Induced Mutation by Gamma Radiation of Penicillium brevicompactum to enhance Production of Mycophenolic Acid

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ABSTRACT

The ability of *Penicillium brevicompactum* to produce mycophenolic acid has been studied since a long time. Different methods to improve the productivity have been exploited. Strain improvement is a milestone to obtain maximum productivity by low investment. In the present study, strain improvement was done by subjecting it to different doses of gamma radiation. Least survival rate of 15.38% was obtained at the dose of 250Gy. The three dosages with least survival rate were further assessed for mycophenolic acid productivity. Mutants 150G4 and 150G3 showed the maximum productivity of 11.012 mg/g and 10.454 mg/g leading to an increase in productivity by 25% and 20 % respectively. Stability studies indicate that the mutants were robust over the six generations studied.

Keywords: *Penicillium brevicompactum*, Gamma Radiation, Mycophenolic acid, Strain improvement

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INTRODUCTION

Mycophenolic acid (MPA) is a promising drug owing to its immunosuppressive and biological activity. It was isolated first from Penicillium by Gosio in 1896. MPA ($C_{17}H_{20}O6$) and its derivatives such as mycophenolate mofetil (MMF) and sodium mycophenolate has been approved by food and drug administration (FDA) as immunosuppressive drugs. These reduce the risk of graft rejection after organ transplantation and are widely used for treating various autoimmune diseases like psoriasis [1,2]. It prevents the proliferation of B and T lymphocytes by inhibiting the enzyme inosine monophosphate dehydrogenase (IMPDH). This halts the purine pathway decreasing the titer of the immune cells and attenuates the rejection mechanism [3].

On the basis of the broad clinical utility of MPA, fermentation processes have been carried out using submerged batch cultures of several species of *Penicillium* particularly, *P. brevicompactum and P. stoloniferum* [4]. Commercial production of MPA has gained a lot of attention in the recent studies. In the current years lot of improvement both quantitative and qualitative has been initiated. Media optimization and strain improvement are the key study areas for the enhancement of yield. Strain improvement can be carried out by mutation, resulting in better yielding strain as compared the wild strain [5]

Mutation for strain improvement is generally carried out through physical and chemical mutagens. Mutations give rise to genetic variation resulting in the differences in the DNA sequences caused either by an insertion, deletion, duplication or inversion of DNA fragments [6].

Such improved strains can reduce the cost of the industrial processes in association with increased productivity and may also possess some specialized desirable characteristics [7].

The study focuses on the mutation of Penicillium brevicompactum through gamma radiation for enhancing the productivity of MPA.

MATERIAL AND METHODS

Microorganism

Penicillium brevicompactum was used throughout the study. It was grown on potato dextrose agar slants at 25ºC and 15 days. The spores were harvested and maintained as cryovials.

Gamma Irradiation

Gamma radiations are reported to be the most efficient ionizing radiation for producing mutants [8]. A gamma ray is a packet of electromagnetic energy(photon) emitted by the nucleus of some radionuclides following radioactive decay. After gamma-irradiation and the breaking of the DNA double-strands, the cell can repair the damaged genetic material in the limit of its capability and genetic improvement may occur [9].

Inducing Mutagenesis

The irradiation process was carried out at Bhabha Atomic Research Centre (BARC), Mumbai, Maharashtra. The spore suspension prepared as described was exposed to

different doses of Co60. Various doses of gamma radiation were selected which were 25, 50, 100, 150, 200, 250 Gy. After treatment with different doses of γ-rays, the exposed spores were transferred to PDA plates. The plates were incubated at 25º C for 15 days.

Selection of the mutant

The percentage of survival rate in each plate was calculated using the equation mentioned below, and mutants with the lowest survival rates were selected from the plate.
Survival rate = No. of colonies after treatment / No.of colonies without treatment (M/C) × 100%
Here, C= No. of colonies without treatment (Control)
M = No. of colonies after treatment (Mutant) [10].
Colonies from each of these were selected and tested for MPA production.

Screening of colonies for MPA productivity

The selected colonies were picked and crushed in 5ml normal saline. 1 ml of the spore suspension was inoculated in seed media. Seed media comprises of dextrose 50g//l, soya peptone 15g/l, yeast extract 10 g/l, malt extract 10 g/l, magnesium sulphate 1 g/l, potassium dihydrogen phosphate 1 g/l and sodium nitrate 2.5 g/l, pH 5.8. 1 ml of the harvested spores suspension was inoculated in 35ml seed in 250 ml erlenmeyer flasks. Seed flasks were incubated at 26ºC at 240 rpm on shaking incubator for 40 hrs. 8% of the seed was transferred to 250 ml flasks containing 35ml of production media. Basal production media was used for the optimization. Production media composition: sucrose 40 g/l, cotton seed meal 10 g/l, soya flour 25 g/l, casein enzyme hydrolysate 25 g/l, magnesium sulphate 1g/l, potassium dihydrogen phosphate 2.5 g/l, PPG 1 g/l, ammonium sulphate 2 g/l. Flasks were incubated at 26ºC and 240 rpm. The yield was assessed through HPLC

HPLC analysis of MPA

Mycophenolic acid produced in the culture broth was determined by HPLC. The culture broth of 2.5 gm was taken in 25 ml volumetric flask with 10 ml methanol and sonicated for 20 minutes and the volume was made up with methanol. The resulting extracted solution was injected into the HPLC (Waters 2496) having C-18 column (Hypersil ODS, 5u C18 (250 mm X 4.6 mm) for the estimation of mycophenolic acid. Concentration of MPA was calculated by comparison of peak areas with those standard mycophenolic acid and subsequently MPA activity was calculated.

Stability Studies

The selected mutant spores were further cultured for five generations to check the retention of mutation.

RESULTS AND DISCUSSION

Gamma radiation was used to induce mutation in *Penicillium brevicompactum* with an aim to improve the strain and finally the MPA productivity. A range of doses (25-250Gy) were used for the study. The survival rate in terms of percentage for each dosage is shown in table 1

Table 1: Survival rate at different dose of γ radiation

Gamma Dosage (Gy)	Test cfu/ml	Control cfu/ml	% Survival
25	11	15	73.33
50	9	14	64.29
100	7	12	58.33
150	6	14	42.86
200	3	14	21.43
250	2	13	15.38

The least survival rate of 15.38 % was observed at 250Gy dose. The colonies from the last three doses that is 150, 200 and 250Gy were selected for further to detect the productivity.

The different colonies and their yield are depicted in table 2

Table 2: Colonies from different doses with MPA productivity

Dosage (Gy)	Mutants	MPA productivity (mg/g)
150 (6 colonies)	150G1	8.746
	150G2	9.627
	150G3	10.452
	150G4	11.012
	150G5	9.402
	150G6	10.016
200 (3 colonies)	200G1	9.846
	200G2	10.271
	200G3	9.146
250 (2 colonies)	250G1	8.114
	250G2	7.215
Control	C1	8.502
	C2	8.765

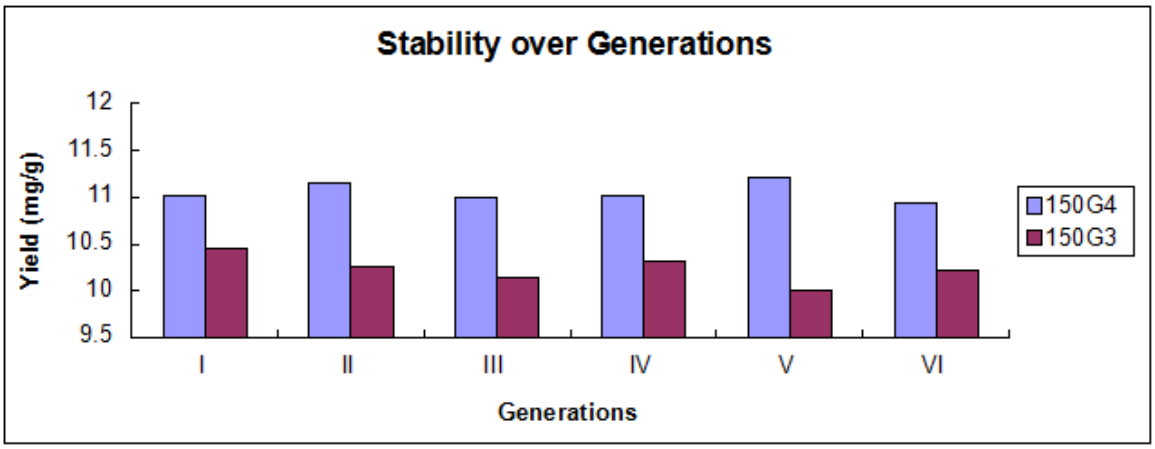
Maximum productivity of MPA was seen in mutant 150G4 having 11.012 mg/g yield followed by 10.452 mg/g in mutant 150G3 at a dose of 150Gy. Mutant 200G2 showed high yield of 10.271 mg/g than control. The mutants produced through 250Gy dosage show very low yield as compared to control.

150G4 showed an increase of 25% in the productivity whereas 150G3 increased the yield by 20%. The mutant 200G2 increased the productivity by 18 %.

Stability Studies

The mutant strains obtained after the treatment of gamma radiation that had maximum productivity were analyzed for stability for 6 generations. The stability analysis of the mutants for the MPA productivity over the generations is depicted in figure 1

Figure 1: Stability analysis of MPA producing gamma mutants



The result clearly showed that the strain 150G4 and 150G3 were stable throughout the six generations. They retained the mutation and thus the productivity over the generations.

CONCLUSION

Mutant fungal strain of *Penicillium brevicompactum* (150G4, 150G3) were created by exposing the wild strain to gamma radiation. The mutants 150G4 and 150G3 showed an increase of 25% and 20 % in mycophenolic acid productivity respectively. The stability studies over six generations showed retention of mutation for all the generations and thus productivity. Since MPA has broad clinical applications, this study of strain improvement is a step forward to achieve commercial large scale production of MPA in a cost effective way.

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