GSC Biological and Pharmaceutical Sciences, 2018, 04(03), 001–006

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(RESEARCH ARTICLE)

Effect of ultraviolet C irradiation on growth and antibacterial activity of *Fomitopsis betulina* (Bull.) B.K. Cui, M.L. Han and Y.C. Dai

Krupodorova Tetiana, Barshteyn Victor *, Kizitska Tetiana, Kvasko Hanna, Andriiash Hanna and Tigunova Olena

Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine, Ukraine, Osipovskogo 2a, 04123, Kyiv, Ukraine.

Publication history**:** Received on 29 July 2018; revised on 10 August 2018; accepted on 16 August 2018

Article DOI: https://doi.org/10.30574/gscbps.2018.4.3.0073

Abstract

The using of ultraviolet C (UVC) irradiation is nowadays one of the effective ways of obtaining a new mutant mushroom species with higher yield and synthesis of biologically active metabolites. Of particular interest is the acquisition by mutant mushrooms the new therapeutic properties. Effect of UVC irradiation on the mycelium growth and antibacterial activity of *Fomitopsis betulina* culture liquid has been studied. The cultures of *F. betulina* grown in glucose-peptone-yeast agar culture medium (GPY) three and ten days were exposed to UVC radiation (λ=254 nm) with durations from five to sixty minutes at distance of 0.3 m and twelve-days-old cultures were subcultured on GPY for further study of biomass production and inhibition of bacteria growth. *F. betulina* mycelium growth increased after 15 min of UVC exposure but not significantly changed by among all treatments, as well as color and odor. This duration of UVC radiation exposure with dose of radiation 0.85 kJ/sm^2 caused a stimulating effect of biomass production irrespective of the growth phase of irradiated culture (at the beginning of growth or at actively growing period). Antibacterial activity of *F. betulina* culture liquid against *Bacillus subtilis* decreased with increase in the time of exposure. The highest action against *Staphylococcus aureus* recorded after 5 min of UVC exposure (0.28 kJ/sm^2 radiation dose), and then also decreased. Antibacterial ability of *F. betulina* culture liquid against *Escherichia coli* increased significantly compared to the control and the highest action was found after UVC irradiation for 15 min (0.85 kJ/sm^2 radiation dose). The obtained knowledge can be applied to obtain new mushroom strains with better therapeutic properties.

Keywords: Ultraviolet C; Mycelium growth; *Fomitopsis betulina*; Cultural liquid; Antibacterial activity

1. Introduction

A general trend in biotechnology research is towards the development of easy techniques that are directed to increase the productivity of the target product. Of particular interest is using UVC irradiation (since it has more energy) for obtaining a new mutant mushroom species with better characteristics, higher yield and synthesis of biologically active metabolites. Investigations are mostly focused on effect of UVC irradiation on protoplast, spores, mycelium and fruit bodies. Protoplasts obtained from mycelial culture of the mushroom *Volvariella volvacea* were found to be highly sensitive to the killing action of UVC irradiation of relatively low intensity (1 J/m^2 per s) [1]. Several changes in growth and sizes were found in mycelium of the UV mutant strains of *Pleurotus* species obtained by protoplast fusion technology [2]. Increasing of productivity and adaptability to a wide range of temperatures were received by UV action on mycelium and basidiospores of five strains of oyster mushrooms [3]. Some studies have established induction of mutation aimed to the reducing of sporulation in basidiospores of *Pleurotus* spp.: *Pleurotus eryngii* [4], *P. florida* and *P. sajor-caju* [5], *P. ostreatus* [6, 7], *P. ostreatus var. florida* [8]. Obtained sporeless mushroom strains can be very in demand at commercial

*Corresponding author

E-mail address: barmash14@gmail.com

cultivation due potential absence of various respiratory allergies. Also it was shown that UV-induced mutations in spores of *Pleurotus* spp. caused to the change in mycelial morfological growth, color of sporophore [7].

Of great interest is the investigation of using the UV irradiation to the production of significant amounts of vitamin D_2 in cultivated species such as *Agaricus bisporus*, *A. bitorquis*, *Lentinula edodes*, *P. ostreatus*, *V. volvacea* [9-11]. UV irradiation as a means of increasing fungal synthesis of vitamin D_2 and topics related to its biovailability as well as clinical studies evidencing the health benefits were successfully summarized in two reviews [10, 11]. The application of UVC irradiation on quality of *A. bisporus* [12] has been also studied. The effects of mutagenesis (using UV radiation as mutagen) on linear mycelia growth rate of *L. subnudus* at different agar media have been investigated [13]. Only few studies devoted to the effects of UV irradiation on manifestation of various therapeutic properties of mushrooms. Previous reports showed that the Ultraviolet B irradiation of *P. ostreatus* can converted ergosterol to vitamin D_2 without affecting the mushroom biological activities including antioxidation, tyrosinase inhibition and cytotoxicity [14]. It was established hormetic doses for subcultures of Ultraviolet A irradiated cultures of *Ingoldiella hamata* in enhancing antibacterial activity [15]. Obtained mutant strains of *P. pulmonarius* and *P. ostreatus* produced exopolysaccharides which possessed better antibacterial activity against different pathogenic microbes compared with some wild type [16]. Thus, a small number of investigations of the effect of UVC irradiation on the therapeutic properties of fungi have been carried out. Given the relevance of such investigations, the aim of this study was to evaluate the effect of different doses of UVC irradiation on the growth of mycelium and antibacterial activity of *F. betulina* culture liquid.

2. Material and methods

2.1. Mushroom and bacterial strains

Fomitopsis betulina (formerly *Piptoporus betulinus*) (Bull.) B.K. Cui, M.L. Han and Y.C. Dai, strain 327 was kindly supplied by the Culture Collection of Mushrooms (IBK) of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine [17]. Stock cultures were maintained on beer-wort-agar slants at 4 °C.

The bacterial cultures *Bacillus subtilis* ATCC 6633, *Escherichia coli* 06, and *Staphylococcus aureus* 209 were kindly supplied by the Culture Collection of Microorganisms of the Department of Industrial Biotechnology of the National Technical University of Ukraine (Igor Sikorsky Kyiv Polytechnic Institute). Tested microorganisms were activated in Mueller Hinton agar (MHA) (37 °C, 24 h) and also used to confirm the absence of contamination and the validity of the inocula. Each microorganism was suspended in sterile saline and diluted to 10^6 colony forming units (CFU) per ml. Afterwards, Petri dishes containing MHA were inoculated with the bacterial suspensions.

2.2. Culture media and conditions

Mycelial cultures were initially grown in petri dishes on glucose-peptone-yeast agar culture medium (GPY) with pH 6.0, composed of (g/L): 20.0 glucose, 3.0 yeast extract, 2.0 peptone, 1.0 K_2HPO_4, 1.0 KH_2PO_4, 0.25 $MgSO_4 \cdot 7H_2O$, and 20.0 agar. Also the liquid culture medium GPY without agar was sterilized by autoclaving for 20 min at 121 °C. Flasks (250 mL) with 50 mL liquid medium were inoculated with three mycelial plugs of 8 mm diameter cut from the Petri dishes using a sterile borer at the stage of actively growing mycelia. Mycelia were grown at static cultures (without agitation and in the dark) in flasks for 14 days at 26 ± 2 °C.

2.3. Exposure of mycelia of *F. betulina* to UVC radiation

Fungal discs (sized 8 mm diameter) of *F. betulina*, were inoculated in the center of petri dishes containing 20 ml GPY solid media and incubated at 26 ± 2 °C. UVC irradiation was applied at the beginning of growth on 3-th day and for ten-days-old actively growing cultures. Each group of three plates was then placed under UVC irradiation (two bactericidal lamps Philips TUV 30 W with wave length of 254 nm) to receive doses at different times for 5, 15, 30, 45 and 60 min and one group of plates was untreated and used as the control. The distance between exposed Petri dishes and UVC source was 0.3 m. The irradiated and non-irradiated Petri dishes with *F. betulina* were incubated at 26 ± 2 °C**.** After 12 days of incubation mycelial plugs were transferred into 250 mL flasks with liquid GPY medium.

2.4. Determination of dry weight

Mycelium was separated from the medium by filtration through Whatman's filter paper No. 4 and washed several times with distilled water. For determination of dry weight mycelium then was dried at 105 °C (Snol**-**58/350, UMEGA, Lithuania) to a constant weight. The culture liquid was concentrated ten times by evaporation using a sand bath.

2.5. Screening of antibacterial activity

The antibacterial activity was determined by the agar disk diffusion method. Sterile paper disks (8 mm) impregnated with concentrated cultural liquid were placed into the petri dishes with MHA previously inoculated with the bacterial suspensions. The inoculated petri dishes have been incubated overnight at 37 °C. Antibacterial activity assessed by measuring of the inhibition zone diameter (in mm) – clear zones formed around each disc. Antibacterial activity was recorded in case when the zone of inhibition was larger than 8 mm. The distilled water was used as negative control.

2.6. Data analysis

All experiments were carried out in triplicate. The data were analyzed by Excel statistical functions using Microsoft Office XP software the Statistical Package for Social Sciences, Program 11.5 Version (SPSS, Inc., 2002). Values are presented as means ± standard error of the mean (SEM). Differences at $P \leq 0.05$ were considered to be significant.

Following equation was used for calculation of irradiated surface area (S), radiation density (E), and radiation doses (H) [18]:

$$S = \pi \cdot r^2 = 3.14 \cdot 4.5^2 = 63.59 \text{ sm}^2,$$

Where, r – radius of petri dishes (4.5 cm).

$$E = \frac{F}{S} = \frac{60}{63.59} = 0.94 \text{ W/sm}^2,$$

Where, F – total radiation capacity (two bactericidal lamp Philips TUV 30 W).

$$H = E \cdot t$$

3. Results and discussion

Various mutagens are promising and can used for selection of the perspective high yielding strains. Ultraviolet light exerts its mutagenic effect by exciting electrons in molecules. Our results indicated that there were no changes in mycelial growth type, color or odor for all irradiation parameters. This observation is in line with data of UVC irradiation of *P. columbinus* mycelium [19], in contrast to data showing UV-induced mutations in spores of *P. ostreatus* in change of mycelial growth type for one irradiated isolate [7]. Mycelium of the UV mutant strain of *Pleurotus* species obtained by protoplast fusion technique were not only significantly faster in growth but also larger in size than the parental strains [2]. It was also observed that there were variations in the mycelial growth rate of ultraviolet induced mutants of *Lentinus subnudus* at all the three media used [13].

The maximum growth of *F. betulina* mycelium was observed at 15 minutes UVC exposure, but it was insignificantly different from the results of the other experiments (Table 1). This duration of UVC irradiation with a radiation dose of 0.85 kJ/sm^2 caused stimulating growth effects irrespective of the growth phase of the irradiated culture (at the beginning of growth or at actively growing period).

Table 1 The influence of UVC irradiation duration on *F. betulina* biomass growth

Exposure duration, min	The yield of biomass, g/l (a.d.w.)	
	Irradiation on 3-th day of growth	Irradiation on 10 day of growth
0 (control)	3.2 ± 0.1	
5	3.3 ± 0.1	2.9 ± 0.3
15	3.6 ± 0.0	3.7 ± 0.3
30	3.3 ± 0.0	3.2 ± 0.3
45	3.3 ± 0.1	3.3 ± 0.1
60	3.3 ± 0.1	2.6 ± 0.2

The main difficulty in comparing the results of studies (with the aim of correcting the irradiation time to increase the growth of fungi) is the absence of a single research protocol, including using a different distance between the UV lamp and the object of investigation, the wave length, the total radiation capacity, and the radiation doses. Nevertheless, our findings generally agree with some investigations of *Pleurotus* species. Twenty minutes irradiation dose showed the best growth of *Pleurotus columbinus* [19]**.** In another studies it was found that with the increase in duration of exposure, the growth of *Pleurotus* spp. mycelium retards [5, 7].

Limited information is reported in the literature about effect of UVC light on antibacterial properties of mushrooms. In this study the different antibacterial activity levels – from 10.2 mm in diameter of inhibition zone to full inhibition of test bacteria growth have been established (Table 2). It has been found that irradiation caused decreased action of *F. betulina* culture liquid against Gram-positive bacteria when compared with the control value. Antibacterial activity of *F. betulina* culture liquid against *B. subtilis* decreased with increase of the time of exposure. The highest action against *S. aureus* recorded after 5 min of UVC exposure (0.28 kJ/sm^2 radiation dose), and then also decreased. Other tendency was obtained in case of Gram-negative bacteria. It was noticed that antibacterial activity of *F. betulina* culture liquid against *E. coli* appears compared to the control, increases significantly and the highest action was found after UVC irradiation for 15 min (0.85 kJ/sm^2 radiation dose).

Table 2 The influence of different UVC irradiation duration on antibacterial activity of *F. betulina* culture liquid

Exposure duration, min	Zone of inhibition, mm		
	***Escherichia coli* 06**	***Bacillus subtilis* ATCC 6633**	***Staphylococcus aureus* 209**
0 (control)	–	FI	FI
5	16.0±4.0	16.0±4.0	FI
15	22.1±1.8	15.5±1.3	18.0±2.1
30	16.8±1.3	11.5±0.8	12.8±0.4
45	10.2±0.7	11.5±0.9	14.0±1.0
60	18.7±1.7	10.0±0.0	19.0±1.0

Note: «–» – the lack of antibacterial activity; FI – full inhibition of bacterial growth (≥ 25 mm in diameter).

There is only few information to accurately compare our findings with similar reports. However, investigation of the effect of UV irradiation on the antibacterial activity of *Ingoldiella hamata* shown its positive effect at small doses: the inhibition of gram-positive (*B. subtilis* and *S. aureus*) and gram-negative cultures (*E. coli* and *E. aerogenes*) was maximally increased after 5 min and after 10 min of UV exposure, respectively [14]. The isolated exopolysaccharides from *Pleurotus pulmonarius* and *P. ostreatus* strains exposed to UV radiation also inhibited growth against *E. coli* and *S. aureus* at different levels [15].

4. Conclusion

Our results confirm that the using of UVC irradiation could be a perspective way to obtain a new mutant mushroom species with better characteristics and productivity. It can be concluded that this factor increased biomass production after 15 min of UV exposure with 0.85 kJ/sm^2 radiation dose and positively influenced on antibacterial activity against *E. coli* and *S. aureus* in small doses: 0.85 and 0.28 kJ/sm^2 after 15 and 5 min of UV exposure, respectively. This obtained knowledge can be applied to other species of mushroom specifically for development of new varieties with better therapeutic properties.

Compliance with ethical standards

Acknowledgments

The authors express their sincere gratitude to Deputy Director for R&D, Institute of Food Biotechnology and Genomics Dr. Sergiy Shulga for his kind consent to use the equipment of his laboratory.

Disclosure of conflict of interest

All the authors declare that they have no competing interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

References

[1] Mukherjee M and Sengupta S. (1986). Mutagenesis of protoplasts and regeneration of mycelium in the mushroom *Volvariella volvacea*. Applied and Environmental Microbiology, 52(6), 1412-1414.

[2] Aswini L, Arunagirinathan and Kavitha M. (2014). Strain improvement of *Pleurotus* species by protoplast fusion. International Journal of Advancements in Research and Technology, 3(8), 32-38.

[3] Beejan PHF and Nowbuth R. (2009). Use of irradiation for the induction of mutations in oyster mushrooms for improvement of strains. In: International Symposium on Induced Mutations in Plants, Vienna, Austria, 12-15 Aug 2008, 289-292. Available on-line: http://www.fao.org/docrep/012/i0956e/I0956e.pdf. Last accessed on 05/07/2018.

[4] Obatake Y, Murakami S, Matsumoto T and Fukumasai-Nakai Y. (2003). Isolation and characterization of a sporeless mutant in *Pleurotus eryngii*. Mycoscience, 44(1), 33-40.

[5] Ravishankar S, Pandy M, Tewari RP and Krishna V. (2006). Development of spore less/ low sporing strains of *Pleurotus* through mutation. World Journal of Microbiology and Biotechnology, 22(10), 1021-1025.

[6] Karvan R and Singh T. (2014). Development of *Pleurotus ostreatus* with ethyl methyl sulfonate and UV light as mutagens. International Journal of Mycology and Plant Pathology, 1 (6), 055–058.

[7] Sharma R and Sharma BM. (2014). Strain improvement in *Pleurotus ostreatus* using UV light and ethyl methyl sulfonate as mutagens. African Journal of Microbiology Research, 8(5), 432-436.

[8] Teimoori BB, Pourianfar HR, Moeini MJ and Janpoor J. (2014). Chemically and physically induced mutagenesis in basidiospores of Oyster mushroom *Pleurotus ostreatus* var. florida. International Journal of Advanced Research, 2(11), 915–921.

[9] Mau GL, Chen PR and Yong GH. (1998). Ultraviolet irradiation increased vitamin D_2 content in edible mushrooms. Journal of Agricultural and Food Chemistry, 46 (12), 5269–5272.

[10] Taofiq O, Fernandes Â, Barros L, Barreiro MF and Ferreira ICFR. (2017). UV-irradiated mushrooms as a source of vitamin D_2: A review trends. Food Science and Technology, 70, 82–94.

[11] Kamweru PK, Tindibale EL. (2016). Vitamin D and vitamin D from ultraviolet-irradiated mushrooms (Review). International Journal of Medecinal Mushrooms, 8(3), 205–214.

[12] Ghasemi-Varnamkhasti M, Yoosefiran SH and Mohammad-Razdari A. (2016). Application of UV-C irradiation on quality and textural properties of Button Mushrooms. International Journal of Nutrition Food Engineering, 10(8), 501–504.

[13] Majolagbe ON, Oloke JK, Deka-Boruah HP and Aina DA. (2013). Effects of mutagenesis on mycelial growth rate (Mgr), total protein, total phenolics and total sugar contents of *Lentinus subnudus* using ultra-violet radiation as mutagen. International Journal of Current Microbiology and Applied Science, 2(5), 18–27.

[14] Banlangsawan N, Sripanidkulchai B and Sanoamuang N. (2016). Investigation of antioxidative, antityrosinase and cytotoxic effects of extract of irradiated Oyster mushroom Songklanakarin. Journal of Science and Technology, 38(1), 31–39.

[15] Sridhar KR. (2012). Antibacterial activity of freshwater fungus *Ingoldiella hamata.* Advanced biotechnology. 11(7), 12–15.

[16] Azuonwu TC, Aina DA, Odutayo FOI, Majolagbe ON, Ezeamagu CO and Aina FO. (2017). Comparative studies of production of exopolysaccharides from wild type, mutants and hybrid of *Pleurotus pulmonarius* and *Pleurotus ostreatus* in submerged culture. Acta SATECH, 9 (1), 121–127.

[17] Buchalo AS, Mitropolskay NYu and Mukchaylova OB. (2011). Culture collections of mushrooms IBK. Altpress, Kyiv, Ukraine, 43 p. (In Ukrainian).

[18] Bilay VI. (1982). Methods experimental mycology. Naukova dumka, Kiev, Ukraine, 550 (in Russian).

[19] El-Fallal AA, El-Sayed AKA and El-Gharabawy HM. (2013). Improving yield and productivity of Oyster mushroom (*Pleurotus columbinus*) using ultraviolet mutation. Journal of Environmental Sciences, 42(2), 241–253.

How to cite this article

Krupodorova T, Barshteyn V, Kizitska T, Kvasko H, Andriiash H and Tigunova O. (2018). Effect of ultraviolet C irradiation on growth and antibacterial activity of *Fomitopsis betulina* (Bull.) B.K. Cui, M.L. Han and Y.C. Dai. GSC Biological and Pharmaceutical Sciences, 4(3), 01-06.