Genetic improvement of fungal β-mannanase and its molecular differentiation

Nivien A. Abosereh[a], Siham A. Ismail[b], Om K.H. Khattab[c], Shaimaa A. Nour[b], Amany A. Abo-Elnasr[c], Amal M. Hashem[b]

[a]Department of Microbial Genetics, Division of Genetic Engineering and Biotechnology, [b]Department of Chemistry of Natural and Microbial Products, Pharmaceutical and Drug Industries Research Division, National Research Centre, Giza, [c]Department of Plant and Microbiology, Science Collage, Helwan University, Helwan, Egypt

Correspondence to Amal M. Hashem, Professor Doctor, Department of Chemistry of Natural and Microbial Products, Pharmaceutical and Drug Industries Research Division, National Research Centre, El Buhouth Street, Dokki, Giza, Egypt.
Tel: +20 106 255 9809;
fax: +20 233 370 931;
e-mail: amal_mhashem@yahoo.com

Received: 9 July 2019
Accepted: 19 August 2019

Egyptian Pharmaceutical Journal 2019, 18:403–410

Background
and objective β-Mannanase is an enzyme that has great potential in many industrial application including feed, food, pharmaceutical, cosmetics, production of mannan and manooligosaccharides, pulp and paper, bioethanol and biodiesel productions, and oil and textile industries. The aim of this study was to describe the potential of gamma and ultraviolet (UV) rays to optimize the production of industrially important β-mannanase enzyme by subjecting *Penicillium citrinium* Egy5LC368457 to these rays.
Materials and methods
Various doses and times of UV and gamma irradiation were used. Genetic diversity was resolved by mistreatment with the Random Amplified Polymorphic Polymer (RAPD-PCR) technique. Ten RAPD oligonucleotide primers amplifying DNA of β-mannanase showed reproducible banding patterns.
Results and conclusion
The results of this study revealed the highest β-mannanase activity was produced by gamma ray 150 Gy (37.42 IU/ml) with 2.27-fold higher than the wild type. A total of 64 bands were obtained from nine of these markers with 44% polymorphic bands. The size of the amplified bands ranged between ~75 and 3000 bp. The genetic polymorphism value of each primer was determined, which ranged between 2 and 9 bands. The primer efficiency of amplification ranged between 3.13 and 23.44%, and the discriminatory power ranged from 4.5 to 25. In conclusion, UV and gamma ray irradiation can induct mutations, which can be carefully acclimatized and commercially propagated under suitable condition. RAPD technique could be successfully applied to the newly β-mannanase and can differentiate mutants.

Keywords:
Penicillium citrinium, random amplified polymorphic polymer, ultraviolet and gamma rays

Egypt Pharmaceut J 18:403–410

1687-4315

Introduction

Microbes are the major source of most enzymes. The demand for these enzymes had occurred owing to growth of sustainable solutions [1,2].

Various fungi can produce β-mannanase, such as *Aspergillus awamori* [3], *Aspergillus oryzae* [4], *Penicillium humicola* [5], *Penicillium oxalicum* [6], *Trichoderma harzianum* [7], and *Rhodothermus marinus* [8]. Many microbic β-mannanase production studies have targeted on the use of pure mannans like locust bean gum and konjac gum as inducers [9,10]. Many wastes have low-value mannan-rich substrates, like coconut meal, palm nut cake, apple pomace and occasional extracts [11], and alternative hemicellulosic biomass like wheat bean and wheat straw, which may also be utilized in bioprocesses [12,13].

Endo-β-1,4 mannanases (E.C.3.2.1.78) are enzymes that hydrolyze the (1,4)-β-D-mannosidic linkages within the main chain of mannans and heteropolysaccharides, consisting mainly of mannose, such as galactomannans and glucomannans, producing manno-oligosaccharides [14].

In fact, mannanases may well be additionally accustomed to cut back the body of occasional extracts [15,16], biobleaching of pulp and detergent trade [5,13,17–20], bioconversion of biomass wastes to fermentable sugars [21,22], and upgrading of animal feed products [17,23]. Furthermore, they are employed in the preparation of manno-oligosaccharides which used as prebiotics (non-nutritional food additives) for selective growth of human beneficial intestinal microflora (*Bifido bacterium* and *Lactobacilli* spp.) [24,25].

For high production of enzyme, microorganisms can be improved by different mutagenesis as a successful method [26].

Ultraviolet (UV) and gamma (γ) irradiations can also be used to obtain mutants yielding higher enzymes production from conidia of thermophilic fungi [27]. The improvement of the mutants for the production of the enzymes occurred by re-constitution of the damaged genes of wild-type strains, with improvement of these properties.

For strain mutation, UV rays are very important inducers. The main effect of these rays is to modify the structure of pyrimidine (cytosine and thymine), causing the formation of thymine dimers, which distort the structure of DNA heliex and block further replication process [28].

Omear *et al.* [29] and Soliman *et al.* [30] have developed the haphazardly amplified polymorphic polymer (RAPD) markers, which have become one-in-all the foremost wide PCR-based DNA markers that can be used to detect the relation between species.

In this work, genetic improvement of fungal β-mannanase by using physical mutagen (UV and gamma) and the differentiation between wild-type strain and their mutant were done.

Materials and methods

Fungal isolate and growth media

The fungal strain *Penicillium citrinium* Egy5 LC368457 was obtained from the Pharoesmomes of Ancient Egyptian Museum, Cairo, Egypt. There is No animal or human experimental. The culture was maintained on potato dextrose agar and incubated at 30°C for 7 days before storage at 4°C with monthly subculturing.

Mutagenesis

Two methods of mutations were employed for strain improvement:

Mutagenesis with gamma rays treatment

In this way, mutagenesis to the spore suspension of *P. citrinium* was carried out using gamma rays produced from Cobalt-60 (Co60) as a source of gamma radiation (by Egyptian Atomic Energy Authority, Nasser City, Egypt).

Five different doses of gamma radiation were employed: 0, 50, 100, 150, 200, and 250 Gy [27].

Mutagenesis with ultraviolet treatment

UV mutagenesis to the spore suspension of *P. citrinium* was treated with UV (power, 30 W, and wavelength, 254 nm), each 15 min intervals, with a distance of 18 cm from the UV light source. Then, the plates were stored in the dark for 2 h. After treatment, mutagenic of each treatment was diluted and plated on potato dextrose agar as complete media and Cazpek media as minimal media at 30°C for 5 days. A single colony from each treatment was subcultured for enzyme production [31].

Culture media

The basal medium for inoculum included the following (g/l): peptone (2); ammonium sulfate (1.5); urea (0.3); $MgSO_4.7H_2O$ (0.5); K_2HPO_4 (10); and locust bean gum (10) [5]. The pH of the medium was adjusted at 5.3 before autoclaving. Each 250 ml Erlenmeyer flask contained 50 ml of the medium and was autoclaved for16 min at 121°C.

Screening of mutated isolates (production medium)

The production medium included the following (g/l): peptone (2.27); ammonium sulfate (1.7); urea (0.34); $MgSO_4 \cdot 0.7H_2O$ (0.6); K_2HPO_4 (7.5); and Coffee waste(3 g/flask). The pH of the medium was adjusted at 4.5 before autoclaving. Each 250 ml Erlenmeyer flask contained 50 ml of the medium and was autoclaved for 16 min at 121°C [5].

An inoculum culture was obtained by culturing the fungal strains in the above medium at 30°C for 48 h with shaking at 120 rpm. The culture flasks were inoculated by 8% of the inoculum and incubated at 30°C in a shaking incubator at 120 rpm for 12 days. Thereafter, the fermented medium was centrifuged, and the filtrate was used as the crude enzyme solution.

Analytical methods

Enzyme assay

An assay was performed by incubating 0.5 ml of appropriately diluted culture filtrate with 1 ml of 1% (w/v) locust bean gum (0.05 mmol/l) sodium citrate buffer at pH 5.0 for 10 min at 50°C [4]. The reducing sugars produced were determined using the Nelson–Somogyi technique [32]. One unit of enzyme activity was defined as the amount of enzyme that released 1 mmol of mannose/ml/min.

Protein determination

To determine the specific enzymatic activity, the quantification of total amount of soluble protein was measured using the Lowry method [33].

Genomic DNA extraction

Genomic DNA was extracted from the most efficient productive strains and their best resulting mutants using Easy Quick DNA extraction kit (Qiagene, Netherlands and Germany) following the manufacturer's instructions.

Random amplified polymorphic DNA (RAPD-PCR)

The RAPD-PCR technique was done using 10 oligonucleotide primers. The PCR was performed according to Plengvidhya *et al.* [34] in a 25 µl reaction volume, and amplification was programmed to 40 cycles after an initial denaturation cycle for 2 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 25°C for 1 min and an extension step at 72°C for 2 min, followed by extension for 10 min at 72°C in the final cycle.

Band analysis

The gels for control and exposed DNA were run for each of the 10 primers (Table 1). A DNA ladder of 100 and 250 bp was also run in each gel. The bands for PCR products were analyzed by Labimage version 5.21 programs. The banding patterns on the gels were transformed into tables of binary characters, where the appearance of a band was given the number one while the absence of the band was denoted by zero. Table 6 was used to first, determine the total number of bands together with their molecular weights that were produced by a primer across all isolates starting from the lowest weight (bottom of the gel) to the highest weight (top of the gel) [35]; second, to determine the monomorphic or common bands (appearing in all isolates) and polymorphic bands (appearing in some isolates only) amplified by a primer; third, to determine the % efficiency of a primer. This was estimated as a percentage of the total number of bands amplified by the primer out of the total number of bands amplified by all primers across all species. This represents the availability of sequences complementary to the primer in the genome; and fourth, to determine the discriminatory power of the primer [36]. This is a percentage of the polymorphic bands amplified by a primer out of the total number of polymorphic bands given by all primers in all isolates.

Table 1 Numbers and sequences of the random amplified polymorphic polymer primers used

Primers	Sequences
OPA-04	5′CCGCATCTAC3′
OPA-05	5′AGGGGTCTTG3′
OPA-07	5′GAAACGGGTG3′
OPA-08	5′GTGACGTAGG3′
OPA-20	5′GTTGCGATCC3′
OPB-12	5′CCTTGACGCA3′
OPC-01	5′TTCGAGCCAG3′
OPC-02	5′GTGAGGCGTC3′
OPC-04	5′CCGCATCTAC3′
OPC-05	5′GATGACCGCC3′

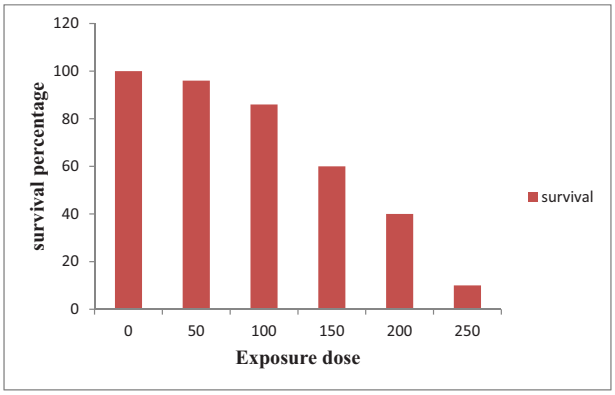
Results and discussion

Screening of β-mannanase activity by gamma-irradiated mutants

β-Mannanase is the most significant catalyst for hemicelluloses digestion, one of abundant groups of chemical compound in nature. This enzyme hydrolyzes mannan yielding mannotriose and mannobiose [37]. The present study is an attempt to improve the yield of enzymes production from *Penicillium* citrinum by gamma rays and/ or UV mutation. A range of doses (50–250 Gy) of gamma radiations were applied to induce mutation in cells of wild strain of *Penicillium* citrinum (Fig. 1). It was found that the survival percentages decreased by increasing exposing dose of gamma rays. The survival percentages were 96, 86, 60, 40, and 10 at doses 50, 100, 150, 200 and 250 Gy, respectively. A total number of 42 mutant strains were subjected to different exposure time of gamma radiation. Approximately 26% of the mutants using 50, 100, 150, 200, and 250 doses of gamma radiation showed β-mannanase activities higher than the parent strain (Table 2). Similar results were revealed by Iftikhar *et al.* [38] who found that with the dosage 140 Gy, MBL-5 showed maximum extracellular lipase production. However, Shahbazi*et al.* [27] reported that gamma causes improvement of the activity of Ccase, CMCase, Avicellase, and Fpase for *Trichoderma* mutagenesis.

Of all the mutants obtained, mutant designated 150-A had the highest increase in the β-mannanases activity (37.42 IU/ml). This is ~2.27 folds of the parent strain, with specific activity above wild type of 2.18 folds. The

Figure 1



The effect of gamma dose at different dose interval on the survival mutation percentage of *Penicillium citrinium.*

mutants coded 200 J, 200 F, and 250 G gave ~0.4 IU/ml. Similarly, an 81% increase in lipase activity has also been reported by *Penicillium expansum* after exposure with gamma irradiation [39].

Table 2 Screening of β-mannanases activity in gamma-irradiated mutants

	IU/ml	Protein	Final pH	Specific activity
Control	16.48±1.29	7.2±0.3	5.4	2.29
50-A	30.65±1.18	6.88±0.01	5.24	4.45
50-B	0.79±0.25	8.01±0.33	5.5	0.09
50-C	2.15±0.52	7.01±0.28	5.56	0.30
50-D	2.1±0.562	8.04±0.27	5.2	0.26
50-E	1.2±0.7	7.91±0.04	5.4	0.15
50-F	1.9±0.53	7.58±0.46	5.6	0.25
50-G	0.79±0.29	8.21±0.15	5.32	0.096
50-H	2.3±0.2	7.08±0.07	5.63	0.32
50-I	9.6±0.85	7.5±0.09	5.4	1.28
50-J	16.6±0.28	6.9±0.13	5.34	2.40
50-K	12.6±1.64	7.58±0.18	5.56	1.66
100-A	20.54±1.74	6.68±0.36	5.23	3.07
100-B	2.7±0.18	8.05±0.4	5.34	0.33
100-C	12.09±1.49	7.86±0.21	5.46	1.53
100-D	21.9±0.38	7.36±0.08	5.3	2.97
100-E	22.63±0.72	7.65±0.12	5.33	2.95
100-F	11.2±2.41	8.8±0.12	5.6	1.27
150-A	37.42±1.84	7.5±0.25	5.34	4.99
150-B	23.2±1.07	8.47±0.48	5.4	2.74
150-C	1.2±0.35	7.12±0.12	5.3	0.16
150-D	22±0.5	7.96±0.70	5.41	2.76
150-E	10.3±0.09	7.31±0.37	5.35	1.40
200-A	4.3±0.32	6.49±0.38	5.23	0.66
200-B	1.6±0.04	6.36±0.48	5.35	0.25
200-C	5.5±0.33	8.73±0.17	5.3	0.63
200-D	1.26±0.10	8.92±0.07	5.32	0.14
200-F	0.4±0.03	7.56±0.21	5.61	0.05
200-G	18.4±0.30	7.34±0.93	5.35	2.50
200-H	2.46±0.16	8.1±0.4	5.4	0.30
200-E	1.38±0.21	7.70±0.16	5.54	0.17
200-I	2.35±0.10	6.98±0.01	5.31	0.33
200-M	1.2±0.31	7.26±0.16	5.36	0.16
200-J	0.43±0.08	8.01±0.20	5.41	0.053
200-K	3.8±1.11	8.1±0.33	5.48	0.46
200-L	13.24±1.09	7.6±0.05	5.29	1.74
250-A	26.13±0.13	7.5±0.38	5.3	3.48
250-B	18.33±0.16	7.25±0.34	5.3	2.52
250-C	6.4±0.17	8.71±0.26	5.24	0.73
250-D	25.03±0.13	7.8±0.12	5.28	3.21
250-E	1.6±0.16	8.22±0.29	5.44	0.19
250-F	6.7±0.26	7.34±0.14	5.41	0.91
250-G	0.49±0.05	6.01±0.09	5.39	0.08

The protein content in all the mutants ranged from 6.01 to 8.92 mg/ml. The mutant coded 50-A, 150-A,150-B, 250-A, and 250-D that gave higher β-mannanases (30.65, 37.42, 23.2, 26.13, and 25.03 IU/ml, respectively) were selected and identified as stable mutants that showed higher stability with five generations (Table 3).

Screening of β-mannanase activity in ultravoilet-irradiated mutants

A total of 22 mutant strains of wild-type strain were developed at intervals of 60 min of exposure to UV irradiation (Table 4). Approximately, 27% of the mutant strains of *P. citrinium* generated from 15, 30, and 45 min of exposure to UV irradiation showed higher increase in β-mannanase activities compared with the wild-type strain. Of all the mutants generated, mutant designated UV30-B had the highest increase in mannanase activity, with ~3-fold higher than the parent strain, whereas the mutant coded UV15-H gave 0.79 IU/ml. The mutant strains 30-B, 45-B, and 45-A showed low stability within three generations (Table 5). It was clear that UV rays were very harmful on the microorganism as only 3.57% germination was reported after 15 min of exposure, and lethal doses were found to be after 60 min (Fig. 2). Our results agree with EL-Bondkly *et al.* [31] who showed that after exposure of *Penicillium roquefortii* strains to UV rays, the lethality percentage sharply increased with the increase in the periods of exposure to UV. Arotupin *et al.* [40] showed also UV irradiation produced higher increase in β-mannanase activities from *Aspergillus glaucaus* and *Rhizopus japonicas.* Prabakaran *et al.* [41] noticed that the highest production of cellulases was observed by *Penicillium chrysogenum* with UV exposure time of 5 min. Moreover, Bapiraju *et al.* [26] observed that UV-induced mutant of *Rhizopus* spp. showed higher lipase activity than the parent strain. Meanwhile, Irfan *et al.* [42] revealed that the UV radiation has increased the CMCase activity up to two times, whereas FPase activity exaggerated to a few times as compared with

Table 3 Stability of gamma mutants

	First generation (IU/ml)	Second generation (IU/ml)	Third generation (IU/ml)	Fourth generation (IU/ml)	Fifth generation (IU/ml)
50-A	30.65	30.62	29.84	31.42	30.68
150-A	37.42	37.85	39.42	39.56	38.22
150-B	23.2	24.52	23.45	23.63	23.69
250-A	26.13	26.87	27.13	26.02	26.28
250-D	25.03	25.00	27.24	26.30	26.05

Table 4 Screening of β-mannanases activity in ultraviolet-irradiated muatnts

Different time	IU/ml	protein	Final pH	Specific activity
Control	16.48±1.3	7.89±0.3	5.23	2.10
15 min				
UV 15-A	18.9±0.62	6.89±0.45	5.69	2.74
UV 15-B	6.5±0.1	6.4±0.21	5.75	1.01
UV 15-C	0.9±0.04	7.5±0.2	5.63	0.12
UV 15-D	9.8±0.23	6.4±0.15	5.72	1.53
UV 15-E	14.36±0.20	6.62±0.44	5.75	2.16
UV 15-F	12.32±0.20	6.3±0.11	5.72	2
UV 15-G	24.26±0.23	6.62±0.82	5.34	3.66
UV 15-H	0.79±0.16	6.23±0.15	5.74	0.13
30 min				
UV 30-A	22.61±0.47	7.88±0.80	5.56	2.87
UV 30-B	48.34±3.13	6.5±0.01	5.63	7.4
UV 30-C	8.33±0.16	7.21±0.10	5.56	1.16
45 min				
UV 45-A	25.84±2.03	6.11±0.19	5.69	4.23
UV 45-B	33.71±1.37	8.49±0.29	5.65	3.97
UV 45-C	17.23±0.11	8.13±0.11	5.70	2.11
UV 45-D	18.47±0.08	7.61±0.17	5.60	2.43
UV 45-E	16.81±0.27	8±0.90	5.56	2.10
UV 45-F	7.8±0.08	8.4±0.44	5.70	0.92
60 min				
UV 60-A	17.38±0.13	6.67±0.1	5.59	2.61
UV 60-B	12.3±0.07	6.6±0.60	5.62	1.86
UV 60-C	13.6±0.06	7.46±0.15	5.72	1.82
UV 60-D	12.2±0.38	6.5±0.29	5.57	1.87
UV 60-E	11.84±0.96	7.3±0.08	5.4	1.62

Table 5 Stability of ultraviolet mutants

Different time	First generation (IU/ml)	Second generation (IU/ml)	Third generation (IU/ml)
30 B	48.34	35.24	25.27
45 B	38.47	24.15	20.47
45 A	33.71	21.48	15.36

the parental strain. However, Burlacu *et al.* [43] reported that UV mutagenesis was a successful method for enhancing the xylanase activity compared with chemical mutagenesis.All of these results indicated that the survival of the parent strain depends on both of the type and period of mutagen treatment and on the microorganism's nature.

Molecular study

The molecular genetic variability among the wild-type and gamma mutants that showed higher productivity and stability (150-A, 150-B, 50-A, 250-Aand 250-D) was evaluated using ten random primers. Nine of ten primers (10-mer random primers: A04, A05, A07, A08, B12, C01, C02, C04, and C05, Table 6) gave positive and detectable bands (Fig. 3). They generated a total of sixty-four RAPD different bands ranging approximately from 75 to 3000 bp size. Band numbers are ranging from

Table 6 Fragments amplified by the nine primers in the wild-type and gamma mutant species and the % efficiency of amplification and discriminatory power of each primer

Primers	Total no of bands	Polymorphic bands	Primer efficiency %	Primer discriminatory power%
OPA-04	9	9	14.06	20.45
OPA-05	6	2	9.38	4.5
OPA-07	15	11	23.44	25
OPA-08	7	5	10.94	11.36
OPB-12	7	3	10.94	6.82
OPC-01	6	2	9.38	4.54
OPC-02	6	5	9.38	11.36
OPC-04	2	2	3.13	4.5
OPC-05	6	5	9.38	11.36
Total	64	44		

two bands for primer OPC-04 to fifteen bands for primer OPA-07 (Fig. 3). However, 13 (23%) bands were monomorphic, and two primers OPC-04 and OPA-04 did not generate monomorphic band.

Additionally, the primer efficiency of amplification ranged between 3.13% (OPC-04) and 23.44% (OPA-07) (Table 6).

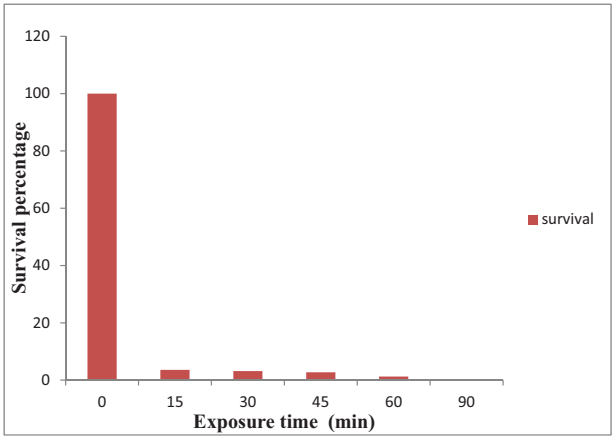
On the contrary, the discriminatory power ranged from 4.5 (OPA-08 and OPA-02) to 25 (OPA-07). Nine of

ten primers gave amplified bands (representative of these PCR products are shown in Fig. 3). All primers succeeded in giving polymorphic and monomorphic bands, except primers OPC-04 and OPA-04. The high potency of the primer is indicative of an oversized space of the order that enhances and permits base pairing between primer and genomic deoxyribonucleic acid [44]. Moreover, discriminatory value of a primer depends only on the number of polymorphic bands produced by the primer relative to the total number of polymorphic bands produced by all primers.

Discriminatory power of a primer is more important than the primer efficiency in determining its DNA fingerprint. In this study, the two primers (OPA-08 and OPB-12) have similar efficiency (10.94), and the same total number of bands (7) gave different discriminatory power of 11.36 and 6.82, respectively.

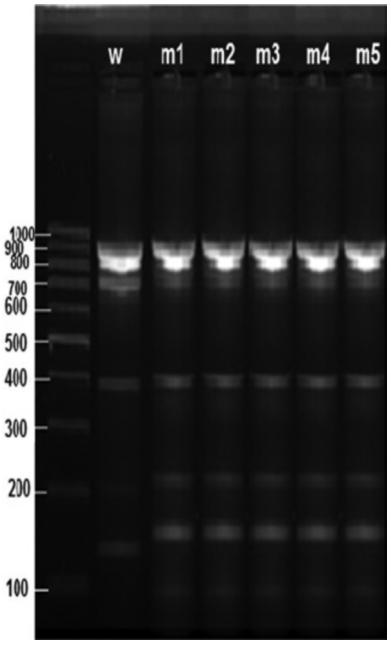
Dendrogram was produced after numerical analysis of the DNA RAPD profiles using the Nei and Lis correlation coefficient, and unweighted pair group

Figure 2



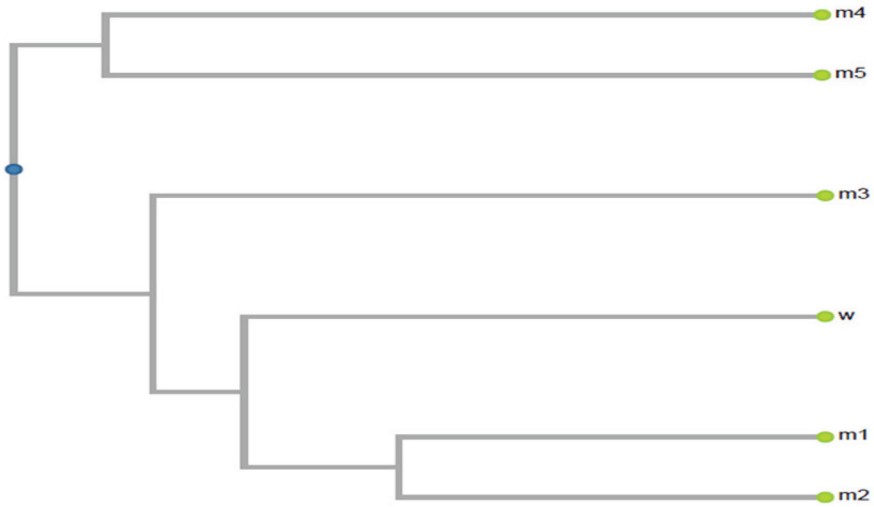
The effect of ultraviolet dose at different time interval on the survival mutation percentage of *Penicillium citrinium.*

Figure 3



Random amplified polymorphic DNA pattern of isolated DNA from *Penicillium citrinium* and their resulting mutants by using nine different random amplified polymorphic polymer primers (OPA-04; OPA-05; OPA-08; OPB-12; OPC-01; OPC-04; OPC-02; OPC-05; OPA-07): W:wild type, m1: 150-A, m2: 150-B, m3: 50-A, m4: 250-A, and m5: 250-D.

Figure 4



Patterns and dendrogram based on unweight pair group method with arithmetic average algorithm (UPGMA) of the DNA Genome patterns of the whole-cells of *Penicillium citrinium* and their mutants. [W:wild type, m1: 150-A, m2: 150-B, m3: 50-A,m4: 250- A ,m5: 250-D].

Table 7 Similarity matrix computed with dice coefficient

	w	M1	M2	M3	M4	M5
W	1	0.576	0.690	0.583	0.560	0.417
M1		1	0.730	0.642	0.545	0.415
M2			1	0.500	0.556	0.423
M3				1	0.455	0.524
M4					1	0.545
M5						1

method with arithmetic averages algorithm (UPGMA) is shown in Fig. 4. Numerical analysis revealed clearly two distinct clusters. The cluster 1 includes m4 and m5. The cluster 2 is divided into two subclusters; the first includes m3 and the second includes w1, m1, and m2. The highest similarity was scored between m1 and m2 (0.7) and the lowest similarity between m5 and m1 (0.415) (Table 7).

Conclusion

UV and gamma ray irradiation can induce mutations, which can be carefully acclimatized and commercially propagated under suitable condition. RAPD technique could be successfully applied to the newly β-mannanases and can differentiate mutants. All of these results indicated that the survival of the parent strain depends on both of the type and period of mutagen treatment and on the microorganism's nature.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 El-Naggar MY, Youseff SA, El-Assar SA, Beltagy EA. Optimization of cultural conditions for β-mannanase from *Aspergillus niger* isolate. Int J Agric Biol 2006; 8:539–545.

2 Adeleke BS, Ojo SO, Oluwafemi YD, Olaniyi OO. Chemical mutagenesis of *Bacillus subtilis* for improved mannanase biosynthesis. J Adv Microbiol 2017; 3:1–6.

3 Kurakake M, Komaki T. Production of b-mannanase and b-mannosidase from *Aspergillus awamori* K4 and their properties. Curr Microbiol 2001; 42:377–380.

4 Hashem AM, Ismail AMS, El-Refai MA, Abdel-Fattah AF. Production and properties of β-Mannanase by free and immobilized cells of Aspergillus oryzae NRRL 3488. Cytobios 2001; 105:115–130.

5 El-Refai MA, Om Kalthoum H, Khattabk H, Ismail SA, Hashem AM, Abo-Elnasr AA, Nour SA. Improved mannanase production from *Penicillium humicola* and application for hydrolysis property. Egypt Pharm J 2014;13:160–167.

6 Chantorn ST, Buengsrisawat K, Pokaseam A, Sombat T, Dangpram P, Jantawon K, Nitisinprasert S. Optimization of extracellular mannanase production from Penicilliumoxalicum KUB-SN2-1 and application for hydrolysis property. JSCI Technol 2013; 35:17–22.

7 Ferreira HM, Filho EXF. Purification and characterization of a β-mannanase from Trichodermaharzianum strain T4. Carbohydr Polym 2004; 57:23–29.

8 Politiz O, Krash M, Thomsen KK, Borriss R. A highly thermostableendo-(1, 4)-b-mannanase from the marine bacterium *Rhodothermus marinus*. Appl Microbiol Biotechnol 2000; 53:715–721.

9 Puchart V, Katapodis P, Biely P, Kremnicky L, Christakopoulos P, Vrsanska M, Bhat MK. Production of xylanases, mannanases, and pectinasesby the thermophilic fungus *Thermomyces lanuginosus*. Enzyme MicrobiolTechnol 1999; 24:355–361.

10 Vijayalaxmi S, Prakash P, Jayalakshmi SK, Mulimani VH, Sreeramulu K. Production of extremely alkaliphilic, halotolerent, detergent, and thermostablemannanase by the free and immobilized cells of *Bacillus halodurans* PPKS-2.Purification and characterization. Appl Biochem Biotech 2013; 171:382–395.

11 keawsompong S. Mannanase. Microbial Enzymes in Bioconversions of Biomass. Bangkok: Department of Biotechnology, Faculty of Agro-Industry, Kasestsart University, 2016; pp.215–229.

12 Kote NV, Patil AGG, Mulimani VH. Optimization of the production of thermostableendo-β-1, 4 mannanase from a newly isolated *Aspergillus niger* gr and *Aspergillus flavus* gr. Appl Biochem Biotechnol 2009; 152:213–223.

13 Soni H, Rawat HK, Ahirwar S, Kango N. Screening, statistical optimized production, and application of β-mannanase from some newly isolated fungi. Eng Life Sci 2017; 17:392-401.

14 McCleary BV. β-mannanase. Methods Enzymol 1988; 160:596–610.

15 Sachslehner A, Foidl G, Foidl N, Gubitz G, Haltrich D. Hydrolysis of isolated coffee mannan and coffee extract by mannanases of *Sclerotium rolfsii*. J Biotecnol 2000; 80:127–134.

16 Nunes FM, Reis A, Domingues MR, Combra MA. Characterization of galactomannan derivatives in roasted coffee beverages. J Agric Food Chem 2006; 54:3428–3439.

17 Van Zyl WH, Rose SH, Trollope K, Görgens JF. Fungal β-mannanases: mannanhydrolysis, heterologous production and biotechnological applications. Pro Biochem 2010; 45:1203–1213.

18 Ahirwar S, Soni H, Rawat HK, Prajapati BP, Kango N. Experimental design of response surface methodology used for utilization of palm kernel cake as solid substrate for optimized production of fungal mannanase. Mycology 2016; 7:143–153.

19 Soni H, Rawat HK, Pletschke BI, Kango N. Purification and characterization of β-mannanase from Aspergillusterreus and its applicability in depolymerization of mannans and saccharification of lignocellulosic biomass. 3 Biotech 2016; 6:136.

20 Germec M, Yatmaz E, Karahalil E, Turhan I. Effect of different fermentation strategies on β-mannanase production in fed-batch bioreactor system. 3Biotech 2017; 7:77.

21 Yokomizo F. Mannose-containing palm kernel meal. United States of America patent 2009; US20040151804 A1.

22 Jørgensen H, Sanadi AR, Felby C, Lange NEK, Fischer M, Ernst S. Production of ethanol and feed by high dry matter hydrolysis and fermentation of palm kernel press cake. Appl Biochem Biotechnol 2010; 161:318–332.

23 Yoon SY, Yang YX, Shinde AR, Choi JY, Kim JS, Kim YW, Kwon IK. Effects of mannanase and distillers dried grain with solubles on growth performance, nutrient digestibility, and carcass characteristics of grower-finisher pigs. J Anim Sci 2010; 88:181–191.

24 Tomotari M. Bifidobacteria and their role in human health. J Indus Microbiol 1990; 6:263–268.

25 Titapoka S, Keawsompong S, Haltrich D, Nitisinprasert S. Selection and characterization of mannanase producing bacteria useful for the formation of prebiotic manno-oligosaccharides from copra meal. World J Microbiol Biotechnol 2008; 24:1425–1433.

26 Bapiraju KVVSN, Sujatha P, Ellaiah P, Ramana T. Mutation induced enhancedbiosynthesis of lipase. Afr J Biotechnol 2004; 3:618–624.

27 Shahbazi S, Ispareh K, Karimi M, Askari H, Ebrahimi MA. Gamma and UV radiation induced mutagenesis in Trichoderma reesei to enhance cellulases enzyme activity. J Fam Alli Sci 2014; 3:543–554.

28 Sambrook J, Russell DW. Molecular cloning: a laboratory manual, 3rd edition. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2001.

29 Omear HA, Al-Assie AH, Dhahi SJ. Application of the randomly amplified polymorphic DNA (RAPD) markers to analyze the genetic variability in species of the fungus Alternaria. Rafidain J Sci 2011; 22(1E):1–16.

30 Soliman EAM, Abo-Aba SEM, Abosereh NA, Aly NA, Aljohani AD. Genetic Variability among High Protease Productive Bacillus Mutants using RAPD-PCR. Int J Curr Microbiol App Sci 2017; 6:1862–1873.

31 EL-Bondkly AM, Keera AA. UV-and EMS-induced mutations affecting synthesis of alkaloids and lipase in *Penicillium roquefortii*. Arab J Biotechnol 2007; 10:241–248.

32 Smogi M. Notes onsugardetermination. J Biolchem 1952; 195:19–23.

33 Lowry OH, Rosebrough NH, Farr AL, Randall R. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–275.

34 Plengvidhya V, Breidt F Jr, Fleming HP. Use ofRAPD-PCR as a method to follow the progress of starter cultures in sauerkraut fermentation. Int J Food Microbiol 2004; 93:287–296.

35 Cao W, Scoles G, Hucl P, Chibbar RN. The use of RAPD analysis to classifyTriticumaccessions. Theor Appl Genet 1999; 98:602–607.

36 Grundman H, Scheidre C, Hartung D, Daschner FD, Pitt TL. Descriminatorypower of three DNA based typing techniques for *P. aeruginosa*. J Clin Microbiol 1995; 33:528–534.

37 Ademark P, Varga A, Medve J, Harjunpää V, Drakenberg T, Tjerneld F, Stålbrand H. Softwood hemicellulose-degrading enzymes from *Aspergillus niger*: purification and properties of a β-mannanase. J Biotechnol 1998; 63:199–210.

38 Iftikhar T, Niaz M, Abbas SQ, Zia MA, Ashraf I, Lee KJ, Haq I. Mutation induced enhanced biosynthesis of lipases by *Rhizopus oligosporus* var. microsporus. Pak J Bot 2010; 42:1235–1249.

39 Sheng CL, Wu JY, Chen CY, Liang T. Semicontinuous production of lipase by *Acinobacter radioresistens* in presence of non-woven fabric. Appl Biochem Biotechnol 2000; 87:73–80.

40 Arotupin DJ, Akinyele BJ, Olaniyi OO. Influence of UV mutagenesis on β-mannanase production potential of *Aspergillus glaucaus* and *Rhizopus japonicus*. Br Microbiol Res J 2015; 5:466–473.

41 Prabakaran MV, Thennarasu R, Ayeswariya M, Bharathidasan R, Chandrakala N, Mohan N. Comparative studies on the enzyme activities of wild and mutant fungal strains isolated from sugarcane fields. Indian J Sci Technol 2009; 2:46–49.

42 Irfan M, Javed J, Syed Q. UV mutagenesis of *Aspergillus niger* for enzyme production in submerged fermentation. Pak J Biochem Mol Biol 2011; 44:137–140.

43 Burlacu A, Israel-roming F, Cornea CP. Fugal strains improvement for xylanase over production through physical and chemical mutagenesis. Agro Life Sci J 2017; 6:40–47.

44 Karp A, Edwards KJ. DNA markers a global overview. DNA Markers 1997; 1–13.