Short Communication:

Effect of Gamma Irradiation on *Aspergillus tamarii* FNCC 6151 to enhance Alpha-Amylase and Glucoamylase Production

Safitri Ika Octariyani[1], Megasari Kartini[2], Sardjono[1], Widada Jaka[3] and Cahyanto Muhammad Nur[1]*
1. Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta, INDONESIA
2. Polytechnic Institute of Nuclear Technology, Yogyakarta, INDONESIA
3. Department of Agricultural Microbiology, Faculty of Agriculture, Gadjah Mada University, Yogyakarta, INDONESIA
*mn_cahyanto@ugm.ac.id

Abstract

Molds can carry out amylase production, but the production of the enzyme is affected by a carbon catabolite repression system, thus reducing amylase production. This study aimed to increase amylase production in Aspergillus tamarii FNCC 6151 with irradiation by gamma rays. Spore suspensions were irradiated at 100, 200, 300, 400 and 500 Gy and the survival of the spores was determined. The spore suspension irradiated at 400 Gy was diluted and grown on medium plates containing starch as a carbon source and 2% glucose as a repressor. After staining with iodine, colonies showing clear zones around them were purified and grown in liquid media for four days and their alpha-amylase and glucoamylase activities were analyzed.

The mutant Mut1 showed a clear zone. This mutant produced 1.127 U/mL of alpha-amylase and 0.011 U/mL of glucoamylase. Meanwhile, the parental strain produced 0.68 U/mL of alpha-amylase and 0.079 U/mL of glucoamylase. The random mutagenesis using gamma-ray into A. tamarii FNCC 6151 increased alpha-amylase and glucoamylase production by 1.8- and 1.4-fold respectively.

Keywords: Alpha-amylase, Glucoamylase, *Aspergillus tamarii* FNCC 6151, Mutagenesis, gamma rays

Introduction

Amylases are commonly used in the food industry as catalysts. Amylases break starch molecules into monomers or oligomers such as glucose, maltose, maltotriose, or dextrin. They are widely applied in corn, maltose, glucose, fruit juice, alcohol, bakery and food additive industries[13]. Molds are living organisms that can produce amylase on an industrial scale such as *Aspergillus oryzae*, *A. niger*, or *A. awamori*[20]. Molds are suitable for solid-state fermentation and are more efficient for producing extracellular enzymes due to their hyphal growth, resistance to low water activity and high osmotic pressure[22].

The production of polymer-degrading enzymes in molds is affected by carbon catabolite repression (CCR). In the CCR system, the amylase synthesis is repressed by the end-product of the degradation, glucose[1]. The addition of 2% glucose as a carbon source inhibited glucoamylase production in *A. niger*[15]. Production of alpha-amylase was also inhibited with the addition of 0.5% glucose in *A. niger*[5]. Repression of alpha-amylase production also occurred with the addition of 0.1% glucose in *Sulfolobus solfataricus*[7] and 0.5% in *Bacillus* sp.[19]

To increase enzyme production, CCR should be eliminated by mutation of DNA responsible for CCR. Previous studies showed that random mutations using gamma rays produced 1.3-fold higher alpha-amylase than control in *Bacillus subtilis*[8]. Gamma irradiation also increased alpha-amylase activity 12-fold in *A. oryzae*[2] whereas mutation using UV increased alpha-amylase production 1.6-fold in *A. flavus*[16].

Our previous study showed that indigenous mold *A. tamarii* FNCC 6151 produced amylases at a relatively high amount, although the amylase production was subject to CCR by glucose[17]. Random mutagenesis by gamma irradiation of the strain was carried out to increase amylase production. The effect of mutagenesis on alpha-amylase and glucoamylase production by the mutant was described.

Material and Methods

A. tamarii FNCC 6151 was provided by the Biotechnology Laboratory at the Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University. The strain was isolated from koji produced in Bantul, Yogyakarta, Indonesia.

Growth medium: The medium used in this work consisted of Mandel's salt solution[12] and starch as the sole carbon source. The plating medium was supplemented with 17.5 g/L of agar and 0.1% Triton X-100.

Effect of glucose on amylase production: The effect of glucose on amylase production was investigated in plate media containing ten g/L of starch. The medium was supplemented with glucose at 0%, 1%, 2% and 3%. The samples were incubated at 28°C for 3 days, then at 50°C for 18 h and then stained with a 1% iodine solution.

Effect of the irradiation dose on spore viability: The spores were irradiated with gamma rays at 100, 200, 300, 400 and 500 Gy and the viability of the spores was measured

afterward. The irradiation dose that killed the spores by 1 log cycle was employed for further experiments.

Selection of mutants resistant to CCR: The mutant suspension was diluted, spread on potato dextrose agar plates and incubated at 28°C for 2 days. After replica plating on medium plates containing 2% glucose and incubation at 28°C followed by 18 h at 50°C, the plates were stained with 1% iodine solution. The colonies showing clear zones were considered resistant to CCR and were selected for further experiments.

Amylase production of the mutant in liquid medium: For 4 days, the selected mutant was grown in a liquid medium containing 100 g/L of starch at 35°C. The culture was centrifuged for 10 min at 4°C and 21,000 × *g* and alpha-amylase and glucoamylase activities were measured in the supernatant.

Enzyme assay: The alpha-amylase and glucoamylase were analyzed using the Ceralpha method and the amyloglucosidase assay reagent (R-AMGR3, Megazyme Ltd., Republic of Ireland) respectively. The analyses were carried out according to the manufacturer's instructions.

Statistical analysis: The experiment was statistically analyzed using the one-way ANOVA method using Duncan's multiple-range test (DMRT) at a significance level of 99%. This analysis was performed using SPSS v.25 (SPSS Inc., USA).

Results and Discussion

Effect of glucose on amylase production in solid medium: Amylase production by *A. tamarii* FNCC 6151 on a plate medium showed a clear zone after staining with iodine; however, the growth of the strain on a medium containing glucose showed a smaller clear zone or none (Figure 1). The clear zone around the colony was significantly smaller in a medium with 1% glucose than in a medium without glucose. There was no clear zone around the colonies when the strain was cultivated in a medium containing 2% or more glucose.

The previous study showed that there was no clear zone surrounding colonies of *Aspergillus oryzae* when it was grown in a plate medium containing starch as the sole carbon source and 1% glucose.[9] When they grew the strain in a liquid medium, the strain showed significantly reduced α-amylase activity by up to 50%. The addition of 0.1% glucose to the medium reduced amylase production in *Sulfolobus solfataricus* by 92%[7]. The supplementation of 1% glucose to the growth medium also reduced amylase production by 98% in *Aspergillus niger*[15] and 51% in *Thermomyces lanuginosus*[11].

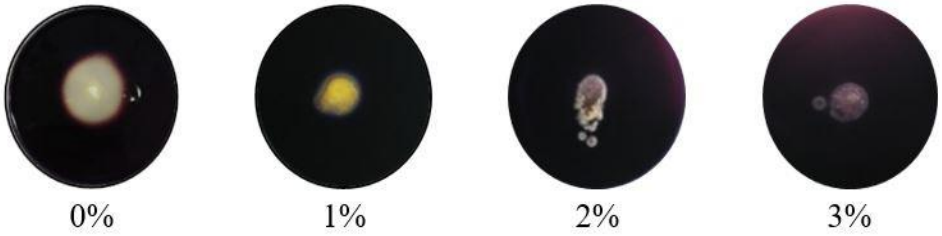

Figure 1: **Clear zones around colonies of A. tamarii FNCC 6151 grown on plate medium supplemented with different amounts of glucose**

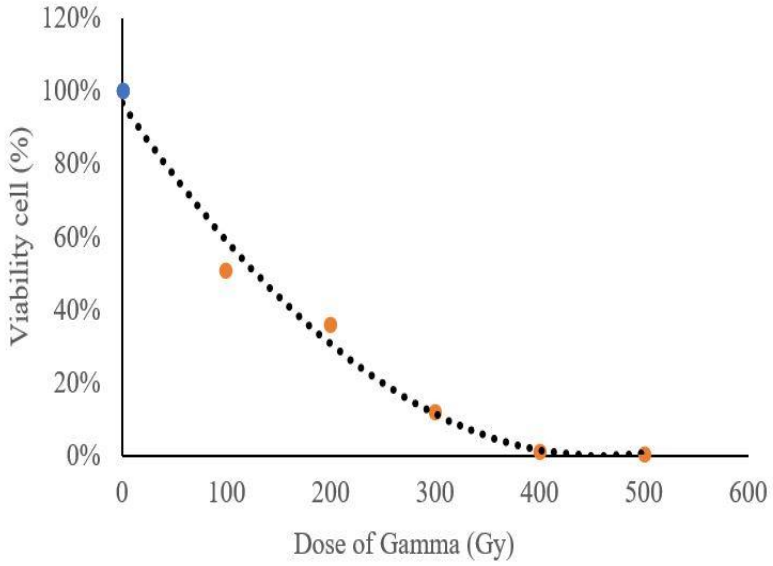

Figure 2: Viability of *Aspergillus tamarii* FNCC 6151 after irradiation with gamma-ray at different dose

The glucose concentration that brought about repression to amylases production was species dependent. Colony of *Aspergillus tamarii* FNCC 6151 on the plate medium supplemented with 1% glucose showed a considerably smaller clear zone than the one grown on the medium without glucose. However, no clear zone was observed around the colony of the strain grown on the medium supplemented with 2% glucose (Figure 1). These results indicate that the production of amylases by *Aspergillus tamarii* FNCC 6151 was inhibited in the presence of 2% glucose. For the selection of mutant strains that have lost their CCR, a plate medium supplemented with 2% glucose was used.

Effect of irradiation dose on spore viability: To enhance amylases production, the strain was irradiated by gamma rays to bring about a mutation in the DNA and hopefully, there was a mutation that eliminated the CCR for amylase production. The irradiation dose used should cause mutagenesis in the DNA, but the degree of mutation was not too severe to degrade the character of the strain. Many workers used irradiation doses that gave one cycle reduction in cell population to obtain the desired mutants[4,6,10,14,18,21]. Figure 2 showed that irradiation of *Aspergillus tamarii* FNCC 6151 spores using 400 Gy of gamma rays killed 90% of the spore population. Mutants produced by this irradiation dose were screened for ones that showed no clear zone around the colony after staining with iodine.

Selection of mutants and amylases production: The mutants produced by gamma irradiation at 400 Gy were screened for those that gave no clear zones on the plate medium containing 2% glucose after staining with iodine solution. One of the mutants, Mut1, showed the criterion (Figure 3). The Mut1 gave a clear zone on the plate medium containing 2% glucose, although the size of the clear zone was smaller than that on the medium without glucose.

Mut1 was grown in a liquid medium containing 0%, 1%, 3% and 5% glucose for 4 days. The activities of alpha-amylase and glucoamylase in the medium were assayed and compared to the one of the parental strains (Table 1). The mutant produced 1.127 U/mL of alpha-amylase and 0.011 U/mL of glucoamylase, which are higher than production of parental strain (0.624 U/mL of alpha-amylase and 0.080 U/mL of glucoamylase). Compared to the parental strain, the mutant produced 1.8-fold more alpha-amylase and 1.4-fold more glucoamylase. This finding indicated that the mutant remained to produce alpha-amylase and glucoamylase, although the growth medium was supplemented by glucose up to 5%.

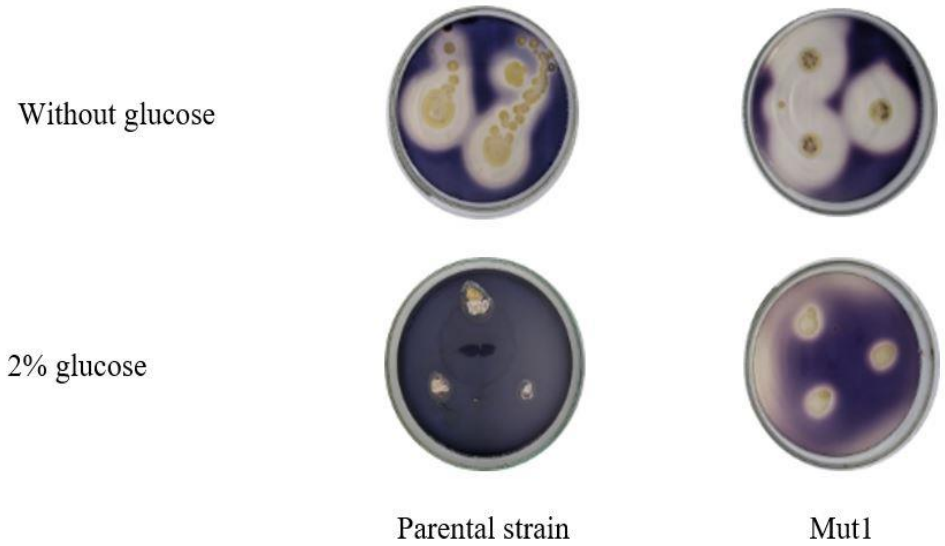

Figure 3: The presence of a clear zone surrounding mutant Mut1 when the strain was grown on a 2% glucose plate medium

Table 1
Production of alpha-amylase and glucoamylase in *A. tamarii* strains FNCC 6151 and Mut1 in liquid medium supplemented with different amounts of glucose.

Strain	Glucose concentration	Alpha-amylase (U/ml)	Glucoamylase (U/ml)
Aspergillus tamarii FNCC 6151	0%	0.625 ± 0.01	0.08 ± 0.00
Aspergillus tamarii Mut1	0%	1.127 ± 0.13	0.011 ± 0.00
	1%	0.869 ± 0.03	0.006 ± 0.00
	3%	0.939 ± 0.00	0.008 ± 0.00
	5%	0.610 ± 0.27	0.009 ± 0.02

Bacillus subtilis was also mutated using gamma rays with a 3 kGy dosage which enhanced amylase activity by 1.3-fold[8]. When *Aspergillus niger* was exposed to 1.2 kGy of gamma rays, its alpha and beta-galactosidase activity increased 2-fold[3].

This study found that gamma-ray irradiation increased the production of alpha-amylase and glucoamylase in *A. tamarii* FNCC 6151 and the mutant retained the enzyme production even when glucose was added to the growth medium. It indicated that the mutant's CCR for alpha-amylase and glucoamylase production was eliminated.

Conclusion

Random mutagenesis of *Aspergillus tamarii* FNCC 6151 using gamma rays with a 400 Gy dosage enhanced alpha-amylase and glucoamylase productions by 1.8-folds and 1.4-folds respectively. The increase of the enzymes produced by the mutant might be due to the removal of CCR to produce the enzyme. This research provides information on applying gamma rays as a potential method for increasing the production of the enzyme amylase by indigenous fungi.

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References

1. Adnan M., Zheng W., Islam W., Arif M., Abubakar Y.S., Wang Z. and Lu G., Carbon catabolite repression in filamentous fungi, *International Journal of Molecular Science*, **19(48)**, 1–23 **(2018)**

2. Aleem B., Rashid M.H., Zeb N., Saqib A., Ihsan A., Iqbal M. and Ali H., Random mutagenesis of super koji (*Aspergillus oryzae*): improvement in production and thermal stability of α-amylases for maltose syrup production, *BMC Microbiology*, **18(1)**, 1–13 **(2018)**

3. Awan M.S., Tabbasam N., Ayub N., Babar M.E., Mehboob-Ur-Rahman R.S.M. and Rajoka M.I., Gamma radiation induced mutagenesis in *Aspergillus niger* to enhance its microbial fermentation activity for industrial enzyme production, *Molecular Biology Reports*, **38(2)**, 1367–1374 **(2011)**

4. Blank G. and Corrigan D., Comparison of resistance of fungal spores to gamma and electron beam radiation, *International Journal of Food Microbiology*, **26(3)**, 269–277 **(1995)**

5. Gupta A., Gupta V.K., Modi D.R. and Yadava L.P., Production and characterization of α-amylase from *Aspergillus niger*, *Biotechnology*, **7(3)**, 551–556 **(2008)**

6. Haq U.I., Nawaz A., Mukhtar H., Mansoor Z., Riaz M., Ahmed M. and Ameer S.M., Random mutagenesis of *Aspergillus niger* and process optimization for enhanced production of glucose oxidase, *Pakistan Journal of Botany*, **46(3)**, 1109–1114 **(2014)**

7. Haseltine C., Rolfsmeier M. and Blum P., The glucose effect and regulation of α-amylase synthesis in the hyperthermophilic archaeon, *Sulfolobus solfataricus*, *Journal of Bacteriology*, **178(4)**, 945–950 **(1996)**

8. Ibrahim H.M.M. and Bashandy A.S., Effect of gamma radiation on alpha-amylase production by *Bacillus subtilis* utilizing potato peel waste, *Isotope & Radiation Research*, **42(4)**, 1071–1082 **(2010)**

9. Ichinose S., Tanaka M., Shintani T. and Gomi K., Improved α-amylase production by *Aspergillus oryzae* after a double deletion of genes involved in carbon catabolite repression, *Applied Microbiology and Biotechnology*, **98(1)**, 335–343 **(2014)**

10. Kostyleva E.V., Sereda A.S., Velikoretskaya I.A., Burtseva E.I., Veselkina T.N., Nefedova L.I., Sharikov A.Y., Tsurikova N.V., Lobanov N.S. and Sinitsyn A.P., Development of schemes of induced mutagenesis for improving the productivity of *Aspergillus* strains producing amylolytic enzymes, *Microbiology*, **86(4)**, 493–502 **(2017)**

11. Kunamneni A., Permaul K. and Singh S., Amylase production in solid state fermentation by the thermophilic fungus *Thermomyces lanuginosus*, *Journal of Bioscience and Bioengineering*, **100(2)**, 168–171 **(2005)**

12. Mandels M., Hontz L. and Nystrom J., Enzymatic hydrolysis of waste cellulose, *Biotechnology and Bioengineering*, **16(11)**, 1471–1493 **(1974)**

13. Mojsov K., Microbial alpha-amylases and their industrial applications: a review, *International Journal of Management, IT and Engineering*, **2(10)**, 583–609 **(2014)**

14. Ottenheim C., Werner K.A., Zimmermann W. and Wu J.C., Improved endoxylanase production and colony morphology of *Aspergillus niger* DSM 26641 by γ-ray induced mutagenesis, *Biochemical Engineering Journal*, **94**, 9–14 **(2015)**

15. Rajoka M.I. and Yasmeen A., Induction and production studies of a novel glucoamylase of *Aspergillus niger*, *World Journal of Microbiology and Biotechnology*, **21(2)**, 179–187 **(2005)**

16. Ruaida A. and Karim K.M.R., Effect of chemical and physical mutagens on amylase producing potentiality of *Aspergillus flavus* NSH9, *Journal of Advanced Biotechnology and Experimental Therapeutics*, **4(1)**, 53–59 **(2021)**

17. Safitri I.O., Suroto D.A., Widada J., Sardjono and Cahyanto M.N., Effect of glucose as carbon catabolite repressor on alpha-amylase and glucoamylase production in Indonesia indigenous fungi, *Malaysian Journal of Microbiology*, **18(3)**, 322-330 **(2022)**

18. Saleh Y.G., Mayo M.S. and Ahearn D.G., Resistance of some common fungi to gamma irradiation, *Applied and Environmental Microbiology*, **54(8)**, 2134–2135 **(1988)**

19. Santos E.D.O. and Martins M.L.L., Effect of the medium composition on formation of amylase by *Bacillus* sp., *Brazilian Archives of Biology and Technology*, **46(1)**, 129–134 **(2003)**

20. Sundarram A. and Murthy T.P.K., α-amylase production and applications : a review, *Journal of Applied & Environmental Microbiology*, **2(4)**, 166–175 **(2014)**

21. Toyoshima Y., Takahashi A., Tanaka H., Watanabe J., Mogi Y., Yamazaki T., Hamada R., Iwashita K., Satoh K. and Narumi I.,

Lethal and mutagenic effects of ion beams and γ-rays in *Aspergillus oryzae*, *Mutation Research Fundamental and Molecular Mechanisms of Mutagenesis*, **740(1–2)**, 43–49 **(2012)**

22. Varalakshmi K.N., Kumudini B.S., Nandini B.N., Solomon J., Suhas R., Mahesh B. and Kavitha A.P., Production and characterization of alpha-amylase from *Aspergillus niger* JGI 24 isolated in Bangalore, *Polish Journal of Microbiology*, **58(1)**, 29–36 **(2009)**.

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