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DOSE RESPONSE FOR *Auricularia auricula-judae* AGAINST ACUTE GAMMA IRRADIATION

HING JAN NIE[1*], AZHAR MOHAMAD[1], ZAITON ABDUL KADIR[1,2], WAN SAFINA WAN ABDUL RAZAK[1,2], IBRAHIM MAHMOOD[1,2] and HISHAM HAMZA HUSSAIN[1,2]

[1]*Agrotechnology and Bioscience Division, Malaysian Nuclear Agency, Bangi, 43000 Kajang, Selangor D.E., Malaysia*
[2]*School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor D.E, Malaysia*
[*]*E-mail: hing@nm.gov.my*

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ABSTRACT

Auricularia auricula-judae is a jelly and ear-like mushroom well known as part of traditional Chinese medicine and cuisine. Issues in mushroom cultivation are related to sustaining sufficient amount of strains with genetic variations for selection and obtaining quality strains for mother cultures. Strain selections by growers are hampered by the narrow genetic variations of existing mushrooms. Induced mutations through gamma radiation was found to significantly generate genetic variations in mushroom. This study aims to determine dose response of *A. auricula-judae* when irradiated with gamma radiation. Samples grown on semi solid PDA (Potato Dextrose Agar) media were exposed to gamma radiation from Cs-137 source in Biobeam GM8000. Doses for acute gamma irradiation ranged from 0 Gy, 0.1 kGy, 0.2 kGy, 0.3 kGy, 0.4 kGy, 0.5 kGy, 0.6 kGy, 0.7 kGy, 0.8 kGy, 0.9 kGy, 1.0 kGy, 1.5 kGy, 2.0 kGy and 4.0 kGy at dose rate 0.013 kGy/min. Visual observations and diameter growth measurements of mycelia were observed 2 days' interval for 8 days. Results revealed mycelia density and growth performance decreases with increasing radiation doses. Other morphological characters of irradiated mycelia remained the same with control. LD_{50} for *A. auricula-judae* was determined at 1.5 kGy. Findings in this study are important for induced mutation studies of *A. auricula-judae* and benefiting to the mushroom industry.

Key words: mushroom, mutagenesis, induced mutation, Cs-137, LD_{50}

INTRODUCTION

Auricularia auricula-judae has long been part of traditional Chinese medicine and cuisine with its distinctive ear and jelly shape (Luo, 1993; Mau *et al.*, 2001; Luo *et al.*, 2009). This mesophilic fungus requires 22-30ºC for optimal mycelia growth (Luo, 1993). Basidiomycetes like *A. auricula-judae* often have medicinal properties in their substances (Wasser & Weis, 1999). Among the medicinal effects from *A. auricula-judae* include antitumour activity, anti-inflammatory, hypocholesterolemia, hypoglycemic, blood pressure regulation, cardiovascular disorders, antioxidant activity, anticoagulant activity and chronic bronchitis (Mau *et al.*, 2001; Luo *et al.*, 2009).

One of the issues present in mushroom cultivation is the difficulty in sustaining large collections of mushroom strains and species to maintain availability of large genetic variations for mushroom breeders. Extinction of either one strain or species will mean loss of thousands of genes for breeding quality strains (Chang, 2008). Quality strains are needed by mushroom growers to achieve high productivity in their farms. Characteristics of quality strains include quickly invading substrates, shorter incubation period and reach fruiting stage faster (Sánchez, 2004).

Due to the current small pool of genetic variations, breeders have come up with ways to increase genetic variations by induced mutations (Jain, 2010). As oppose to in nature, where genetic variations in plants occur through spontaneous mutations; induced mutations by ionizing radiations and chemical agents increase frequency of mutations and produce higher amount of genetic variations (Jain, 2010). The type of radiation most frequently used for induced mutations is gamma

* To whom correspondence should be addressed.

radiation due to repeated success and can cause mutations over a wide spectrum (Nakagawa, 2009; Jan *et al.*, 2012). Ionizing radiations have high energy and ionizing effect to induced mutations by breaking DNA molecules and cause alterations in bases (Djajanegara & Harsoyo, 2009). Besides, gamma radiation was also used for mushroom irradiation for consumption to enhance shelf life and eliminate pathogens (Akram & Kwon, 2010). As in food irradiation it is a safe process (Sommer, 2008).

In studies on induced mutations, determining the correct range of radiation doses is necessary (Ramchander *et al*., 2015). Doses too high will kill the specimens; the LD_{50} (Median Lethal Dose) values assist in establishing the correct dose range (Jain, 2010; Ramchander *et al.*, 2015). This experiment aims to determine the dose response and LD_{50} of *A. auricula-judae* through acute gamma irradiation.

MATERIALS AND METHODS

Sample materials

A. auricula-judae used in this work was obtained from Malaysian Nuclear Agency mushroom collection. Mycelia used were the third subculture and grown on semi solid Potato Dextrose Agar (PDA, OXOID).

Irradiation of samples

Plates grown with *A. auricula-judae* were exposed to gamma radiation from a Cs-137 source in gamma irradiation device, Biobeam GM8000 at 25-28ºC. The 14 doses range from 0 Gy, 0.1 kGy, 0.2 kGy, 0.3 kGy, 0.4 kGy, 0.5 kGy, 0.6 kGy, 0.7 kGy, 0.8 kGy, 0.9 kGy, 1.0 kGy, 1.5 Gy, 2.0 kGy and 4.0 kGy for acute gamma irradiation at dose rate 0.013 kGy/min. Each 14 plates were irradiated with different doses. A 6 mm diameter sterile cork-borer was used to produce 30 agar plugs covered with mycelia (mycelia plugs) on each irradiated sample plates.

Growth performance evaluation

Visual observations on homogeneity of mycelia growth were made on to 2 plates (10 agar plugs in 1 plate) and for another 10 plates (1 agar plugs per plate). Additional measurements on mycelia diameter growth were conducted for each dose. Observations were made every 2 days for 8 days. Plates were kept at 23-25ºC.

RESULTS AND DISCUSSION

Based on visual observations shown in Figure 1, homogeneity in mycelia growth and morphology was consistent among the 10 mycelia plugs for each doses. Thus, the difference in growth between increasing radiation doses can be attributed to the received gamma radiation and not genetic/strain instability. Screening is vital as genetic instability often occurs among fungal strains cultured in the laboratory even without external mutagen (Li *et al.,*

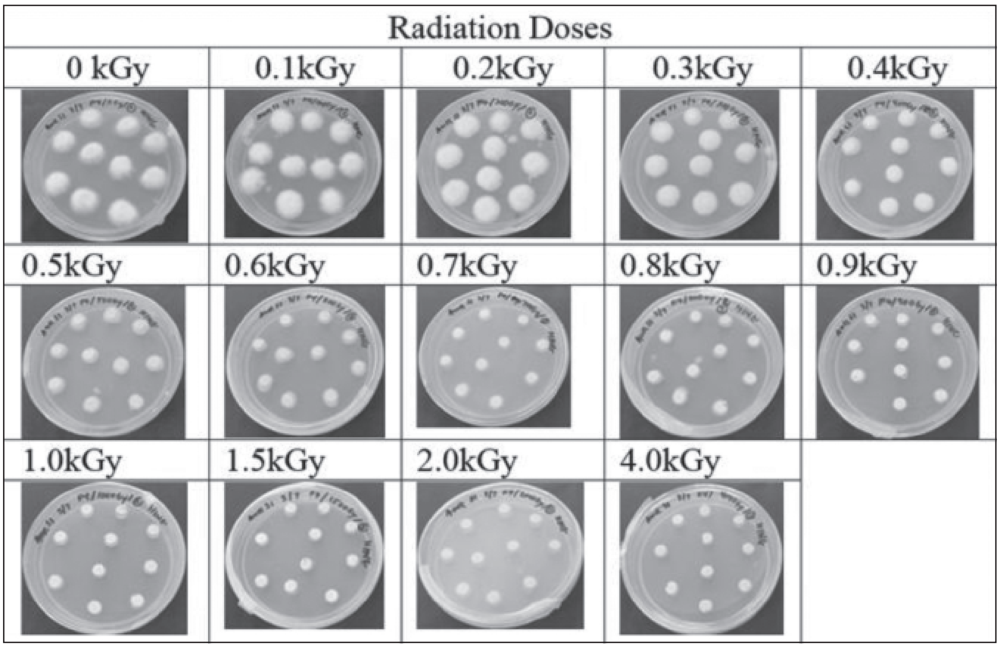

Fig. 1. *A. auricula-judae* mycelia 2 days after acute gamma irradiation. Mycelia were irradiated with 14 different doses 0 Gy, 0.1 kGy, 0.2 kGy, 0.3 kGy, 0.4 kGy, 0.5 kGy, 0.6 kGy, 0.7 kGy, 0.8 kGy, 0.9 kGy, 1.0 kGy, 1.5 Gy, 2.0 kGy and 4.0 kGy. Homogeneity was observed among the 10 agar plugs in each plate.

1994; Kudryavtseva *et al.,* 2011). Morphological variations such as sectors found on PDA grown mycelia can be due to genetic instability (Li *et al.,* 1994).

Further growth and genetic comparisons with other strains are required to determine genetic homogeneity of *A. auricula-judae*. Therefore, deciding the possibility results obtained in this experiment can be a general representative of cultivated *A. auricula-judae* strains in Malaysia. Studies on other mushroom species suggested genetic homogeneity is common among cultivated strains. Chiu *et al* (1996) described genetic homogeneity among cultivated *Lentinula edodes* in China. Xu *et al* (1997) reported genetic diversity among *Agaricus bisporus* in various regionals in the world but also determined genetic homogeneity among cultivar-like isolates of *A. bisporus*.

Observations were conducted on samples not only to determine effects of increasing gamma radiation doses but also to detect any abnormalities in morphology and growth compared to control that can be an indication of mutation. In this experiment, morphological characters of mycelia remained the same with control except for mycelia density and growth. Figure 2 illustrated mycelia density decreases as radiation doses increases. From 0.3 kGy mycelia density was observed to decrease compared to control (0 kGy). Mycelia density and growth was severely affected starting from 0.9 kGy with minimum or no growth observed for 2.0 kGy and 4.0 kGy.

Mycelia diameter growth decreases as gamma radiation doses increases as in Figure 2 and 3. Growth and mycelia density decrease were due to inhibition by higher doses and thus more damaging gamma radiation (Jan *et al*., 2012). Results obtained were similar with other studies on mushrooms mycelia irradiated with radiation. Gamma radiation affected mycelia growth of *Pleurotus ostreatus* and cause genetic differences in a study by Lee and Chang (1999). Patel *et al* (2013) reported decreased in mycelia growth of *Pleurotus sajor-caju* exposed with ultraviolet radiation.

Apart from comparisons on mycelia growth and morphology, a method to further study effects of radiation doses is to grow the mycelia on substrates to collect their fruit bodies. Thus, more comparisons can be made such as time required for mycelia to fully colonized substrate bags, time needed for fruit

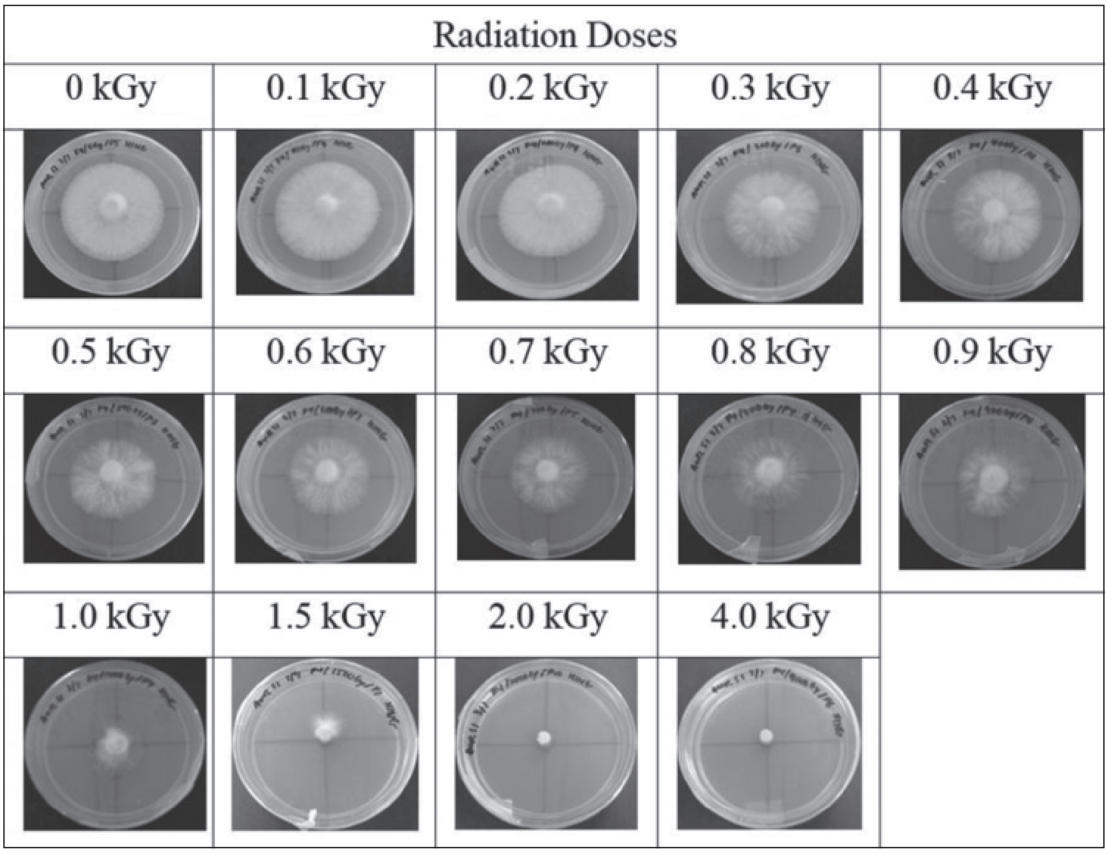

Fig. 2. *A. auricula-judae* mycelia 6 days after acute gamma irradiation. Mycelia were irradiated with 14 different doses 0 Gy, 0.1 kGy, 0.2 kGy, 0.3 kGy, 0.4 kGy, 0.5 kGy, 0.6 kGy, 0.7 kGy, 0.8 kGy, 0.9 kGy, 1.0 kGy, 1.5 Gy, 2.0 kGy and 4.0 kGy. Radial growth were measured and plates photographed every 2 days. Decreasing mycelia growth and density observed with higher doses.

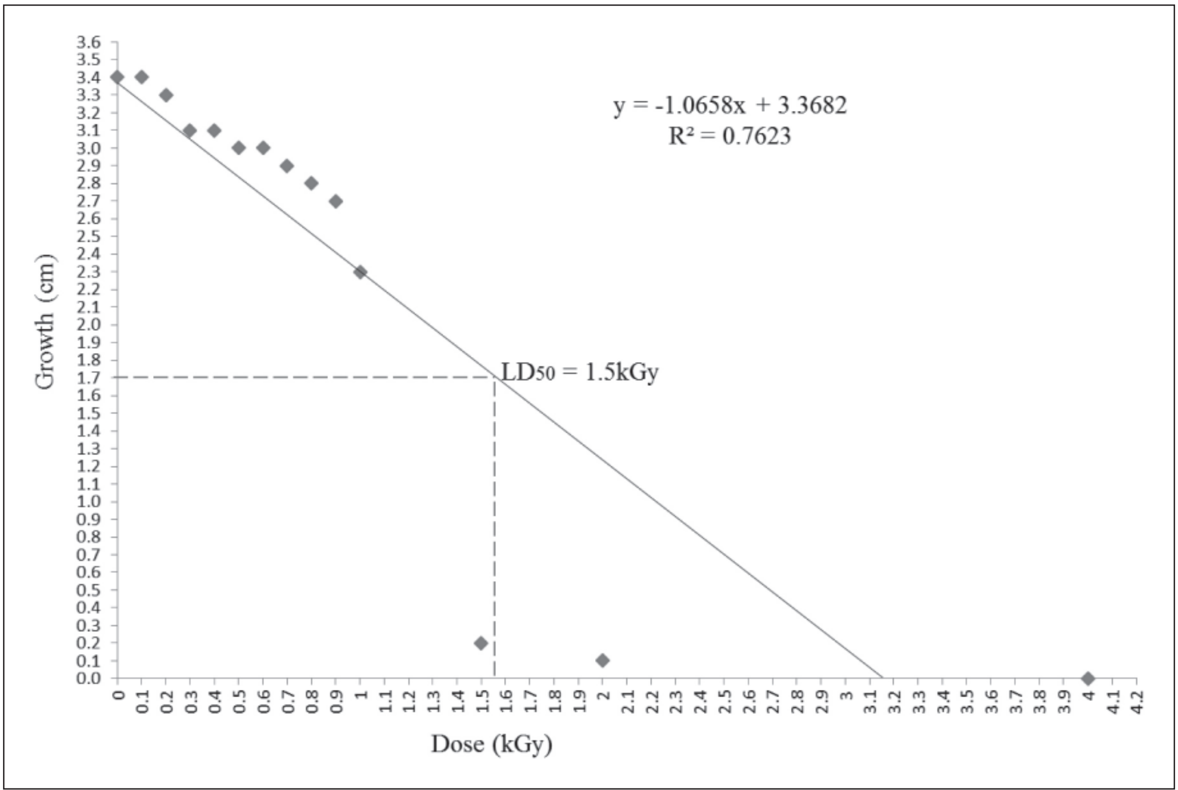

Fig. 3. Gamma radiation dose response of *A. auricula-judae* mycelia after 8 days. Mycelia radial growth decreases as gamma radiation doses increases. LD_{50} was determined at 1.5 kGy.

bodies formation, yield from each substrate bag and morphologies of the fruit bodies (Djajanegara & Harsoyo, 2009; Ibrahim *et al.,* 2015).

The LD_{100} was 4.0 kGy and LD_{50} for *A. auricula-judae* was determined to be 1.5 kGy through the linear equation, y=-0.0011x + 3.3682 generated from the plotted dose response graph in Figure 3. LD_{50} was calculated by using the 50% of the maximum mycelia growth on PDA plates on day 8 which was 1.7cm (y) radial growth. Therefore, the suggested range of optimum dose for future mutagenesis studies on *A. auricula-judae* would be slightly above or below 1.5 kGy. It was suggested each mushroom species have different optimum dose ranges as Rashid *et al* (2014) reported optimum dose range below 2.2 kGy for *Pleurotus sajor-caju*.

CONCLUSION

Mycelia growth of *A. auricula-judae* decreases as dose of gamma radiation increases. LD_{50} for *A. auricula-judae* was observed at 1.5 kGy. Information from this study can be used as reference for strain improvement specific to *A. auricula-judae* species.

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