Phenotypic variation and characterization of mutant matting in shiitake

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`Abstract

Shiitake (*Lentinula edodes*) is an edible mushroom that has many uses such as: pharmaceutical, nutraceutical and cosmeceutical industries. In this study, we will induce Shiitake to create the genetic variation via exposing the spores of shiitake to gamma (ϒ) ray at different doses (0-700 Gy) then make the matting between two different monokaryon mycelium (MM). poatato dextrose agar (PDA), this media will be used for spore germination and monokaryon mycelium subculturing during this study. The compatibility of the matting will be observed macroscopically (observing on the plates of PDA) and microscopically (by observing the clamps test under the microscope (Olympus brand)). The finding of this study, there is no significant changing in the growth performance of irradiated monokaryon mycelium in comparing with non-irradiated mycelium. From 108 matting only 15 were compatibles. This study, the physical mutagen will be used followed by mating as a normal stage of life cycle for creating potential strain of shiitake with alteration in phenotypic characterization of dikaryon mycelium(DM) as a compatible mating for two MM.

Key words: monokaryon mycelium, Shiitake, PDA.

Introduction

Shiitake is a mushroom known as *Lentinula edodes*. The name Shiitake was derived from two Japanese words *shii* name of tree Castanopsis cuspidata and *take* mean mushroom. (Wasser S. 2004). Shiitake have a medical importance due to the activities of active compound in the cell wall of the mushroom, those activities are anti-cholesterol, anti-oxidant, anti-inflammatory, anti-microbial, immune enhancing, and effects cancer as anti-cancer (Wasser, S.P., and Weiss,A.L., 1999)..In Japan evidence of significant epidemiology cleared correlation between low rate of cancer mortality and mushroom exhaustion (Borchers 1999).

The ϒ-rays was used as physical mutagen on the spores (Crow & Abrahamson, 1997; Djajanegara, 2008). The spores were subcultured on potato dextrose agar (PDA), then germinated to form the monokaryon mycelium (MM). The matting between two different MM resulted in formation of dikaryon mycelium (DM). The affinity between to different MM resulted in formation of clamps connection to exchange the genetics information during creating DM (Kothe., 2001). The formation of DM is the normal stage of shiitake life cycle, which contain many clamps connection to proceed for the formation of fruit body (Heitman 2006). The aim of this study is to investigate the phenotypic alteration in DM of shiitake by using the physical mutagen (ϒ-rays) and hypothesising the creating of potential strain from shiitake.

Material and method

Sample collection and radiation

Lentinula edodes matured fruity bodies were collected from Kundasang Sabah. Spore print was made by capsized the fruity body on trace paper for two hours and kept at -20 ºC for storage purposes. Then the spores were diluted in tube (1.5 ml) with 100µl of sterilize distilled water (DW), then exposed the tubes to ϒ-radiation by using the caesium 137 (C^{137}), at different doses (0, 100, 200, 300, 400, 500, 600 and 700 Gy) with 13.1 $Gy.min^{-1}$ as a dose rate.

PDA

After radiation the spores were germinated on freshly prepared potato dextrose agar (PDA) media was used for mushroom mycelium growing. 39 g was PDA dissolved in 1 litter DW and stir the mixture until completely powder dissolve and autoclave at 121 ^{o} C for 20 min and at 1.03 bar of pressure.

Clamps connection identification

A small piece of carefully cut mycelium (without the agar) was mounted on a glass microscope slide. Then, 40 µl of distilled water was mounted on the slides before covered with a cover slip for observation. A microscope (OLYMPUS) with 40X objective magnification lens was used to observe clamp connections.

Matting observation

The irradiated spores were sub-cultured on PDA, then single spore was isolated from different doses and continuously subculture until the third subculture. Then use two monokaryon mycelium from germination of irradiated spores for matting. From each dose three single spore were isolated and the matting were achieved at three different groups of monokaryon mycelium from different doses. 108 matting were evaluated in this study. As shown in the table.1.

Table.1 Matting between the monokaryon mycelium with different doses.

	Control*	100 Gy	200 Gy	300 Gy	400 Gy	500 Gy	600 Gy	700 Gy
Control*	control-control							
100 Gy	control-100	100-100						
200 Gy	control-200	100-200	200-200					
300 Gy	control-300	100-300	200-300	300-300				
400 Gy	control-400	100-400	200-400	300-400	400-400			
500 Gy	control-500	100-500	200-500	300-500	400-500	500-500		
600 Gy	control-600	100-600	200-600	300-600	400-600	500-600	600-500	
700 Gy	control-700	100-700	200-700	300-700	400-700	500-700	600-700	700-700

*Control is non-irradiated monokaryon mycelium

Results and discussion

This study, shows the phenotypic alteration in dikaryon mycelium formation after the matting of compatible monokaryon mycelium isolated from spores exposed to ϒ-radiation. From 108 matting crossed only 15 crosses were showed compatibility (14% were compatible), as shown table.2.

Number	Sample ID	Growth rates / 12 days
1	CX7mLTN	7.88±0.66
2	3mX7mLTN	8.11±0.68
3	1mX2mLTN	7.45±0.62
4	5mX7mLTN	7.70±0.64
5	CX1mLTN	7.64±0.64
6	2mX7mLTN	7.60±0.63
7	CX7mLTN	7.69±0.64
8	1mX2mLTN	8.40±0.70
9	1mX5mLTN	8.11±0.68
10	5mX7mLTN	8.19±0.68
11	3mX7mLTN	8.22±0.69
12	2mX6mLTN	8.40±0.70
13	6mX7mLTN	7.67±0.64
14	3mX5mLTN	8.20±0.68
15	2mX7mLTN	8.40±0.70

Macroscopically results for matting can be observed by naked eyes. The observation was depending on the expanding between two monokaryon mycelium toward each other. As figure 1 shows the compatible matting does not form the line in between unlike the incompatible which clearly showed the line between two monokaryon mycelium, farther more the sub-culturing of compatible matting forming the typical circle while the sub-culturing of the incompatible matting yield in forming atypical circle (butterfly like), that was due to the compatible matting forming dikaryon mycelium, while the incompatible still two monokaryon mycelium with different growth performance.

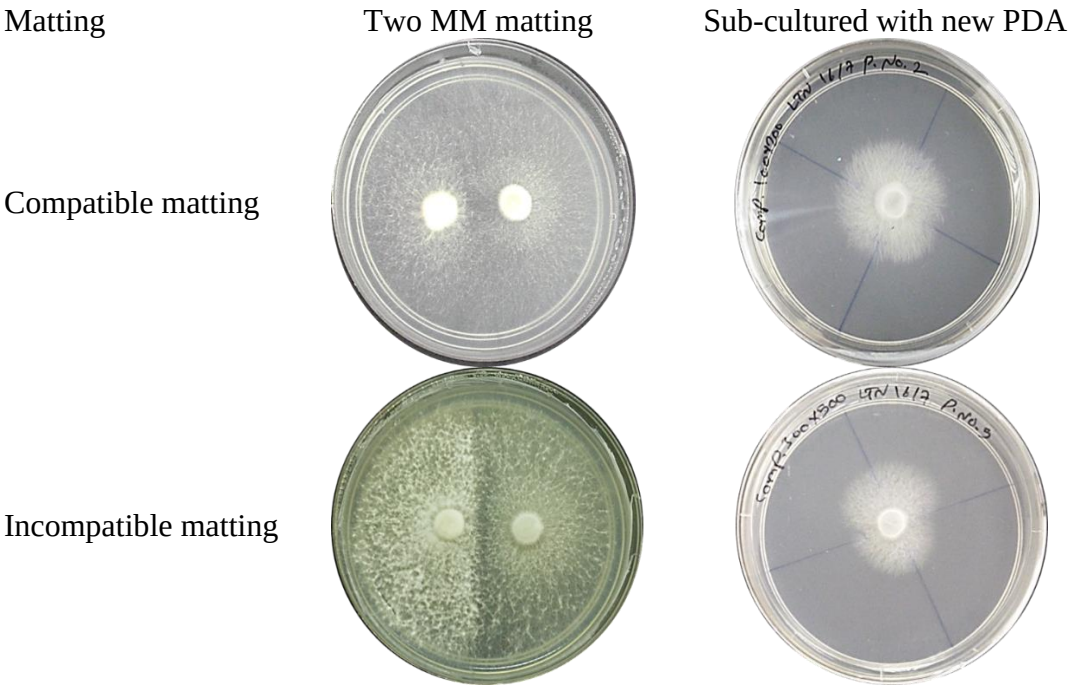

Figure 1. Identification of compatibility macroscopically between two monokaryon mycelium (MM) on the left, and on the right the subculturing of both (compatible and incompatible) with butterfly formation.

Proceeding to the microscopically test and the investigation for the existence of the clamps connection. This study, shown the clamps connection of dikaryon mycelium were record higher counting in comparing with monokaryon mycelium. (As show in Figure 2.).

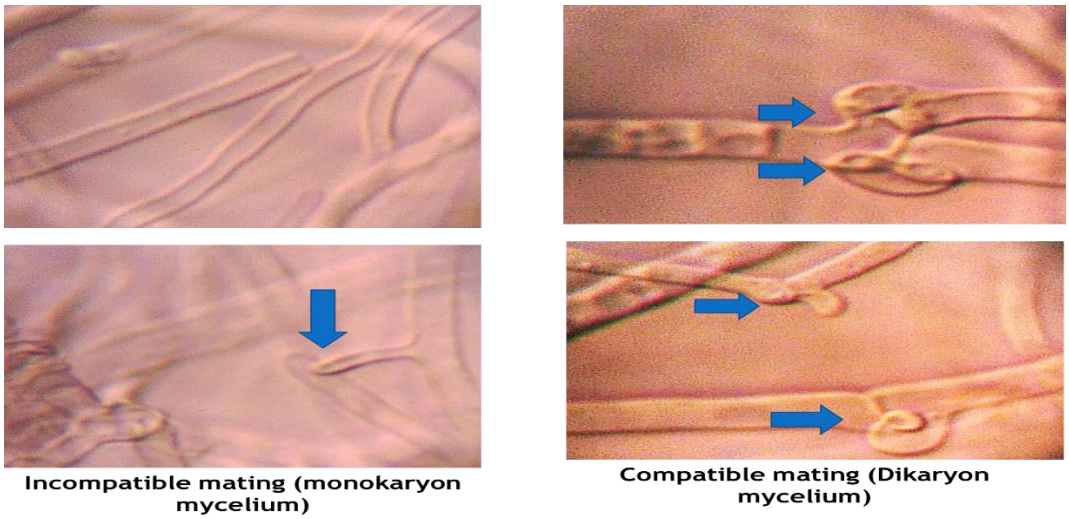

Fig. 2. The clamps connection in both of incompatible matting or monokaryon mycelium (on the right), and compatible matting or dikaryon mycelium (on the left).

From 108 matting only 15 matting were compatible and shows the positive polarity between two monokaryon mycelium and grown to form the dikaryon mycelium. The growth rates of the compatible samples were shown different growth performances, and some of them were shown higher growth performance in comparing with control, as shown in Fig.3.

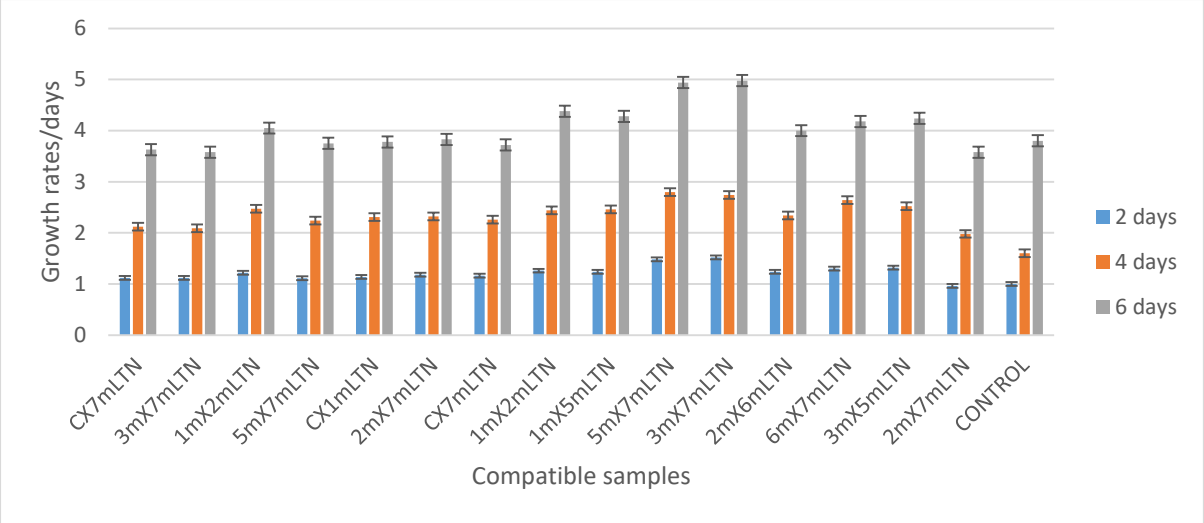

Figure.3. Growth rates of 15 compatible samples in comparing with control of 2, 4 and 6 days.

Figure.4 shows the phenotypically variation of the compatible samples in comparing with control. The variation were observed with dikaryon mycelium by using the naked eyes. The mycelium of compatible samples were more vigorous and thicker.

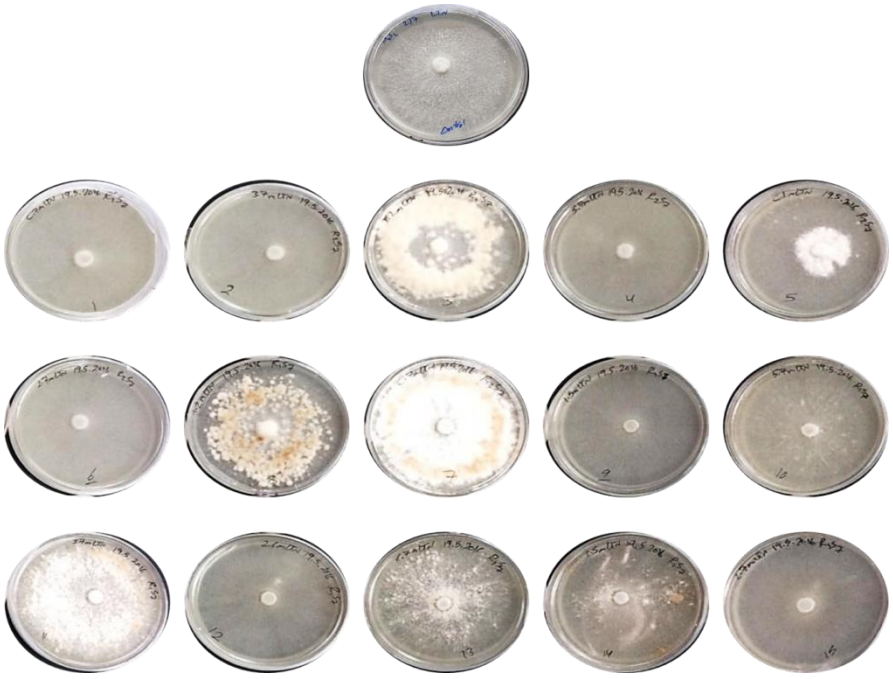

Figure.4. Compatible mattings of 15 hybrid samples from this study showed phenotypic variation as compare to control at maturity stage. The samples will grow on PDA media incubated at 26 C in slightly dark place.

Conclusion

ϒ-radiation creates variation in monokaryon spores at both genetic and phenotypic levels based on mycelium evaluation. These variations can be observed form their growth performance and mycelium morphology by only naked eyes. By mating the monokaryon spores, good traits as fast growing mycelium can be transferred and improved the variation of the samples derived from the radiation. However, strains selection should be carried out via fruiting the hybrids strains to select a novel strain accordingly.

References

Borchers, A. T., Stern, J. S., Hackman, R. M., Keen, C. L., & Gershwin, M. E. (1999). Mushrooms, tumors, and immunity. Proceedings of the Society for Experimental Biology and Medicine, 221(4), 281-293.

Crow, James F., and Seymour Abrahamson. "Seventy years ago: mutation becomes experimental." Genetics 147.4 (1997): 1491.

Djajanegara, I. 2008. White oyster mushroom (Pleurotus florida) mutant with altered antioxidant content. BIOTROPIA-The Southeast Asian Journal of Tropical Biology 15 (1):65-73.

Heitman, J. 2006. Sexual Reproduction and the Evolution of Microbial Pathogens. Current Biology 16(17): R711-R725

Kothe, E., 2001. Mating-type genes for basidiomycete strain improvement in mushroom farming. *Applied Microbiol Biotechnol.* 602-612.

Wasser, Solomon P., and Alexander L. Weis. "Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives (review)." International Journal of medicinal mushrooms 1.1 (1999).

Wasser, S. P., Coates, P., Blackman, M., Cragg, G., Levine, M., Moss, J. & White, J. 2005. Encyclopedia of Dietary Supplements, Taylor & Francis.