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Production of Cellulases and Xylanase by Thermophilic and Alkaliphilic Bacterial Strains Isolated from Agricultural Wastes

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Abstract: Seven agricultural wastes named wheat bran (WB), rice straw (RS), wheat straw (WS), corn cob (CC), sugarcane bagasse (SCB), potato peal (PP) and banana peal (BP) were used for isolation of bacterial strains and as substrate for cellulases (CMCase, FPase and avicelase) and xylanase production. Five of the isolated strains were thermophilic and alkaliphilic. These bacterial isolates were tested to determine their abilities to produce cellulases and xylanase on the different agricultural wastes. The results revealed that isolate MAM-38 produced highest CMCase and avicelase (360 and 354 U/ml) on WB, while MAM-29 produced the highest FPase, xylanase and protein (54 and 294 U/ml and 894 µg/ml) respectively on the substrate. Bacterial isolate MAM-38 produced the highest CMCase, FPase, xylanase and extracellular protein (248, 38, 227 U/ml and 850 µg/ml), respectively on RS, while the highest avicelase produced (27 U/ml) was by MAM-29 on RS also. Isolate MAM-38 produced the highest CMCase (292, 561, 99 and 653 U/ml) on CC, SCB, PP and BP respectively. Isolate MAM-29 produced the highest FPase and avicelase (45 and 14 U/ml) on CC. The highest xylanase (503 U/ml) was produced by MAM-51 on SCB. So, the most potent isolates MAM-29 and MAM-38 were selected to determine their dose response curve. Gamma radiation (15 kGy) reduced the viable count of MAM-29 by 5.7 log cycles while reduced 4.7 log cycles for MAM-38. This means that MAM-38 was more resistant to gamma radiation than MAM-29.

Key words: CMCase · FPase · Avicelase · Xylanase · Isolation · Survey · Radiation

INTRODUCTION

The existence of pollution problems associated with agro-industrial wastes, scarcity of places for its disposal, costlier treatment options and increased need to save valuable resources have forced to encourage the utilization and bioconversion of waste into high value industrially useful products. The huge amount of residual plant biomass considered as "waste" can potentially be used to produce various value added products like biofuels, animal feeds, chemicals, enzymes …*etc*. The demand for industrial enzymes, particularly of microbial origin is ever increasing owing to their applications in a wide variety of processes [1]. Globally large amount of agricultural residues are produced, most of which is burnt as waste disposal and small amount is used for mulching, for fuel or as fodder. Three types of energy can be produced from lignocellulosic residues by thermo-chemical or biochemical processing, liquid fuels such as ethanol or pyrolysis oil, gaseous fuels such as biogas (methane) and electricity [2]. The bioconversion of cellulose to fermentable sugars requires the synergistic action of complete cellulase system comprising of endoglucanase (EC 3.2.1.4) which act randomly on soluble and insoluble cellulose chains, exoglucanase (cellobiohydrolases, EC 3.2.1.91) which liberate cellobiose from the reducing and non-reducing ends of cellulose chains and β-glucosidases (EC 3.2.1.21) which liberate glucose from cellobiose [3,4].

The hemicellulase system involves among other endo-1, 4-β-D-xylanase (EC 3.2.1.8), which cleaves internal bonds in the xylan chain, β-xylosidases (EC 3.2.1.37), which cleaves xylooligosaccharides to produce xylose [5]. Xylanase (endo-1, 4-β-D-xylan xylanohydrolase, EC 3.2.1.8) catalyzes the hydrolysis of xylan to produce a mixture of shorter xylo-oligosaccharides, xylose and

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xylobiose [6]. There is an increasing demand for cellulases in the market for various applications, among which the bioconversion of lignocellulosic biomass for ethanol production is the major one [7]. Besides this, cellulases have many other potential applications as well, for example, formulation of washing powder, animal feed production [8], textile industry, pulp and paper industry, starch processing, grain alcohol fermentation, malting and brewing, extraction of fruits and vegetable juices [9]. The thermostability characteristics of cellulases enzyme system is a key to industrial interest of cellulose hydrolysis. Thermostable cellulolytic enzymes have wide applications in food and sugar industries where high temperature process such as pasteurization is used [10]. The majority of commercial cellulases are extracellular enzymes produced by mesophilic microorganisms. Since the use of cellulose degrading enzymes is related to industrial processes operating at high temperatures, application of thermostable enzymes produced by thermophilic microorganisms appears to be advantageous [11]. Colombatto *et al*. [12, 13] examined benefits from adding extracellular enzymes derived from thermophilic microorganisms to ruminant diets in view of the advantages of thermostable enzymes, called thermozymes, compared to their mesophilic counterparts.

As lignocellulosic materials are found in abundance in nature in the form of agricultural and industrial residues, they could be exploited as potential substrates for growing the microorganisms [6]. Rice straw (RS) is one of the most abundant lignocellulosic crop residues in the world. Its annual production is about 731 million tons which is distributed in Africa, Asia, Europe and America. This amount of RS can potentially produce 205 billion liters of bio-ethanol per year [14]. RS is composed of cellulose (41%) and hemicellulose (20%) which is bound to lignin (12%) by hydrogen and covalent bonds. The content of lignin in RS is less than that in other common feedstocks, such as corn stover and wheat straw. Cellulase production from bacteria can be an advantage as the enzyme production rate is normally higher due to bacterial high growth rate [15]. Reports on bacterial hydrolytic enzymes by SSF, however, are primarily confined to only *Bacillus* spp. [16-19] which could be attributed to their ability to the substrate particles to produce filamentous cells for penetration and to their specific need for water activity. Among these, *Bacillus licheniformis* enzymes are reported to have a marginally higher thermostability [20, 21], however, there are very few reports on xylanase production in SSF to date. Solid state fermentation (SSF) systems have generated much interest in recent years because they offer several economical and practical advantages over submerged cultivation equipment, improved product recovery, reduced waste water output, higher product concentration, lower capital investment, lower plant operational costs [22, 23], lower energy, a simple fermentation medium, has superior productivity and does not require a rigorous control of fermentation parameters [24, 25], less effluent generation, low catabolic repression [23], higher product stability, cultivation of microorganisms specialized for the water insoluble substrates or mixed cultivation of various fungi and last but not least, lower demand on sterility due to the low water activity used in SSF [26].

This study aims to get bacterial isolates having the ability to produce battery of cellulases and xylanase which tolerate harsh conditions (high temperature and alkalinity) to be used in industry and technology under extreme conditions.

MATERIALS AND METHODS

Agricultural Wastes: Seven agricultural wastes named, wheat straw (WS), rice straw (RS), wheat bran (WB), corn cob (CC), sugarcane bagasse (SCB), potato peal (PP), banana peal (BP) were collected from Upper Egypt Governorates.

Preparation of Agricultural Wastes: The lignocellulosic materials (agricultural wastes) were dried and milled into small pieces (3-5 mm). The milled agricultural wastes were used for isolation of bacterial strains and for SSF as substrates.

Isolation of Bacterial Strains: Ten grams of milled agricultural wastes were added to 90.0 ml sterile saline in 250 ml flasks under aseptic condition. The flasks were shaked vigorously for 15 min. The agriculture waste suspensions were serially diluted. From an appropriate dilution, 0.1 ml of three successive dilutions was plated on the surface of Luria-Bertani (L.B) agar plates (10.0 g tryptone, 5.0 g yeast extract, 5.0 g NaCl and 20.0 g agar in 1L of distilled water) [27] in duplicates. The plates were incubated for 48 h at 37°C. The well grown separated single colonies were picked up and subcultured on L.B slants. Slants were kept at 4°C.

Screening for Cellulolytic Activity of the Bacterial Isolates: Basal medium [28] supplemented with 1% carboxymethyl cellulose (CMC) product of Sigma, St.

Louis, MO, USA was sterilized by autoclaving at 121°C for 15 min. The basal medium was solidified before sterilization by 2% agar. The basal medium was poured in plates. The plates were inoculated by streaking the different isolates on their surfaces. The plates were incubated at 37°C for 48 h. The bacterial isolates which could grow on these plates were picked up as presumptive indication for possessing cellulase activities (promising isolates).

Screening for Thermophilic and Alkaliphilic Bacterial Isolate: The promising isolates were steaked on the surface of L.B agar plates and incubated at 50°C for 72h. The well grown bacterial isolates were picked up as thermophilic bacterial isolates. These thermophilic isolates were streaked on L.B agar plates with pH 10.0 and incubated at 37°C for 48 h. The well grown bacterial isolates were picked up as thermophilic and alkaliphilic isolates. These isolates were examined by Gram stain under light microscope (Leica, LEITZ, LABOR LUXS, Germany).

Selecting the Best Substrate for Cellulases and Xylanase Production by SSF: The thermophilic and alkaliphilic bacterial isolates were used to inoculate flasks (250 ml) containing 10.0 grams of the milled agricultural wastes after moisting by 20.0 ml distilled water and autoclaving. Five ml of bacterial culture of the selected isolates were used to inoculate every flask. Three replicates were used for each isolate onto each substrate. The flasks were incubated at 37°C for 48 h stagnant.

Enzyme Extraction: According to Abo-State [29], the enzymes were extracted from the fermented flasks with 100 ml of distilled water. The whole content was filtered and squeezed through muslin cloth. The filtered extract was centrifuged at 8000 rpm for 15 min. by cooling centrifuge (4°C). The clear supernatant was used as crude enzymes for enzyme assay and extracellular protein determination.

Enzyme Assay:

CMCase Assay: Endoglucanase, Carboxymethyl cellulase (CMCase) activity was determined according to Wang *et al.* [30]. 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 [31] by dinitrosalisylic acid (DNS) reagent (product of Sigma/Aldrich, USA). The resulted reducing sugars were determined at 540 nm by spectrophotometer (LW-V-200RS UV/VIS, Germany). The concentration of resulted reducing sugars was determined using glucose standard curve. One unit of CMCase is the micro mole of glucose liberated per ml of culture filtrate (crude enzyme) per minute.

Fpase Assay: Total cellulase (FPase) activity was the crude enzyme supernatant was determined as described by Gadgil *et al.* [32]. 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 h at 50°C, the resulted reducing sugars were determined by DNS reagent as previously mentioned. One unit of FPase is the micromole of glucose librated per ml of culture filtrate per minute.

Avicelase Assay: Avicelase activity was determined according to Li and Gao [33]. One ml of the crude enzyme supernatant was incubated with 1 ml of 2% (w/v) Avicel (product of Sigma, St. Louis, USA) in 0.1M phosphate-citrate buffer (pH 6.6) at 40°C for 2h. The resulted reducing sugars were determined by DNS reagent as previously mentioned. One unit of Avicelase is the micromole of glucose librated per ml of culture filtrate per minute.

Xylanase Assay: Xylanase assay was determined according to Chaplin [34]. One ml of the crude enzyme supernatant was mixed with 1 ml of 2% xylan from brichwood (product of Sigma/Aldrich, St. Louis, USA) in sodium acetate buffer (pH 5.5) and incubated at 50°C for 30 min. The released reducing sugar was determined by DNS reagent as previously mentioned. Standard curve was determined by xylose. One unit of xylanase is the micromole of xylose librated per ml of culture filtrate per minute under the assay conditions.

Protein Determination: Protein was determined according to Lowry *et al.* [35]. 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 (product of Fluka, Switzerland) was added to the previous mixture. The tubes were leaved for 20 min. at room temperature and the absorbance was measured at 720 nm by spectrophotometer.

Effect of Gamma Irradiation on the Most Potent Isolates: The most potent isolates (MAM-29 and MAM-38) were inoculated in L.B broth medium and incubated for 48 h at

37°C in shaking incubator. The bacterial cultures were centrifuged at 8000 rpm for 15 min. at 4°C. The pellets was washed with sterile saline and resuspended into saline to form pool for each isolate. Five ml aliquiot was distributed in clean sterile screw cap test tube. The tubes of each isolate were exposed to different doses of gamma irradiation (1, 2, 4, 6, 8, 10 and 15 kGy) from the Indian chamber (Co-60) at National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The dose rate was 1 kGy/20 min. and 9 seconds at the time of experiment at room temperature. The viable count was determined according to Abo-State [29]. The irradiated and non irradiated (control) were serially diluted and spreaded on the surface of L.B plates. Three replicates were used for each dose for each isolate. The plates were incubated at 37°C for 48 h, the count were recorded to determine the dose response curve. Colonies exposed to different doses of gamma irradiation with differences in morphology (shape, colour, margine, surface or size) were picked up and used to determine their cellulolytic and xylanolytic activities by SSF technique.

RESULTS AND DISCUSSION

Isolation of Different Bacteria: The aim of this study was to isolate strains of thermophilic and alkaliphilic bacteria with improved cellulases and xylanase production and had potential industrial application. Fifty-nine bacterial isolates were isolated from different agricultural wastes. Most of the isolates, obtained from agricultural wastes, were *Bacillus* sp. which was probably rather dominant in these wastes.

Selection of the Most Promising Isolates: In the present study, fifty-nine bacterial isolate were isolated from different agricultural wastes. Thirty-seven isolate only had cellulolytic activity (nine of them with faint growth). Five isolates (MAM-29, MAM-38, MAM-50, MAM-51 and MAM-53) were found to have alkaliphilic and thermophilic activities as described before and they were isolated from (PP, PP, CC, RS and RS) respectively. These Five isolates were selected for studying cellulases and xylanase production on different agricultural wastes as indicated in Table 1. Two of them gave high cellulases and xylanase production on RS when used as a sole carbon source. These isolates (*Bacillus* sp. MAM-38 and *Bacillus* sp. MAM-29) were isolated from the potato peal. The SSF system using RS as support is a feasible and economical method for the production of cellulases and xylanase based on the fact that RS is one of the cheap and abundant agro-waste.

Production of Cellulases on Different Agricultural Wastes: The ability of the five isolates to produce cellulases and xylanase were determined. Production of endoglucanase (CMCase), total cellulase (FPase), exoglucanase (Avicelase), xylanase and extracellular protein on SSF of eight different agricultural wastes (WS, RS, WB, CC, SCB, PP, BP, mixture of WB & RS) had been shown in Fig. 1-8. Results reveled that *Bacillus* MAM-29 gave best production for (FPase 54U/ml, xylanase 294 U/ml and extracellular protein 894 µg/ml) on WB as showed in Fig. 1. However, isolate *Bacillus* MAM-38 gave highest CMCase (360 U/ml) and avicelase (35 U/ml). The isolate MAM-38 was found to give the highest production on RS as indicated in Fig. 2 (CMCase 248 U/ml, FPase 38 U/ml and xylanase 227 U/ml) followed by isolate MAM-29 which gave CMCase (191 U/ml), FPase (37 U/ml) and xylanase (174 U/ml). However, isolate MAM-29 gave highest avicelase (27 U/ml) followed by isolate MAM-38 which gave (23 U/ml) on RS. Rice straw was selected as a substrate in this study because it produced every year by millions of tons and this biomass is managed predominantly through Rice Straw Burning (RSB). RSB leads to significant air pollution and has been banned in some regions, whereas stubble and straw incorporation into wet soil during land preparation is associated with enhanced methane emissions. Therefore, both strategies have important deleterious environmental effects and fail to take advantage of the huge energy potential of rice straw [36].

A number of approaches have been adopted, aiming towards reducing the cost of enzymes production, these have included the use of different lignocellulosic wastes including sawdust [37], corn cob [38], bagasse [39], wheat straw [40, 41], rice straw [42] and wheat bran [38] as examples of low cost materials which have been successfully used as substrates for cellulase fermentation by fungi mainly *Aspergillus* sp. and *Trichoderma* sp. When using WS as a substrate, isolate MAM-50 gave highest CMCase (306 U/ml) and isolate MAM-51 gave highest FPase (60 U/ml) and avicelase (36 U/ml). However, isolate MAM-38 gave highest xylanase (304 U/ml) and extracellular protein production (1189.7µg/ml) as indicated in Fig. 3. In case of CC, the isolate MAM-38 gave highest CMCase (292 U/ml) and isolate MAM-29 gave best FPase (45 U/ml) and avicelase (14 U/ml). While, isolate MAM-51 gave highest xylanase production (219 U/ml) and isolate MAM-53 gave best production for extracellular protein (877.4 µg/ml) as illustrated in Fig. 4. Also, isolate MAM-53 gave highest CMCase (571 U/ml) and FPase production (227.7 U/ml) on SCB. However, isolate MAM-51 gave best avicelase (23 U/ml) and xylanase (503 U/ml) as revealed in

Table 1: CMCase produced by different bacterial isolates

Isolate code	Colony description	1% CMC	pH 10	Temp 50°C
MAM-29	Medium size, creamy, obaque, irregular margine and flat.	++	+++++	++
MAM-38	Large size, creamy, flat, smooth obaque and irregular margine.	+++	++++	+++
MAM-50	Large size, white, flat, smooth, obaque and irregular margine.	+++	+++++	++
MAM-51	Large size, white, flat, smooth, obaque and irregular margine.	+++	++++	+++
MAM-53	Medium size, creamy, smooth, obaque, irregular margine and concave.	+++	++++	++++

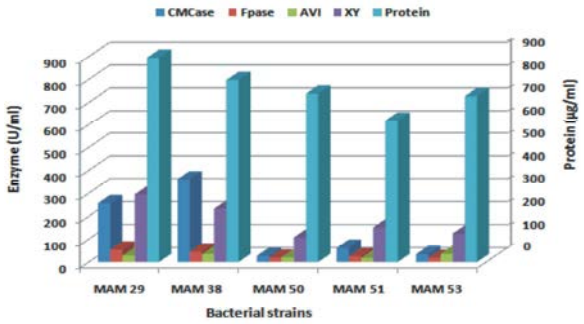

Fig. 1: Cellulases and xylanase production by the five isolates on WB using SSF.

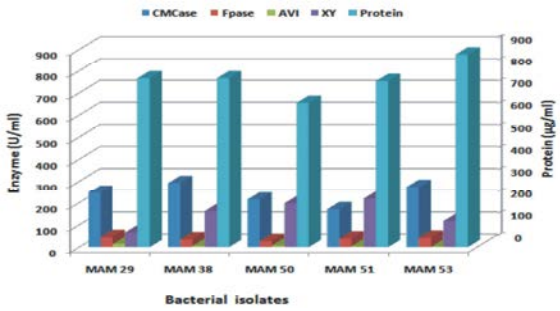

Fig. 4: Cellulases and xylanase production by the five isolates on CC using SSF.

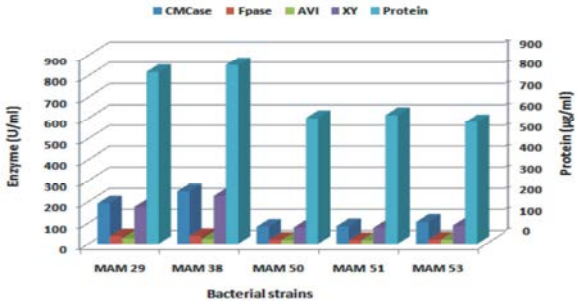

Fig. 2: Cellulases and xylanase production by the five isolates on RS using SSF.

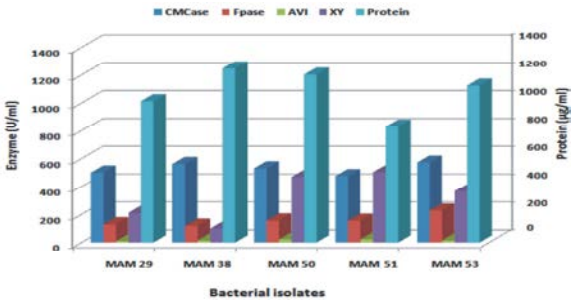

Fig. 5: Cellulases and xylanase production by the five isolates on SCB using SSF.

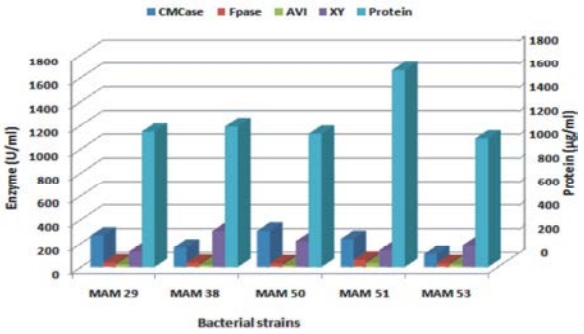

Fig. 3: Cellulases and xylanase production by the five isolates on WS using SSF.

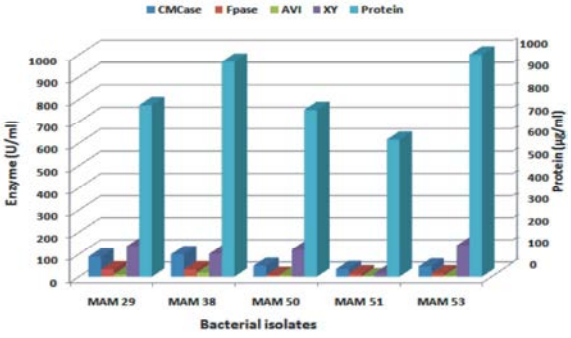

Fig. 6: Cellulases and xylanase production by the five isolates on potato peal using SSF.

Fig. 5.When using potato peal as a substrate, isolate MAM-38 gave highest CMCase, FPase and avicelase (99 U/ml, 32 U/ml and 17 U/ml respectively) but isolate MAM-53 gave best xylanase (139 U/ml) as indicated in Fig. 6. In case of using banana peal as a substrate, *Bacillus* isolate MAM-38 gave the best CMCase and Fpase (653 U/ml and 119 U/ml, respectively) while, the highest xylanase was given by isolate MAM-50 (417 U/ml) and the highest avicelase produced by MAM-53 (42 U/ml) as showed in Fig. 7. Among the few easily available lignocellulosic substrates tested banana fruit stalk gave the maximum cellulose production when

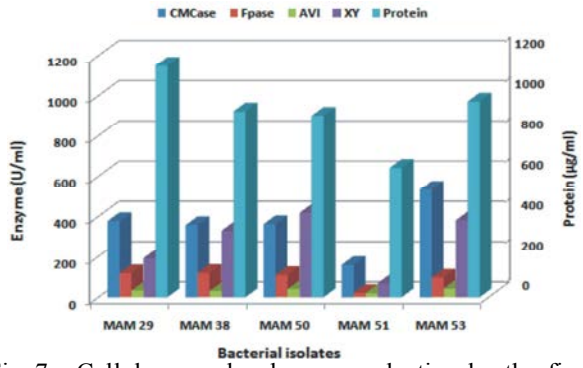

Fig. 7: Cellulases and xylanase production by the five isolates on banana peal using SSF.

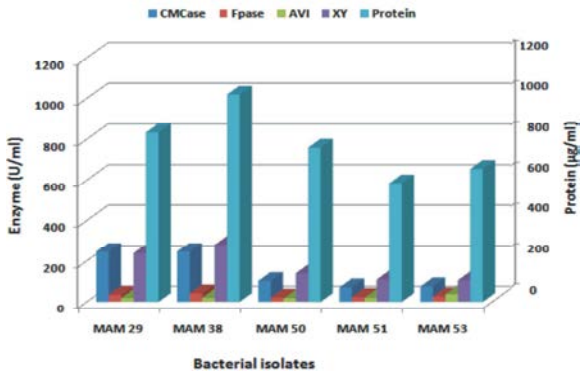

Fig. 8: Cellulases and xylanase production by the five isolates on mixture of WB and RS using SSF.

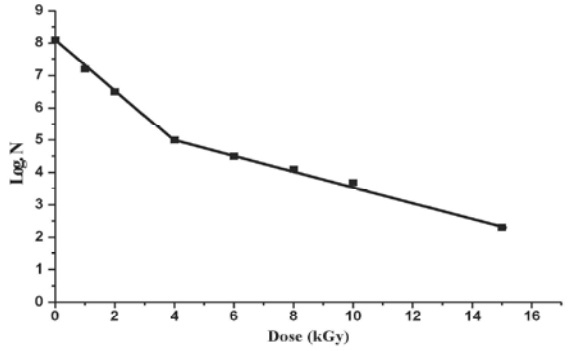

Fig. 9: Effect of gamma radiation doses on the viable count of *Bacillus* sp. MAM-29.

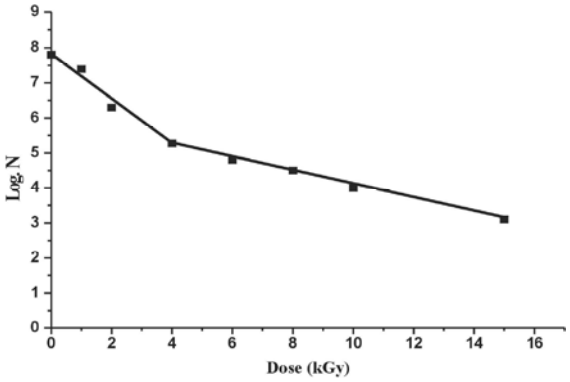

Fig. 10: Effect of gamma radiation doses on the viable count of *Bacillus* sp. MAM-38.

fermented with *Bacillus subtilis* under SSF. The total enzyme titres were 4-10 folds higher than those when WB, rice bran and RS were used as substrates [43]. Results of this study revealed that banana peal is the best substrate for CMCase production among the substrates tested. It gave maximum CMCase production (653 U/ml). The total titres were (2-25) fold higher than those when WB was used as a substrate using SSF.

Finally, when using mixture of RS and WB as a substrate, isolate MAM-38 gave highest CMCase, FPase and xylanase (246 U/ml, 42 U/ml and 280 U/ml respectively) but isolate MAM-53 gave highest avicelase (38 U/ml) as indicated in Fig .8. Results of this study revealed that the use of mixture of RS and WB as a substrate for the production of these enzymes enhance the production of avicelase and xylanase. This may be because the WB consists of considerable amount of soluble sugar like glucose (42.5% dry wt.), xylose (15.4% dry wt.), arabinose (3.1% dry wt.) and galactose (2.7% dry wt.) required for the initiation of growth and replication of the microorganism as explained previously by Lequart *et al*. [44].

Effect of Gamma Radiation on the Isolated Bacteria Viability: In the present study, trials had been carried out to enhance the productivity of CMCase, FPase, avicelase and xylanase by the two selected bacterial isolates (*Bacillus* MAM-29 and MAM-38) through their treatment by gamma rays to induce mutant strains having increased enzymatic activities. To achieve this goal, the bacterial pool of the selected isolated strains, *Bacillus* MAM-29 and MAM-38 were exposed for increasing doses of gamma radiation. Gamma radiation reduced the viable count of the two isolates gradually by increasing the dose as indicated in Fig. 9 and 10. As the gamma doses increased, the viable count decreased to some extent. Until 15 kGy, the bacterial cells of the two isolates still viable. Selection of mutants results from different doses of gamma radiation was on the basis of any changes in the morphological characters of the colony (size, colour, margine, pigmentation, texture or surface…*etc*.). Ionizing radiation reduced the viable count of bacteria and fungi. As the dose increased, the viable count decreased gradually [29, 45, 46, 47]. These results confirmed by Aziz

Table 2: Cellulases and xylanase production of *Bacillus* sp. MAM-29 mutants on SSF

Mutant number	Dose (kGy)	CMCase U/ml	Fpase U/ml	Avi U/ml	Xyn U/ml	Protein µg/ml
Control	0	583.7	286.8	122.9	353.0	484.9
1	1	534.9	293.9	102.3	335	423.8
2	2	408.9	222.8	131.6	373.6	345.6
3	2	500.1	189.7	121.8	356.3	480.0
4	2	480.6	314.4	135.7	372.6	465.3
5	10	518.6	316.0	143.0	345.4	509.3
6	1	539.2	117.1	128.1	359.5	475.1
7	1	487.1	148.6	115.9	342.2	553.3
8	1	587.0	274.2	131.3	384.5	499.5
9	1	406.0	238.9	97.4	360.6	387.2
10	8	551.5	284.0	129.8	372.6	453.1
11	8	542.8	221.0	158.7	241.2	484.9
12	2	604.7	253.6	147.6	350.8	475.1
13	2	383.2	140.5	112.6	331.3	475.1
14	1	519.0	257.6	118.8	351.9	509.3
15	1	480.9	219.6	146.8	372.6	497.1
16	8	558.0	191.4	134.0	334.6	433.6
17	8	541.4	156.1	97.6	330.2	432.1
18	2	566.4	254.4	123.1	367.1	514.2
19	8	582.6	211.5	104.7	397.5	448.2
20	8	571.8	214	128.3	367.1	377.4
21	8	506.6	222.3	127.5	350.8	470.2
22	10	534.9	211.5	92.2	338.9	499.5
23	10	420.9	285.5	149.3	385.6	396.9
24	10	578.3	245.3	125.1	350.8	379.8
25	10	391.5	235.6	95.3	338.9	555.7
26	2	667.3	217.1	134.7	366.0	396.9

Table 3: Cellulases and xylanase production of *Bacillus* sp. MAM-38 mutants on SSF

Mutant number	Dose (kGy)	CMCase U/ml	FPase U/ml	Avi U/ml	Xyn U/ml	Protein µg/ml
Control	0	512.2	187.4	123.4	260.4	499.5
27	15	464.1	206.4	110.3	278.8	399.4
28	15	479.3	216.2	112.2	263.6	477.6
29	15	507.5	183.6	87.8	284.2	443.4
30	15	621.5	178.2	134.8	260.4	399.4
31	15	462.7	183.1	109.3	239.7	470.2
32	6	534.3	146.7	103.5	245.2	482.2
33	2	538.7	161.9	97.3	221.3	448.2
34	15	607.1	142.4	117.9	251.7	465.3
35	15	588.6	176.6	110.6	299.4	538.6
36	15	559.3	184.2	106.0	253.8	514.2
37	10	570.2	226.0	132.1	265.8	516.6
38	10	553.9	140.7	101.1	221.3	572.8
39	2	481.1	168.4	96.7	236.5	563.1
40	2	512.6	147.8	82.9	245.2	546.0
41	2	551.7	19.7	110.6	308.1	587.5
42	2	363.3	122.0	109.2	278.8	509.3
43	4	523.0	191.5	102.4	292.9	567.9
44	2	605.5	200.7	123.0	297.3	570.4
45	2	657.6	122.3	96.8	266.9	519.1
46	8	542.5	161.9	105.2	188.7	538.6
47	2	545.7	173.3	115.5	268.0	526.4
48	2	530.5	133.5	100.6	245.2	585.0
49	2	503.4	124.3	93.2	265.8	509.3
50	6	490.4	72.3	103.3	277.7	467.8
51	6	605.5	201.2	114.7	275.6	438.5

and Mahrous [48]. Rajoka [49] reported 1.6 fold enhanced productivity of extracellular endoglucanase over the mutant parent.

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. *Bacillus* MAM-29, when exposed to different gamma doses gave 26 mutants with different abilities to produce cellulases and xylanase when compared with the control (non-irradiated strain). The results revealed that in case of *Bacillus* MAM-29, the best mutant in CMCase production was mutant No. "26" which exposed to 2kGy but the best FPase production had been recorded by mutant No. "5" which exposed to 10 kGy. Also the best avicelase production was by mutant No. "11" which exposed to 8 kGy and the highest xylanase production was by mutant No. "19" which exposed to 8 kGy. The best extracellular protein was by mutant No. "25" which exposed to 10 kGy as indicated in Table 2. In case of *Bacillus* MAM-38, the results revealed that, the best mutant for CMCase was mutant number "45" which exposed to 2 kGy, while the best FPase producing mutant was No. "37" which exposed to 10 kGy. Also the best mutant in avicelase production was No. "30"which exposed to 15 kGy. And the best xylanase and extracellular protein production was by mutant No. "41" which exposed to 2 kGy as indicated in Table 3.

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