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Isolation Cellulolytic Fungi from Plants and Animals Wastesin Iraq

Ayad Jaber Kubba* ***Adel saadi salman AL-Saadi*****

*Assist Professor, Institute of Genetic Engineering and Biotechnology, University of Baghdad, Baghdad, Iraq.
** Directorate of Environmental and Water, Ministry of Science and Technology, Baghdad, Iraq.

E-mail: adilmyco@yahoo.com

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Abstract:

134 samples of plants and animals wastes were taken from three different regions outside Baghdad and three different regions in Baghdad. 24 cellulolytic isolates fungi AO, C1, TH1, AN1, R1, TV, PG, AF, B1, L1, AP, TH, AP1, AN3, AO2, A, A1, C, F, AO1, C2, F1, CL and AP2 independent were chosen out of 48 selected fungi. The best optimal conditions for growth were 30ºC and pH 7. The isolates were identified and screened according to the colony diameter, biomass and density of spores in addition of capability to produce the hydrolytic enzymes for cellulose.

Key words: fungi, plant and animals wastes, cellulose.

Introduction:

The fungi plays an important role as a biocatalysts for the production of nutrition, chemicals materials and biofuels. It is important in improving of solar energy technology, biodegradation and bioremediation[1]. Fungi are chemotrophs, obtaining energy from chemicals, and heterotrophs, using organic compounds as carbon source [2]. They secrete extracellular enzymes to degrade polymers outside the cell and absorb the released nutrients and transport water through the cell membrane[3]. Most of fungi that grow on dead trees (saprotrophic fungi), and their role in natural ecosystem is by decomposition of wood and recycle the nutrients of wood. More distribution Wood degrading fungi are into white-mold and brown-mold fungi. Wood maceration mechanisms of both groups depend on enzymes (radical formation), decrease pH, and the production of organic acids[4]**.** Consisting the plant materials of three main units, cellulose, hemicellulose and lignin Lignocellulosic materials including agricultural wastes, forestry remnants, herbs and woody materials have great opportunity for bio-fuel production. Consist the agricultural lignocellulosic biomass is of almost10–25% lignin, 20–30% hemicellulose, and 40–50% cellulose[5]. Most cellulose are utilized in industry as a raw material in

paper and chemical production[6]**.** Renewable resource on earth is cellulose the most common organic[7]**.** From two stages composed the conversion of cellulose into glucose throught enzyme system of fungi beta-1, 4 glucanase breaks the glucosidic linkage to cellobiose, and then beta-1, 4 glucosidic linkages is broken by beta-glucosidase[8]. Fungi are hetrerotrophic organism depending on carbon compounds synthesized by other living organism[9]. In commercial biofuel production the fungi may have a important role, by degrading lignocellulose material and many of them are good producers of solvents. Trichoderma spp. is one of filamentous fungi that has been evaluated for their ability to produce biofuels which is a cellulose-degrading isolated from fungus soil and lytic plant material throughout the world, and produce fermentable sugars easily then converted to ethanol[10]**.**The aim of this research is to isolate and diagnose fungus analyst for Cellulose by using natural cellulosic sources as a basic step to isolate and select the celluloses production microorganisms successfully.

Materials and Methods:

Samples collection

The 134 samples in this study were collected from agriculture, plants and animals waste in the period from December 2014 to February 2015 which included plant leaves, bark, Roots, plant residuals, animal waste, rumen livestock from different regions in Baghdad city. The samples were taken from three regions outside of Baghdad (farms, Basaateen Al –Doora. farms, Basaateen Al-Siaafia and Basaateen Al-Greaat) and three regions from inside Baghdad (Baghdad University gardens and groves,Basaateen Al-Jaadria and Zawraa´s gardens). The samples were kept in clean poly ethylene bags and transformed to the laboratory and immediately used for the isolation process.

Isolation of fungi:

In isolated of fungi uses a serial dilution technique[11]. A sample suspension was prepared by adding 10 gm of sample to100 ml normal salin and shaking for 15 minutes in an orbital shaker[12]**.** Immediately, into the fifth dilution each suspension was serially diluted. From these diluents, 0.1 ml was transferred into plates with potato dextrose agar media, Roe Bengal agar and malt extract agar spread with a glass spreader and incubated for 5-7 days at 27±2 °C for observation. Each colony that appeared on the plate was considered as one colony forming unit (CFU).

Identification of isolated fungi

The isolated fungi were stained with Lactophenol cotton blue, lactic acid 10% and observed by the microscope. The morphology of the fungi was identified by using taxonomic keys[13,14]**.**

Growth capability (qualitative evaluation)

Thefungi was placed on two plates of Czapek Dox agar supplemented with carbox- ymethyl cellulose (1.2% w/v) and cellulose(0.6 w/v). The czapex dox agar was prepared according to[15].After an appropriate incubation period of 5- 7 days growth of fungi and cellulolytic activity was detected by appearance of clear zone around the colonies. Hydrolytic zones around the growing colonies were recorded for carboxymethyl cellulose activity. To enhance the visibility of hydrolytic zones, the plates were treated according to[16], the carboxymethyl cellulase activity was revealed by the presence of clearing zone around colonies, making duplicate for each isolate.

Result and Discussion:

Characterization of isolated fungi

48 isolates of fungi (18 genera) from 134 samples from plants and animals waste were isolated. The fungus isolates were differed accordingly to different places and varieties as shown in the table (1).

Table (1) showed the genus of fungi isolated from samples.

Genus	Genus
Cladosporium spp.	***Metarhizium spp.***
Aspergillus spp.	***Rodotorula***
Penicillium spp.	***Clestothesium***
Yeast colonies	***Geotrichumcandidum***
Alternaria spp.	***Bipolaris spp.***
Fusarium spp.	***Aerobasidium spp.***
Mucor spp.	***Scopularopsis spp.***
Trichoderma spp.	***Rhizopus ssp.***
Mycelia sterilia	***Emericella***

The distribution of these fungi in the agricultural and plant waste may be due to their presence in large numbers in the soil, which is the main source of spores of microorganisms and their ability for exploitation components to live and growth. The fungal species could utilize cellulose containing in this waste as carbon source[17]**.** This results agree with[18] which isolated and characterized many genus of fungi *Penicilium, Alternaria,* yeast, *Rhizopus, Fusarium*and*Aspergillus* from different soil samples including: (agricultural, fields, gardens, petroleum soil and cattle soil) in Baghdad. Furthermore, three fungi *Fusarium, Penicillium* and *Aspergillus* from mangrove leaves, mangrove wood and cotton leaves have been isolated[19].On the other hand, it is reported the presence of two genera *Asp. flavus* and *niger, Penicilium chrysogenum* in animal waste[20].

Screening of Cellulolytic isolated fungi

The results showed in table (2) that the growthof48 fungal isolates belonging to 18 isolating fungi were culturing upon Czapek Dox agar media supplemented with carboxymethyl cellulose (1.2% w/v) as a single source of carbon in order to make sure of the ability of fungus growth on media limited only for carboxymethyl cellulose as a source of carbon, and upon culturing Czapek Dox agar media supplemented cellulose(0.6 w/v) as a source of complex carbon. The best isolates growth on Czapek Dox agar with CMC and cellulose at alone (TH1, F2, TV, TH, R1, F, CL, A, L1, C, C2, PG and P), depended on the colony diameter and biomass and spores density. The diameter growth of the colonies upon Czapek Dox agar media supplemented with CMC was high and the biomass, spores density were generally less as for diameter growth of the colonies upon Czapek Dox agar media supplemented with cellulose. The biomass and spores density are a light compared with CMC. This result is normal because of the difficulty of degrading the cellulose by this isolates. The growth rate was less in isolates AO, C1, GC, AF, B1, E1, FS, AO2, M, AO1, A1 and F1. According to the same table the diameter growth of the colonies upon Czapek Dox agar media supplemented with CMC and the biomass, spores density generally is less. The diameter growth of the colonies upon Czapek Dox agar media supplemented with cellulose were less than the biomass and spores density. This is confirmed the ability of the isolates to produce cellulase enzymes on one hand and perhaps the production of other enzymes act synergistically such as hemicellulase enzymes and ligninase enzymes. The biomass and spores density to isolates AN1, AP, AP1, AP2, AN3 , E2, S1, MH, RO, RS, AT, AN2, E, M2, PG2, AT1 and AN4 are a light or

very light although the diameter area the colonies may be higher. The isolates Y, PG1, CM, R, PV and B2 in the table did not show any growth upon Czapek Dox agar CMC and cellulose. The developing fungi on these media are abundant in the environment, and have no special nutritional requirement and can be cultivated on organic and inorganic nutrients. It is widespread and utilizes a wide range of nutrients because of its ability to product a large number of digestive enzymes[21]**.** The PDA media supplemented with carboxy-methyl cellulose (1% w/v) were used as a single source of carbon to detection of Cellulolytic isolates *Aspergillus, Penicillium, Trichoderma* and *Fusarium* [17]**,** while [22] has used minimal medium supplemented with 1% (W/V) of cellulose as a model compound for polysaccharides to isolate the species of yeasts.

Table (2) showed Susceptibility growth of isolates on Czapek Dox agar with carboxymethyl cellulose (CMC) and Czapek Dox agar with cellulose /size in mm with different temperatures

s.	Abbr.	Name of genus	Cmc			Cellulose		
			27C°	30°C	34C°	27C°	30C°	34C°
1	AO	*Aspergillus ochraceus*	31 +	38 + +	18 +	27 +	30 +	21+
2	C1	*Chladsporium 9*	22+ +	25+ + +	16 +	11+ +	16+ + +	9 +
3	GC	*Giotricum candidum*	50+ +	54+ +	49 +	18 +	22+	16 +
4	TH1	*Metarhizium spp. 15*	76 + +	80+ + +	71 ++	76+ +	80+ +	75+ +
5	AN1	*Aspergillus niger 3*	44+	46+	43+	62+	72+	54+
6	R1	*Clestothecium spp.9*	67+++	68++++	60++	53+	56+	51+
7	TV	*Trichoderma viride*	80++	80+++	78++	78+	80++	77+
8	PG	*Penicillium glabrum*	25++	26++	21+	30+	33+	23+
9	AF	*Aspergillus flavus 4*	52++	54+++	52+	38+	46+	34+
10	MH	*Mucor hemalis 10*	80++	80++	80+	76+	80+	65+
11	RO	*Rhizopus oryzae 13*	78+ +	80++	73+	29+	33+	22+
12	RS	*Rhizopus stolonifer 7*	76++	80++	79+	40+	44++	38+
13	AT	*Aspergillus terreus 13*	37++	40++	38++	24+	30+	22+
14	B1	*Bipolaris spp 9*	55++	56+++	51+	33+	36++	31+
15	S1	*Scopularopsis spp*	23+	22++	22+	-ve	-ve	-ve
16	E1	*Emercella spp 22*	40+++	44+++	32+	26+	32++	25+
17	L1	*Mycelia stereilia 8*	31+++	34++++	25+++	23+	26++	21+
18	Y	*Yeastes*	-ve	-ve	-ve	-ve	-ve	-ve
19	PG1	*Penicillium glabrum 3*	-ve	-ve	-ve	-ve	-ve	-ve
20	AP	*Arobasidium pullulas 21*	23+	26++	22+	-ve	-ve	-ve
21	B2	*Bipolaris spp.*	-ve	-ve	-ve	-ve	-ve	-ve
22	M	*Mucor spp. 11*	79++	78+++	72+	55+	65++	46+
23	CM	*Chladosporium marcrcum*	2+	-ve	-ve	-ve	-ve	-ve
24	R	*Rhodotorula 5*	-ve	-ve	-ve	-ve	-ve	-ve
25	TH	*Trichoderma harazinum*	80++	80+++	76+	62+	65++	62+
26	AN2	*Aspergillus niger 15*	64+	80+	33+	-ve	-ve	-ve
27	FS	*F. sporotrichiodes15*	40+++	42+++	32+	31+	34++	28+
28	AP1	*Aspergillus parasiticus*	33++	33++++	31++	10+	12+	7+
29	AN3	*Aspergillus niger 6*	30+	30++	24+	-ve	3+	-ve
30	E	*Emericella spp. 18*	9++	11+++	7+	11+	18++	-ve
31	AO2	*Aspergillus ochraceus 20*	22++	23+++	20+	12+	16++	7+
32	A	*Alternaria alternate 19*	37+++	40+++	36++	28+	33++	26+
33	M2	*Mucor spp. 16*	74++	76++	71+	55+	60+	54+
34	A1	*Alternaria spp.20*	43++	51+++	40++	20+	22++	19+
35	C	*Chladspoirium spp.19*	18+	20+++	16+	3+	5++	-ve
36	F	*Fusarium spp.12*	56++	60+++	44+	27+	32+++	30+
37	AO1	*Aspergillus ochracus 12*	12++	14+++	11+	8+	8+	4+
38	P	*Penicillium spp.18*	18++	27+++	21+	9+	11++	4+
39	PG2	*Pencillium glabrum 4*	8++	10++	-ve	6+	10++	-ve
40	C2	*Chladsporium spp.20*	2+	17+++	10+	9++	11 ++	8 ++
41	F1	*Fusarium spp 22*	54+	73+	56+	54+	60+	48+
42	CL	*Mycelia sterilia 20*	35++	40+++	32+	32+	33++	28+
43	F2	*Fusarium spp. 19*	74++	80+++	70+	66+	80++	64+
44	E2	*Emercella spp. 20*	54+	55++	41+	37+	43+	33+
45	PV	*Penicillium variable 24*	12+	-ve	-ve	7+	-ve	-ve
46	AP2	*Aspergillus parasiticus 23*	11++	14+++	9+	7+	10+	6+
47	AT1	*Aspergillus terruse*	23+	30+	22+	18+	-ve	-ve
48	AN4	*Aspergillus niger 21*	24+	28++	20+	8+	10++	5 +

- No growth, + Poor growth, ++ Moderate growth, +++ Good growth, ++++ Massive growth.

Screening of fungal isolates for cellulytic activity

In table (3) the results showed the maximal zone of clearance was shown for TH1, TH, TV, AP, AO, R1, AF, AO2, CL , C1, AN1, PG, AT, B1, L1, AP1, A, C, F, AO1, C2, AP2 and AT1 which were recorded the best zone around the colony, while the isolates S1, E1, FS, AN3, E, A1, PG1, F1, E2,and AN4 were recorded less than 5 mm zone around the colony . 15 fungi isolates results did not show zone surrounding the colony as GC, MH, RO, RS, Y, PG1, B2, M, CM, R, AN2, M2, P, F2 and PV . The disparity in the ability to produce the enzyme may be due to variation in the genetic structure of the isolates or may be due to differences in environments that isolates collection from and may have been normalized to some environmental conditions (pH, temperature) differences from conducting test conditions**[**23]. The cellulase production ability of fungi assessed by estimating zone surrounding the colony formed because the ability of fungal isolates to hydrolyse cellulose. Glucose units one of the smaller sugar components produce by cellulases are a group of hydrolytic enzymes capable of hydrolysing cellulose. The nature's biodegradation processes product by cellulolytic enzymes where plant lignocellulosic materials are efficiently degraded by cellulolytic fungi [24]. This result agreed with the study [25] regardingan Training to isolate the fungi from the undiscovered soils of (Western Ghats) and screen them for the production hydrolytic enzymes as cellulase and CMCase. They were shown the fungal isolates *Aspergellus sp., Penecillium sp., Trichoderma sp., Alternaria sp*. and *Cladosporium sp*. zone surrounding the colony in Carboxymethyl cellulose media by production of extracellular enzymes. Also the susceptibility of fungal isolates showed *Penicillium sp، Alternaria alternata، Aspergillus niger, Trichoderma viride، Fusarium solani، Rhizopus sp* and *Aureobasidium pullulans* for the production of cellulase enzyme in the solid media (Carboxymethyl Cellulose agar) which collected from soil and leaves of citrus trees in Mosul city [26].

Table (3) measurement results for the clear zone of fungal isolates

s.	Abbr.	Zone /mm	s.	Abbr.	Zone/mm
1	AO	10	25	TH	40
2	C1	7	26	AN2	0
3	GC	0	27	FS	2
4	TH1	40	28	AP1	7
5	AN1	9	29	AN3	3
6	R1	10	30	E	3
7	TV	40	31	AO2	10
8	PG	7	32	A	6
9	AF	10	33	M2	0
`10	MH	0	34	A1	3
11	RO	0	35	C	5
12	RS	0	36	F	8
13	AT	6	37	AO1	9
14	B1	7	38	P	0
15	S1	2	39	PG1	2
16	E1	3	40	C2	8
17	L1	7	41	F1	3
18	Y	0	42	CL	10
19	PG1	0	43	F2	0
20	AP	24	44	E2	3
21	B2	0	45	PV	0
22	M	0	46	AP2	6
23	CM	0	47	AT1	5
24	R	0	48	AN4	4

Determine the pH optimum and temperature optimum

PH has a direct impact in activity to the growth in the metabolism of fungus. The effect of three levels of acidity testing (5.8, 7, and7.8) was tested on the isolates. The optimum growth to all isolates appeared in the pH 7, except for isolates Y and B2 which are not growing in this rate. Because the effect of acidity number on the growth of fungus, and their impact on the salts solubility in the medium and ionization of nutrition and transport, it showed the readiness and facilitate the developing microorganism [23]. In order to investigate the role of temperature on

development and growth the fungus, different incubation temperatures were used (27, 30, and 34 C°). The highest level of growth was obtained at 30 C° and the less level of growth was obtained at 34C° and in some of isolates fungal at 27C° as AN1, PG, AT, S1, E1, R and A1. The effect of temperature in the identification activity of various microorganisms including fungi especially the growth and metabolism is a fundamental way to control all activities catabolism and metabolism of these microorganisms [27].

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عزل الفطريات المحلله للسليلوز من المخلفات النباتية والحيوانية في العراق

اياد جابر كبة * **عادل سعدي السعدي ****

* استاذ مساعد، معهد الهندسة الوراثية والتقنيات الاحيائية للدراسات العليا، جامعة بغداد، بغداد ، العراق.
** دائرة البيئة والمياه ، وزارة العلوم والتكنلوجيا، بغداد ، العراق.

الخلاصة:

تم أخذ 134 نموذج لمخلفات نباتية وحيوانية من ثلاث مناطق مختلفة خارج مدينة بغداد وثلاث مناطق مختلفة اخرى داخل المدينة . تم انتخاب 24 عزلة فطرية (AO, C1, TH1, AN1, R1, TV, PG, AF, B1, L1, AP, TH, AP1 AN3 AO2, A, A1, C, F, AO1, C2, F1, CL, AP2,) محللة للسيليلوزمن 48 عزلة فطرية. وكانت الظروف المثلى للنمو عند درجة حرارة 30 م° ودالة حامضية 7. شخصت العزلات وغربلت حسب قطر المستعمرة النامية، الكتلة الحيوية للمستعمرة والكثافة السبورية اضافة الى القدرة على انتاج الانزيمات المحلله للسيليلوز.

الكلمات المفتاحية: الفطريات، مخلفات نباتية وحيوانية ، سيليلوز.