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USE OF MUTATION AND PROTOPLAST FUSION STRATEGIES IN *Aspergillus foetidus* NRRL-337 FOR GENETIC IMPROVEMENT OF CELLULASE PRODUCTION

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ABSTRACT

Fifty two stable mutants were induced from *Aspergillus foetidus* NRRL- 337, as auxotrophs and/or morphological variants after irradiation with UV light for 3,6,9 and 12 minutes. These mutants were tested for productivity of cellulase components CMCase, FPase and β-glucosidase. Seven mutants showed more than three fold CMCase activities of the parental strain, whereas five mutants showed more than 2 fold increase in activities for FPase, and three mutants showed more than 2 fold increase of β-glucosidase activities compared to the wild type isolate.

The promising mutant No. 40 was used as a common parent (A) for protoplast fusion with the original strain (B), mutant No. 22 (C) and mutant No. 45 (D). The induced stable fusants were evaluated for cellulase activity. Out of these fusants (15), two (No. 2 and No. 12) fusants over yielded the higher parent (A) in the CMCase activity, two (No.11and 14)showed (F P)ase activity less than their lower parent (D), one fusant (No. 8) overyielded its higher parent (A) in β-glucosidase activity, while one fusant (No. 15) showed β-glucosidase activity less than that of its parent (D).

Key words: *Aspergillus foetidus, cellulase activity, fungal enzymes, mutants, protoplast fusion.*

1. INTRODUCTION

Cellulase is a multienzyme system, mainly endo, exo,- and β-glucosidase, which are essential for the complete hydrolysis of insoluble cellulose. A major barrier for the application of this enzyme to convert of cellulose to glucose is the high cost of the enzyme. A large number of fungi is reported to have cellulolytic activity such as *Aspergillus Spp.*. and *Trichoderma Spp.* It has been shown that, for the complete hydrolysis of insoluble cellulose, synergistic action between cellulase components is required. Although *Trichoderma* cellulases are most effective in hydrolysing crystalline cellulose, the predominant end product is cellobiose and not glucose.

Aspergillus species are better sources of β-glucosidase than *Trichoderma* and their β-glucosidases are less susceptible to end-product inhibition (Araujo *et al.*, 199i). Kirimura *et al.*, (1997) reported that the fusion frequency was 10^{-5}- 10^{-4} for the interspecific protoplast fusion between *A.terreus* and *A. usamii*, in which protoplast formation was $3.9x10^6$ and $3.2x10^6$/ml, respectively.

Ogawa *et al.*, (1987) made intraspecific hybridization in *Trichoderma reesei* upon protoplast fusion using colour mutants and obtained some heterakaryon as well as some diploid fusants. They reported that CMCase activity for the heterokaryons reached 10.72 u/ml, whereas the parental strain produced 13.78 u/ml and diploids gave 10.89 u/ml, whereas β-glucosidase activity was 0.295 u/ml for heterokaryons compared to 0.398 u/ml for the parental strain and 0.297 u/ml for diploid fusants. They concluded that it is possible to use colour mutants to monitor the successful fusion of protoplast in the fungi.

Schimenti *et al.*, (1983) isolated some nystatin resistant mutants from *Trichoderma reesei* which showed increased cellulase yields. Certain mutants derived from the hypercellulolytic strain RUT-C30 overyielded their parental isolate in cellulase production, but these strains proved to be unstable and reverted to the basal enzyme yield. Mishra and Gopalkrishnan (1984) obtained some cellulase constitutive mutants in *Trichoderma reesei* and reported that the mutant strain C-5 showed filter paper degrading activity (FPA) on 1% sucrose reached 0.48 *U*/ml compared to 0.01 *U*/ml for its original strain QM 9414. When this mutant grows on 1% cellulose (Solka-Floc) as a carbon source, it gave 1.8 *U*/ml compared to 1.5 *U*/ml for

the parental strain. With regard to β-glucosidase activity, the mutant strain C-5 showed 0.68 *U*/ml, whereas its wild type strain gave 0.66 U/ml. When this mutant grows on 2% cellulose, it showed 4.5 *U* /ml for (FPA) and 1.1 *U* /ml for β-glucosidase activity under the same conditions. Araujo and D'Souza (1986) studied cellulolytic enzyme components produced by *Aspergillus terreus* ATCC 52430 and its mutant UNGI-40 and reported that after the cellulase was resolved into various cellulolytic fractions, their purity was judged by analytical disc electrophoresis. They stated that β-glucosidase, endoglucanase and exoglucanase of the wild strain differed in their molecular weights from those of the mutant strain. They added that the enzyme activities differed according to differences in molecular weights. Durand *et al.*, (1987) isolated a number of strains of the fungus *Trichoderma reesei* suitable for cellulase production upon six mutant generations, each generation fulfilling the three criteria, improved productivity compared to the previous generation. However, high stability and ability need to be further improved. They selected the mutant strain CL 847 which exhibited a four-fold increase in cellulase productivity in cellulose media as compared to the starting strain QM 9414, with increased β-glucosidase activity. Strauss and Kubicek (1990) obtained a mutant strain from *Trichoderma reesei* after γ-irradiation of the strain QM 9414, namely M8 which did not form β-glucosidase when grown on glucose, whereas it secreted β-glucosidase upon growth on cellobiose with an activity of 0.370 compared to 0.300 *U*/ml in the parental strain. This mutant was also able to grow on cellulose, but secrets β-glucosidase with a longer lag activity reaching 0.140 *U* /ml compared to 0.230 I. *U*/ml in its parental strain.

Araujo *et al.*, (1991) used mutation induction strategies on *Aspergillus terreus* to increase cellulase production of the strain ATCC 52430. They applied UV –rays as a mutagen on spores using a UV lamp with a wavelength of 250 nm at 30 cm distance for 30, 60 and 90 second. They isolated a mutant strain having 3.5, 4.6 and 3.3 fold increases in filter paper, β-glucosidase and carboxmethyl cellulase activity, respectively, compared to the original strain. They added that cellulase activity *U* /ml reached 0.52, 0.61 and 0.46 for FPA, 0.84, 1.02 and 0.90 for β-Gl-Case and 8.7, 9.5 and 9.4 for CMCase at 30,60 and 90Sec., respectively as an average activities of

4 isolates in each. Cellulase activity (*U*/ml) for the control strain was 0.28(FPA), 0.33 (β-GLCase) and 5.2 (CMCase). Gadgil *et al.* (1995) isolated a cellulase hyperproducing mutant upon treatment of *Trichoderma reesei* QM 9414 using a combination of UV light and sodium nitrate treatment. Enzyme activity in liquid culture reached 0.36 *μ*/ml for the parental strain compared to 0.54 in the mutant strain for FPA reaction, 0.30 in the parent and 0.32 in the mutant for β-glucosidase, 3.6 in the parent compared to 6.5 in the mutant for CMCase activities.

2. MATERIALS AND METHODS

In this investigation, an introduced strain of the fungus *Aspergillus foetidus* namely NRRL 337 was used for the induction of mutations. The aim of the present work is to evaluate the induced mutants concerning production of the enzyme cellulase. Protoplast fusion between the higher producing mutants for this enzyme to obtain high productive fusants was also one of the aims of this study.

2.1. Mutagenic treatment

A Phillips TUV 30 W Lamp (type number 57413 P140) was used as the source of radiation. Samples from spore suspension of *A. foetidus* NRRL 337 were irradiated at 20 cm distance from the lamp for 3,6,9 and 12 minutes. Colonies, which gave growth on (CM) but not on (MM) plates, were considered auxotrophs. In addition, some morphological mutants were isolated according to colony shape and colour to be tested.

2.2. Media

1- Minimal medium (MM) Czapek's medium was used for autotrophic strain as described by Ainsworth and Bisby (1945).
2- Complete medium (CM): Contains Czapek's medium supplemented with 0.5% peptone and 0.5% yeast extract.
3- Production medium (PM): as described by Haapala *et al.* (1995) which consists of : Solka Floc (Cellulose Floc-division BW, USA) 30 g, KH_2PO_4 10 g, $(NH_4)_2 SO_4$ 5g , $Mg SO_4 . 7H_2O$ 0.3g, $Ca Cl_2 . 2H_2O$ 0.4g, $Fe SO_4 . H_2O$ 0.005 g, Urea 1.5 g, proteose peptone 3g, yeast extract 1 g , Tween 80 1 ml and 5 ml trace elements solution ($Mn SO_4 . H_2O$ 1.6 g , $Zn SO_4 . 7H_2O$ 1.4 g and $CoCl_2$ 2g) completed

to 1 liter with distilled water. The pH of the growth and production media after sterilization was 5.3.

2.3. Production of Cellulase

Conical flasks containing 50 ml of PM medium were inoculated with 5 ml of spore suspension from 5 day old slant cultures of parent and mutant strains separately. The flasks were incubated at 30°C on a rotary shaker (200 rpm) for 10 days. The content of each flask was centrifuged at 5000 rpm for 10 min at 0°C to remove mycelium and release the enzyme in the clear supernatant, which was tested on the spectrophotometer for the filter paper activity (FPA), carboxymethyl cellulase (CMCase) activiy and β-glucosidase activity.

2.3.1. The filter paper activity (FPA)

This test was performed as described by Mandels *et al.* (1976) as follows: ¼ ml diluted culture supernatant was added to ¼ ml citrate buffer along with 25 mg of Whatman No.1 filter paper. After incubation for 1h at 50°C, the reaction was terminated by adding 1.5 ml dinitrosalicylic acid (DNS) reagent and boiling for 10 min, then 2 ml distilled water were added to the solution and, after cooling, absorbance was measured at 540 nm. One unit of FPA (1μ/ml) activity was expressed as 1μ mol glucose equivalent released per min.

2.3.2. Carboxymethyl cellulase (CMCase) activity)

According to Saddler (1982), this test was made as follows: ¼ ml of the supernatant (enzyme extract) was added to ¼ ml of 1% carboxymethyl cellulose and ¼ ml of citrate buffer 0.1 M, pH 4.8, at 50°C for 30 min. The reaction was terminated by adding 2 ml of DNS reagent and boiling for 15 min . After cooling, 2 ml distilled water was added and then , measuring the absorbance at 540 nm.

One unit of CMC ase activity (I *U*) is equivalent to the released 1μ mol glucose equivalent per min.

2.3.3. β-glucosidase activity

It was conducted as described by Bailey and Nevalainen (1981) as follows: 0.1 ml of enzyme extract was added to 0.9 ml of (NPGP)4-Nitrophenyl-β-D-Glucopyranosice. After incubation for10 min at 50°C,the reaction was terminated by adding1ml of1M Na_2CO_3,

then diluted by adding 2 ml distilled water . The colour formed, due to the 4-nitrophenol liberated in the alkaline environment, was measured at 400 nm against a blank containing buffer solution.

One unit of enzyme activity (*U* /ml) represnts the amount which liberated 1μ mol of 4- nitrophenol from the reaction mixture per min.

2.4. Protoplast fusion

Protoplast fusion was performed according to the method outlined by Kirimura *et al.* (1986). For this purpose, the original strain of *A.. foetidus* NRRL 337 as well as two morphologically different mutants were chosen for protoplast fusion, individually with the highest cellulase productive mutant strain (No. 40) as a common parent. Conidia from each of these isolates were inoculated in 60 ml liquid growing medium and then cultivated with shaking (120 rpm) in flasks (250 ml) at 30°C for 24 hours. Growing mycelium was collected and washed twice, then suspended in 10 ml of lytic enzyme (Novozyme 234) and 0.7 M KCl, as an osmotic stabilizer, in 0.05 M potassium phosphate buffer at pH 6.0 . After 3.5 hours of incubation with shaking (90 rpm) at 30°C, formed protoplasts were collected by low centrifugation and washed three times with a stabilized buffer.

Protoplast formation was examined and counted under the microscope using 0.1 ml of the dilute (10^{-5}) protoplast suspension on the hemocytometer.

Protoplasts prepared from each of the three parental strains (W.T., No. 22 and 45) were mixed individually with protoplasts from the common parent strain (No.40) by which three mixtures were performed. Each mixture was slowly diluted with stability buffer, then incubated at 30°C for 15 min. Regeneration of protoplasts was performed by dilution to 10^{-1}. Samples of 0.2 ml were plated on 20 ml of the hypertonic medium and incubated at 30°C for 7 days.

Regeneration frequency was estimated as the ratio between the number of colonies formed to the number of protoplasts plated. Stable fusant colonies for growth shape and colour along with their parental mutants were tested for cellulase productivity.

3. RESULTS AND DISCUSSION

3.1.Mutation percentages

Application of UV light treatments on *A. foetidus* NRRL 337 at different exposure times resulted in different survival rates as well as different mutation percentages as shown in Table (1). At 3 minute treatment, survival rate reached 32.31% accompanied with mutation percentage of 25% , whereas at 6 min., survival was 27.54% with mutation rate of 26.92%. The treatment for 9 min., gave survival rate of 22.83%, whereas the treatment for 12 min had survival rate of 18.72% only accompanied with mutation percentages of 22 .23% and 30.35%, respectively.

As shown in Table (1) out of 400 tested colonies, only 52 stable mutants were obtained, 11 of which after the treatment at 3 min., 14 at 6 min., 10 at 9 min. and 17 at 12 min. Some of these mutants were selected as morphological variants which differed from the original strain, others were chosen as auxotrophs, or both.

Table (1): Survival and mutant rates in *A.foetidus* NRRL-337 after treatment with UV light.

Exposure time (min)	Survival %	No. of tested colonies	No. of stable mutants	Mutant %
Control	100.00	150	0.0	0.0
3	32.31	44	11	25.00
6	27.54	52	14	26.92
9	22.83	45	10	22.22
12	18.72	56	17	30.36
Mean	25.38	47.25	13.0	26.12

3.2. Productivity of Cellulase components by *A.foetidus* strain and its mutants

As shown in Table (2) the original strain NRRL-337 showed CMCase activity of 1.08 *U*/ml in the fermented broth. However, out of the 52 induced mutants, 2 lost their productivity of CMC ase, one of them (No.7) from the treatment 3 min and the other (N0.20) from the treatment 6 min . In addition , 14 mutants showed production

capacity lower than the original strain, from treatments 3,6 and 12 min. exposure time. Furthermore, 11 isolates produced this enzyme in a capacity similar to their parental strain. On the other side, 25 isolates showed enzyme activities more than the parental strain, 7 of which showed activity three times more than the original strain (No. 27, 30, 32, 38, 40, 42 and 45). These promising 7 mutants were induced after UV treatment for 9 or 12 min. Their productivity reached 3.31, 3.37, 3.40, 3.32, 4.12, 3.57 and 3.64 U/ml, respectively. Araujo *et al.* (1991) obtained a mutant strain of *Aspergillus terreus* upon UV treatment which produced 3.3 fold increase in CMCase activity. Moreover, they isolated 4 mutants which gave an avaerge of 8.7, 9.5 and 9.4 *U* /ml for CMCase activity at 30, 60 and 90 sec.UV-treatments, respectively compared to 5.2 *U* /ml in the control strain. Gadgil *et al.* (1995) selected a mutant strain of *Trichoderma reesei* which showed CMCase activity of 6.5 compared to 3.6 *U* /ml in the wild type.

With respect to FPase activities, Table (2) shows that 13 mutants failed to produce this enzyme, whereas 19 mutants had activities lower than the wild type as it gave 0.48 U/ml of the enzyme activity. Meantime, 3 mutants (No. 7, 43 and 51) gave activities as the original strain, while the remainder 17 mutants overyielded their parental isolate. Most of these high productive mutant strains were obtained from the treatment 12 min (7 mutants) and the treatment 6 min. (5 ones). The most promising five mutants (showing more than 2 fold increase activity) No. 2, 40, 45, 46 and 47 produced 1.02, 1.62, 1.32, 1.02 and 1.00 *U* /ml, respectively. In *Trichoderma reesei*, Mishra and Gopalkrishnan (1984) obtained the mutant strain C-5 which showed FPase activity 0.48 compared to 0.01 *U* /ml for its original strain. When this mutant was grown on 2% cellulose it showed 4.5 *U* /ml FPase activity. Araujo *et al.*, (1991) isolated a mutant strain from *Aspergillus terreus* having 3.5 fold increase in FPase activity upon UV irradiation. They selected 4 mutants which averaged 0.52, 0.61 and, 0.46 *U* /ml FPase activities the 30, 60 and 90 sec-UV-treatments, respectively compared to 0.28 *U* /ml for the control strain . Also, Gadgil *et al.*, (1995) obtained a mutant strain of *Trichoderma reesei* which showed FPase activity of 0.54 compared to 0.36 *U* /ml for the parental strain.

Table (2): Cellulase activity for *A. foetidus* NRRL-337 and its induced mutants on fermentation media. (IU/ml).

Isolates and time of exposure	CMCase	F Pase	β-glucosidase
WT.	1.08	0.48	1.45
3 min			
1	1.53	0.00	1.15
2	2.08	1.02	0.77
3	1.10	0.16	0.97
4	0.75	0.00	1.62
5	0.83	0.00	0.96
6	0.92	0.63	1.86
7	0.00	0.48	2.92
8	1.18	0.30	1.94
9	1.77	0.69	0.21
10	0.91	0.00	1.47
11	1.71	0.15	2.72
6 min			
12	1.20	0.42	2.21
13	1.56	0.30	2.09
14	1.07	0.32	1.93
15	1.14	0.63	2.00
16	1.12	0.60	1.02
17	1.06	0.14	2.16
18	0.42	0.14	1.77
19	1.10	0.60	1.85
20	0.00	0.32	1.42
21	1.10	0.61	1.53
22	1.10	0.70	2.98
23	1.17	0.30	2.48
24	0.75	0.15	1.19
25	0.86	0.12	0.43
9 min			
26	1.53	0.00	1.92
27	3.31	0.00	1.84
28	2.91	0.18	1.36
29	2.87	0.70	0.24
30	3.37	0.00	2.21
31	2.72	0.00	1.02
32	3.40	0.63	0.62
33	2.81	0.15	0.59
34	2.70	0.39	1.42
35	2.13	0.00	1.94
12 min			
36	0.52	0.70	2.17
37	1.66	0.21	1.56
38	3.32	0.00	2.56
39	1.98	0.00	1.17
40	4.12	1.62	3.45
41	0.92	0.12	1.66
42	3.57	0.00	1.30
43	2.70	0.48	0.57
44	0.83	0.75	1.72
45	3.64	1.32	1.92
46	2.17	1.02	0.72
47	0.82	1.00	1.50
48	0.16	0.00	1.26
49	1.33	0.63	1.68
50	0.37	0.36	1.14
51	0.58	0.49	1.18
52	1.07	0.34	2.28

Regarding β-glucosidase activities, the W.T strain had an activity of 1.45 *U*/ml, whereas 20 mutants showed an activity lower than it (Table 2) . In addition, out of the induced mutants, 4 had enzyme activity similar to their parent, while the remainder (28 mutants) overyielded the original strain, most of which from the treatments for 6 min. (9 mutants) and 12 min. (10 mutants). Out of these high yielding isolates, 3 of them (No. 7, 22 and 40) had β-glucosidase activities 2 fold more than the wild type activity, producing 2.92, 2.98 and 3.45 U/ml, respectively . In *Trichoderma reesei,* Mishra and Gopalkrishnan (1984) reported that the mutant strain C-5 showed β-glucosidase activity of 1.1 *U*/ml compared to 0.66 *U* /ml only in its parental strain. Strauss and Kubicek (1990) obtained the mutant strain M8 after γ- irradiation of *Trichoderma reesei* which secreted β-glucosidase with an activity of 0.370 compared to 0.300 *U* /ml in the wild type. In *Aspergillus terreus,* Araujo *et al.* (1991) isolated a mutant strain which produced 4.6 fold increase in β-glucosidase activity after UV treatment. They selected four mutants which averaged 0.84, 1.02 and 0.90 (*U* /ml) for β-glucosidase activity at 30, 60 and 90 sec treatments, respectively compared to 0.33 *U* /ml for the W.T. Also Gadgil *et al.* (1995) isolated a mutant strain of *T. reesei* which gave β-glucosidase activity of 0.32 compared to 0.30 *U*/ml for the original strain.

Table (3): Protoplast formation in the 4 parental isolates of *A. foetidus* after 3½ hours of lytic enzyme treatment.

Parental isolate	Number of protoplasts formed	Protoplast yield /ml
A (40)	11	$11X10^6$
B (W.T)	12	$12X10^6$
C (22)	10	$10X10^6$
D (45)	9	$9X10^6$
Mean	10.5	$10.5X10^6$

3.3. Protoplast fusion and cellulase production

It is well known that protoplast fusion technique may produce stable diploid recombinants from which geneticists can select the desired genotype.Therefore,the three mutants No. 22 ,40 and 45 along with the original strain were chosen as parental isolates

(designated C, A, D and B, respectively) according to their cellulase activities and morphological features. Colour and shape of colonies of these parental isolates are shown in Figure 1 (the upper 4 colonies, from right to left mutant No. 40 (A), W.T (B), mutant No. 22 (C) and mutant No. 45 (D), respectively).

Table (4): Percentages of fusion and regeneration frequency between the four parental isolates.

Fusion system	Fusion frequency	No. of stable fusants	Regeneration frequency
A x B	1.60 %	6	0.37 %
A x C	1.45 %	4	0.27 %
A x D	1.55 %	5	0.32 %
Mean	1.50 %	5	0.33 %

Table (5) shows cellulase components productivity for these parental strains along with the estimated mid-parent values.

Protoplast fusion between the high productive mutant No. 40 (A) as a common parent and each of, the W.T (B), No. 22 (C) and No. 45 (D), was carried out to obtain new recombinants (fusants) with high cellulase production capacity. Stages of protoplast formation in mycelia of *A. foetidus* strains are shown in Figure (2). Protoplast formation in *Aspergillus foetidus* isolates used in this study averaged $10.5x10^6$/ml upon using mycelia of 24 hr. age (Table 3). Kirimura *et al.* (1997) obtained $3.2x10^6$/ml protoplasts in 24 hr. aged mycelia of a mutant from *A. usamii.*

Results presented in Table (4) indicated that fusion frequency ranged from 1.45% to 1.60% with an average of 1.50%, though regeneration frequency ranged from 0.27% to 0.37% with an average of 0.33%. A total of 43 colonies were obtained from the three fusion systems, 15 of which were morphologically stable. Some of the stable fusants resembled one of their parents morphologically, whereas others showed intermediate phenotypes while the remainder gave new recombinant phenotypes (Figure I).

With regard to enzyme production in the 15 stable fusants, data in Table (5) showed that CMCase activity for fusant no.2 reached 4.66 *U* /ml exceeding the higher parent A (No.40) which gave 4.12 *U*/ml. On the other side, fusant no .4 showed CMC ase activity

Plate : a

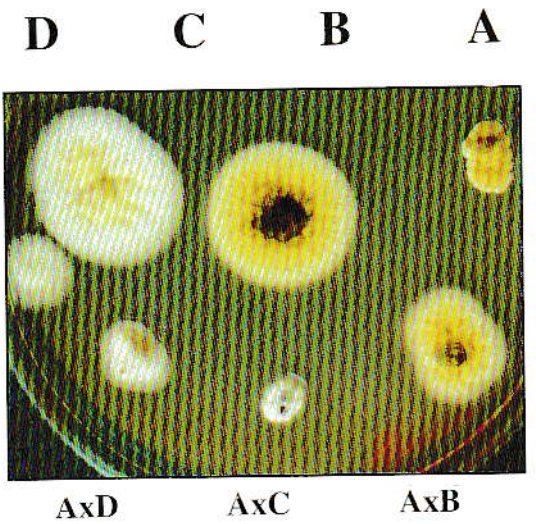


Plate: b

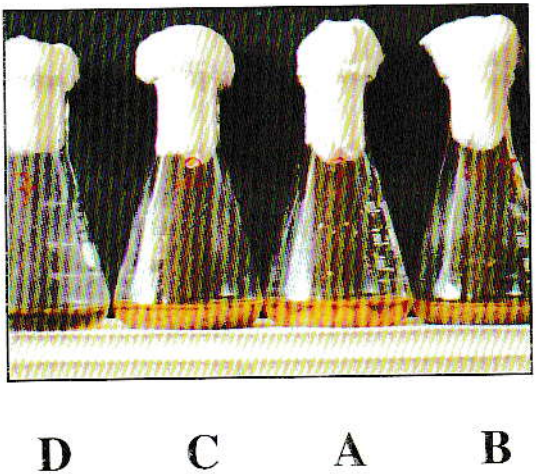
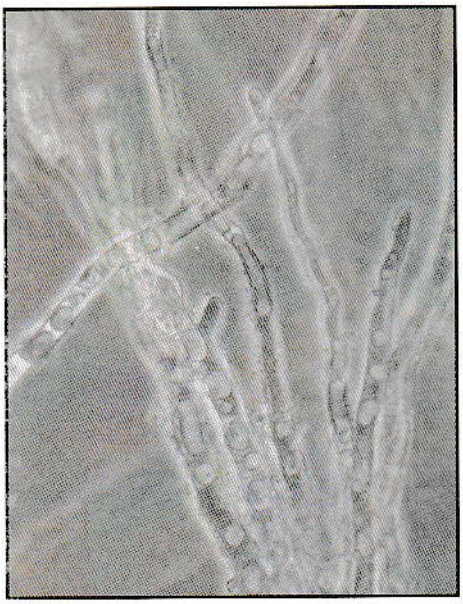


Figure (1): Plate (a): colour and shape of colonies for the parental isolates used in protoplast fusion (the upper 4 from right to left A, B, C and D) along with some of their fusants (the lower 3 from right to left AxB , AxC and AxD)

Plate (b):pellets size and colour for the parental isolates (from right to left B, A, C and D).



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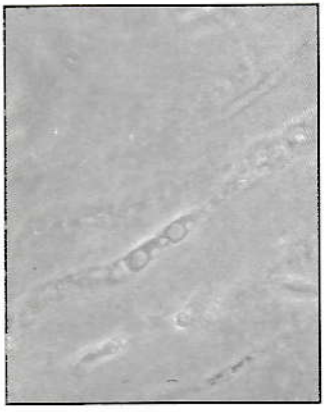

Plate: b

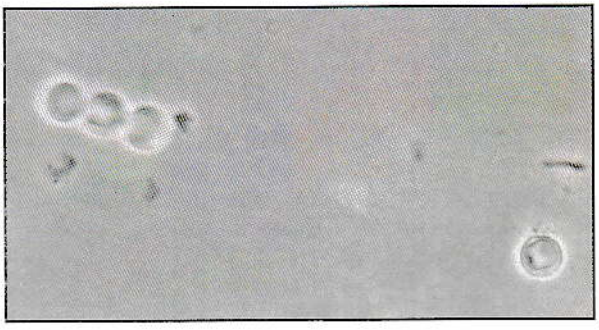

Plate: c

Figure (2): Stages of protoplast formation from mycelia of *A. foetidus* isolates after treatment with the lytic enzyme
Plate a: mycelia before lytic digestion
Plate b: mycelia after 2 hr of lytic digestion
Plate c: free protoplast after 3 hr of lytic digestion

similar to its lower parent B (W.T). The remaining 4 fusants of the combination AxB (no. 1,3,5 and 6) showed activities within the parental range for this enzyme (Table 5).

Table (5):Cellulase component activity in the parental strains used in protoplast fusion (*U*/ml) along with the 15 stable fusants.

Strains and Fusants	CMCase	F.Pase	β-glucosidase
40 (A)	4.12	1.62	3.45
W.T (B)	1.08	0.48	1.45
22 (C)	1.10	0.70	2.98
45 (D)	3.64	1.32	1.92
AxB	**2.06**	**1.05**	**2.45**
1	2.13	1.12	1.44
2	4.66	0.93	1.82
3	2.77	0.65	2.98
4	1.11	1.05	1.65
5	2.18	0.48	2.54
6	3.70	1.34	1.98
AxC	**2.61**	**1.16**	**3.21**
7	2.98	0.74	2.86
8	1.12	0.65	3.75
9	3.18	0.84	1.85
10	1.91	1.21	2.35
AxD	**3.88**	**1.47**	**2.68**
11	3.67	0.64	1.98
12	4.25	1.31	2.86
13	2.70	1.25	1.97
14	3.74	0.37	2.12
15	3.85	1.47	1.70

* mid parent value

The system AxC produced 4 fusants, one of which (no. 8) gave an activity similar to its lower parent C, whereas three (no. 7, 9 and 10) had activities within their parental range (Table 5). The combination AxD gave 5 stable fusants, one of which (no. 12) exceeded its higher parent A in this respect producing 4. 25 *U*/ml , while fusant

(no.13) produced an enzyme less than its lower parent D, whereas (no. 11, 14 and 15) gave productivities within the parental range (Table 5). With regard to F. P ase activity in the 6 fusants of the system AxB, one (no.5) showed 0.48 *U* /ml like its lower parent (B), whereas the remaining ones (no.1,2,3,4 and 6) gave activities within their parental range (Table 5). Out of the 4 fusants of the system AxC, 3 showed activities similar to that of the lower parent C (no. 7,8 and 9) whereas the fourth fusant (no. 10) had a moderate activity of 1.21 *U*/ml. The system AxD gave five stable fusants, two of which (no.12 and 13) showed activity near that of their lower parent D, two fusants gave activity less than it (no.11 and 14), whereas the remainder one (no.15) had activity similar to the mid-parent value (Table 5). With respect to β-glucosidase activity, the system AxB had 6 stable fusants one of which (no.1) showed activity like its lower parent B, whereas the remainder ones had activities within their parental range (Table 5).

The system AxC gave 4 stable fusants, one of which (no.8) overyielded its higher parent A giving 3.75 *U* /ml, whereas the remainder ones (no.7, 9 and 10) showed activities less than that of their lower parent. In the combination AxD, out of the 5 fusants, one (no.15) had activity less than its lower parent D, whereas 2 fusants gave similar activity as this parent (no.11 and 13), while the remaining 2 (no. 12 and 14) had activities within their parental range (Table 5). In *Trichoderma reesei* Ogawa *et al.* (1987) obtained some heterokaryons as well as some diploid fusants upon protoplast fusion using colour mutants. Activity of CMCase reached 10.72 μ/ml for the heterokaryons compared to 10.89 U/ml in the diploids, whereas the parental strain gave 13.78 *U* /ml. They reported that β-glucosidase activity was 0.295 *U* /ml for heterokaryons compared to 0.398 (*U* /ml) for the parental isolate and 0.297 (*U* /ml) for diploid fusants. They concluded that it is possible to use colour mutants to monitor the successful fusion of protoplast in fungi.

From our results it can be concluded that the induction of mutations in *A.foetidus* may lead to genetic changes by which mutants differed morphologically and physiologically. Also, protoplast fusion between the induced mutants of this fungus caused some genetic changes in genes affecting cellulase component production. It is possible, however, that changes may be a consequence of parasexual recombination in the fungal genome.

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إستراتيجيات استخدام الطفرات والدمج الخلوى فى فطر *Aspergillus foetidus* للتحسين الوراثى فى انتاجيه انزيم السليوليز

فاطمة نبيه طلخان

قسم الوراثة والسيتولوجى – المركز القومى للبحوث – الدقى – القاهرة

ملخص

أجريت هذه الدراسة بغرض إحداث تغيرات وراثية فـــى الســـلالة 337- NRRL من فطر الأسبرجلس فيوتيدس من خلال استراتيجيات استحداث طفـــرات من المعاملة بالأشعة فوق البنفسجية وكذلك استخدام تكنيك الدمج الخلوى بين بعض هذه الطفرات مع إختبار العزلات الأكثر ثباتاً سواء من الطفـــرات أو المندمجـــات الخلوية من حيث القدرة على انتاج مكونـــات انزيـــم الســـليوليز (CMCase β- glucosidase – F.P ase) و مقارنتها بالسلالة الأصلية.

وتشير أهم النتائج المتحصل عليها الى ما يلى :

1- أمكن استحداث وعزل 52 طـــافر مختلـف عـن السـلالة الأصليـة مورفولوجياً وفسيولوجياً معظمها لا يستطيع النمو على بيئة العوز وينمـو فقط على البيئة الكاملة وهذه الطفرات هى الأكثر ثباتاً بين حوالى 400 مستعمرة ناتجة من المعاملة بالأشعة .

2- أختبرت هذه الطوافر لإنتاجيتها من مكونات إنزيم السـليوليز المختلفـة فأظهرت 7 طفرات منها قدرة عالية على انتـاج المكـون الإنزيمـى CMC ase بكميات تزيد على ثلاثة أمثال انتاجية السلالة الأصلية لـهذا الإنزيم.

3- أظهرت 5 طفرات قدرة عالية على انتاج المكون الإنزيمـى ال F.Pase واعطت زيادة تعادل اكثر من مرتين قدر انتـاج السـلالة الأصليـة . وكذلك اعطت 3 طفرات أخرى زيادة تعادل مرتين قدر انتـاج السـلالة الأصلية من المكون الأنزيمى β-glucosidase .

4- لإجراء الدمج الخلوى تم إختيار الطافر رقم (40) كأب مشترك من ناحية للدمج مع السلالة الأصلية ومع كلا من الطافر رقم 22 ، 45 من الناحية الأخرى للحصول على بعض العزلات المدمجة من هذه التوليفات الثلاثـة – وبعد حدوث الدمج تم اختبار اكثر المسـتعمرات ثباتـاً لمزيـد مـن الدراسة عليها .

5- تم انتخاب 15 وحدة مندمجة ثابتة من بين مستعمرات الدمـج الخلـوى منها العزلتين رقم 2 ، 12 اللتين اظهرتا تفوقاً فى انتاج المكون الإنزيمى **CMC ase** على الطافرة الأبوية الأعلى إنتاجية (رقم 40) على حيـن اظهرت العزلتان رقم 11 ، 14 نقصاً فى انتاجيتـهما مـن المكـون الإنزيمى F.P ase أقل من إنتاجيه الطافر الأبوي الأقل انتاجـاً (رقـم 45)

6- أظهرت العزلة رقم 8 تفوقاً على الأب الأعلى (الطافررقم 40) فـى انتاج المكون الإنزيمى β-glucosidase على حين ان الوحدة المندمجـة رقم 15 أظهرت إنتاجية تقل عنها فى الطافر الأبوى الأقل انتاجـاً لـهذا المكون الإنزيمي (رقم 45) .

يمكن من هذه النتائج القول أن تكنيك الدمج الخلـوى بجـانب اسـتحداث الطفرات يمكن أن ينتج توليفات وراثية جديدة ثابتة من فطر الاسبرجلس فيوتيدس يكون بعضها اعلى انتاجاً لبعض مكونات إنزيم السليوليز عن السلالة الأصلية .