ARTICLE

ISSN: 9 772303 337008

UTILIZATION OF ELECTRIC PULSED POWER ON FRUITING OF EDIBLE MUSHROOMS

Shoji Ohga
Division of Forest Environmental Sciences, Department of Agro-environmental Sciences, Kyushu University, Fukuoka 811-2415, Japan
ohga@forest.kyushu-u.ac.jp

ABSTRACT

Effect of pulsed power was investigated on fruit body formation of 10 edible mushrooms, *Lentinula edodes, Glifola frondosa, Pholiota nameko, Flammulina velutipes, Hypsizygus marmoreus, Pleurotus ostreatus, Pleurotus eryngii, Pleurotus abalones, Agrocybe cylindracea* and *Sparassis crispa*. Pulsed power of 100-170 kV was directly charged to the substrate just before fructification. The effect of the pulsed power resulted to promote for 10 edible mushrooms fructification. The treatment especially stimulated the fructification on *Pleurotus* species.

INTRODUCTION

Cultivation of edible mushrooms on sawdust-based substrate is steadily improving to effective methods. Especially, efficient techniques are useful for this cultivation method. Biological efficiency has been improved by optimizing various factors, such as substrate formula, strain type, culture maturity, water condition and other environmental conditions of the cultivation room.

Electric power utility is now applied to various agricultural crops, especially in horticultural fields. Cultivation system has been improved with electricity utilization in the field of systematic house crops such as tomato, lettuce, strawberry and various kinds of flower. Effect of air ions on plants have experimented. Fruit body production was promoted by the electric impulse treatment on the logwood or sawdust substrate (Ohga et al, 2001) of *L. edodes*. We investigated here effect of the pulsed power on the fruit body formation in the sawdust-based substrate of 10 edible mushrooms.

MATERIALS AND METHODS

Strains

Ten edible mushroom species used in this study, *Lentinula edodes, Grifora frondosa, Pholiota nameko, Flammulina velutipes, Hypsizygus marmoreus, Pleurotus ostreatus, Pleurotus eryngii, Pleurotus abalones, Agrocybe cylindracea, Sparassis crispa*. All of these strains were isolated from fruit bodies of commercial sources. Strains were maintained on a potato dextrose agar medium (Difco) at 4^0C. Especially, as for *L. edodes* four strains belonging to 3 different fruiting types were examined in this work. These were KS-1, KS-2 (for year-round cultivation), KS-6 (for cultivation in autumn) and KS-12 (for cultivation in spring). The substrate of *L. edodes* had three flushes in the fruiting stage. First flush occurred spontaneously just after movement of the substrates. Second and 3rd flushes were induced by soaking treatment of 16 hr (130-day substrate) and 20 hr (170-day substrate), respectively.

Culture media and growth conditions

Fruit body production was tested on a plastic bag or bottle. The plastic bag contained 1.2 kg substrate, and the plastic bottle (800 ml) contained 500 g substrate. *G. frondosa* was cultivated on the large bag filled with 2.5 kg substrate, and *P. nameko* was cultivated on the bottle filled with 700 g substrate. The medium was sterilized by autoclaving at 120^0C for 30 min and then allowed to cool to room temperature. The sawdust spawn was inoculated at the surface of the plastic bag and bottle substrates, respectively.

All cultures were cultured in the dark during the early phase of fungal growth until 14 days from inoculation, and then were exposed

to 500 lux intensity of 12 hr intervals of cool-white fluorescent illumination. Incubation days for mycelial growth and fruiting treatment were specific for various mushrooms. The plastic bag and bottle cultures were continuing incubated longer to continue vegetative mycelial growth. The fructification stimulation, kinkaki treatment (removal of both spawn and the uppermost layer of the medium) was done with the fully matured cultures of *F. velutipes, H. marmoreus, P. ostreatus, P. eryngii, P. abalonus, and A. cylindracea*. Chilling treatment (shift down of temperature) was given to all species cultures after various incubation days. Fruit body production was measured under the low temperature conditions for various days.

Measurement of culture maturity

Ergosterol content and water potential of the substrate were measured in order to estimate the culture maturity. Ergosterol measurement was done according to the method as described previously (Ohga and Donoghue, 1998; Ohga and Wood, 2001). Water potential (ψ) of the cultures was measured by the thermocouple psychrometer (Wescor HR-33T micro voltmeter coupled to C-52F sample chamber).

Pulsed power treatment

Pulsed power treatments were done just before the fruiting stages. Pulsed power of 100-170 kV (1.0 kWS) was directly shot through the electrodes from the instrument (Yushin Tech. Co. Ltd) to the mature sawdust-based substrates.

RESULTS AND DISCUSSION

Maturity of substrate at the electric impulse treatment Ergosterol content

Mycelial growth and physiological condition were judged from the ergosterol content and water potential at the time of electric impulse treatment. Ergosterol content is suitable indicator for judging the substrate maturity. The substrates were moved to the production room on day 90. First, ergosterol content was measured at this phase. Ergosterol content of the strain KS-6 was high, and followed by the strains KS-1 and KS-2. Low level of ergosterol content in the strain KS-12 suggests insufficient maturation of the sawdust-based substrate. Ergosterol content of the substrate in the full grown was more than 2000 μg/g. Second flush was induced on day 130 by the water soaking treatment. Ergosterol content of all substrates has already reached to 2000 μg/g at this stage. Third flush was induced on day 170 by the water soaking treatment. Ergosterol content decreased by the fruit body formation during first and second production stages. The substrate of the strains for autumn and year-round cultivation were seemed to full maturity. Only the strain for winter cultivation maintained useful culture maturity at this stage.

Water Potential (Ψ)

Water condition of the sawdust-based substrate is one of the most important factors on the fruit body formation. The ψ of the substrate has clear relationship to capacity of fruit body formation. The value of ψ for spontaneous fruiting was from –0.5 to –0.7 MPa. The substrates of three strains except for the strain KS-12 were suitable for fruit body formation on the day 90. The strain KS-12 has not reached to the sufficient maturation at day 90 under the culture conditions. On day 130, all of the substrates were suitable for fruit body formation. The values of ψ indicated about –0.5 MPa at the growth phase. On day 170, the values of ψ indicated quite high because of the senescence of the substrates. The capacity of water holding might be decreased at the growth phase. The strain KS-12 maintained the suitable water condition for fruiting even on day 170.

Effect of pulsed power on fruit body production

First flush spontaneously occurred at 5 to 15 days after the pulsed power treatment. Pulsed power resulted to more production of fruit-body in all tested strains. Difference between the pulsed

power and control was already recognized in the primodia formation stage. Number of primodia on the treated substrate was more than that of control. Shapes of pilei and stipe of the fruit body were normal in the treated substrates. Second flush was induced by water soaking for 16 hr on day 130. Fruit body formation occurred at 5 to 20 days after the pulsed power treatment. Effect of the treatment appeared more clearly than the 1st flush. Third flush was also induced by water soaking for 20 hr on day 170. Fruit body formation occurred at 10 to 30 days after the pulsed power treatment. Effect of the pulsed power in this time was the strongest of all period. The fruiting capacity was actually promoted by the pulsed power. Though, decrease of fruit body production in the 3rd flush is usual pattern on the sawdust-based substrate of *L. edodes*, the pulsed power treatment improved the yield.

Treatment of pulsed power promoted the fruit body formation of *L. edodes*. The effect was clearer on the aged substrates at 3rd flush. Pulsed power treatment will be contributed to the sawdust-based cultivation of *L. edodes*. We have also reported the possibility of enhancement of fruit body formation of the mycorrhizal fungi (*Laccaria laccata*) with pulsed power in nursery and plantations (Ohga and Iida, 2001). Further works are needed to clarify the relationship between fungal physiology and the pulsed power.

Pulsed power resulted to more production of fruit body in all tested mushrooms. Fructification occurred at 5 to 10 days after the pulsed power treatment. Difference between the pulsed power treatment and control was already recognized in the primodia formation stage. Number of primodia on the treated substrate was more than that of control. Shapes of pilei and stipe of the fruit body were normal in the treated substrates.

Pleurotus abalonus was most sensitive for the pulsed power stimulation. Growth ratio (yield on treated substrate / yield on control) indicated 173% by the pulsed power treatment. The value was 168% in *P. ostreatus*, and 150% in *P. eryngii*. In general, the treatment may be effective to *Pleurotus* sp. judging for the significant differences at $p < 0.01$. Growth ration of *A. cylindracea* and *H. marmoreus* were 156% and 136%, respectively. These 2 mushrooms revealed significant differences at $p < 0.05$. There was no significant difference on the substrate of *G. frondosa, P. nameko* and *F. velutipes* in spite of yield increase by the treatment.

The pulsed power treatment also effective to the fructification of *Sparassis crispa* (medicinal mushroom). This mushroom is recently reported for quite high content of β1,3-glucan, and contributed to cancer disease. Fruit body yield increased 130-180% under pulsed power stimulation compared with control.

Difference for the pulsed power treatment was recognized according to fruit body fructification. Single fruiting type was more sensitive for the pulsed power stimulation than gather fruiting type.

The reason of positive effect of pulsed power for fructification is not understand in detail. The cracking phenomenon of mycelia was observed by the pulsed power treatment on the log wood of *L. edodes*. The cracking was concluded the main effect of fructification promotion of *L. edodes*. Pulsed power stimulation was obviously effective to the yield of some edible mushrooms tested here. Various factors have to examine for the positive effect of these edible mushrooms fructification.

Pulsed power treatment has a possibility to apply the large scale industrial production site. Some application tools for convenient pulsed power supply on the substrate will be also improved in the field of technology.

REFERENCES

Ohga S and Donoghue JD. 1998. Evaluation of methods for determining microbial activity and maturity of sawdust-based cultures of shiitake (*Lentinula edodes*). Mushroom Sci. Biotechnol. 6: 115–123

Ohga S and Wood DA. 2000. Efficacy of ectomycorrhizal basidiomycetes on Japanese larch seedlings assessed by ergosterol assay. Mycologia 92: 394–398

Ohga S and Iida S. 2001. Effect of electric impulse on sporocarp formation of ectomycorrhizal fungus *Laccaria laccata* in Japanese red pine plantation. J. For. Res. 6: 37-41

Ohga S, Iida S, Koo CD and Cho NS. 2001. Effect of electric impulse on fruit body production of *Lentinula edodes* in the sawdust-based substrate. Mushroom Sci. Biotechnol. 9: 7-12