American-Eurasian J. Agric. & Environ. Sci., 8 (4): 402-410, 2010
ISSN 1818-6769

Enhanced Production of Cellulase(S) By *Aspergillus* spp. Isolated From Agriculture Wastes by Solid State Fermentation

[1]M.A.M. Abo-State, [1]A.I. Hammad, [2]M. Swelim and [1]R.B. Gannam

[1]Department of Microbiology,
National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt
[2]Faculty of Science, Benha University, Egypt

Abstract: Twenty nine fungal strains were isolated from agriculture wastes. *Aspergillus* spp. was the predominant genera in these agriculture wastes. The most potent cellulase producers were selected for studying their cellulase productivities on Wheat Straw (WS), Wheat Bran (WB), Rice Straw (RS) and Corn Cob (CC) as cheap, renewable agriculture wastes by solid state fermentation (SSF). Five *Aspergillus* spp. and standard strain *Trichoderma viride* were grown on the agriculture wastes and CMCase, FPase, Avicelase and soluble protein were determined. *T. viride* produces the highest CMCase on WS (555U/ml), while the highest FPase (141U/ml) and Avicelase (46U/ml) were produced on WB. The isolated strain *Aspergillus* MAM-F35 gave the highest CMCase (487U/ml), FPase (79U/ml) and Avicelase (35U/ml) on WS. However, the isolated strain *Aspergillus* MAM-F23 gave the highest CMCase (309U/ml) on RS, while the highest Avicelase (45U/ml) on WS. So, the highest cellulases were produced on the agriculture wastes in the order WS> WB> RS> CC. The most potent strains were exposed to increasing doses of gamma radiation to determine their dose response curve. Gamma radiation reduced the viable count of *Aspergillus* MAM-F23 and 35 gradually, as the dose increased, the viability decreased. 5.0 and 4.0 kGy reduced the viability of *Aspergillus* MAM-F23 and 35 completely. Mutant No. ”4" of *Aspergillus* MAM-F23 which exposed to 0.5 kGy produced higher cellulases (CMCase 372U/ml, FPase 64U/ml and Avicelase 39U/ml) than the parent strain (CMCase 305U/ml, FPase 48 U/ml and Avicelase 29U/ml). However, mutant No. “1" of *Aspergillus* MAM-F35, which exposed also to 0.5 kGy, gave the highest cellulases than the parent strain.

Key words: Isolation · CMCase · FPase · Avicelase · Gamma radiation

INTRODUCTION

Cellulase is a complex enzyme composed of cellobiohydrolases, endoglucanases and β-glucosidases which all act synergistically to convert complex carbohydrates present in lignocellulosic biomass into glucose efficiently [1]. Cellulase(s) are industrially important enzymes that are sold in large volumes for use in different industrial applications, for example in starch processing, animal feed production, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp and paper industry and textile industry [2-6]. There are growing market for cellulases in the field of detergent industry and saccharifaication of agriculture wastes for bioethanol technology [7-10].

Lignocellulosic wastes are the largest group of wastes present on this plant causing environmental pollution [11]. It is estimated that the photosynthetic process produced 1.5 x 10^{11} ton (150 billion tons) of dry material annually with respect to carbon of which about 50% is cellulose [12]. Wheat straw represents 149 million tons per year in Europe according to FAO [13]. Submerged fermentation (SmF) is used for industrial production of cellulases. The cost of production and low yield of these enzymes are the major problems for industrial applications [14]. It has been reported that solid state fermentation (SSF) as an attractive process to produce cellulose(s) which is economical due to its lower capital investment and lower operating expenses [15,16]. Production of cellulases by fungi in SSF using agriculture wastes has been reported [9, 17-20].

Corresponding Author: M.A.M. Abo-State, Department of Microbiology,
National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt.

Therefore, the aim of this work is to get rid of agriculture wastes by a safety manner not aggressive to the environment and to produce valuable enzymes from cheap, renewable raw material to achieve sustainable development.

MATERIALS AND METHODS

Strains Isolation: Agriculture wastes, wheat straw (WS), rice straw (RS), wheat bran (WB) and corn cob (CC), were collected from Upper Egypt Governorates which used for isolation of fungi. Ten grams were transferred to aliquots of 90.0 ml sterile saline in 250 ml flasks. They were shaken vigorously at constant speed for 15 min.

The agriculture waste suspensions were then subjected to serial dilutions. From the appropriate dilutions, 0.1 ml was spreaded on the surface of PDA [21] plate in duplicate. The plates were incubated for 5 days at 28°C. The well grown spreaded single colonies were picked-up and subcultured on PDA slants. The mold genera were reported.

Substrates: The lignocellulosic materials (wheat straw, rice straw, wheat bran and corn cob) were firstly dried and milled into small pieces (3-5mm). These milled agriculture wastes were used for solid state fermentation.

Preparation of Spore Suspensions: The isolated fungal strains maintained on PDA slants were used to prepare spore suspensions as described before by Abo-State [22]. The spore suspensions count was $\simeq 10^7$ spores/ml.

Screening for Cellulolytic Activities of the Isolated Fungi: Basal medium, [23] supplemented with 1% carboxy-methyl cellulose (CMC) (Sigma chemical Co., St Louis, MO, USA) and sterilized by autoclaving 121°C for 15 min. was used. These basal medium was inoculated with 4.0 ml spore suspensions. Three replicates were used for each fungal isolates and the standard strain *Trichoderma viride*. The inoculated flasks were incubated at 28°C as stationary culture for 7 days. After incubation, 10 ml of the cultures were centrifuged at 8000 rpm for 10 min by cooling centrifuge. The supernatants were used for determination of reducing sugars, carboxymethyl cellulase (CMCase) and protein.

Inoculation and Culture Conditions of Solid State Fermentation: Ten grams of each agriculture wastes were mixed with 25 ml distilled water as moisting agent into 250 ml Erlenmeyer flasks. The flasks were all sterilized for 30min. at 121°C. Four ml of prepared spore suspensions were inoculated and incubated at 28°C under static condition.

Enzyme Extraction: The solid substrate culture broth was prepared by adding 10-fold (V/W) distilled water and shaking (180 rpm) at 28°C for 60 min. Then the solid materials and fungal biomass were separated by centrifugation (10.000 rpm for 15 min.). The clarified supernatant used for enzyme assays.

Enzyme Assays

CMCase Assay: Endoglucanase, Carboxymethyl cellulase (CMCase) activity was determined according to Wang *et al.* [24]. One ml of the crude enzyme supernatant was incubated with 1 ml of 1% CMC in 0.1 M sodium acetate buffer solution pH 5.0 for 30 min at 63°C. The resulted reducing sugars were determined according to Miller [25] by dinitrosalisylic acid (DNS).

Fpase Assay: Total cellulase (FPase) activity was determined as described by Gadgil *et al.* [26]. One ml of the crude enzyme supernatant was incubated with 2 ml of 0.1 M citrate buffer (pH 4.8) containing 50 mg Whatman No. 1 filter paper. After incubation for 1 hour at 50°C, the resulted reducing sugars were determined.

Avicelase Assay: Avicelase activity was determined according to Li and Gao [27]. One ml of crude enzyme supernatant was incubated with 1 ml of 2% (W/V) Avicel (Sigma) in 0.1 M phosphate-citrate buffer (pH 6.6) at 40°C for 2 hours. The resulted reducing sugars were determined. One unit of CMCase, FPase and Avicelase activity was expressed as 1 μ M glucose equivalents released ml^{-1} min^{-1}.

Protein Determination: Protein was determined according to Lowry *et al.* [28]. One ml of the crude enzyme supernatant was used and 5.0 ml reaction mixture was added in a clean dry test tube. The tubes were kept at room temperature for 10 min. Then 0.5 ml of Folin reagent (Fluka) was added to the previous mixture. The tubes were leaved for 20 min. at room temperature and the absorbance was measured at 720 nm.

Effect of Gamma-Irradiation on the Viable Count of Some Isolates and Cellulases Production: Spore suspensions (2×10^7 spores/ml) were exposed to different doses, 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 and 6.0 kGy by the Indian gamma cell of Co-60 located at the National Center for Radiation

Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The dose rate was 1 kGy/12.5 min. at the time of experiment at room temperature. The viability was determined as previously mentioned by Abo-State [29]. The irradiated and non irradiated (control) were serially diluted and spreaded on the surface of Sabroud, [21] plates. Three replicates were used for each dose for each isolate. The plates were incubated at 28°C for 5 days, the count were recorded. Colonies exposed to different doses of gamma irradiation with difference in morphology (shape, colour, margin, surface or size) were picked up and used to determine their cellulolytic activities by solid state fermentation technique.

RESULTS AND DISCUSSION

Isolation of Different Fungi: The aim of this study was to isolate strains of fungi with improved cellulases production and had potential industrial application. Twenty-nine fungal isolates were isolated from different agriculture wastes as indicated in Table 1. Most of the isolates, obtained from agriculture wastes, were *Aspergillus* spp. which was probably rather dominant in these wastes.

Selection for the Most Potent Isolates: Five of these isolates were selected, four of them gave high CMCase production and one gave low CMCase for further studies about cellulases production on solid state fermentation. These isolates were *Aspergillus niger* MAM-F5 and 13; *Aspergillus* spp. MAM-F 23, 35 and 40. These five strains produced different amount of CMCase, reducing sugars and soluble protein. Isolate MAM-F23 gave the highest CMCase (233U/ml) and reducing sugar (1270µg/ml), followed by isolate *Aspergillus* MAM-F35 which gave CMCase (207 U/ml) and reducing sugar (1050µg/ml). However, isolate *Aspergillus niger* MAM-F5, gave the same amount of reducing sugar (1050µg/ml) as isolate MAM-F35 but its CMCase was very low (46U/ml). Soluble protein also varied greatly, they ranging between 8 and 124µg/ml as indicated in Table 1. Gao *et al*. [20] isolated a new thermophilic fungus *Aspergillus terreus* M11 from compost containing cellulose for cellulase production.

Production of Cellulases on Different Agricultural Wastes: Production of endoglucanase (CMCase), total cellulase (FPase), exoglucanase (Avicelase) and protein on solid state fermentation of four different agriculture wastes (WS, RS, WB and CC) had been shown in Fig.1

Table 1: Reducing sugars and Carboxymethyl cellulase produced by different fungal isolates

Isolate code	Reducing sugar (U/ml)	CMCase (U/ml)	Soluble protein (µg/ml)
Aspergillus sp. (MAM-F1)	640	89	92
Penicillium sp. MAM-F2	720	148	108
Aspergillus niger (MAM-F3)	680	148	70
Aspergillus sp. (MAM-F4)	620	87	88
Aspergillus niger (MAM-F5)	1050	46	77
Aspergillus sp. (MAM-F6)	680	1o2	81
Penicillium sp. (MAM-F8)	880	122	75
Pencillium sp. (MAM-F9)	900	72	88
Aspergillus niger (MAM-F12)	700	96	67
Aspergillus niger (MAM-F13)	240	157	93
Penicillium sp. (MAM-F14)	290	80	42
Pencillium sp. (MAM-F15)	910	119	63
Aspergillus sp. (MAM-F16)	720	85	17
Aspergillus sp. (MAM-F17)	1130	119	8
Fusarium sp. (MAM-F18)	1100	135	14
Fusarsium sp. (MAM-F19)	1120	157	14
Asp. Niger (MAM-F20)	850	156	11
Fusarium sp. (MAM-F21)	680	102	92
Aspergillus sp. (MAM-F23)	1270	233	80
Aspergillus sp. (MAM-F24)	1170	39	8
Penicillium sp. (MAM-F25)	1220	167	124
Penicillium sp. (MAM-F26)	940	148	114
Aspergillus sp. (MAM-F29)	550	91	61
Aspergillus sp. (MAM-F30)	350	70	75
Aspergillus sp. (MAM-F31)	450	74	81
Penicillium sp. (MAM-F32)	490	76	75
Aspergillus sp. (MAM-F34)	710	30	8
Aspergillus sp. (MAM-F35)	1050	207	34
Aspergillus sp. (MAM-F40)	200	167	93

for the standard strain *T. viride*. The results revealed that WS was the best substrate for CMCase (555 U/ml) while WB was the best for FPase and Avicelase (141 and 46 U/ml), respectively.

In case of *Aspergillus niger* MAM-F13, CMCase and Avicelase were best produced on WS (396 and 41 U/ml) respectively as indicated in Fig. 2. However, the best substrate for FPase and protein was recorded by WB (120 U/ml and 456 µg/ml), respectively. While, the results of *Aspergillus niger* MAM-5 revealed that the best substrate for FPase and Avicelase (65 and 24 U/ml) respectively, was WS as indicated in Fig. 3. The best CMCase and protein had been recorded for WB (333 U/ml and 439 µg/ml), respectively.

The other *Aspergillus* spp., revealed that *Aspergillus* MAM-F23 gave the highest Avicelase production (45 U/ml) on WS, while the highest CMCase (309 U/ml) on RS and Fpase (83 U/ml) on CC as indicated in Fig. 4.

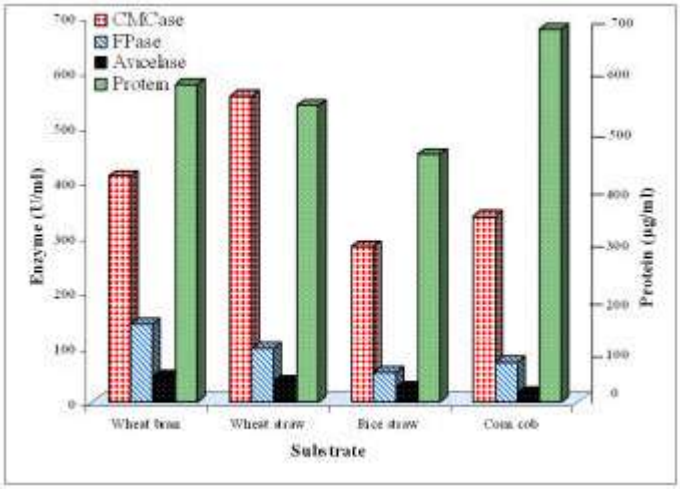

Fig. 1: Cellulase production of *T. viride* on different agriculture wastes by SSF

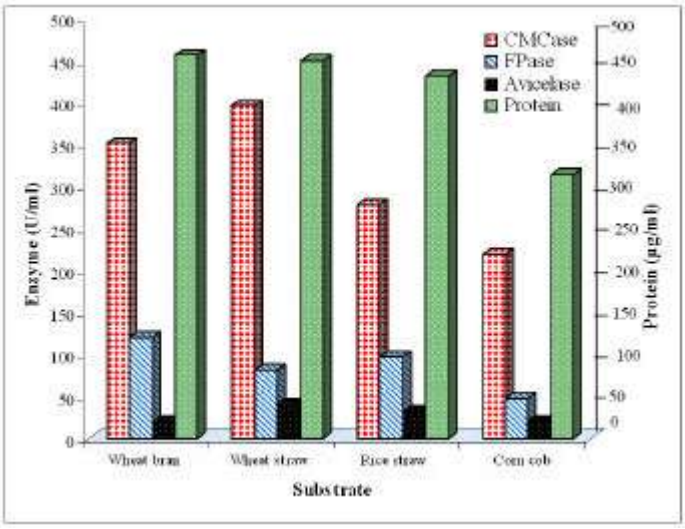

Fig. 2: Cellulase production of *A. niger* MAM-F13 on different agriculture wastes by SSF

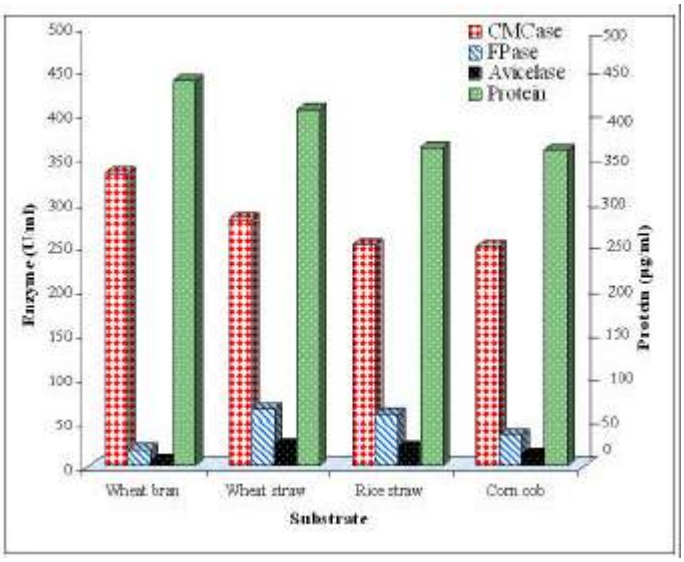

Fig. 3: Cellulase production of *A. niger* MAM-F5 on different agriculture wastes by SSF

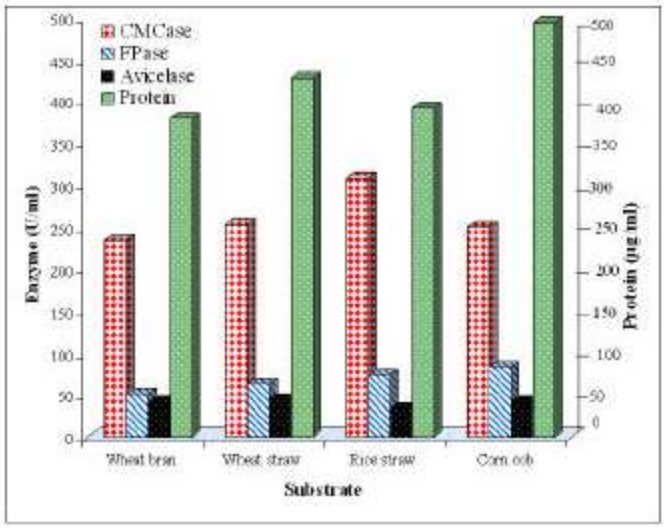

Fig. 4: Cellulase production of *Aspergillus* MAM-F23 on different agriculture wastes by SSF

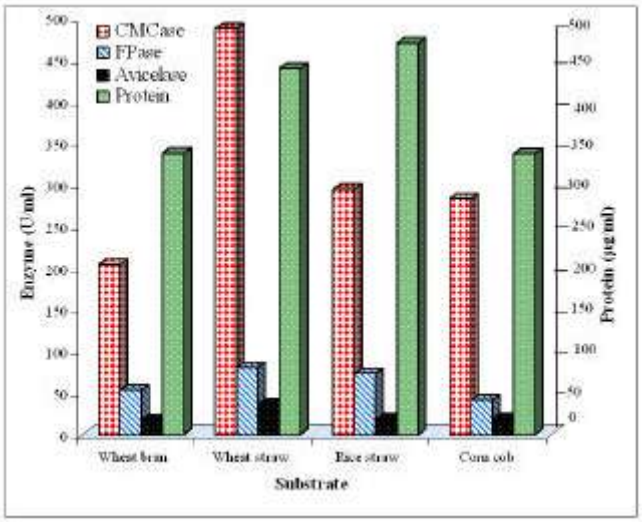

Fig. 5: Cellulase production of *Aspergillus* MAM-F35 on different agriculture wastes by SSF

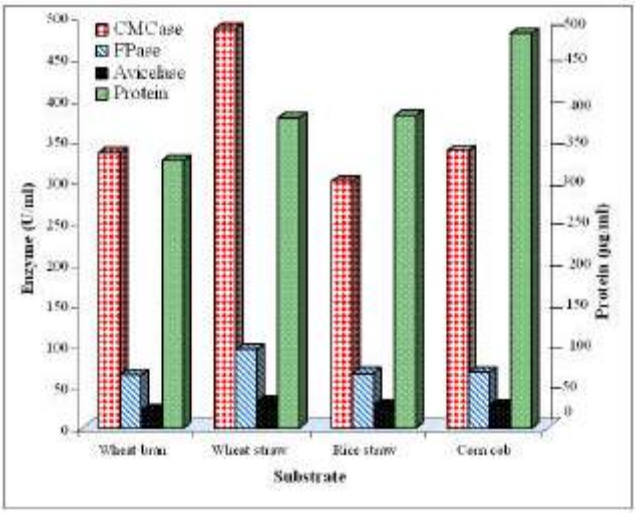

Fig. 6: Cellulase production of *Aspergillus* MAM-F40 on different agriculture wastes by SSF

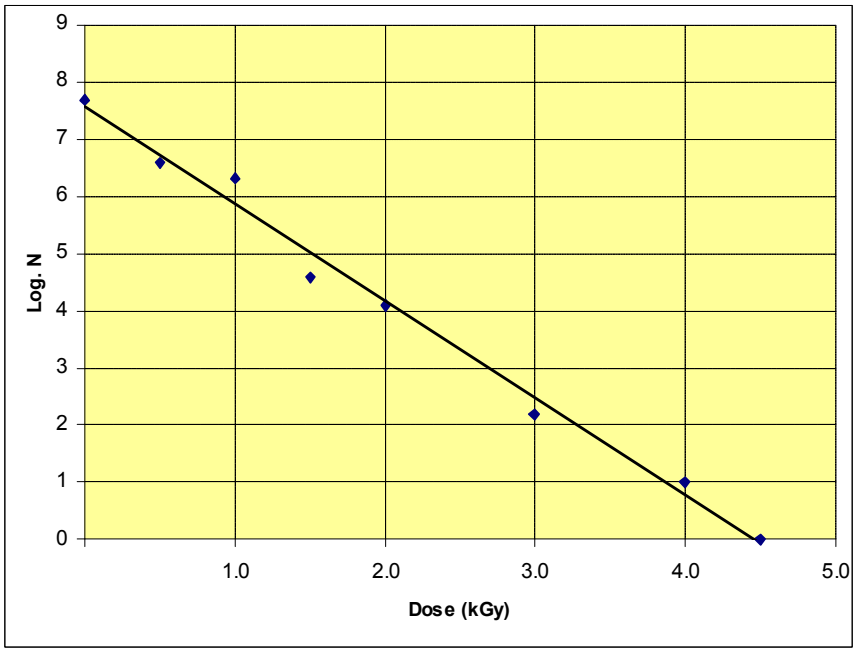

Fig. 7: Effect of gamma-radiation doses on the viable count of *Aspergillus terreus* MAM-F23

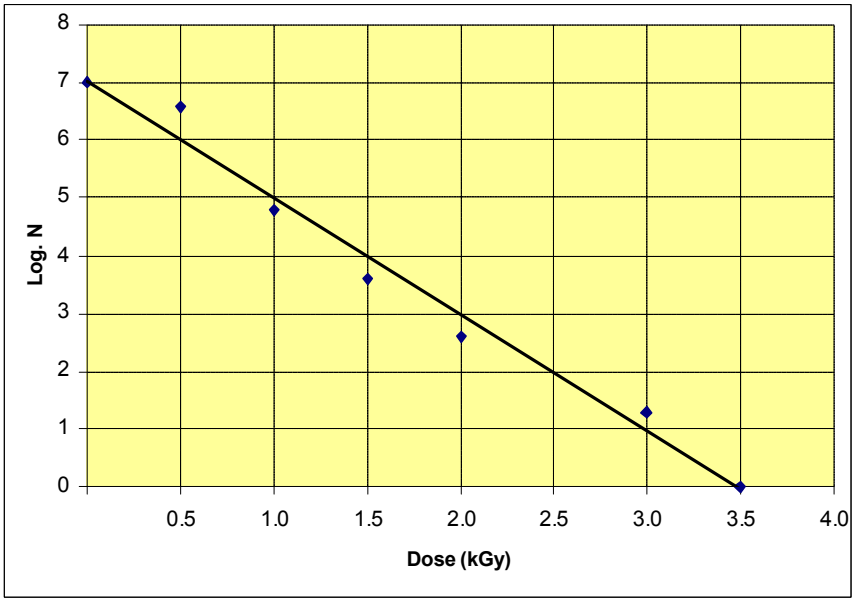

Fig. 8: Effect of gamma-radiation doses on the viable count of *Aspergillus flavus* MAM-F35

In case of *Aspergillus* MAM-F35 and 40, the highest production of the three types of cellulases had been recorded on WS as indicated in Figures 5 and 6. CMCase, FPase and Avicelase of MAM-F35 were 487, 80 and 36 U/ml and that of MAM-F40 were 485, 95 and 32 U/ml, respectively.

From the previous results, it was found that wheat straw supported the production of different cellulases followed by wheat bran followed by rice straw at the last was corn cob, i.e. WS>WB>RS>CC. Adsorption of enzymes and the formation of enzyme substrate complexes are considered to be critical steps in the enzymatic hydrolysis of cellulose. Cellulose fibers contain both amorphous and crystalline regions. Crystalline regions are considered to be more difficult to be degraded than the amorphous regions [30]. The highest

productivity of the three cellulases (Endoglucanase, exoglucanase and FPase) on wheat straw means that these enzymes adsorbed efficiently on wheat straw. Also, means that wheat straw contains the two forms, amorphous and crystalline cellulose. Also, the high production of Avicelase on wheat straw means that wheat straw contains a considerable amount of crystalline cellulose (Avicel). These results were confirmed by the results of Jatinder *et al.* [18, 31].

In fact, the comparisons of cellulase activities produced by different laboratories is not readily made in quantitative manner as no standard conditions of cellulase activity assay have been adopted by Gao *et al.* [20]. Also the difficulty in comparison between cellulose(s) activities depends on the difference between strains used in production, condition of production (SmF or SSF), assay determination and other physical factors. The thermophilic fungus, *Melanocarpus* sp. MTCC 3922, produced CMCase (142 U/g) and FPase (40 U/g) under SSF [18]. However, under SSF, also but another thermophilic fungus, *Scytalidium thermophilum* produced 62.5 U/g CMCase and FPase (3.0 U/g) [31]. Another, thermophilic fungus, *Aspergillus terreus* M11, when grown on lignocellulosic materials in SSF produced 581 U/g CMCase and 243 U/g FPase [20]. But in case of avicelase, Jatinder *et al.* [18, 31] found that, Avicel-adsorbable endoglucanase (AAEG) and Avicelase were ranging from 5.3 to 30.9 and 0.16 to 0.87 U/g, respectively.

Effect of Gamma Radiation on the Isolated Fungi Viability: Gamma radiation reduced the viable count of the spores of *Aspergillus* MAM-F23 and 35 gradually as indicated in Figures 7 and 8. As the gamma dose increased, the viable count decreased. 5.0 and 4.0 kGy reduced the viability of the two isolated strains MAM-F23 and 35, respectively. Ionizing radiation reduced the viable count of bacteria and fungi. As the dose increased, the viable count decreased gradually [22, 29, 32, 33]. These results, also confirmed by Aziz and Mahrous [34]. They recorded that the dose required for complete inhibition of fungi ranged from 4.0 to 6.0 kGy.

Induction of Hyper Producer Mutant: In the development of more economical production of cellulases, an efficient approach is the isolation of cellulase-hyper producing mutants. *Aspergillus* MAM-F23, when exposed to different gamma doses gave 11 mutants with different abilities to produce cellulases when compared with the control (non-irradiated strain) as indicated in Table 2. The results revealed that, the best mutant for CMCase and FPase was mutant No. "4" which exposed to 0.5 kGy, while the best Avicelase producing mutant was No. "8" which exposed to 2.0 kGy, which was the best also for protein production. Mutant No. "4" produced 31% Avicelase, 21% CMCase and 34% FPase more than the parent strain (control).

In case of *Aspergillus* MAM-F35, five mutants were selected as indicated in Table 3. The best mutant in the three kinds of cellulases (CMCase, FPase and Avicelase) was mutant No. "1" which exposed to 0.5 kGy. Its enzymatic production was superior than that of the control, but the best protein production had been recorded by mutant No. "3" which exposed to 1 kGy. So Mutant No. "1" was the best mutant in cellulase production. It produced 26% Avicelase, 24% CMCase and 46% FPase more than the parent strain (control). These results were confirmed by the previous results of El-Batal and Abo-State [35]. They found enhanced productivity in CMCase, FPase, Avicelase, xylanase, pectinase, α-amylase and protease by gamma-irradiation at dose 1.0 kGy with present increase 8%, 20%, 10%, 4%, 31%, 22%

Table 2: Cellulase production of *Aspergillus terreus* MAM-F23 mutant on SSF

Mutant number	Dose (kGy)	CMCase (U/ml)	FPase (U/ml)	Avicelase (U/ml)	Protein (µg/ml)
Control Parent strain	0	306	48	30	427
1	0.5	319	50	31	413
2	0.5	315	51	34	422
3	0.5	209	45	33	414
4	0.5	372	65	39	452
5	0.5	339	48	30	449
6	1.0	333	50	31	439
7	1.5	337	50	36	446
8	2.0	365	56	54	435
9	2.0	350	63	37	455
10	2.0	265	37	24	406
11	4.0	350	57	36	442

Table 3: Cellulase production of *Aspergillus flavus* MAM-F35 mutant on SSF

Mutant number	Dose (kGy)	CMCase (U/ml)	FPase (U/ml)	Avicelase (U/ml)	Protein (µg/ml)
Control Parent strain	0	246	30	19	346
1	0.5	307	44	24	366
2	0.5	270	40	25	366
3	1.0	280	38	24	414
4	1.0	291	32	24	410
5	1.0	287	34	22	345

and 34% respectively as compared with un-irradiated control. Also, the highest CMCase activity was recorded for *Fusarium neoceras* mutant No. "1" and No. "6" which exposed to 1 min UV-radiation. While, the highest CMCase of *F. oxysporum* was mutant No. "4" which exposed to 4 min. UV-radiation [22]. Mutant No. "36" which exposed to 10 kGy produced the highest extracellular protein and xylanase activity (700 µg/ml and 9993 U/g). This hyper producer mutant which exposed to 4 min UV-irradiation produced 10,350 U/g xylanase compared with the parent strain which produced 9651 U/g [29]. Rajoka [36] reported 1.6 fold enhanced productivity of extracellular endoglucanase over the mutant parent. After the optimization, the FPA in *T. reesei* MCG77 mutant was increased by 2.5 folds compared to that of *T. reesei* QM9414 mutant [19]. *Acremonium cellulotylicus* C-1 was subjected to mutagensis using UV-irradiation and N-methyl-N-nitro-N-nitrosoguanidine (NTG) and strain CF-2612 was isolated. This strain exhibited higher Fpase activities (18 U/ml) than that of the parent strain (12 U/ml) [37].

REFERENCES

1. Hölker, U., M. Höfer and J. Lenz, 2004. Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. Appl. Microbiol. Biotechnol., 64: 175-186.
2. Ögel, Z.B., K. Yarangümeli, H. Dürdar and I. Ifrij, 2001. Submerged cultivation of *Scytalidium thermophilum* on complex lignocellulosic biomass for endoglucanase production. Enzyme and Microbial. Technol., 28: 689-695.
3. Roopesh, K., R.K. Sumetra, M.S. Nampoothiri, G. Szakacs and A. Pandey, 2006. Comparison of phytase production on wheat bran and oil cakes in solid-state fermentation by *Mucor racemosus*. Bioresour. Technol., 97: 506-511.
4. Adsul, M.G., K.B. Bastawde, A.J. Varma and D.V. Gokhale, 2007. Strain improvement of *Penicillium janthinellum* NCIM 1171 for increased cellulase production. Bioresour. Technol., 98: 1467-1473.
5. Kaur, J., B.S. Chadha, B.A. Kumar and H.S. Saini, 2007. Purification and characterization of two endoglucanases from *Melanocarpus* sp. MTCC 3922. Bioresour. Technol., 98: 74-81.
6. Papinutti, V.L. and F. Forchiassin, 2007. Lignocellulolytic enzymes from *Fomes sclerodermeus* growing in solid-state fermentation. J. Food Eng., 81: 54-59.
7. Shih, I.L., C.Y. Kuo, F.C. Hsieh, S.S. Kao and C. Hsieh, 2008. Use of surface response methodology to optimize culture conditions for itorin A production by *Pacillus subtilis* in solid state fermentation. J. Chin. Inst. Chem. Eng., 39: 635-643.
8. Camassola, M. and A.J.P. Dillon, 2009. Biological pretreatment of sugarcane bagasse for the production of cellulases and xylanases by *Penicillium echinulatum*. Ind. Crops and Products, 29: 742-647.
9. Dogaris, I., G. Vakontios, E. Kalogeris, D. Mamma and D. Kekos, 2009. Induction of cellulases and hemicellulases from *Neurospora crassa* under solid-state cultivation for bioconversion of sorghum bagasse ethanol. Ind. Crops and Products, 29: 404-411.
10. Sukumaran, R.K., R.R. Singhania, G.M. Mathew and A. Pandey, 2009. Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production. Renewable Energy, 34: 421-424.
11. Rani, D.S. and K. Nand, 2000. Production of thermostable cellulase-free xylanase by *Clostridium absonum*. Process Biochem., 36: 355-362.
12. Persson, I., F. Tjerneld and B. Hahn-Hägerdahl, 1991. Fungal cellulolytic enzyme production part of: Persson, I. Production and utilization of cellulolytic enzymes in aqueous two-phase systems. Thesis University of Lund, Sweden, 1989.
13. FAO., 2004. Statistical yearbook production. Food and Agriculture Organization of the United Nations, Rome.

14. Kang, S.W., Y.S. Park, J.S. Lee, S.I. Hong and S.W. Kim, 2004. Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. Bioresour. Technol., 91: 153-156.
15. Yang, Y.H., B.C. Wang, Q.H. Wang, L.J. Xiang and C.R. Duan, 2004. Research on solid-state fermentation on rice chaff with a microbial consortium. Colloid Surf., 34: 1-6.
16. Singhania, R.R., A.K. Patel, C.R. Soccol and A. Pandey, 2009. Recent advances in solid-state fermentation. Biochem. Eng. J., 44: 13-18.
17. Umikalsom, M.S., A.B. Arrif, Z.H. Shamsuddin, C.C. Tong, M.A. Hassan and M.I.A. Karim, 1997. Production of cellulase by a wild strain of *Chaetomium globosum* using delignified oil palm empty-fruit-bunch fibre as substrate. Appl. Microbiol. Biotechnol., 47: 590-595.
18. Jatinder, K., B.S. Chadha and H.S. Saini, 2006a. Optimization of medium components for production of cellulases by *Melanocarpus* sp. MTCC 3922 under solid-state fermentation. World J. Microbiol. Biotechnol., 22: 15-22.
19. Latifian, M., Z. Hamidin-Esfahani and M. Barzegar, 2007. Evaluation of culture conditions for cellulase production by two *Trichoderma reesei* mutants under solid-state fermentation conditions. Bioresour. Technol., 98: 1-4.
20. Gao, J., H. Weng, D. Zhu, M. Yuan, F. Guan and Y. Xi, 2008. Production and characterization of cellulolytic enzymes from thermoacidophilic fungal *Aspergillus terreus* M11 under solid-state cultivation of corn stover. Bioresour. Technol., 99: 7623-7629.
21. Oxoid, 1982. Manual of Culture Media, Ingredients and other Laboratory Services. Published by Oxoid Limited, Wade Road, Basingstoke, Hampshire RG24 OPW. UK.
22. Abo-State, M.A.M., 2003. Production of carboxymethyl cellulase by *Fusarium oxysporium* and *Fusarium neoceras* from gamma-pretreated lignocellulosic wastes, Egypt. J. Biotecnol., 15: 151-168.
23. Bahkali, A.H., 1995. Production of cellulase, xylanase and poly-galacturonase by *Verticillium tricorpusaon* on different substrates. Bioresour. Technol., 35: 171-174.
24. Wang, C., T. Hseu and C. Huang, 1988. Induction of cellulase by cello-oligosaccharides in *Trichoderma konigii* G-39. J. Biotechnol., 9: 47-60.
25. Miller, G.L., 1959. Use of Dinitrosalysilic acid reagent for the determination of reducing sugars. Anal. Chem., 31: 426-428.
26. Gadgil, N.J., H.F. Daginawala, T. Chakakrabarti and P. Khanna, 1995. Enhanced cellulase production by mutant of *Trichoderma reesei*. Enzyme Microb. Technol., 17: 942-946.
27. Li, X. and P. Gao, 1997. Isolation and partial properties of cellulose decomposing strain of *Cytophaga* sp. LX-7 from soil. J. Appl. Microb., 82: 73-80.
28. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.
29. Abo-State, M.A.M., 2004. High-level xylanase production by Radio- resistant, thermophilic *Bacillus megaterium* and its mutants in solid-state fermentation. Egypt. J. Biotechnol., 17: 119-137.
30. Arifoğlu, N. and Z.B. Ögel, 2000. Avicel-adsorbable endoglucanase production by the thermophilic fungus *Scytalidium thermophilum* type culture *Torula thermophila*. Enzyme Microb. Technol., 27: 560-569.
31. Jatinder, K., B.S. Chadha and H.S. Saini, 2006b. Optimization of culture conditions for production of cellulases and xylanases by *Scytalidium thermophilum* using Response Surface Methodology. World J. Microbiol. Biotechnol., 22: 169-176.
32. Abo-State, M.A.M., 1991. Control of *Bacillus cereus* isolated from certain foods. M.Sc. Thesis, Fac. Sci., Cairo Univ.
33. Abo-State, M.A.M., 1996. Study of genetic background and effect of radiation on toxin production by *Bacillus cereus*. Ph.D. Thesis, Fac. Sci., Cairo Univ.
34. Aziz, N.H. and S.R. Mahrous, 2004. Effect of γ-irradiation on aflatoxin B_1 production by *A. flavus* and chemical composition of three crop seeds. Nahrung-Food, 48: 234-238.
35. El-Batal, A.I. and M.A. Abo-State, 2006. Production of cellulase, xylanase, pectinase, α-amylase and protease enzyme cocktail by *Bacillus* spp. and their mixed cultures with *Candida tropicalis* and *Rhodotorula glutinis* under solid-state fermentation. Egypt. J. Rad. Sci. Applic., 19: 139-156.
36. Rajoka, M.I., 2005. Double mutants of *Cellulomonas biazotea* for production of cellulases and hemicellulases following growth on straw of a perennial grass. World. J. Microbiol. Biotechnol., 21: 1063-1066.
37. Fang, X., S. Yano, H. Inoue and S. Sawayama, 2009. Strain improvement of *Acremonium cellulolyticus* for cellulase production by mutation. J. Biosci. Bioeng., 107: 256-261.