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Compost Microflora and Screening of Fungi for Cellulotic Capability

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ABSTRACT. Compost microflora, mostly thermophilic, isolated during various stages of composting municipal refuse in Kuwait (mainly composed of food waste and cardboard), consisted of dominant bacteria in counts of 10^4-10^9 g^{-1}, followed by actinomycetes at 10^4-10^8 g^{-1} and fungi at 10^5-10^8 g^{-1}. Among these microbial groups, some isolates were identified to the species level, some to the genus level. Based on semiquantitative test of extracellular protein accumulation, the total number of fungal isolates (21) was reduced to six prospective ones. Further screening of fungi was based on assays of cellobiase, carboxymethylcellulase (CMCase), filter paper and cotton activity, following growth on different carbon sources. A comparison was also made with other available fungi or with published data. Cellobiase and CMCase activities of some KISR strains were comparable or superior to those published, other activities were rather low.

Composting is principally a biological process achieved by the microbial degradation of organic material; it involves aerobic respiration and passes through a thermophilic stage with compost as the stabilized end-product. Household refuse is the major constituent of municipal refuse (garbage) in Kuwait, so it is basically organic in nature. Natour *et al.* (1982a) reported on the composition of household solid waste as: food waste, 41.7-60.5%; paper and cardboard, 11.4-29.4%; inorganic and miscellaneous material, about 20%. In another survey, the amount of municipal refuse in 1982 was estimated to be 900-1000.10^3 t/yr; this was divided into 540.10^3 of organic waste and 210-293.10^3 of cellulosic waste (Razzaque and Salman 1982).

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The microflora in the household refuse is usually composed of different bacteria, fungi, actinomycetes and protoza. Their exact role and mode of action in the process of decomposition of organic matter is not fully known. In this paper, compost microflora is partially characterized and results on the cellulose-degrading capability of isolated fungi is reported. Details may be found in Natour *et al.* (1982b).

Material and Methods

Compost Process

The microorganisms were isolated from compost made from two different composting processes: municipal and experimental. Municipal composting carried out by the Municipal Organic Fertilizer Plant in Kuwait yielded a rather low quality compost because of the many inadequacies in the process (low moisture content [35-40%] and low aeration, height of compost heaps [up to 4.5 m], and composting period [33-51 days]). More intensive composting was achieved in 1 m^2 experimental bins, either naturally or artificially aerated, with more controlled conditions. With naturally aerated bins, high quality compost was obtained in 9-10 days of composting. In artificially aerated windrowing trials (by turning bins mechanically every three to five days), good compost was yielded within 26 days.

Isolation of Microorganisms; Their Taxonomy

Municipal Compost

Actinomycetes were isolated on yeast/malt extract glucose agar at 28°C. No pH adjustment is required. Using this medium, one obtains maximum production of spores with minimal production of vegetative mycelium (Harrold 1950). Their counts were taken after three to five days of incubation. Bacteria were isolated on either peptone-beef extract agar or nutrient agar, with the pH adjusted to 7, and incubated at 37°C for five to six days and fungi on potato-dextrose agar. Culture media were asceptically poured into 95 mm petri dishes containing 0.1 ml of a 10^{-5} dilution of the compost sample. Compost dilutions were prepared by blending 10 g of compost in 400 ml sterile distilled water mixed in a Waring blender for 1 min. The volume was then made up to one liter with sterile distilled water. Tenfold dilutions were prepared by mixing 10 ml of the compost dilution into 90 ml sterile distilled water. The experiment was repeated four times (four heaps 5 m in dia. and 4.5 m high).

Experimental Compost

The microbial populations in the compost piles were determined initially and after 3, 8, 13 and 21 days of composting. Microorganisms were isolated by culturing on aseptic media from 0.1 ml of 10^{-5} dilutions of compost material at 24, 34, and

52°C. Bacteria were isolated on nutrient agar medium, fungi on potato-dextrose agar, and actinomycetes on Jensen or starch-casein media. The experiment was repeated four times (four bins 1 m^2 at the base and with 30 cm high walls).

Analysis of compost material for protozoan biomass was made by direct microscopical count during composting in the naturally aerated bins in the first, third, sixth, eighth and ninth days of composting. Counts were made from composite samples taken at depths of 10 and 30 cm.

The taxonomy of the bacterial and fungal isolates was determined by the Commonwealth Mycological Institute (Kew, Surrey, UK).

Cultivation of Fungi and Cellulase Screening

Organisms

Based on preliminary screening, the following strains were tested for cellulose activities: *Absidia corymbifera* (Cohn) Sacc. and Trotter, *Acremonium strictum* Gams, *Aspergillus flavus* Link ex Fries, *Aspergillus fumigatus* Fresenius, *Cladosporium cladosporiodes* (Fres.) de Vries and *Neurospora sitophila* Shear & Dodge. An unknown mixed thermophilic culture of fungi isolated from a composting trial after 10 days was also used. The following three mesophilic standard strains were compared: *Trichoderma reesei* NRRL 3653 (= QM 9123), *Trichoderma reesei* NRRL 6156 (= QM 9414) and *Trichoderma reesei* NRRL 11236 (= MCG 77).

Media

These strains were grown in three kinds of media with the following cellulosic materials as carbon sources: filter paper, cotton, carboxymethylcellulose (CMC), cellobiose and cardboard. Potato-dextrose agar was used for maintenance of fungi. Cultures were grown at 28°C in dim light to encourage sporulation (Mandels and Weber 1969). After one week, slants were stored in the refrigerator until needed. Transfers were made every month. For shake flask cultures on a rotary shaker, either *T. viride* medium (Mandels and Weber 1969) or Czapek's medium (Raper *et al.* 1973) supplemented with 1% (w/w) powdered cellulose source (CMC, cellobiose, filter paper, cotton, cardboard) were used.

Assays

Reducing sugars in the culture supernatant as glucose were estimated by DNS method (Miller 1959, Rosenberg and Oberkotter 1977) and glucose by GLU-CINET (I.S.C.T., Sclavo, SpA, Siena, Italy), based on the colorimetric determination of glucose using an enzymatic oxidation of glucose by glucose oxidase. Hydrogen peroxide, a product of the reaction, reacts with hydroxy benzoate-4-aminophenazone to yield a red quinone. Protein was estimated by the Lowry method (Lowry *et al.* 1951). The cellobiase assay (β-glucosidase, E.C.3.2.1.21), carboxymethylcellulase-CMCase (endoglucanase; C_x; 1,4-B-D-glucan glucanohy-

drolase; E.C.3.2.1.4), filter paper assay (as a measure of both C_1 and C_x activity; C_1 is denoted as exoglucanase or 1,4-B-D-glucan cellobiohydrolase; E.C.3.2.1.91) and cotton assay (as a measure of C_1, but C_x must be present) were estimated by methods described in Rosenberg and Oberkotter (1977). The cellobiase assay was carried out with the help of GLU-CINET reagent. Cellobiase and CMCase activities (μmol glucose min^{-1} ml $supernatant^{-1}$), filter paper activity (mg glucose $ml^{-1}h^{-1}$) and cotton activity (mg glucose $ml^{-1}h^{-1}$) were expressed in I.U. Residual cellulose after degradation of substrate by fungi was determined by a method of Romanelli *et al.* (1975) based on mild digestion of the fungal biomass by an alkali.

Results and Discussion

Compost Microflora

The viable counts per gram of municipal compost (dry weight) were: 10^4-10^8 for actinomycetes, 10^4-10^9 for bacteria and 10^3-10^8 for fungi (Table 1). Since the temperature was usually 40°C or above during composting (range 30-60°C, depending on heap height), the isolated microorganisms were mostly thermotolerant or thermophilic.

Table 1. Microbial counts (viable cells) in municipal compost per one gram (dry weight).

Heap No.	Micro-organisms	Interval of Study (week)			
		2	3	4	5
1	Actinomycetes	*	1.8×10^8	4.2×10^8	5.4×10^6
	Bacteria	*	7.7×10^6	8.5×10^7	1.4×10^4
	Fungi	*	2.3×10^5	2.8×10^5	*
2	Actinomycetes	*	4.4×10^7	3.0×10^7	3.9×10^5
	Bacteria	1.4×10^8	4.6×10^8	8.4×10^8	2.3×10^4
	Fungi	1.6×10^7	1.9×10^7	*	1.0×10^3
3	Actinomycetes	1.8×10^7	*	3.5×10^4	6.7×10^5
	Bacteria	9.2×10^8	5.8×10^8	1.1×10^7	6.6×10^5
	Fungi	1.4×10^6	6.2×10^6	*	5.0×10^8
4	Actinomycetes	*	6.3×10^5	5.9×10^4	3.3×10^8
	Bacteria	2.1×10^7	5.8×10^5	2.7×10^4	1.5×10^9
	Fungi	*	*	1.7×10^4	2.8×10^8

Note: Heaps were 5 m in dia. and 4.5 m high.
* No samples have been taken.

Table 2. Microbial counts (viable cells) in experimental compost per one gram (dry weight).

Compost trials	Microflora	Interval of study (d)														
		0			3			6			13			21		
		24°C	34°C	52°C	24°C	34°C	52°C	24°C	34°C	52°C	24°C	34°C	52°C	24°C	34°C	52°C
Bin 2*	Bacteria	9.5×10^8	2.6×10^8	1.5×10^8	2.8×10^7	4.4×10^8	2.4×10^8	2.0×10^7	2.9×10^7	1.1×10^7	1.6×10^9	1.1×10^8	2.4×10^7	2.75×10^9	2.1×10^8	1.1×10^6
	Fungi	5.0×10^7	7.0×10^7	1.5×10^6	0.0	2.7×10^6	0.0	2.2×10^7	2.0×10^7	7.0×10^6	1.1×10^8	1.1×10^7	2.2×10^7	4.0×10^6	1.2×10^6	4.0×10^5
	Actinomycetes	5.7×10^8	5.3×10^8	1.5×10^6	1.7×10^9	1.9×10^9	2.8×10^7	0.0×10^8	7.0×10^8	1.1×10^8		5.0×10^7	6.5×10^7	1.9×10^9	1.1×10^8	7.0×10^5
Bin 3**	Bacteria	9.5×10^8	2.6×10^8	1.5×10^8	1.66×10^7	4.0×10^8	2.4×10^8	4.7×10^6	2.6×10^7	5.0×10^7	2.8×10^9	3.5×10^7	1.2×10^9	3.4×10^9	1.2×10^8	5.5×10^7
	Fungi	5.0×10^7	7.0×10^7	1.5×10^6	4.7×10^8	9.5×10^6	2.4×10^6	7.1×10^6	7.1×10^6	4.0×10^6	0.0	1.0×10^7	0.0	0.0	3.5×10^6	2.5×10^5
	Actinomycetes	5.7×10^8	5.3×10^8	1.5×10^6	1.6×10^9	1.3×10^7	3.8×10^7	1.8×10^9	3.0×10^9	1.0×10^8	5.0×10^9	1.0×10^8		1.3×10^9	7.1×10^8	1.5×10^6
Bin 4	Bacteria	9.5×10^8	2.6×10^8	1.5×10^8	7.7×10^6	9.1×10^8	2.3×10^8	1.0×10^7	3.5×10^7	1.5×10^7	4.0×10^7	1.2×10^8	2.2×10^6	2.4×10^9	4.0×10^7	3.3×10^7
	Fungi	5.0×10^7	7.0×10^7	1.5×10^6	6.6×10^6	1.5×10^7	3.3×10^6	4.1×10^6	6.3×10^6	4.2×10^6	0.0	2.0×10^7	7.5×10^6	0.0	1.5×10^6	5.5×10^5
	Actinomycetes	5.7×10^8	5.3×10^8	1.5×10^6	4.3×10^8	1.1×10^9	1.2×10^7	1.5×10^9	3.5×10^8	9.8×10^7	2.5×10^9	2.0×10^8		1.2×10^9	9.5×10^8	1.1×10^5
Bin 5	Bacteria	9.5×10^8	2.6×10^8	1.5×10^8	5.3×10^8	9.3×10^6	1.1×10^8	1.1×10^7	5.7×10^7	1.0×10^8	2.7×10^6	7.0×10^8	3.3×10^7	2.6×10^6	9.0×10^7	3.5×10^6
	Fungi	5.0×10^7	7.0×10^7	1.5×10^6	0.0	3.8×10^6	0.0	2.2×10^6	1.1×10^7	2.2×10^6	1.3×10^8	4.6×10^6	0.0	0.0	2.0×10^6	2.2×10^5
	Actinomycetes	5.7×10^8	5.3×10^8	1.5×10^6	2.5×10^9	7.3×10^8	7.1×10^7	1.1×10^7	3.0×10^7	1.0×10^8	2.3×10^8	3.0×10^8		1.0×10^8	7.5×10^7	7.5×10^6

* An addition of urea to adjust C/N.

** An addition of a complex fertilizer to adjust P; N; K.

Table 3. Protozoan populations per one gram (dry weight) of experimental compost (10 cm depth, naturally aerated bins)*.

Interval of study (d)	F	R	C	Total
1	8300	1360	150	9810
3	20500	3170	50	23720
6	37800	11980	20	49800
8	40300	15600	00	55900
9	43300	23325	00	66625

F = Flagellates
R = Rhizopodes
C = Ciliates
* Averages of various counts from three bins.

The microbial counts per gram of experimental compost (dry weight) were somewhat higher than in the municipal compost; the order of magnitude was: 10^5-10^9 for actinomycetes, 10^6-10^9 for bacteria and 0-10^8 for fungi (Table 2), depending on the incubation temperature (24, 34 or 52°C). Counts were more or less constant regardless of whether the compost was treated with urea or with fertilizer, or of intervals between treatments. This was mainly because the composting finished before complete stabilization.

Analysis of the municipal and experimental compost revealed that both were rich in protozoan populations, particularly in the flagellated and rhizopode types. Because of the rapid increase in compost temperature with compost height, protozoan populations were found only 10 cm deep. An example of protozoan counts in the experimental compost is shown in Table 3.

Fungal and bacterial isolates from municipal compost were further characterized by taxonomy (Table 4). The fungi spectrum and counts were very similar to those documented in the literature (Poincelot 1974, 1975; Finstein and Morris 1975, Gray *et al.* 1971).

Cellulase(s) Activity of Fungi

Screening of Fungi

The excretion of enzymes to the culture supernatant was the basis for screening the fungi listed in Table 4. The total protein content of the supernatant (compared

Table 4. Bacterial and fungal isolates from municipal compost.

Bacteria	*Acinetobacter calcoaceticus* (Beijerinck) Baumann, Doudoroff and Stainer
	Alcaligenes faecalis Castellani and Chalmers
	Bacillus sp.
	Bacillus subtilis (Ehrenberg) Cohn
	Corynebacterium sp.
	Enterobacter aerogenes (Kruse) Hormaeche and Edwards
	Enterobacter cloacae (Jordan) Hormaeche and Edwards
	Micrococcus sp.
	Serratia marcescens Bizio
	Staphylococcus sp.
	Staphylococcus aureus Rosenbach
Ascomycetes	* *Neurospora sitophila* Shear & Bodge
Hyphomycetes	* *Acremonium strictum* Gams
	Alternaria alternata (Fr.) Keissler
	* *Asperigillus flavus* Link ex Fries
	* *Aspergillus fumigatus* Fresenius
	Aspergillus nidulans Eidam
	Aspergillus niger van Tieghem
	Cephalosporium sp.
	* *Cladosporium cladosporioides* (Fres.) de Vries
	Humicola sp.
	Paecilomyces variotti Bainier
	Penicillium chrysogenum Thom
	Penicillium cyclopium Westling
	Trichoderma sp.
	Trichosporon sp.
Basidiomycetes	*Coprinus cinereus* (Fres.) S.F. Gray
Zygomycetes	* *Absidia corymbifera* (Cohn) Sacc. & Trotter
	Mucor circinelloides van Tieghem
	Mucor pusillus (Lindt) Schipper
	Rhizopus nigricans
	Rhizomucor miehei (Cooney and Emerson) Schiffer.

* Isolates further tested for specific cellulase activity.

with the protein content of the homogenized culture) obtained from fungal cultures was a measure of extracellular cellulase activity. The isolates of fungi were grown on Czapek's medium, using filter paper Whatman No. 1, cotton or dry ground compost as a carbon source and incubated at 45°C at a shaking frequency of 150 rpm for 9 days. Protein in the supernatant was determined by Lowry's method. From this preliminary investigation, it was found that any observed activity was dependent on the carbon source used. On cotton, neither growth nor enzyme

production was noted with any of the strains. On the basis of results obtained with filter paper or compost, the number of fungi was reduced to six prospective strains (Table 5). The rest of the fungal isolates exhibited negligible activity and were omitted from further study.

Production of Cellulases on Various Substrates

The fungi were grown on Czapek's medium using either filter paper or absorbent cotton as a carbon source (nine days at 40°C, shaking frequency 150 rpm). Both cotton and filter paper activities were determined *via* production of reducing sugars (DNS method) and were lower (Table 6) than values obtained from the literature (Table 10). They did not correlate with a capability to produce extracellular protein (Table 5).

CMCase activities were estimated for fungi grown on both Czapek's and *T. viride* media supplement with 1% CMC. Maximal titres were achieved after nine days of cultivation. Table 7 compares two media for CMCase activity. *T. viride* medium gave consistently higher activity, *Cladosporium* sp. was the best strain (see Fig. 1 A-F).

β-glucosidase activities were assessed on *T. viride* medium supplemented with 1% cellobiose or 1% filter paper as a carbon source, being, in general, higher on the latter substrate; Fig. 2A-F presents a time course of the activity for filter paper as a carbon source.

Table 5. Total protein in the supernatant and culture homogenate.

Fungal isolate	Protein ($\mu g\ ml^{-1}$)								
	Whatman filter paper			Cotton			Compost		
	Supernatant	Homogenate (total)	%	Supernatant	Homogenate (total)	%	Supernatant	Homogenate (total)	%
Absidia corymbifera	19.6	144.9	13.5	0.0	0.0	0.0	26.9	42.2	63.7
Acremonium strictum	3.6	36.0	10.0	0.0	0.0	0.0	13.3	41.2	32.3
Aspergillus flavus	0.0	90.0	0.0	0.0	0.0	0.0	3.0	46.0	6.5
Aspergillus fumigatus	2.0	30.4	6.6	0.0	0.0	0.0	0.0	0.0	0.0
Cladosporium sp.	45.4	189.5	23.9	0.0	0.0	0.0	36.3	94.0	38.0
Neurospora sitophila	33.5	195.7	17.1	0.0	0.0	0.0	14.2	66.4	21.5

Note that percentage denotes the percentage of supernatant protein in the total protein (in a homogenate; mechanical homogenization).

Table 6. Cotton (CT) and filter paper (FP) activities in culture supernatants.

Fungal isolate	CT (I.U.)	FP (I.U.)
Absidia corymbifera	0.0	0.05
Aspergillus flavus	0.0	0.004
Aspergillus fumigatus	0.0	0.0
Cladosporium sp.	0.0	0.0
Neurospora sitophila	0.02	0.0

Table 7. Comparison of extracellular CMCase activity for *T. viride* and Czapek's media after nine days of cultivation.

Fungal isolate	CMCase (I.U.)		
	T. viride medium (%)	Czapek's medium (%)	Czapek's medium *T. viride* medium (%)
Absidia corymbifera	1.70	0.89	52.0
Acremonium strictum	1.31	0.12	9.0
Aspergillus flavus	1.05	0.62	59.0
Aspergillus fumigatus	1.29	0.57	44.0
Cladosporium sp.	1.75	0.71	40.0
Neurospora sitophila	1.6	0.0	–

In another experiment, the six thermophilic fungal isolates used above, mixed unknown fungal isolates from compost and three standard mesophilic *Trichoderma* strains were grown on *T. viride* medium with 1% ground cardboard and 0.1% glucose (to initiate growth) as carbon sources. Temperatures of 45°C, for thermophilic strains and 30°C, for mesophilic strains, were used at 150 rpm on a rotary shaker for ten days. The culture filtrates were assayed for CMCase and cellobiase activity, for protein accumulation and for cellulose utilization. The total protein in the supernatant was highest for *Acremonium strictum* (more than 1 mg ml^{-1}) on the tenth day of cultivation.

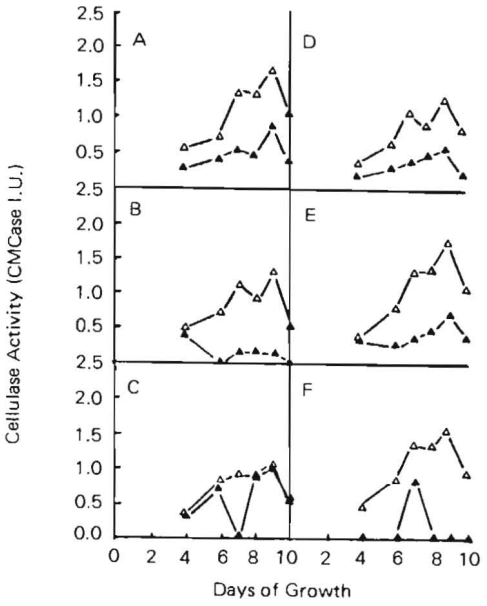

Fig. 1A-F. Comparison of extracellular CMCase activity for *T. viride* and Czapek's media.
△—△ In *Trichoderma viride* medium
▲—▲ In Czapek's medium

A. *Absidia corymbifera*
B. *Acremonium strictum*
C. *Aspergillus flavus*
D. *Aspergillus fumigatus*
E. *Cladosporium* sp.
F. *Neurospora sitophila*

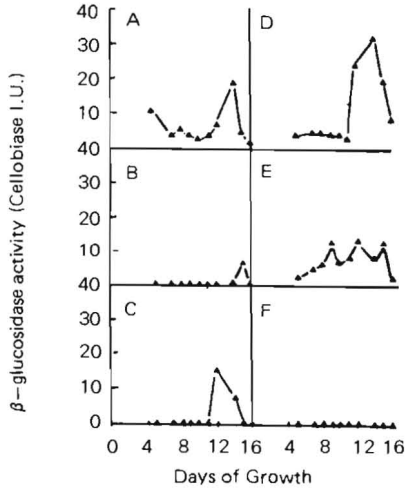

Fig. 2A-F. β-glucosidase production with filter paper as carbon source in *T. viride* medium.

A. *Absidia corymbifera*
B. *Acremonium strictum*
C. *Aspergillus flavus*
D. *Aspergillus fumigatus*
E. *Cladosporium* sp.
F. *Neurospora sitophila*

Table 8. Cellobiase and CMCase activities in cardboard-utilizing cultures.

Days of growth / Fungi isolate	3		4		5		6		7		10	
	a	b	a	b	a	b	a	b	a	b	a	b
Absidia corymbifera	0.23	0.34	0.30	0.30	0.30	0.38	0.33	0.32	0.87	0.43	0.57	0.39
Acremonium strictum	0.20	0.11	0.30	0.10	0.44	0.34	0.35	0.27	0.20	0.43	1.55	0.42
Aspergillus flavus	0.75	0.26	0.74	0.04	0.25	0.05	0.30	0.11	0.63	0.50	0.53	0.37
Aspergillus fumigatus	0.23	0.34	0.30	0.33	0.37	0.39	0.33	0.33	0.63	0.42	0.57	0.39
Cladosporium sp.	0.30	0.33	0.30	0.30	0.34	0.38	0.33	0.35	0.87	0.47	0.60	0.39
Neurospora sitophila	0.37	0.35	0.44	0.29	0.25	0.40	0.10	0.32	0.30	0.53	0.57	0.43
T. reesei NRRL 3656	0.20	0.04	0.23	0.34	0.34	0.04	0.31	0.38	0.54	0.42	0.85	0.39
T. reesei NRRL 6156	0.27	0.18	0.34	0.34	0.37	0.40	0.35	0.39	0.73	0.50	1.50	0.35
T. reesei NRRL 11236	0.27	0.18	0.30	0.08	0.37	0.35	0.31	0.28	0.54	0.36	1.14	0.04
Mixed fungal culture	0.34	0.01	0.20	0.0	0.30	0.0	0.31	0.01	0.35	0.0	0.37	0.0

[a] Cellobiase activity (I.U.)
[b] CMCase activity (I.U.)

Table 9. Loss of cellulose in cardboard-utilizing cultures.

Days of growth / Fungi isolate	Loss of cellulose (%)					
	3	4	5	6	7	10
Absidia corymbifera	25.7	50.1	55.2	61.3	63.8	67.4
Acremonium strictum	26.0	30.0	33.5	42.6	46.2	65.2
Aspergillus flavus	8.6	15.7	17.3	18.4	19.7	58.4
Aspergillus fumigatus	41.2	40.4	64.3	63.3	64.0	67.4
Cladosporium sp.	37.8	43.8	58.6	63.5	64.8	68.9
Neurospora sitophila	28.7	41.9	56.2	62.4	69.2	70.4
T. reesei NRRL 3656	16.6	45.0	56.8	57.6	61.1	62.0
T. reesei NRRL 6156	38.2	41.8	44.6	62.2	65.9	65.0
T. reesei NRRL 11236	30.0	45.3	59.0	63.0	65.0	66.0
Mixed fungal culture	6.5	6.7	8.2	8.2	10.4	15.7

Table 10. Some reported maximal cellulase activities for different microorganisms and comparison with KISR strains.

Organism	FP[1]	CT[2]	CMCase[3]	CB[4]	Author	Comments (substrate)
Aspergillus terreus	0.75	–	0.70	–	Garg and Neelakantan (1981)	Alkali-treated bagasse
Chaetomium cellulolyticum	0.54	–	2.23	–	Fähnrich and Irrgang (1981)	Newspaper
Chaetomium cellulolyticum	1.20	0.40	0.33	–	Moo-Young *et al.* (1977)	Sawdust
Sporotrichum thermophile	1.80	5.0	5.0	0.01	Canevasci and Gattlen (1981)	Pure cellulose
Aspergillus phoenicis	–	–	–	11.6	Sternberg *et al.* (1977)	Different cellulosic material
Trichoderma reesei NRRL 6156	2.60	4.50	0.78	–	Moo-Young *et al.* (1977)	Sawdust
Trichoderma reesei NRRL 6156	7.20	44.0	21.0	0.007	Ganevasci and Gattlen (1981)	Solka-floc
Trichoderma reesei NRRL 6156	65.0	–	–	2.5	Sternberg and Dorval (1979)	Spruce pulp cellulose
Trichoderma reesei NRRL 6156	36.0	–	–	–	Ghose and Sahai (1979)	Powdered cotton cellulose, fed-batch
Trichoderma reesei NRRL 3653	9.8	5.5	–	–	Griffin *et al.* (1974)	Feed-lot waste
Trichoderma reesei NRRL 11236	28.1	–	–	–	Ryu *et al.* (1979)	Lactose, two-stage chemostat
Trichoderma sp.	8.2	13.4	–	–	Zhu *et al.* (1982)	Rice straw, wheat bran
Trichoderma reesei	–	–	8.3	–	Gottwaldova *et al.* (1982)	Micro-crystalline cellulose; double fed-batch
KISR strains' maximal activity	0.05	0.02	1.8	31.2	This paper	Cellobiose, filter paper, CMC, cardboard, cotton

[1]FP = filter paper activity in mg glucose ml^{-1} h^{-1}
[2]CT = cotton activity in mg glucose ml^{-1} d^{-1}
[3]CMCase = carboxymethylcellulase in μmol glucose ml^{-1} min^{-1}
[4]CB = cellobiase activity in μmol glucose ml^{-1} min^{-1}

The profile of cellobiase and CMCase activities for all tested strains are given in Table *8*. In Table 8, it is shown that activities at the end of cultivation were comparable for all strains except *Aspergillus flavus* and *T. reesei* NRRL 6156, which exhibited higher activities. When results were compared with those in Fig. 2 A-F, cardboard does not seem to be as good a substrate for cellobiase production as filter paper (or cellobiose). On the other hand, CMCase activities were lower than those obtained on defined media (Table 7 and Fig. 1 A-F). The cardboard utilization efficiency was around 65-70%, except for the mixed culture (Table 9).

A comprehensive comparison of KISR strains with standard ones is presented in Table 10. Local strains exert high CMCase and cellobiase but low filter paper and cotton activities. Activities were particularly depended on the types of substrate used; some substrates may serve as inducers of a given activity. Filter paper Whatman No. 1 used as substrate for cellulase and CMC for CMCase. The uniquely high cellobiase activity of some KISR strains may be used to produce soluble sugars from waste cellulose or as a sweetener. *A. flavus* produces the potent carcinogen Aflatoxin. The production of enzymes has not so far been optimized, especially in the direction of temperature and cellulosic substrate. The major contribution of this work is to show that new thermophilic fungi are available to degrade cellulosic material. Other bacterial and actinomycete isolates remain to be tested.

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الأحياء المجهرية فى السماد العضوى المصنوع من المخلفات ومقدرة بعض الفطريات فيه على تحليل السيليلوز

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قسم البيوتكنولوجيا - معهد الكويت للأبحاث العلمية ص.ب ٢٤٨٨٥ - الصفاة - الكويت

تقوم الكويت بصناعة السماد العضوى من مخلفات البلدية التي تتكون في الغالب من مخلفات الطعام والكرتون. ونظرا لوجود أعداد هائلة من تلك الكائنات التي يعيش معظمها على درجات حرارة مرتفعة نسبيا، إذ إن أعداد الكائنات المختلفة في السماد تراوحت في الجرام الجاف ما بين 10^{4}-10^{9} للبكتيريا، 10^{4}-10^{8} للأكتينوما يسيتس، 10^{5}-10^{8} للفطريات. ولقد تم تصنيف تلك الكائنات الى رتبة الجنس أو النوع.

وبناء على تحديد مقدار إفراز الفطريات للبروتينات بصورة شبه كمية، اختيرت إحدى وعشرون عزلة من مجموعة الفطريات ومن ثم قلصّ هذا العدد إلى ست عزلات فقط، تمت دراسة مقدرتها على تحليل السيليلوز وذلك بتحديد كمية إفرازاتها لإنزيمي السليوبييز والكاربوكسي مثيل سيليوليز، وكذلك درجة تحليلها لورق الترشيح والقطن أثناء تنميتها على مصادر كربون مختلفة.

وبالمقارنة مع فطريات أخرى ومع ما تم نشره في هذا المجال، تبين أن فعالية أنزيمي السلوبييز والكاربوكسي مثيل سيليوليز لديها كانت مشابهة أو أعلى من فعاليات ما نشرعن فطريات أخرى.