Mesner and Kubik, 1990, who were studying formation and secretion of cellulase compound of Trichoderma reesie in different sources of carbon, could approve different amount of sugar in the next days after inoculation.. A high protein production is occured after cmc inoculation, probably because of fungal initial growth [17].They assayed the amount of sugar by dinitrosalicylic acid (DNS). The presence of reductive sugar could approve the existence of enzyme in the medium. Furthermore, based on Giemba and Brandelli, 2002, researches, with isolating different soil borne fungi from different place, they could identified 9 different species which had shown well cellulolytic activities in four lineages enzymatic groups. Two of them that had noticable activities were Trichoderma (B17) and Aspergillus (A23). However, Lotfi (2008) reported that the highest celluloytic activity was occurred in 14 days post incubation by T. harzianum but this rate was recorded 15 days post incubation for our isolates. Protein assay showed that there was significant difference in released protein between tested genera and species but the amount of this increase was less than sugar. The time point of protein increscent was early of sugar that it could be due to fungi preparation to cellulose degradation and provide necessary enzyme for cellulose degradation. Our Results showed that between protein and sugar released was an correlation that it confirm above suggestion.

CONCLUSION

Screening of soil boil fungi to know their ability for cellulose activity and related protein assay is the first step to introduce them for industry. Fungi have a high ability to be use for enzymatic process in nature without any pollution for natural environment and residue. It also could utile to discover their sugar degradation activity and help us to choice more activity isolates to introduce its as a renovate resources for fuel production. In this study we attempted to screen our available isolates to determine the better isolates finally we present *Trichoderma harzianum* species as a foremost fungi candidate to cellulose degradation application.

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