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ABSTRACT

The cellulase activities of 8 isolates of fungi obtained from agricultural soils of Hidalgo County, TX were investigated by measuring the changes in the viscosity of a buffered solution of carboxymethylcellulose (CMC), produced by the fluids obtained from liquid cultures of these isolates. The change in the viscosity of the reaction mixture incubated at constant temperature (30 C) was measured with Cannon–Fenske routine viscometers. A buffered cellulase solution was used as a control.

Due to the variability that existed among the isolates investigated, it was possible to select active producers of cellulase with the method followed in this investigation. The cellulase activities of 7 of the isolates were higher than the activity of the cellulase control solution. The production of cellulase by the fungi was higher when the isolates were grown in liquid media containing CMC than when glucose was used as the carbon source. Whereas all isolates produced detectable cellulase in the presence of CMC, only 7 of 8 did so in the presence of glucose.

INTRODUCTION

Soils under cultivation are the usual habitat of many species of fungi which may live there as saprophytes, parasites of the root systems of cultivated crops, or in mycorrhizal associations with the roots of some species of perennial plants. While most of the fungi that form ectotrophic mycorrhizae do not have the ability to decompose cellulose or lignin (Garrett, 1956) many other species of fungi from the soil are strongly cellulolytic (Alexander, 1961).

Some of the plant pathogenic fungi that live permanently in the soil or overwinter in this medium are capable of direct penetration into the root tissues of their respective hosts (Agrios, 1978). When plant pathogens that have the ability to decompose cellulose come in close contact with the roots of susceptible plants, penetration into the root tissues may be accomplished by the softening or destruction of the plant cell walls by cellulases or lignin degrading enzymes (Agrios, 1978).

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The main objectives of this investigation were: (1) to isolate cellulase-producing species of fungi from agricultural soils, and (2) to assess the cellulase activity of these species.

MATERIALS AND METHODS

Soil Samples

Several soil samples were collected from agricultural fields of Hidalgo County, TX. Samples were taken from the upper 2.5 cm of the ground and placed in sterile test tubes (150 x 16 mm) which were covered with plastic caps. The soil collections were allowed to dry at room temperature for 1 wk.

Selective Medium

Dried soil samples were pulverized in a mortar and transferred to petri plates containing a selective medium of the following composition: 0.55% KNO_3, 0.16% KCl, 0.10% KH_2PO_4, 0.04% $MgSO_4 \cdot 7H_2O$, 1% carboxymethylcellulose sodium salt (CMC by U.S. Biochemical Corporation, Cleveland, OH), 1.5% agar and distilled water to make 1 ℓ. A small amount of pulverized soil (0.01 - 0.10 g) was taken up on the flattened end of a nichrome needle and mixed directly in 10 ml of the cooled medium (Warcup, 1950).

Fungi Isolates

Isolations from the fungal colonies that grew on the selective medium were made, identified, and then maintained in test tubes of selective medium or potato-dextrose-agar, PDA Difo (B13). All cultures were incubated at 25 C. The growth of all isolates was excellent when cultivated on PDA or in liquid media containing glucose instead of CMC and all other components as described below for the cellulase production medium.

Liquid Cultures

Fungal isolates were cultivated in 250-ml Erlenmeyer flasks containing 50 ml of a liquid medium for cellulase production of the following compostion: 0.55% KNO_3, 0.16% KCl, 0.10% KH_2PO_4, 0.04% $MgSO_4 \cdot 7H_2O$, 0.04% $ZnSO_4 \cdot 7H_2O$, 0.02% $MnSO_4 \cdot H_2O$, 1.0% CMC, and distilled water to make 1 ℓ: Before sterilizing (121 C, 15 min, 15 psi) the medium had a pH of 5.5. Each isolate was aseptically transferred into a separate flask of sterile liquid medium. The inoculum consisted of a 5-mm disk containing hyphae and spores that was cut from a 7-day-old culture of the isolate grown in a PDA petri plate (Reid, 1966). The culture of each isolate was replicated twice.

Culture Fluid Samples

Samples of the culture medium used to grow each isolate were taken after 5, 10 and 15 days of growth by pipetting 8 ml/sample. The fluids were then

centrifuged at 6650 x g, at 20 C for 15 min. After centrifugation the upper 5 ml of the fluids were decanted into sterile test tubes and frozen until the cellulase assays were made.

Cellulase Assays

Samples were assayed for cellulase activity be measuring the change caused in the viscosity of a cellulose derivative test solution when the fluid obtained from each isolate was mixed with it and the mixture was incubated at constant temperature (Levinson and Reese, 1950). The test solution consisted of 1% CMC dissolved in 0.05 M sodium citrate buffer. The changes in viscosity of the reaction mixture were determined with Cannon-Fenske routine viscometers (Induchem Lab Glass Co., NJ). The reaction mixture consisted of 8 ml of the CMC test solution and 2 ml of the culture fluid. All tests were made at 30 C, at 20-min intervals for 60 min. After each incubation time, the viscosity of the reaction mixture was determined by the time in seconds required for the meniscus to fall from the upper to the lower line of the viscometer (Kelman and Cowling, 1967). Each assay was repeated twice. The existence of 2 cellulose degrading enzymes (C_1 and C_X) has been suggested before (Levinson and Reese, 1950). (C_1 acts on cellulose to allow further enzymatic hydrolysis. C_X hydrolyzes soluble cellulose derivatives.) In this work only the activities of the C_X enzyme were determined. Each unit of enzyme activity represents 1% decrease in the viscosity of the reaction mixture after 60 min of incubation at 30 C (Ferrari and Arnison, 1974; and Pesis, *et al.*, 1978).

Cellulase Control Solution

A 1.25% cellualse control solution was prepared with Cellulase (ICN Nutritional Biochemicals, Cleveland, OH) dissolved in 0.05 M sodium citrate buffer.

RESULTS AND DISCUSSION

The fungal isolates were identified to 5 genera and 7 species (Table 1). A code number for identification was assigned to each isolate.

The cellulase activity of the fluids obtained from each of the isolates after 5 days of growth in the liquid medium and after 60 min of incubation in the reaction mixture was compared (Table 1) with the activity of the cellulase control solution. The cellulase activity of 4 of the isolates investigated was higher than the activity (75.72 units) determined for the cellulase control solution. Isolate 12351-4 of *Fusarium oxysporum* had the highest cellulase activity (87.25 units) measured in this work, whereas the lowest activity (6.18 units) corresponded to isolate 7132-2 of *Aspergillus niger.*

The cellulase activity of the culture fluids obtained after 5 days of growth of the isolates was determined after 20, 40 and 60 min of incubation in the reaction mixture (Table 1). The maximum increment of cellulase activity was observed

TABLE 1

Cellulase activities[a] of Soil Fungi Grown for 5 Days in Liquid Medium Containing Carboxymethylcellulose and After 20, 40, and 60 Min of Incubation at 30 C

Genus and Species	Code Number	Incubation Time (Min)		
		20	40	60
Fusarium oxysporum	12351-4	71.04	83.61	87.25
Fusarium solani	12351-5	60.20	76.63	82.85
Aspergillus terreus	7131-4	66.00	74.00	78.00
Fusarium episphaeria	12351-6	50.04	65.22	75.80
Cellulase control solution		65.72	72.86	75.72
Mucor sp.	5112-3	53.91	65.80	73.25
Alternaria humicola	12331-2	26.60	43.52	54.95
Chaetomium globosum	8311-1	32.05	46.16	53.85
Aspergillus niger	7131-2	2.47	3.71	6.18

[a] C_x units. Each value is the mean of 4 determinations.

during the first 20 min of incubation of the fluids obtained from all isolates. Maximum cellulase activity in all isolates was measured after 60 min of incubation in the reaction mixture.

The cellulase activity of the fluids obtained from each of the isolates of this investigation was determined after 5, 10 and 15 days of cultivation and after 60 min in the reaction mixture (Table 2). The activity of 5 of these isolates reached its maximum level during the first 5 days of cultivation. The activity of these isolates was reduced at the end of 15 days of growth, from a reduction of 2.10% of isolate 12351-5 of *F. solani* to 30.40% of isolate 5112-3 of *Mucor* sp. The activity of the other 3 isolates increased after the first 5 days of growth in liquid medium (Table 2). After 10 days of growth, the activity of isolate 7131-2 of *A. niger*

TABLE 2

Cellulase Activities[a] of Soil Fungi Grown for 5, 10 and 15 Days in Liquid Medium Containing Carboxymethylcellulose and After 60 Min of Incubation at 30 C

Genus and Species	Code Number	Growth Period (Days)		
		5	10	15
Fusarium oxysporum	12351–4	87.25	76.75	76.32
Fusarium solani	12351-5	82.85	74.41	81.11
Aspergillus terreus	7131-4	78.00	80.77	87.30
Fusarium episphaeria	12351-6	75.80	72.52	72.80
Cellulase control solution		75.72	75.72	75.72
Mucor sp.	5112-3	73.25	50.82	50.98
Alternaria humicola	12331-2	54.95	40.63	42.65
Chaetomium globosum	8311-1	53.85	77.78	82.50
Aspergillus niger	7131-2	6.18	9.80	7.50

[a] C_x units. Each value is the mean of 4 determinations.

increased 58.57% over the previous determination. Isolate 7131-4 of *A. terreus* increased its activity by 11.92% at the end of 15 days of cultivation, while isolate 8311-1 of *Chaetomium globosum* increased its activity by 53.20% at the end of 15 days of growth.

Attempting to determine if the production of cellulase was induced by the carbon source of the medium, each isolate was grown in liquid medium of the mineral composition described above, with glucose (1.5%) instead of CMC. The cellulase activities of the isolates grown for 5 days in this medium were determined after 60 min of incubation at 30 C.

Mandels and Reese (1957) and Norkrans (1963) indicated that glucose used as a carbon source in the medium does not induce the synthesis of cellulases in most species of fungi. However, the results of this experiment (Table 3) indicated that in 7 of the isolates investigated the synthesis of the enzyme (C_x) in measurable amounts proceeds when glucose is incorporated as the sole carbon source in the growing medium. The production of cellulase in isolate 12351-4 of *F. oxysporum* seems to be strictly dependent on induction by a cellulosic substrate. A comparison between the results of Table 1 and Table 3 indicates that in 7 of the isolates, CMC is stronger than glucose as an inducer of cellulase production. However, isolate 7131-2 of *A. niger* produced over 3 times as much cellulase (20.29 units) when grown in the medium containing glucose than when cultivated in the medium containing CMC (6.18 units).

TABLE 3

Cellulase Activities[a] of Soil Fungi Grown for 5 Days in Liquid Medium Containing Glucose and After 60 Min of Incubation at 30 C

Genus and Species	Code Number	Cellulase Activity (%)
Cellulase control solution		75.72
Chaetomium globosum	8311-1	42.35
Alternaria humicola	12331-2	20.84
Aspergillus niger	7131-2	20.29
Mucor sp.	5112-3	7.15
Fusarium solani	12351-5	5.59
Aspergillus terreus	7131-4	4.11
Fusarium episphaeria	12351-6	1.41
Fusarium oxysporum	12351-4	0.00

[a]C_x units. Each value is the mean of 4 determinations.

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