MJFAS MALAYSIAN JOURNAL OF FUNDAMENTAL AND APPLIED SCIENCES
PRINT ISSN: 2289-5981 | ONLINE ISSN: 2289-599X

RESEARCH ARTICLE

Potential mutant of *Lentinula edodes* with high yield of (1-3), (1-6-), β-D-glucan

Ibrahim Mahmood [a], Azhar Mohamad [c], Fauzi Daud [a], Babul Airianah Othman [a, b], Douglas Law [e], Yew Hoong Cheah [d], Mushrifah Idris [b], Azwan Mat Lazim [a, b], Seng Joe Lim [a], Nur Hidayah Jamar [a, b], Herryawan Ryadi Eziwar Dyari [a, b], Nik Marzuki Sidik [e], Shazrul Fazry [a, b, *]

[a] *Fakulti Sains dan Teknologi, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia*
[b] *Pusat Penyelidikan Tasik Chini, Fakulti Sains dan Teknologi, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia*
[c] *Malaysian Nuclear Agency, Bangi 43000, Kajang, Selangor, Malaysia*
[d] *ZACH Biotech Depot Sdn. Bhd., 43300 Cheras, Selangor, Malaysia*
[e] *Faculty of Agro Based Industry, Universiti Malaysia Kelantan, Jeli Campus, Locked Bag 100, 17600 Jeli, Kelantan, Malaysia*

* *Corresponding author: shazrul@ukm.edu.my*

Article history
Received 28 December 2018
Revised 22 February 2019
Accepted 4 April 2019
Published Online 15 May 2019

Abstract

Lentinula edodes or better known as Shiitake mushroom contains β-1,3;1,6-glucan as part of the cell wall components and studies have shown that it has the ability to enhance the immune system and work as anticancer. The aim of this study was to create potential strain of *L. edodes* with the high content of β-1,3;1,6-glucan, which has the potential for biotechnological purposes. *L. edodes* spores were irradiated with gamma ray (ɣ-rays) and incubated, which formed monokaryon mycelium (MM). Compatible mating of mutated monokaryon formed mutant line dikaryon mycelium (MLDM) that was selected for genotypic and phenotypic comparisons with the wild-type line dikaryon mycelium (WLDM). The concentration of β-1,3;1,6-glucan was measured using a commercial β-Glucan Megazyme Assay Kit (Yeast & Mushroom). Three MLDM (A37, A26, and C07) with 38.8, 36.0 and 34.5% (w/w) of 100 mg, respectively, significantly produced higher amount of β-1,3;1,6-glucan in comparison with WLDM (20.2% (w/w) of 100 mg).

***Keywords*:** DM, MM, mutation, ISSR-PCR, *Lentinula edodes*

INTRODUCTION

L. edodes fruit body contains 88-92% water, lipids, proteins, carbohydrates, vitamins and minerals (Solomon, 2004). The ratio of these components is different, depending on parameters used such as the substrate, cultivation method, temperature, humidity, darkness ratio and fruiting condition (Mata *et al.*, 2016), thus affecting the production of bioactive compounds and nutrition value of the mushroom. Dried *L. edodes* contains 58-60% carbohydrates, 9-10% fibres, 20-23% proteins, 3-4% lipids and ash 4-5%, and it is different as compared to the raw *L. edodes*. *L. edodes* is well-known with the higher source of vitamins especially vitamin B_{12} (Bisen *et al.*, 2010) and D2 (Hiroki *et al.*, 2014). Furthermore, water soluble polysaccharides, known as lentinan have the ability to act as anticancer (Wang *et al.*, 2017).

It is well known that mushroom cell wall is mainly composed of two types of polymers, which are β-glucan and chitin (Shida *et al.*, 1981; Du *et al.*, 2014). However, mushroom cell wall consists of three layers: heteropolysaccharide and β-1,3;1,6-glucan as outside layer, β-(1-6)-glucan with a small number of β-(1-3) branches as middle layer, and the inner layer that consisted of β-glucan and chitin (Bak *et al.*, 2014). The production of β-glucan is affected by potential interferences agents that presented during fermentation (Crognale *et al.*, 2007).

A previous study showed that the *L. edodes* has the bioactive compound of the β-glucans (specifically β-1,3;1,6-glucan) (Sahasrabudhe *et al.*, 2016). However, the β-glucans produced are low in solubility, thus chemical modification is done to improve the bio-viability of the polysaccharide. Improvement in the functional properties of β-glucan is done by using the chemical and physical crosslinking reactions (Hatada *et al.*, 1995). β-glucans can be modified chemically to obtain various derivatives for medicinal and/or potential industrial uses (Synytsya & Novák, 2013). A previous study reported that the modification in the β-glucan chemical structure could affect the bioactive properties either negatively or positively (Zeković *et al.*, 2005; Makkar *et al.*, 2007).

The existence of β-glucan in the cell wall of microorganisms (yeast, bacteria and fungi) and plants has been reported by (Roemer & Bussey, 1991). Two classes of alkali-insoluble β-glucan are existed in *Saccharomyces cerevisiae*, the first is (1-3)- β-glucan which makes up 25% of the dried cell wall and the second is (1-6)- β-glucan which makes up to 7% of the dried cell wall (Pérez & Ribas, 2013). Usually, bacteria have different branching classes of β-glucan which are (1-4)-β-glucan and (1-6)-β-glucan (17). The (1-3)- β-glucan is considered as the most common cell wall component in fungi(Roemer & Bussey 1991). In plants, β-glucan has been found as (1-3)-β-glucan callose of cell wall (Roemer & Bussey, 1991).

There are various techniques that have been applied to produce fungal mutants, for example, mating between monokaryon hyphal (Fukuda *et al.*, 1995), fusion of protoplast between two cells (Yan *et al.*, 1996) and transformation of genes (Godfrey *et al.*, 1994). However, the biggest problem in getting the compatible types of

mating in fungi is the low regeneration of protoplast (Kawasumi *et al.*, 1988).

The mutation by ɣ-ray has the ability to alter the bases of DNA sequence without changing the genomic sequence of the organism (Sato *et al.*, 2006; Lee *et al.*, 2015). Several studies have shown the changes of specific genes in fungi upon radiation with the ɣ-rays such as filamentous fungi (Zolan *et al.*, 1988; Boominathan *et al.*, 1990). In this study, ɣ-ray was used to induce mutation in *L. edodes* in order to get mutants with high content of β-1,3;1,6-glucan, enabling them to have a great prospect in the biotechnology industry.

EXPERIMENTAL

Sample preparation and single spore isolation

Fruit bodies were collected from Kundasang Sabah, Malaysia. After that, the spore print was made from the cap. The spores were scraped and diluted with 100µL of distilled water, then exposed to ɣ-ray with Caesium-137 (by Malaysian Nuclear Agency). Different doses of strength (0, 100, 200, 300, 400, 500, 600 and 700 Gy) were used whereas the dose rate was fixed at 13.1 $Gy.min^{-1}$. The spores were germinated on freshly prepared potato dextrose agar (PDA) (OXOID, USA), where the media were prepared according to manufacturing manual (39g of powder was dissolved in one litre of distilled water and autoclaved at 1.05 bar for 15 min at 121°C). Next, single spore was isolated using cylinder puncher (0.7 mm). Then, monokaryon mycelium was isolated from germination of single spore and prepared for mating test (Kaur & Sodhi, 2015).

Mating test

The mating was carried out by the subculture of two different monokaryon mycelium (MM) on the same PDA plate. By using three different groups of spores, 108 crossings were achieved.

Extraction and measuring of β-glucan

Polysaccharides extraction was carried out by using boiling technique from dried mycelium (DM) (Szwengiel & Stachowiak, 2016). The concentration of β-1, 3;1,6-glucan was determined as described in manual β-Glucan Assay Kit (Yeast & Mushroom) (Megazyme). Calculation of the total glucan was done using the equation below:

Total glucan = α-glucan +β-glucan

DNA extraction and purification

Genomic DNA (gDNA) was extracted from *L.* edodes mycelium as previously described by (Singh *et al.*, 2016), where 0.5g of freshly collected DM was used for gDNA extraction. Purified gDNA was quantified using (NanoDrop 1000) THERMO SCIENTIFIC$^{CO.}$ and validated using 1% agarose gel electrophoresis.

ISSR-PCR amplification

Ten ISSR primers were optimized and used for the detection of polymorphisms (Table 1). The ISSR-PCR analysis was carried out using 4 µl of DNA (50 µg/ml), 1 µl of dNTPs (10 mM), 2 µl of forward and reverse primers (10 mM), 1.5 µl of $MgCl_2$ (25 mM), 0.1 µl of Taq DNA polymerase (1.25 units), 5 µl of 5X Reaction Buffer, and 11.4 µl of nuclease-free water (Sigma). The PCR program was as follows: 2 min at 94°C, followed by 30 amplification cycles (1 min at 94°C, 50 sec for primer annealing temperature, and followed by 1 min at 72°C) and 5 min at 72°C as final extension. The amplified products, which were in 1.5 % agarose gel electrophoresis, were used for PCR products detection (Rashid *et al.*, 2013; Zhao *et al.*, 2016).

Mutants selection of *L. edodes*

Mutant line dikaryon mycelium (MLDMs) were selected based on the changes or presence of ISSR-DNA bands while the growth performance of mushrooms was compared to wild-type line dikaryon mycelium (WLDM). Growth performance assay was carried out as described previously by (Ibrahim *et al.*, 2017). All data was analysed with ANOVA using SPSS v.22.

Table 1. List of ISSR Primers and melting temperature.

Primer no.	Primer sequencing (5'-3')	Tm (^{0}C)
IS1	(CAC)7T (22 mer)	45
IS 10	BDBT(CCT)6 (18 mer)	63
IS11	HVH(TCC)6 (20 mer)	45
IS 12	(AG)8T (19 mer)	45
IS 13	(AG)8G (17 mer)	45
IS 14	(GA)8T (17 mer)	45
IS16	(GA)8A (17 mer)	45
	(TG)8A (17 mer)	45
IS 47	(ACC)6 (18 mer)	45
IS 72	5' (GTC)5 3' (15 mer)	45

Notes: B = C, G, T; D = A, G, T; H = A, C, T; R = A, G; V = A, C, G; Y = C, T

RESULTS AND DISCUSSION

Growth performance of single spore

The irradiated spores did not show any differences in growth performance after 14 days of germination. The ɣ-rays did not affect the growth performance of MM isolated from spores.

Mating of MM

In this study, 15 MLDM were successfully produced by crossing 108 irradiated MM. Compatibility test was carried out by observing the migration of MM to each other on PDA plate, as well as by using the microscope to observe the formation of clamps (Figure 1). The compatible mating had a higher number of clamps formation as compared to incompatible mating. Normally, compatible mating would produce 5-6 number of clamps/HPF whereas incompatible mating would only produce 0-2 number of clamps/HPF. (Lee *et al.*, 2000) suggested that the higher number of clamps would increase the formation of fruit body. Besides, incompatible mating would grow and form an atypical circle (butterfly-like), producing two different circles, and thus, reducing the chances of mating and the formation of clamps (Figure 2).

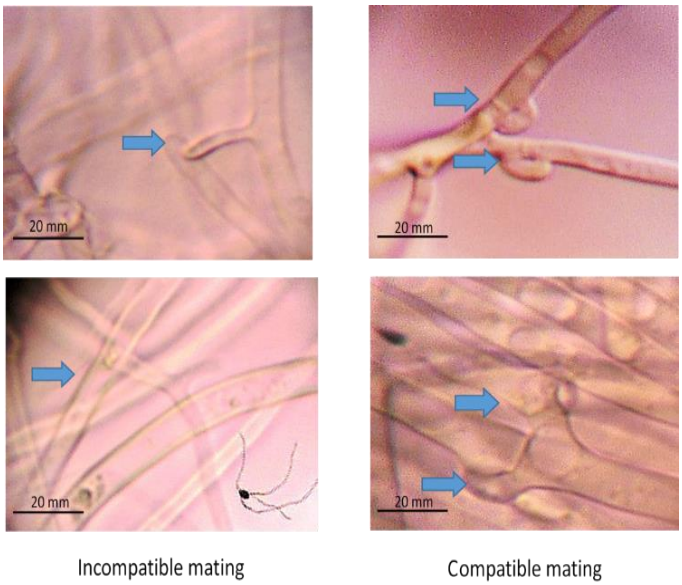

Fig. 1 Observation of clamps formation. The left pictures show the low clamps formation of incompatible mating, the right pictures show the high clamps formation of compatible mating. (The blue arrows refer to the clamps in right and hocking of mycelium in left). The clamps were observed at 40X magnification power. Unlike the incompatible mating, compatible mating showed higher number of clamps.

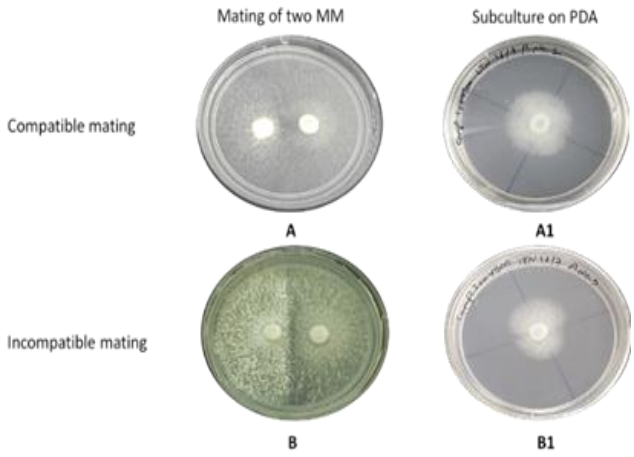

Fig. 2 Macroscopically observation for mating. (A) The morphology of compatible mating of monokaryon mycelia (no separation of mycelia was observed in the compatible mating culture), (B) Incompatible mating of mycelia (distinct "butterfly" morphology was observed due to the rejection of two mycelia at the mid line which would prevent mating, (A1) Continuous sub-culturing formed a typical circle, and the last (B1) Continuous sub-culturing formed an atypical circle (butterfly phenomena). The sub-culture was done by using freshly prepared PDA and 0.7mm cylinder puncher.

Growth performance

WLDM has slower growth performance as compared to the 15 isolated MLDM. The growth performance of shiitake was 8.4 cm/14 days, while certain potential mutant lines (MLDM) showed faster growth performance in comparison with WLDM. The faster growth performance of some MLDM was due to the effects of ɣ- rays which produced some changes in the genetic materials of the mushroom (Fu *et al.*, 2008). Besides, previous study has also shown that the growth performance of *L. edodes* was associated with the cell wall content, especially laccase and cellulose although the expressions of both these enzymes were not similar at the same time (Synytsya & Novák, 2013).

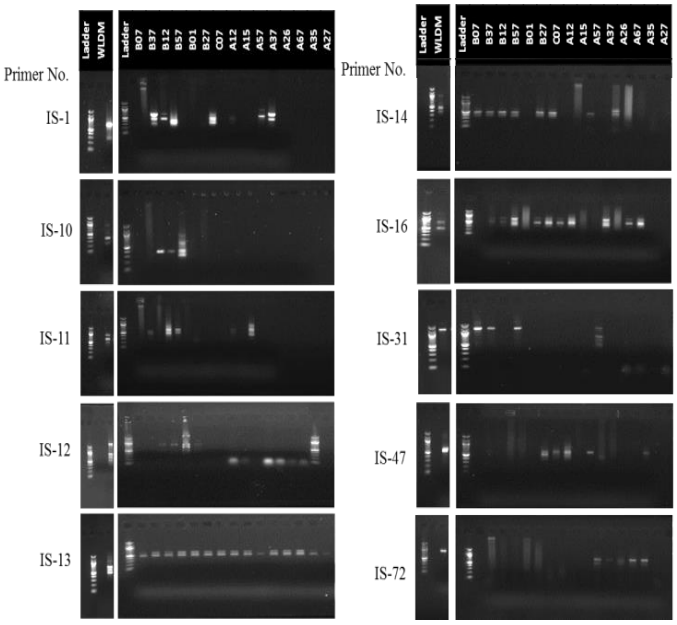

Fig 3 Electrophoresis result of ISSR-PCR. The PCR products were run with 1.5% agarose gel, after mixing 20 µl of products with 5µl 6X blue dye (PROMEGA). The results were determined by observing the bands (presence, absence and shifting).

Observing the phenotypic and genotypic variation of DM

Genetic variation was determined by observing the presence, absence and shifting of bands of ISSR-PCR products (Figure 3). According to (Malekzadeh *et al.*, 2014), ISSR primers were powerful enough for the detection of polymorphism among closely related genotypes which could be used for fingerprinting and legal ownership. It has been used as a molecular tool to identify the genotype variation in different strains of button mushroom (*Agaricus bisporus*) and this method could compare the differences between WLDM and MLDM by observing the existence of specific bands from different primer combinations. The results have shown that all 15 MLDM were different in comparison with WLDM (Figure 2). Interestingly, phenotypic variations of some MLDM which showed the fluffy and rigid DM (mutant B12, B01, C07, A12 and A37) were different from the WLDM (Figure 4). Study on irradiated *Pleurotus Ostreatus* which produced fluffy mycelium did not affect the growth performance in comparison with wild mycelium (Sharma & Sharma, 2014). Furthermore, mycelium rigidity was due to the change in the lignin composition of mycelium which affected the mycelium structure (Saidu *et al.*, 2015).

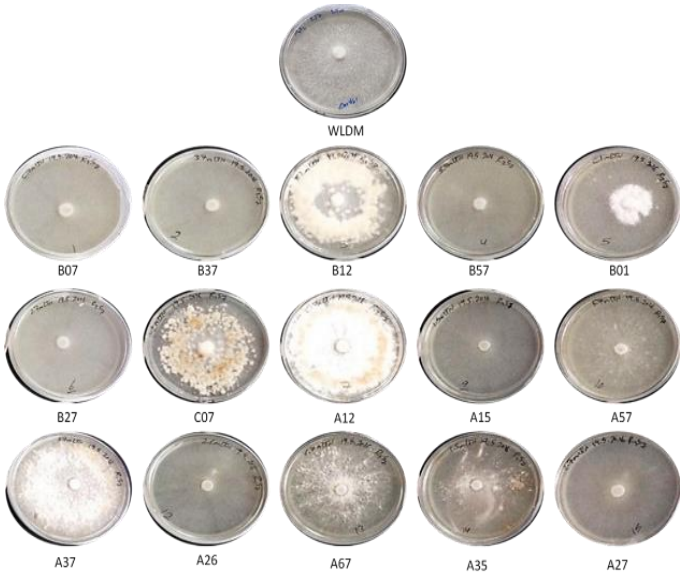

Fig. 4 Phenotypic variation. All DM at 25 days after subculture, some of the WLDM showed phenotypic differences in comparison with WLDM. B12, B01, C07, A12, and A37 were shown to be rigid, fluffy and white mycelium in comparison to WLDM.

Determination of β-1,3;1,6-glucan

Dried metabolites were extracted from dried mycelium to investigate the effects of ɣ-rays on the production of β-1,3;1,6-glucan. The findings showed noteworthy results by comparing the concentration of β-1,3;1,6-glucan from three of the MLDM, which were 38.8, 36.0 and 34.5 % w/w of 100 mg dm, whereas the WLDM produced 20.2 % w/w of 100 mg dm (Table 2). Recent study has also shown similar results for the wild-type *L. edodes* (Sari *et al.*, 2017). Exposing the ɣ- rays to 2.0 KGy could soften cell wall structure by changing the activity of several enzymes of the fruit body, either by decreasing (negative effects) or increasing (positive effects) the concentration of β-1,3;1,6-glucan, and thus, affecting its production (Jiang *et al.* 2010). Our results showed the highest amount of β-1,3;1,6-glucan in A37 mutant (positive effects) with 38.8 % w/w of 100 mg dm, while the lowest amount was in A35 mutant which produced 13.3 % w/w of 100 mg dm (negative effects).

CONCLUSION

Three *L. edodes* mutants (A37, A26 and C07) were successfully mutated to produce a higher amount of β-1,3;1,6-glucan (at least 1.5 times more) compared to the wild-type strain via ɣ-ray radiation. We proposed that it was easier, faster and cheaper to use ɣ-rays in order to produce new mushroom breed with higher amount of desired active compound (in this case β-1,3;1,6-glucan) for future purposes.

Table 2. Total glucan concentration of *Lentinus edodes* mutants and wild lines.

Sample no.	Total glucan	α-glucan	β-glucan
B07	27.6	2.7	24.9
B37	19.1	1.8	17.3
B12	19.0	1.3	17.7
B57	21.1	2.0	19.1
B01	15.4	1.4	14.0
B27	18.9	1.7	17.2
C07	38.1	3.6	34.5
A12	27.3	2.9	24.4
A15	34.7	3.2	31.5
A57	28.0	2.5	25.5
A37	43.0	4.2	38.8
A26	40.0	4.0	36.0
A67	32.4	3.2	29.2
A35	14.8	1.5	13.3
A27	15.0	1.6	13.4
WLDM	22.4	2.2	20.2

* concentration measurement unites (%w/w for 100mg)

ACKNOWLEDGEMENT

We would like to acknowledge the Malaysian Nuclear Agency and ZACH Biotech Depot Sdn. Bhd. for their cooperation and permission to access to their facilities. We would also like to thank Universiti Kebangsaan Malaysia (UKM) and Pusat Penyelidikan Tasik Chini (PPTC), UKM for providing us the grant (LIV-2015-05) and space for our mushroom farm.

REFERENCES

Bak, W. C., Park, J. H., Park, Y. A., Ka, K. H. 2014. Determination of glucan contents in the fruiting bodies and mycelia of *Lentinula edodes* cultivars. *Mycobiology*. 42, 3, 301–305.

Bisen, P. S., Baghel, R. K., Sanodiya, B. S., Thakur, G. S. & Prasad, G. 2010. *Lentinus edodes*: A macrofungus with pharmacological activities. *Curr Med Chem*. 17, 22, 2419–2430.

Boominathan, K., Dass, S., Randall, T. 1990. Nitrogen-deregulated mutants of *Phanerochaete chrysosporium*—A lignin-degrading basidiomycete. *Arch. Microbiol.* 153, 6, 521–527.

Crognale, S., Bruno, M., Fidaleo, M., Moresi, M. & Petruccioli, M. 2007. Production of β-glucan and related glucan-hydrolases by *Botryosphaeria rhodina*. *J Appl Microbiol*. 102, 3, 860–871.

Du, B., Bian, Z., Xu, B. 2014. Skin health promotion effects of natural beta-glucan derived from cereals and microorganisms: A review. *Phytother Res.* 28, 2, 159–166.

Fu, H. -W., Li, Y. -F., Shu, Q. -Y. 2008. A revisit of mutation induction by gamma rays in rice (*Oryza sativa* L.): Implications of microsatellite markers for quality control. *Mol. Breed.* 22, 2, 281–288.

Fukuda, M., Harada, Y., Imahori, S., Fukumasa-Nakai, Y., Hayashi, Y. 1995. Inheritance of mitochondrial DNA in sexual crosses and protoplast cell fusions in Lentinula edodes. *Curr. Gene.* 27, 6, 550–554.

Godfrey, B., Akileswaran, L., Gold, M. 1994. A reporter gene construct for studying the regulation of manganese peroxidase gene expression. *Appl Environ Microbiol.* 60, 4, 1353–1358.

Hatada, M. H., Lu, X., Laird, E. R., Green, J., Morgenstern, J. P., Lou, M., Marr, C. S., Phillips, T. B., Ram, M. K., Theriault, K. 1995. Molecular basis for interaction of the protein tyrosine kinase ZAP-70 with the T-cell receptor. *Nature*. 377, 6544, 32–38.

Hiroki, Sh., Catherine, P., Randi, V., Paolo, M., Bojana, P., Jianqing, X., John, P.O., Kazunori, H., Shinji, S., Kazuhisa, T., Dominic, C., Marie-Paule L. K. I., 2014. Antibody informatics for drug discovery. *Biochim Biophys Acta Proteins Proteom.* 1844, 11, 2002-2015.

Ibrahim, M., Mohamad, A., Daud, F., Kadir, Z. A., Nie, H. J., Hussain, H. H., Safina, W., Razak, W. A., Airianah, O. B., Fazry, S. 2017. Variants production of *Lentinula edodes* spores, monokaryon mycelium and dikaryon mycelium by ɣ- irradiation. *Malays Appl Biol.* 46, 1, 77–82.

Jiang, T., Luo, S., Chen, Q., Shen, L., Ying, T. 2010. Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (*Lentinus edodes*). *Food Chem.* 122, 3, 761–767.

Kaur, A. & Sodhi, H. S. 2015. Characterization of single spore isolates of *Volvariella volvacea* (Bulliard: Fries) Singer. *J. Appl. Hortic.* 17, 3, 236–240.

Kawasumi, T., Baba, T. & Yanagi, S. 1988. Protoplast fusion of incompatible mating type combinations of *Lentinus edodes* (Shiitake) auxotrophs. *Agr Biol Chem.* 52, 12, 3197–3199.

Lee, K. J., Kim, J. B., Choi, H. I., Ha, B. K., Kang, S. Y., & Kim, D. S. (2015). Selection of soybean mutant lines with altered seed coat colour and their antioxidant activity. *Plant Breeding*, 134(5), 573-579.

Lee, Y. K., Chang, H. H., Kim, J. S., Kim, J. K. & Lee, K. S. 2000. Lignocellulolytic mutants of *Pleurotus ostreatus* induced by gamma-ray radiation and their genetic similarities. *Rad. Phys. Chem*. 57, 2, 145–150.

Makkar, H. P. S., Siddhuraju, P. & Becker, K. Glucosinolates. Humana Press, Totowa, 2007, p.55.

Malekzadeh, K., Mohsenifard, E. & Jalalzadeh, M. 2014. Identification and strain-typing of button mushroom using ISSR, ITS and IGS markers. *Mod Genet J*. 2014, 2014, 343–352.

Mata, G., Salmones, D. & Pérez-Merlo, R. 2016. Hydrolytic enzyme activities in shiitake mushroom (*Lentinula edodes*) strains cultivated on coffee pulp. *Revi. Argen. de Microb.* 48, 3, 191–195.

Pérez, P. & Ribas, J. 2013. *Fungal cell wall analysis*. Laboratory Protocols in Fungal Biology, Springer, New York.

Rashid, R. A., Mohamad, A., Awang, M. R., Mutaat, H. H., Mohamad, S. A., Hasan, A. A., Maskom, M. M. and Nahar, S. K. M. (2013). Isolation and optimization of inter-simple sequence repeat (ISSR) technique for Pleurotus sajor caju towards enviromental study in support of the country's nuclear power programme. *Jurnal Sains Nuklear Malaysia*, 25(1), 1-8.

Roemer, T. & Bussey, H. 1991. Yeast beta-glucan synthesis: KRE6 encodes a predicted type II membrane protein required for glucan synthesis in vivo and for glucan synthase activity in vitro. *Proc Natl Acad Sci U S A.* 88, 24, 11295–11299.

Sahasrabudhe, N. M., Tian, L., van den Berg, M., Bruggeman, G., Bruininx, E., Schols, H. A., Faas, M. M. & de Vos, P. 2016. Endo-glucanase digestion of oat β-Glucan enhances Dectin-1 activation in human dendritic cells. *J Funct Foods.* 21, 104–112.

Saidu, M., Busari, A., Yuzir, A. & Salim, M. 2015. Effect of oyster mushroom on biodegradation of oil palm mesocarp fibre. *Int J Environ Ecol Eng.* 9, 10, 1248–1252.

Sari, M., Prange, A., Lelley, J. & Hambitzer, R. 2017. Screening of beta-glucan contents in commercially cultivated and wild growing mushrooms. *Food Chem.* 216, 2017, 45–51.

Sato, Y., Shirasawa, K., Takahashi, Y., Nishimura, M. & Nishio, T. 2006. Mutant selection from progeny of gamma-ray-irradiated rice by DNA heteroduplex cleavage using *Brassica petiole* extract. *Breed. Sci.* 56, 2, 179–183.

Sharma, R. & Sharma, B.M. 2014. Strain improvement in *Pleurotus Ostreatus* using UV light and ethyl methyl sulfonate as mutagens. *Afr J Microbiol Res.* 8, 5, 432–436.

Shida, M., Ushioda, Y. & Nakajima, T. 1981. Structure of the alkali-insoluble skeletal glucan of *Lentinus edodes*. *J Biochem.* 90, 4, 1093-1100.

Singh, N. M., Singh, W. R. C., Singh, H. B. & Singh, W. N. 2016. DNA extraction from Siroi lily (Lilium mackliniae)-an endangered species using CTAB method. *Euro J Exp Biol.* 6, 4, 38–40.

Solomon, W. P., In: Coates, P. M., Paul, M. C., Blackman, M., Blackman, M. R., Cragg, G. M., Levine, M., White, J. D., Moss, J. & Levine, M. A (Eds.) *Encyclopedia of Dietary Supplements*, CRC Press, Florida, 2004, p. 653-664.

Synytsya, A. & Novák, M. 2013. Structural diversity of fungal glucans. *Carbohydr Polym.* 92, 1, 792–809.

Szwengiel, A. & Stachowiak, B. 2016. Deproteinization of water-soluble ß-glucan during acid extraction from fruiting bodies of *Pleurotus ostreatus* mushrooms. *Carbohydr Polym.* 146, 310–319.

Wang, Y., Xu, G., Tang, X. & Chen, H. 2017. Polysaccharide lentinan extracted from the stipe of *Lentinus edodes* mushroom exerts anticancer activities through the transcriptional regulation of cell cycle. *FASEB J*. 31, Abstract No. lb391.

Yan, K., Zhu, B., Cheng, Y., Li, Q. & Zhao, H. 1996. Transformation of *Pleurotus sapidus* protoplasts by electroporation. *Chin J Biotechnol.* 12, 4, 40–44.

Zeković, D. B., Kwiatkowski, S., Vrvić, M. M., Jakovljević, D. & Moran, C. A. 2005. Natural and modified (1→3)-β-D-glucans in health promotion and disease alleviation. *Crit Rev Biotechnol*. 25, 4, 205–230.

Zhao, H., Wang, Y., Yang, D., Zhao, X., Li, N. & Zhou, Y. 2016. An analysis of genetic diversity in *Marphysa sanguinea* from different geographic populations using ISSR polymorphisms. *Biochem Syst Ecol*. 64, 65–69.
Zolan, M. E., Tremel, C. J. & Pukkila, P. J. 1988. Production and characterization of radiation-sensitive meiotic mutants of *Coprinus cinereus*. *Genetics*. 120, 2, 379–387.