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Bioconversion of Straw Into Improved Fodder: Mycoprotein Production and Cellulolytic Acivity of Rice Straw Decomposing Fungi

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Sixty two out of the sixty four species of fungal isolates tested could produce both exo-β1,4-gluconase (C_1) and endo-β1,4-gluconase (C_x) on pure cellulose and rice straw as carbon source in Czapek's medium. Fifty-eight and fifteen species were able to grow at 25°C and at 45°C, respectively. Eleven species could grow at both 25°C and 45°C while, four species appeared only at 45°C. The most cellulolytic species at 25°C was *Trichoderma koningii* producing 1.164 C_1 (mg glucose/1 *ml* culture filtrate/1 hr) and 2.690 C_x on pure cellulose, and 0.889 C_1 and 1.810 C_x on rice straw, respectively. At 45°C, the most active thermotolerant species were *Aspergillus terreus*, followed by *A. fumigatus*. *Talaromyces thermophilus* was the highest active thermophilic species followed by *Malbranchea sulfurea*. Most of these species were also active in fermentation of rice straw at 25 and 45°C ($P<0.05$). The most active ones were *T. koningii*, *A. ochraceus* and *A. terreus*, which produced 201.5, 193.1 and 188.1 mg crude protein/g dry straw, respectively.

KEYWORDS: Bioconversion, Cellulases, Decomposition, Fodder, Mycoprotein, Rice straw

Filamentous fungi have a greater penetrating power into insoluble substrates and are, therefore, more suitable for solid-state fermentation of lignocellulosic materials (Chahal, 1982; Kang *et al.*, 2004). Cellulose and hemicellulose are the major components of straw. Therefore, the ability to decompose the plant cell wall polysaccharides is likely to be an essential requirement for fungi active in straw decay (Harper and Lynch, 1982a; Morais *et al.*, 1999; Majumdar *et al.*, 2001). These cellulosic and hemicellulosic components of this agricultural residue are potentially available for saccharification or bioconversion to mycoprotein as an improved feed supplement (Amanat, 1987; Rai *et al.*, 1989; Darmwal and Gaur, 1991). A number of studies are being carried out on the use of agricultural wastes as feed supplements. Only few of these studies were focused on microbial treatment of rice straw. Enhanced digestibility of plant residues such as wheat straw and corn straw results from an increase in the concentration of easily soluble substrates as well as from physical and other chemical changes in the straw that occurred by bioconversion with fungi (Zadrazil, 1977; Rai *et al.*, 1989; Ganev *et al.*, 1998; Xuexia *et al.*, 2001). Several studies recommended the use of thermophilic and thermotolerant fungi for mycoprotein production on cellulosic substrates by solid state fermentation because the fungi can simplify the process technology (Srinivasan, 1979; Grajek, 1988; Cronel *et al.*, 1991).

In this study, I have attempted to screen several fungi previously isolated on rice straw substrate for the ability to saccharify and enhance the protein content of untreated rice straw.

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Materials and Methods

Strains. Fungi studied in this investigation were previously isolated from rice straw using nylon net bag technique (House and Stinner, 1987; Wise and Schaefer, 1994) from 10 areas all over Sharkia province, east of Nile Delta, Egypt on ground rice straw-Czapek's agar medium at 5°C, 25°C and 45°C.

Analysis of rice straw. Moisture content of straw was determined by drying a sample in a hot air oven at 105°C till a constant weight was obtained. Hemicelluloses, lignin, and ash content were estimated according to Tappi standards (1957), α-cellulose to Paech and Tracey (1955), silica to Wilde *et al.* (1972), and total nitrogen estimated by the micro-Kjeldahl method (Allen, 1953).

Determination of cellulolytic activity. Spores of each fungus were inoculated into triplicate of reagent bottles (100 *ml*), each containing 1.0 g of pure cellulose or rice straw chopped to lengths of 5~6 cm instead of sucrose in 5 *ml* Czapek's broth medium containing (g/5 *ml* water), $NaNO_3$, 0.3; KH_2PO_4, 0.1; $MgSO_4$, 0.05 and KCl, 0.05. The bottles were incubated at either 25°C or 45°C for 5 days. Activities of exo-β1,4 (C_1) and endo-β1,4 (C_x) cellulases expressed as mg glucose per one ml fungal filtrate per one hour were determined according to the method of Mandels and Weber (1969). Total reducing value (mg/g dry straw) was obtained using glucose as standard by the previously described method (Chaplin and Kennedy, 1987).

Determination of protein content. Soluble protein (mg/g dry straw) with bovine albumin as standard, and total nitrogen (mg/g dry straw) were determined in fermented rice straw as previously described (Lowery *et al.*, 1951; Allen, 1953).

Statistical analysis. The obtained data were analyzed by one-way ANOVA and multiple-way ANOVA (Snedecor and Cochran, 1982) and differences between means were calculated at the 5% probability level using Duncan's new multiple range tests (Duncan, 1955). Bivariate correlation matrix of the obtained data was done by using SPSS software program (ver. 8) as described by Dytham (1999).

Results

Chemical composition of straw. Table 1 showed that α-cellulose was the highest constituent of rice straw; it represents 39.73% (w/w) of the straw, followed by hemicelluloses, representing 25.77% (w/w). This means that both α-cellulose and hemicelluloses, which are collectively known as holocellulose, constitutes about 65.5% of rice straw. Lignin portion was about 13.43%, while ash is 16.66%; about 77.8% of this ash was silica. Lignin and ash, the aggressive compounds in straw, constitute about 30.1% of its weight. However crude protein, the most important constituent, is only 3.33% of straw weight.

Cellulases activities of fungi. The results presented in Table 2 show that both exo-β1,4 gluconase (C_1) and endo-β1,4 gluconase (C_x) were produced from sixty-two fungal isolates out of the sixty-four species examined. The other two species were *Mucor circinelloides* that failed to produce these two enzymes, while *Penicillium chrysogenum* produced both C_1 and C_x on pure cellulose only but not on rice straw although these two species were able to grow on rice straw-Czapek's medium. Generally, C_x enzyme was produced with higher quantities than C_1 enzyme and both enzymes were more highly produced on pure cellulose than on rice straw as carbon source in the medium.

At 25°C, *Trichoderma koningii* and *T. harzianum* were the species, producing the highest amount of cellulases enzymes. *T. koningii* produced 1.164 mg glucose/ml/hr of C_1 and 2.690 mg glucose/ml/hr of C_x on pure cellulose, and 0.889 of C_1 and 1.810 of C_x on rice straw, respectively. *T. harzianum* produced 1.108 of C_1 and 2.294 of C_x on pure cellulase, and 0.809 of C_1 and 1.701 of C_x on rice straw as carbon sources. Members of the genus *Aspergillus* occupied the second position, with *A. ochraceus* the highest active one (C_1 0.810, 0.752 and C_x 1.536, 1.492) followed by *A. terreus*, *A. flavus*, *A. awamori*, and *A. fumigatus*. Two species of the genus *Fusarium* were also

Table 1. Chemical composition of rice straw

Constituent	Composition %
α-Cellulose	39.73±1.01
Hemicelluloses	25.77±0.43
Lignin	13.43±0.81
Crude protein*	3.33±0.23
Ash	16.66±0.33
Silica**	13.0±0.20

*Crude protein = total nitrogen×6.25.
**Silica represents 77.8% of ash content.

Table 2. Cellulases activities (C_1 and C_x) of isolated fungi growing on pure cellulose (C) and rice straw (R) as carbon source at 25 and 45°C

Species	Cellulases activities (mg/glucose/1 *ml* filterate/1 hr)							
	25°C				45°C			
	C_1*		C_x**		C_1*		C_x**	
	C	R	C	R	C	R	C	R
Acremonium fusidioides (Nicot) Gams	0.102	0.090	0.274	0.200	–	–	–	–
A. kiliense Grütz	0.128	0.104	0.295	0.269	–	–	–	–
A. strictum W.Gams	0.121	0.102	0.334	0.291	–	–	–	–
Alternaria alternata (Fr.) Keiss.	0.207	0.206	0.710	0.652	–	–	–	–
A. tenuissima (Kunze ex Pers.) Wilts.	0.347	0.287	0.895	0.785	-	-	-	-
Aspergillus awamori Nakaz.	0.675	0.591	1.056	0.990	0.313	0.292	0.621	0.588
A. flavus Link var. *flavus*	0.816	0.757	1.419	1.077	0.218	0.196	0.503	0.479
A. flavus var. *columnaris* Raper & Fennell	0.752	0.728	0.915	1.231	0.218	0.190	0.459	0.459
A. fumigatus Fr. var. *fumigatus*	0.344	0.299	0.913	0.842	1.000	0.907	2.243	2.181
A. niger Tiegh. var. *niger*	0.708	0.609	1.409	1.352	0.256	0.210	0.590	0.457
A. ochraceus Wilh.	0.810	0.752	1.536	1.492	–	–	–	–
A. sydowii (Bain & Sart.) Thom & Church	0.294	0.198	0.747	0.660	–	–	–	–
A. tamarii Kita	0.312	0.316	0.693	0.650	–	–	–	–
A. terreus Thom var. *terreus*	0.649	0.911	1.508	1.421	0.960	0.822	2.316	2.150
A. ustus (Bain) Thom & Church	0.291	0.218	0.601	0.520	0.306	0.256	0.627	0.582

Table 2. Continued

Species	Cellulases activities (mg/glucose/1 *ml* filterate/1 hr)							
	25°C				45°C			
	C_1*		C_x**		C_1*		C_x**	
	C	R	C	R	C	R	C	R
Botrytis cinerea Per.	0.657	0.524	0.876	0.800	–	–	–	–
Botrytrichum piluliferum Sacc. & Marchal	0.237	0.184	0.392	0.309	–	–	–	–
Chaetomium cochliodes Pall.	0.126	0.204	0.102	0.261	–	–	–	–
C. globosum Kunze ex Stend.	0.329	0.281	0.760	0.708	–	–	–	–
C. spirale Zopf	0.303	0.224	0.462	0.307	0.153	0.156	0.312	0.210
C. thermophilum La Touche	–	–	–	–	0.224	0.197	0.449	0.409
Cladosporium cladosporioides(Fresen.)de Vries	0.093	0.060	0.171	0.145	–	–	–	–
C. herbarum (Pers.) Link	0.316	0.291	0.702	0.638	–	–	–	–
Cochliobolus lunatus Nelson & Haasis	0.128	0.102	0.296	0.283	–	–	–	–
C. sativus (Ito & Kurib.) Drechsler ex Dastur	0.343	0.306	0.862	0.728	–	–	–	–
Colletotrichum dematium (Pers. ex Fr.) Grove	0.153	0.084	0.219	0.188	–	–	–	–
Emericella nidulans (Eidam) Vuillemin var. *Lata* Suberamanian	0.346	0.307	0.623	0.523	0.410	0.851	0.851	0.707
E. quadrilineata (Thom & Raper) Benj.	0.326	0.256	0.509	0.488	0.311	0.470	0.407	0.216
Epicoccum nigrum Link	0.294	0.267	0.510	0.408	–	–	–	–
Fusarium moniliforme Sheld.	0.346	0.303	0.817	0.754	–	–	–	–
F. oxysporum Schlecht.	0.610	0.513	1.214	0.913	–	–	–	–
F. pallidoroseum (Cooke) Sacc.	0.343	0.306	0.632	0.590	–	–	–	–
F. solani (Mart.) Sacc.	0.657	0.509	1.607	1.331	–	–	–	–
Gliocladium catenulatum Gilm. & Abbott	0.710	0.590	1.287	0.866	–	–	–	–
Humicola grisea Traaen var. *grisea*	0.257	0.198	0.509	0.464	–	–	–	–
H. grisea var. *thermoidea* Cooney & Emer.	–	–	–	–	0.116	0.193	0.469	0.245
Malbranchea sulfurea (Miehe) Sigler& Carmich.	–	–	–	–	0.113	0.169	0.547	0.209
Mucor circinelloides Van Tiegh.	–	–	–	–	–	–	–	–
M. racemosus Fresen.	0.060	0.033	0.106	0.074	–	–	–	–
Myrothecium roridum Tode	0.410	0.391	0.909	0.758	–	–	–	–
M. verrucaria (Alb. & Schwein.) Ditmar	0.606	0.504	1.066	0.896	–	–	–	–
Nigrospora sphaerica (Sacc.) Mason	0.103	0.094	0.259	0.203	–	–	–	–
Oidiodendron griseum Robak	0.303	0.295	0.556	0.460	–	–	–	–
Penicillium canescens Sopp	0.117	0.079	0.250	0.168	–	–	–	–
P. chrysogenum Thom	0.223	–	0.388	–	–	–	–	–
P. citrinum Thom	0.331	0.185	0.418	0.355	–	–	–	–
P. corylophilum Dierckx	0.450	0.388	1.034	0.789	–	–	–	–
P. herquei Bain. & Sart.	0.240	0.198	0.475	0.394	–	–	–	–
P. janthinellum Biourge	0.317	0.252	0.507	0.378	–	–	–	–
P. oxalicum Currie & Thom	0.326	0.264	0.787	0.666	–	–	–	–
P. rubrum Stoll	0.236	0.153	0.501	0.310	–	–	–	–
P. verrucosum Dierckx var. *verrucosum*	0.264	0.212	0.497	0.415	–	–	–	–
Phoma herburum Peyronel	0.336	0.299	0.737	0.678	–	–	–	–
Pestalotia sp	0.262	0.212	0.560	0.419	–	–	–	–
Rhizomucor pusillus (Lindt) Schipper	0.088	0.063	0.169	0.155	0.229	0.205	0.457	0.317
Rhizopus stolonifer (Lindt) Schipper	0.156	0.132	0.411	0.285	–	–	–	–
Scopulariopsis brevicaulis (Sacc.) Bain.	0.528	0.189	0.765	0.145	–	–	–	–
Stachybotrys chartarum (Ehrenb. ex Link) Hugh.	0.246	0.181	0.513	0.411	–	–	–	–
S. elegans (Pidopl.) Gams.	0.347	0.265	0.660	0.469	–	–	–	–
Talaromyces thermophilus Stolk	–	–	–	–	0.249	0.212	0.505	0.493
Trichoderma harzianum Rifai	1.108	0.809	2.294	1.701	–	–	–	–
T. koningii Oudem.	1.164	0.889	2.690	1.810	–	–	–	–
Trichothecium roseum (Pers.) Link ex Gray	0.347	0.309	0.556	0.413	–	–	–	–
Verticillum catenulatum (Kamyschko ex Barron & Onions) Gams	0.312	0.231	0.785	0.565	–	–	–	–

*C_1 = exo-β1,4 glucanase.
**C_x = endo-β1,4 glucanase.

Table 3. Total reducing value (RV), soluble protein (SP) and crude protein (CP) of fermented straw at 25° and 45°C by fungi isolated from soil by nylon net bag technique on grinded rice straw - Czapek's agar medium

Species \ Temperature °C	25°			45°		
	RV	SP	CP	RV	SP	CP*
Acremonium fusidioides (Nicot) Gams	2.9	48.3	124.7	–	–	–
A. kiliense Grütz	3.2	54.2	129.7	–	–	–
A. strictum W.Gams	4.0	57.4	135.5	–	–	–
Alternaria alternata (Fr.) Keiss.	4.6	55.1	116.3	–	–	–
A. tenuissima (Kunze ex Pers.) Wilts.	6.0	55.8	126.1	–	–	–
Aspergillus awamori Nakaz.	7.8	72.3	180.1	3.3	46.5	117.1
A. flavus Link var. *flavus*	9.2	77.3	182.2	3.1	47.2	113.4
A. flavus var. *columnaris* Raper & Fennell	8.3	72.3	176.8	3.7	45.9	115.8
A. fumigatus Fr. var. *fumigatus*	5.8	6.8	157.4	10.9	93.7	180.6
A. niger Tiegh. var *niger*	8.7	80.1	188.6	5.9	49.4	136.5
A. ochraceus Wilh.	11.9	82.4	193.1	–	–	–
A. sydowii (Bain & Sart.) Thom & Church	6.5	67.2	156.2	–	–	–
A. tamarii Kita	7.0	73.2	168.4	–	–	–
A. terreus Thom var. *terreus*	10.1	77.0	188.1	10.8	83.3	183.2
A. ustus (Bain) Thom & Church	3.5	46.2	136.1	5.8	64.5	158.5
Botrytis cinerea Pers.	6.4	73.8	170.5	–	–	–
Botrytrichum piluliferum Sacc. & Marchal	5.4	66.2	165.9	–	–	–
Chaetomium cochliodes Pall.	6.5	64.4	150.3	–	–	–
C. globosum Kunze ex Stend.	6.4	70.6	159.8	–	–	–
C. spirale Zopf	6.0	64.4	163.6			
C. thermophilum La Touche	–	–	–	5.0	66.6	157.6
Cladosporium cladosporioides (Fresen.) de Vries	4.3	45.7	123.1	–	–	–
C. herbarum (Pers.) Link	7.3	76.1	180.2	–	–	–
Cochliobolus lunatus Nelson & Haasis	3.2	42.9	119.6	–	–	–
C. sativus (Ito & Kurib.) Drechsler ex Dastur	6.2	70.8	161.2	–	–	–
Colletotrichum dematium (Pers. ex Fr.) Grove	4.1	59.9	139.1	–	–	–
Emericella nidulans (Eidam) Vuillemin var. *Lata* Subramanian	7.5	63.7	178.0	8.6	77.9	177.6
E. quadrilineata (Thom & Raper) Benj.	6.0	62.3	151.8	4.8	53.1	135.6
Epicoccum nigrum Link	5.6	56.8	154.8	–	–	–
Fusarium moniliforme Sheld.	6.0	53.8	142.7	–	–	–
F. oxysporum Schlecht	8.2	77.8	161.0	–	–	–
F. pallidoroseum (Cooke) Sacc.	5.2	57.1	151.3	–	–	–
F. solani (Mart.) Sacc.	8.5	78.0	164.2	–	–	–
Gliocladium catenulatum Gilm. & Abbott	9.2	92.7	182.3	–	–	–
Humicola grisea Traaen var. *grisea*	4.7	56.8	144.6	–	–	–
H. grisea var. *thermoidea* Cooney & Emer.	–	–	–	2.3	39.9	104.4
Malbranchea sulfurea (Miehe) Sigler& Carmich.	–	–	–	7.4	83.4	152.1
Mucor circinelloides Van Tiegh.	3.1	42.1	121.0	–	–	–
M. racemosus Fresen.	3.3	55.9	120.9	–	–	–
Myrothecium roridum Tode	8.2	84.2	173.4	–	–	–
M. verrucaria (Alb. & Schwein.) Ditmar	9.5	81.1	185.1	–	–	–
Nigrospora sphaerica (Sacc.) Mason	2.7	43.9	116.6	–	–	–
Oidiodendron griseum Robak	5.1	69.8	162.4	–	–	–
Penicillium canescens Sopp	6.9	755	168.1	–	–	–
P. chrysogenum Thom	2.5	41.3	110.0	–	–	–
P. citrinum Thom	5.5	67.4	156.1	–	–	–
P. corylophilum Dierckx	8.5	76.1	174.8	–	–	–
P. herquei Bain. & Sart.	6.4	72.5	152.8	–	–	–
P. janthinellum Biourge	4.8	60.5	150.3	–	–	–
P. oxalicum Currie & Thom	6.6	78.4	166.5	–	–	–
P. rubrum Stoll	4.8	50.9	133.0	–	–	–
P. verrucosum Dierckx var. *verrucosum*	6.4	73.6	149.5	–	–	–
Phoma herburum Peyronel	7.4	82.1	162.4	–	–	–
Pestalotia sp	6.4	72.9	150.0	–	–	–

Table 3. Continued

Species \ Temperature °C	25°			45°		
	RV	SP	CP	RV	SP	CP*
Rhizomucor pusillus (Lindt) Schipper	4.2	54.7	117.3	5.1	64.7	132.1
Rhizopus stolonifer (Lindt) Schipper	4.8	59.2	142.6	–	–	–
Scopulariopsis brevicaulis (Sacc.) Bain.	3.6	46.2	100.1	–	–	–
Stachybotrys chartarum (Ehrenb. ex Link) Hugh.	6.3	72.3	143.8	–	–	–
S. elegans (Pidopl.) Gams.	7.7	82.6	161.7	–	–	–
Talaromyces thermophilus Stolk	–	–	–	7.2	63.1	152.1
Trichoderma harzianum Rifai	14.3	90.1	185.1	–	–	–
T. koningii Oudem.	16.6	94.4	201.5	–	–	–
Trichothecium roseum (Pers.) Link ex Gray	6.6	74.4	161.5	–	–	–
Verticillum catenulatum (Kamyschko ex Barron & Onions) Gams	5.0	54.6	123.3	–	–	–

*CP (crude protein) = total nitrogen × 6.25.

highly active: *F. solani* (C_1 0.657, 0.509 and C_x 1.607, 1.331) followed by *F. oxysporum* (C_1 0.610, 0.513 and C_x 1.214, 0.913). The other cellulases active producers were *Penicillium corylophilum*, *Gliocladium catenulatum*, and *Myrothecium verrucaria*.

On the other hand, at 45°C *A. fumigatus* (C_1 1.0, 0.907 and C_x 2.243, 2.181 mg glucose/1 *ml*/1 hr), *A. terreus* (C_1 0.960, 0.822 and C_x 2.316, 2.150), and *Emericella nidulans* (C_1 0.410, 0.851 and C_x 0.851, 0.707) were the most highly active species. Generally these thermotolerant species were higher in cellulases activities than thermophilic species which were arranged descendingly as follows, *Talaromyces thermophilus, Chaetomium thermophilum, Malbranchea sulfurea, Humicola grisea* var *thermoidea* and *Rhizomucor pusillus*. Cellulases activities of other thermotolerant species were lower at 45° than at 25°C.

Protein content and soluble sugars of fermented straw. Total reducing value and soluble protein in culture filtrates and crude protein contents of fermented straw produced by the previously isolated fungi were shown in Table 3. At 25°C the highest crude protein content was detected in straw fermented by *T. koningii* (201.5 mg/g dry straw) followed by *A. ochraceus* (193.1 mg/g) and *A. terreus* (188.1 mg/g). Soluble protein and reducing sugars produced by these fungi in culture filtrates were also high: 94.4, 16.6; 82.4, 11.9 and 77.0, 10.1 mg/g dry straw, respectively.

At 45°C, crude protein of fermented straw by *A. terreus* was the highest (183.2 mg/g dry straw) with reducing sugars 10.8 mg/g and soluble protein 83.3 mg/g. The other thermotolerant and thermophilic species gave lower crude protein in fermented straw than these two species.

Discussion

Rice straw, one of the major agricultural by-products of Egypt, contains 65.5% holocellulose (39.73% cellulose and 25.77% hemicelluloses), and 13.43% lignin, as shown in Table 1. This made it a good fodder for ruminants. Previous studies suggested that, since lignocellulosic crop residues contain appreciable quantities of cellulose, hemicelluloses and lignin, they are potentially good substrates for production of single cell protein as animal feeds (Majumdar *et al.*, 2001; Tengerdy and Szakacs, 2003). However, the low protein content (3.33%) of dry rice straw is the main problem since any crop residue with less 8% crude protein is considered inadequate as a livestock feed (Jackson, 1977).

Fungal isolates at Table 2 are versatile in the ratio of the enzyme components and their cellulases activities seemed to be dependent on the nature of the cellulose source. This was also found by Afzal *et al.* (1983) and Cruz and Halos (1988). The results showed that *T. koningii* and *T. harzianum* were the highest active cellulose producers on pure cellulose and rice straw substrates at 25°C. This was also found on pure cellulose by many workers (Chahal, 1985; Sidhu and Sandhu, 1985; Falih, 1998) and on lignocellulose (Tengerdy and Szakacs, 2003). *A. ochraceus*, *A. terreus* and some other members of the genus *Aspergillus* isolated came in the second position and produced C_1 and C_x on both substrates. *Aspergillus* members were also found to be very efficient producers of cellulases enzymes (Darmwal and Gaur, 1991; Moubasher, 1993; Kang *et al.*, 2004).

The industrialization of cellulases, mycoprotein and compost production is greatly affected by the thermostability of the enzymes (Maheshwari *et al.*, 2000). Therefore, the interest to find and study the thermophilic cellulases producers was greatly needed. Results at Table 2 show that the thermotolerants *A. terreus* and *A. fumigatus* produced more C_1 and C_x than the other thermotolerant and thermophilic species. Several research groups had studied the cellulolytic activities of many fungi under thermophilic conditions and they found that *Chaetomium thermophile*, *Humicola grisea* var *thermoidea*, *A. terreus*, *A. fumigatus*, *A. nidulans* and *Talaromyces emersonii* were able to degrade cellulose (Mandels, 1981; Moubasher *et*

al., 1984; Deacon, 1985). Coronel *et al.* (1991) found that *A. fumigatus* was the most active cellulase producer on rice straw substrate out of the 144 tested strains of thermophilic lignocellulosic-degrading fungi and maximum CMC and FP cellulases activities were produced after 4 days of incubation. Bastawde (1992) used the thermotolerant *A. terreus* to saccharify rice straw and some other cellulosic substrates. It was also found at Table 2 that *M. circinelloides* can not produce C_1 nor C_x on both substrates, while *P. chrysogenum* produced these enzymes only on pure cellulose substrate. This is, however, contrast to the growth of these two species on rice straw as carbon source (Table 3) and their previous appearance on rice straw-Czapek's agar plates. Similarly Harper and Lynch (1985) found the same result for *P. chrysogenum*. This means that their appearance was due to growth on the hemicellulose component of rice straw instead of its cellulose. Harper and Lynch (1982b) mentioned that colonization of straw initially involves growth not on polysaccharides but on simple water-soluble components. These components support growth of primary colonizers whether cellulolytic or noncellulolytic that are considered important in the earliest stages of straw decay (Garrett, 1981; Harper and Lynch, 1985). Cellulolytic fungi capable of growth on these simple soluble components will gain an initial advantage in having colonized the straw prior to the onset of cellulolysis. They may then be successful in challenging subsequent attempts by other fungi to colonize the straw.

Cellulases activities were reflected on fermentation ability of isolated fungi. There is a significant positive correlation ($P<0.05$) between the C_1 and C_x activities and crude protein yield in fermented straw by fungal isolates, soluble sugars and soluble protein in its filtrate, as indicated at Table 4. Results in Table 3 were in harmony with those presented at Table 2 whereas, fungi with high C_1 and C_x activity were also high in production of reducing sugars, soluble protein and crude protein of fermented straw. *T. koningii* followed by *A. ochraceus* has the highest fermentable ability at 25°C raising the crude protein of straw into 201.5 and 193.1 mg/g dry straw respectively, while, *A. terreus* and *A. fumigatus* were the highest active at 45°C producing 183.3 and 180.6 mg/g dry straw respectively. This high fermentable activity of *Aspergillus* and *Trichoderma* species may be attributed to their high xylanolytic activity found by Abdel-Sater and El-Said (2001) and enables these species to colonize the straw and grow well on its cellulose content before other fungi. Araujo and D'Souza (1986) and Youssef and Aziz (1999) used cellulases from *A. terreus* and *T. koningii* for conversion of rice straw into single-cell protein. Srinvasan (1979) and Araujo and D'Souza (1986) mentioned that among three different systems (bacterial, fungal and yeast) of the microbial degradation of cellulose, *A. terreus* system was the most promising technology for single-cell protein production and it's economically feasible on a commercial scale. Thanikachalam and Rangarajan (1992) found that crude protein content of the residues of alkali treated and untreated fermented straw by *Trichoderma* sp., *Aspergillus* sp. and *Fusarium* sp. was 21.85, 18.75 and 25.00 and 17.5, 15.6 and 19.4%. Ganev *et al.* (1998) stated that crude protein content of wheat straw increased 4~5 times compared to the natural straw by bioconversion of wheat straw with *Cephalosporium* sp. or *Pleurotus ostreatus*. Also, Grajek (1988) emphasized that the use of thermotolerant and thermophilic fungi for protein production from cellulosic substrate by solid-state fermentation can simplify the process technology by eliminating the need for heat removal and reducing contamination hazards. So, the following work will be conducted using *A. ochraceus*, *A. terreus* and *T. koningii*.

Table 4. Pearson correlation coefficients (r) showing the relationship between enzyme activity and fungal products (RV, SP and CP) of the fermented straw at different incubation temperature degrees

Products	25°C		45°C	
	C_1	C_x	C_1	C_x
RV	0.837***	0.884***	0.909***	0.905***
SP	0.649***	0.665***	0.851***	0.843***
CP	0.672***	0.621***	0.837***	0.820***

***Correlation is significant at the 0.001 level.

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