Chapter 7

High-Voltage Methods for Mushroom Fruit-Body Developments

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Additional information is available at the end of the chapter

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Abstract

High-voltage electrical stimulation is effective for promotion of fruit-body development in mushroom cultivation. The high voltage applying to cultivation bed of mushroom generates intense electric field inside the bed substrate. The intense electric field accelerates the hypha move owing to the electrostatic force. As a result, some parts of hyphae are cut and scratched. The cutting and scratching of hypha work as stimulation for promotion of the fruit-body development. The promotion effect of high-voltage stimulation to sawdust-based substrate of *L.* and natural logs hosting *Lentinula edodes*, *Pholiota microspora* and *Hypholoma lateritium* are confirmed through the experiment in the cultivation field. The fruit-body formation of mushrooms increases 1.3–2.0 times in terms of the total weight. The accumulated yield of *L. edodes* for four cultivation seasons is improved from 160 to 320 g by applying high voltage of 50 or 100 kV. However, the yield decreases from 320 to 240 g upon increasing applied voltage from 100 to 130 kV. The yield of the other types of mushrooms shows tendencies similar to those of *L. edodes* by applying high voltage. An optimal voltage exists for efficient fruiting body induction.

Keywords: fruit-body development, mushroom cultivation, high-voltage methods, electrical stimulation, *L. edodes*, *Pholiota microspora*, *Hypholoma lateritium*

1. Introduction

Mushrooms such as *Agaricus bisporus* (white button mushroom), *Pleurotus ostreatus* (oyster mushroom), *L. edodes* (shiitake mushroom), *Flammulina velutipes* (enokitake or winter mushroom) are globally cultivated for fresh food or dried food. Some other mushrooms such as

Ganoderma lucidum are cultivated for special medicinal mushroom. Mushroom is fruiting body mainly in basidiomycetous fungi and some ascomycetous fungi. Therefore, mushrooms are developed for spore formation at reproductive growth phase. Mushroom farming is mainly based on two methods: log-grown or fungus bed-culture which using a pot fill with sawdust-based substrate. The later method offers controllable conditions so that effective mushroom growth can be expected. 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.

Physical phenomena in cells caused by external pulsed electromagnetic energy have a variety of applications on biotechnologies [1]. The electrical stimulation can either destroy the cells and plants or promote its growth rate, depending on the degree of stimulation. In nature, mushrooms extraordinary grow-up around a hit point of a lightning have been reported by some mushroom farmers. Early studies of mushroom growth promotion by artificial lightning were carried out on edible mushroom cultivation using an impulse generator [2]. The output voltage of the impulse generator was more than 500 kV. After that, the high-voltage pulsed power supplies were designed to generate an output voltage from 50 to 130 kV for the electrical stimulation on mushroom cultivation bed. The promotion effects of high-voltage stimulation on sawdust-based substrate of *L. decastes* and natural logs hosting *L. edodes, Pholiota microspora* and *Hypholoma lateritium* were evaluated using the developed compact pulsed power generator [3]. Typical stimulation effects are shown in **Figure 1** as a photograph of cultured *L. edodes* taken on the same day. The upper bed-log was used in cultivation without the high-voltage stimulation. The lower bed-log was used in cultivation and a 50 kV voltage was applied 50 times as stimulation. *L. edodes* in the stimulated log grew faster than that in the bed-log without stimulation. The high-voltage electrode is located on the left side of the log. The fruiting bodies mainly grow near the high-voltage electrode. In this chapter, the effect of high-voltage electrical stimulation on induction of fruiting body of mushroom is described.

Figure 1. Typical photograph of the cultured *L. edodes* with (**bottom**) and without (**top**) electrical stimulation.

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2. Mushroom cultivation and stimulation for fruiting body development

Mushroom is fruiting body mainly in basidiomycetous fungi and some ascomycetous fungi. Therefore, mushrooms are developed for spore formation. Multiple environmental factors such as light, temperature, nutrient, gaseous components influence fruiting body induction and development. These environmental factors are used for sensing appropriate conditions for spore formation and dispersal.

Condition for fruiting body induction is one of critical factor for mushroom cultivation. To establish high yield cultivation method, it is very important to understand effects of environmental factors for fruiting body induction. Environmental factors for fruiting body induction are classified into physiological and physical factors. Gaseous condition and nutrient, or hormones are classified as physiological factor, and wounding or striking as physical factors. Light is one of the important factors for fruiting body induction, and blue light is the effective wavelength. For example, light promotes fruiting body induction in *L. edodes*. In contrast, some species can induce fruiting body in complete darkness. Therefore, light can promote fruiting body development in some species, but not really necessary. Temperature is one of the critical factors for fruiting body induction in basidiomycetes. Especially, down shift of temperature stimulates fruiting body induction in many mushroom species. For example, fruiting body of *F. velutipes* can induce temperature down shift (e.g. 23 → 16°C) in complete darkness. Interestingly, fruiting body formed in complete darkness has tiny cap on its head [4]. It is revealed that proteins expressed specifically during fruiting body formation are regulated by temperature but not by light in *F. velutipes*. Nutrient is another critical factor for fruiting body induction. Especially, high concentration of carbon and nitrogen sources inhibits fruiting body induction. Wood decay fungi are major species for commercially cultivating mushrooms, therefore, wood decay is closely related to fruiting body induction.

Wounding or striking are used for commercial cultivation in several mushroom species. For example, scrapping mycelia on surface of the media (so called Kinkaki in Japanese) is used for fruiting body induction in several mushrooms. Striking log wood is used for stimulation of fruiting body induction especially in *L. edodes*. Electrical stimulation is also a physical factor for fruiting body induction similar to Kinkaki or striking. Japanese farmers have their elders' wisdom that lightning comes crashing into the ground provokes a plentiful mushroom harvest. Electrical stimulation used for stimulating fruiting body induction by mimicking the effect of lightning in nature.

3. History of electrical stimulation for mushroom fruiting body development

The application of a pulsed high voltage to improve the yield in edible mushroom cultivation has also been attempted by some research groups. The fruiting capacity of shiitake mushroom (*L. edodes*) was remarkably promoted by applying a high voltage to cultivation bed-log (wood) [3]. This effect was also recognized in *L. edodes* fruiting on a mature saw-dust substrate [5, 6]. The fruiting body (sporocarp) yield in the electrically stimulated substrate was observed to be 1.7 times more than that without the electrical stimulation [6]. This effect was also confirmed in the fruiting body development of edible mushrooms: *Grifola frondosa*, *P. microspora*, *F. velutipes*, *Hypsizygus marmoreus*, *P. ostreatus*, *P. eryngii*, *P. abalones* and *Agrocybe cylindracea* [7, 8]. The fruiting body yield in the electrically stimulated substrate was observed to be 130–180% greater than that without the electrical stimulation [7]. The high-voltage stimulation technique was also applied to ectomycorrhizal fungi such as *Laccaria laccata* and *Tricholoma matsutake* [9, 10].

Many types of electrical power supplies have been employed to provide electrical stimulation. A large scale 1 MV high-voltage impulse generator was used to stimulate *L. edodes* log wood [2]. High-voltage AC was used to stimulate an *L. edodes* sawdust substrate [5]. Inductive energy storage (IES) pulsed power generators have favorable features for mushroom-cultivating applications, for example, they are compact, cost effective, light, and have high-voltage amplification compared with capacitive energy storage generators such as the impulse generator [11]. The yield of *L. edodes* fruiting bodies was improved with high-voltage stimulation generated by the IES pulsed power generators. The effect of the pulsed voltage stimulation on some other types of mushroom such as *P. microspora* and *L. decastes* was also confirmed using an IES generator developed for the improvement of yield of mushroom production [8]. The harvested weight from log wood and/or sawdust substrates for mushroom cultivation was increased by applying a pulsed voltage as an electrical stimulation.

The mechanism driving the increase in the fruiting body formation by applying high voltage is not clear, but researchers have suggested two possible explanations. One is that the mushroom hyphae are ruptured by applying a high voltage. Physical damage to the hypha stimulates fruiting body formation in mushrooms [5, 7]. The other explanation involves the activation of enzymes. Some enzymes are activated by applying a high voltage, and consequently, mushroom fruiting bodies develop abundantly [2]. Some effects of the high-voltage stimulation were recognized using microscopic observation and chemical analysis. A scanning electron microscope observation indicated that the synthesis of crump connections was accelerated with electrical stimulation [2, 5]. Some types of enzymes, including *laccase* and *protease*, were activated by the electrical stimulation [3, 5, 9].

4. Laboratory test using impulse generator

Early stage of the study on mushroom fruiting promotion and large scale impulse generators was used as artificial lightning for stimulation on the mushroom fruiting promotion. In this section, the laboratory test of artificial lightning stimulation for fruiting body induction using impulse voltage is described.

Figure 2 shows typical photograph of an impulse generator [12]. The impulse generator consists of 10–20 capacitors, gap switches and damping resistors [13]. The capacitors are connected

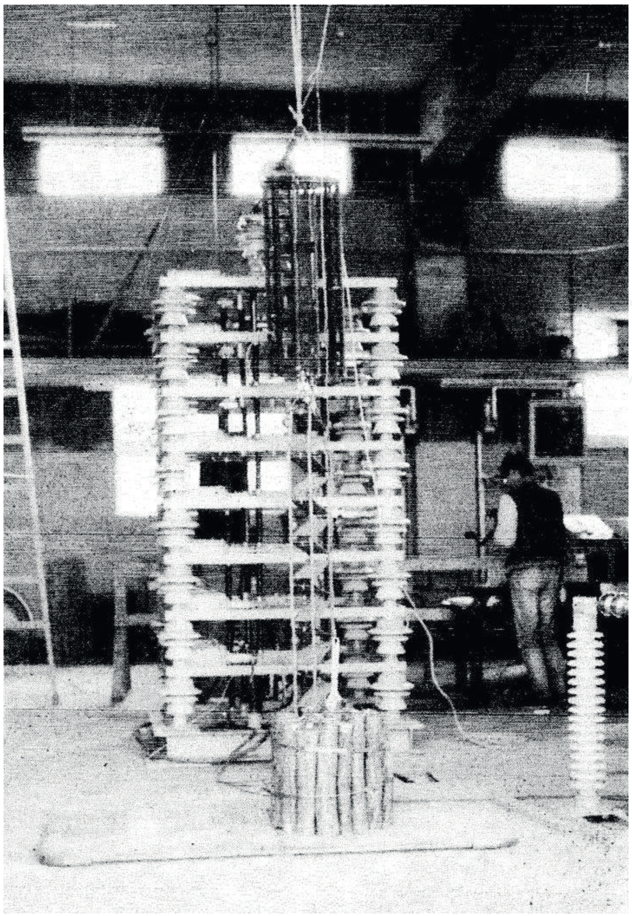

Figure 2. Photograph of impulse generator at stimulation on shiitake mushroom cultivation bed-log [12].

in parallel at charging phase. After charging up the capacitors, the connection of the capacitors is changed from parallel to series using the gap switches. As a result, the output voltage is multiplied by changing the connection of the capacitors. Typical output voltage is in range from 250 kV to 1 MV. The rise time of the output voltage is controlled around the microsecond-order as an artificial lightning stroke voltage. The example of the applied voltage to the bet-log is shown in **Figure 3** [2]. The peak voltage of 288 kV is generated by operating the impulse generator. The rise time of the voltage is close to 0.5 μs as shown in **Figure 3**. In experiments, the bed-logs are connected to high-voltage electrode as shown in **Figure 2**. The bed-logs (Konara oak; *Quercus serrata*) have dimension of 1 m length. The bed-logs 5–9 are bundled or connected in parallel as shown in **Figure 4** for the high-voltage stimulation by impulse generator. The impulse high voltages are applied to the bed-logs bundle or top of the bed-logs connected in parallel. After the stimulation, the bed-logs are cultivated for fruiting body formation. The yielding rates of the fruiting bodies on the bed-logs are monitored for each stimulation condition.

Typical results of the stimulation on yielding rate of *L. edodes* fruiting bodies are shown in **Tables 1** and **2** for various amplitudes of applied voltage. The numbers of the bed-logs are 24 and 21 for each experimental condition. The number of fruiting body formation and total harvested yield increase by stimulating high voltage. In both cases, the fruiting body

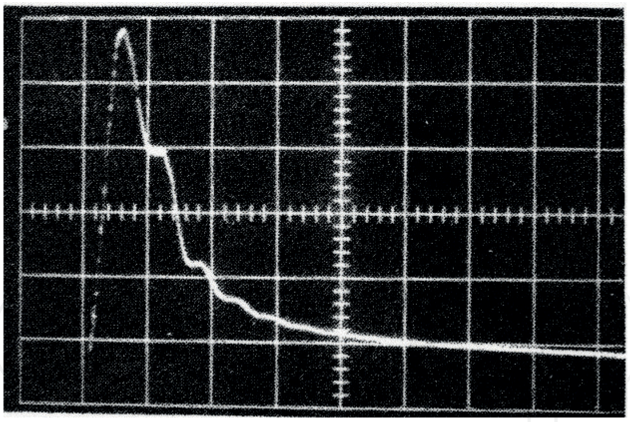

Figure 3. 288 kV output voltage of an impulse generator [2]. X: Time (1 μs/div.), Y: Voltage (50 kV/div.).

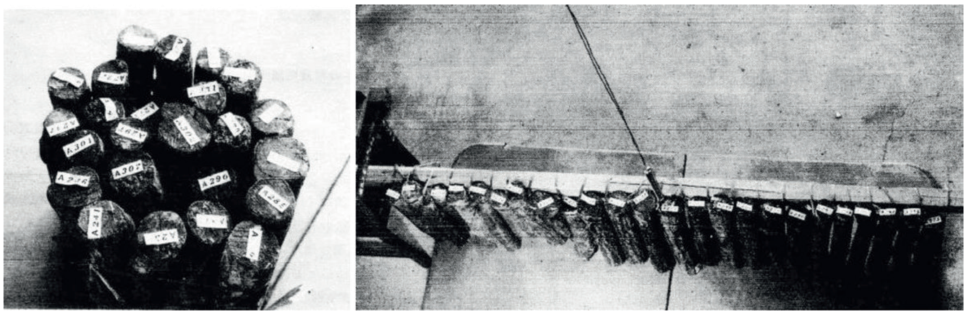

Figure 4. Photographs of setup of bed-logs for impulse high-voltage stimulation [12].

Exp. group.	Number of exp. bed-logs	Fruit-body yield (per 1 m^3 of wood) Number	Dry wt (g)
144 kV	2 4	505.3	1337.0
288 kV	2 4	770.1	2171.4
576 kV	2 4	121.6	558.4
Contd.	2 4	16.9	55.2

Bed-log age: 38 months after inoculation (Yakult haru 2). Water content of bed-logs: 38.9% (mean value of six samples). All exp. groups had 34 mm rainfall in a week after discharge.

Table 1. Fruit-body yield of *L. edodes* of bed-logs using high-voltage stimulation without submergence treatment [2].

yields increase by applying impulse high voltages as stimulation for fruiting body forming. However, the optimum amplitude of impulse voltage for improving fruiting body yield exists as **Tables 1** and **2**. The fruiting body yield at 288 kV impulse voltage is larger than those at 144 and 576 kV applied voltage as shown in **Table 1**. When an electrical field E is generated by applying impulse high voltage to the bed-logs, hyphae will thus be subjected to a Coulomb force f ($f = qE$; q means total charge of the hypha) from the electrical field. As a result, the

Exp. group.	Number of exp. bed-logs	Fruit-body yield (per 1 m^3 of wood) Number	Dry wt (g)
288 kV	2 1	650.8	2100.0
576 kV	2 1	485.8	1648.9
720 kV	2 1	453.8	1427.4
Cont.	2 1	276.2	840.6

Bed-log age: 38 months after inoculation (Yakult haru 2). Water content of bed-logs: 42.3% (mean value of six samples).

Table 2. Fruit-body yield of *L. edodes* of bed-logs using high-voltage stimulation with submergence treatment [2].

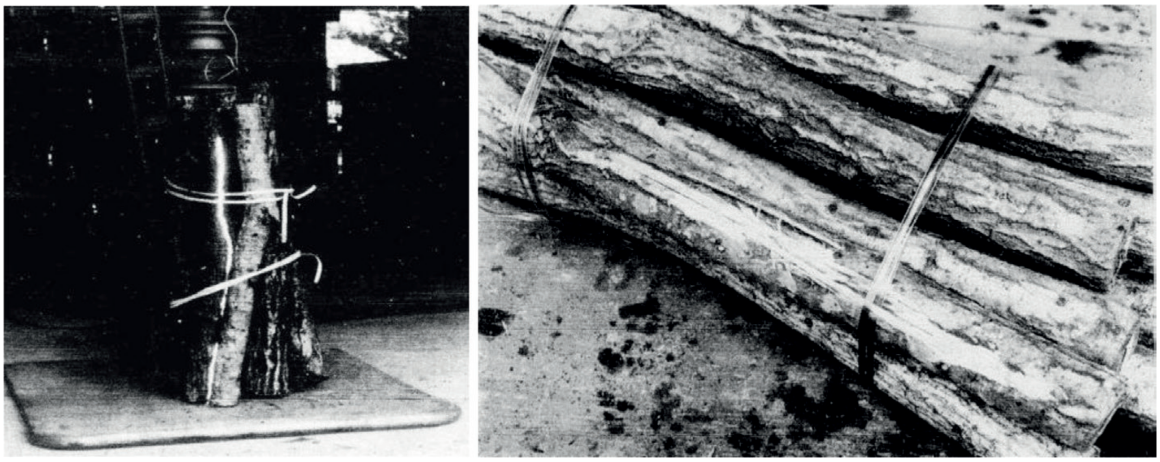

Figure 5. Photographs of electrical discharge on surface of the bed-log and crack of the bed-logs by impulse high-voltage application [12].

hyphae are accelerated towards the positive electrode according to the equation $f = ma$, where m and a mean mass of the hypha and acceleration of the hypha, respectively. The application of electric pulses, resulting in hyphal displacement and sometimes damage, can be considered as a form of physical stress. The physical stress works as trigger to promote the fruiting body formation. However, when the applied voltage is too high compared with the optimum condition, the physical damage of the hypha is too much for stimulation of fruiting body promotion. Sometimes the bed-logs are also damaged by the high pressure wave (shockwave) caused by electrical discharge and impulse high current as shown in **Figure 5** [12].

The frequencies of the fruiting body yield by impulse high-voltage stimulation under same condition with **Table 1** are shown in **Figure 6** [2]. In the control case (without high-voltage stimulation), the fruiting body cannot be harvested for 20 bed-logs (83%). One fruiting body can be harvested from four bed-logs (17%). However, the fruiting bodies can be harvested from 21 bed-logs (except 3 bed-logs; 12%) at 288 kV impulse voltage applying. The decrease of number of the bed-log without *L. edodes* fruiting bodies mainly contributes to increasing yield of mushroom by applying high-voltage shown in **Table 1**.

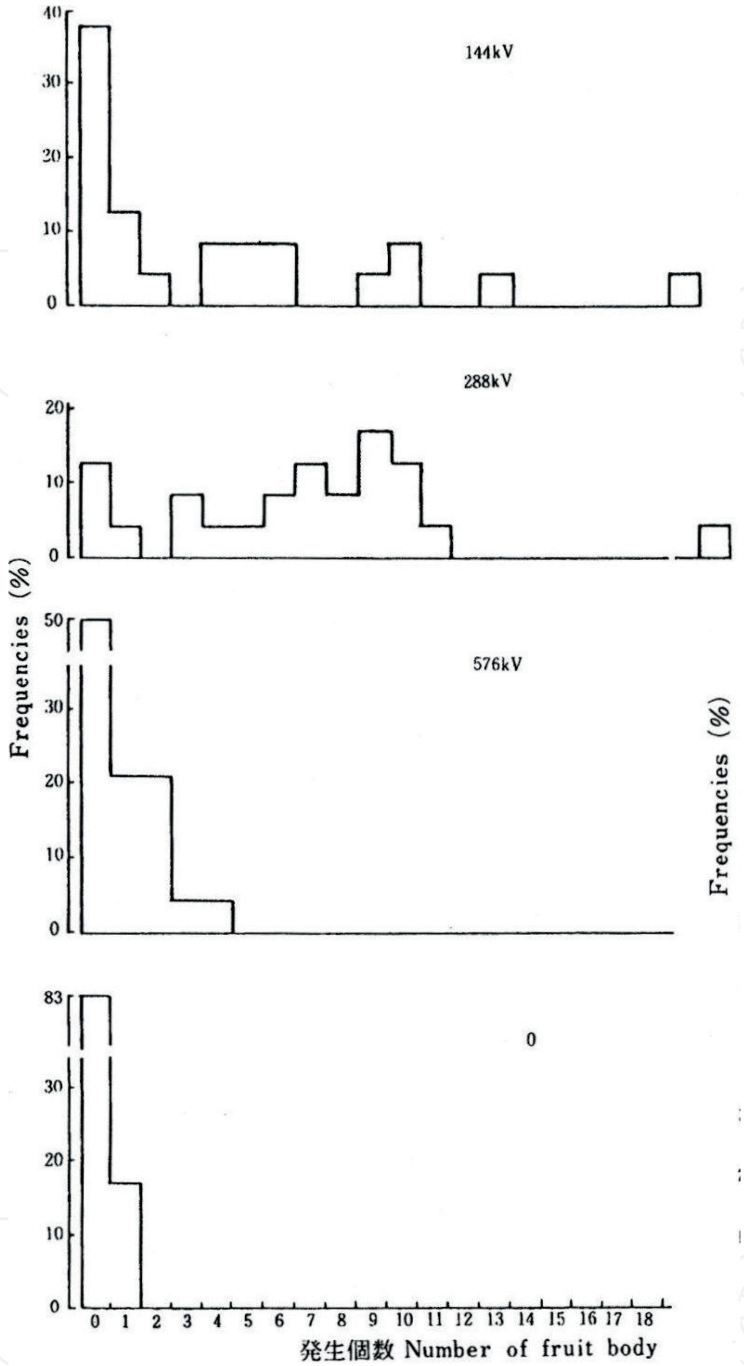

Figure 6. Frequencies of the fruit-body yield by impulse high-voltage stimulation to *L. edodes* of bed-logs without water submerged treatment [2].

5. Field test using compact high-voltage generator

The impulse generator has huge size for utilization in mushroom-cultivating field as shown in **Figure 2**. Some types of compact high-voltage pulse generator were developed for promotion of the fruiting body formation on bed-logs or sawdust bed-blocks (substrate) of mushroom cultivation.

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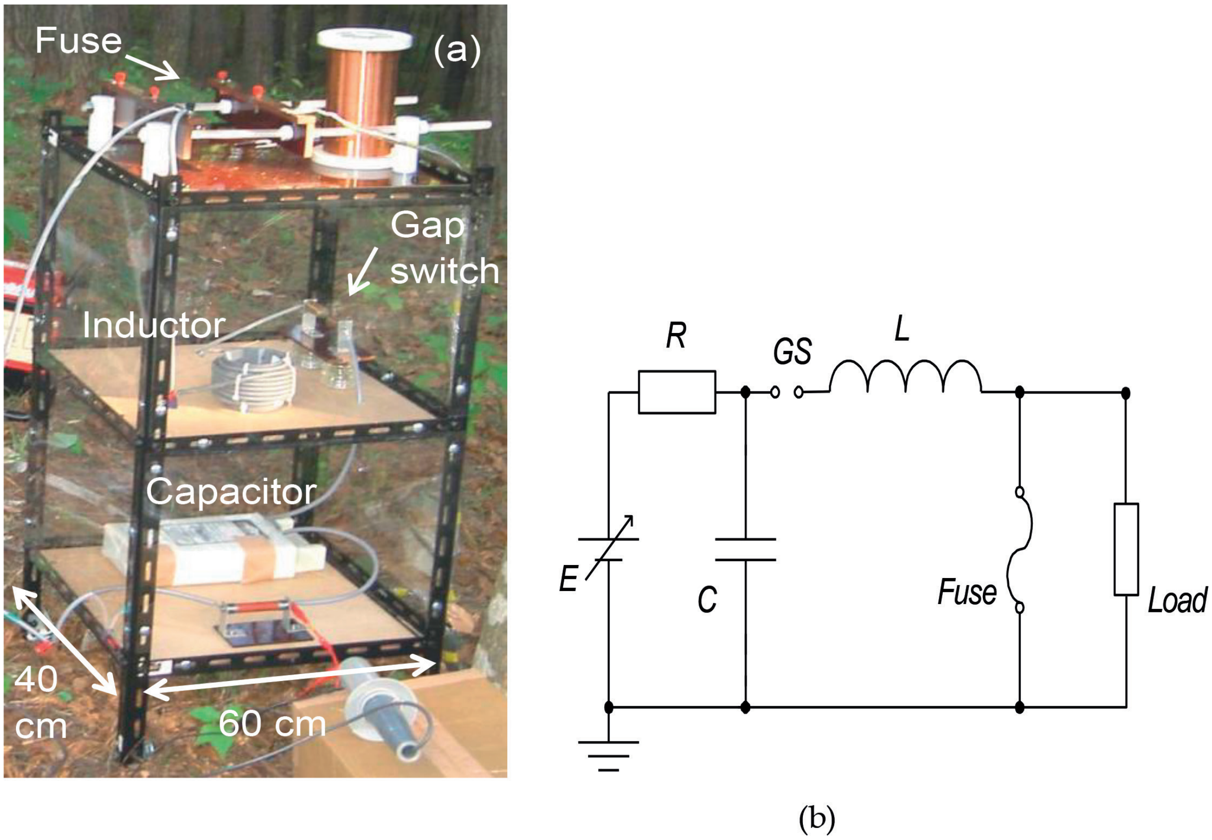

Figure 7. IES pulsed power generator with fuse opening switch; (a) photograph and its circuit. (*C*: Primary energy storage capacitor, *L*: Secondary energy storage inductor) [8].

Figure 7(a) and **(b)** shows photograph and equivalent circuit of a compact pulsed power generator used for promotion of fruit-body formation in natural-log based mushroom cultivation [8]. An inductive energy storage (IES) system consists of a primary energy storage capacitor C, a closing switch GS, a secondary energy storage inductor L and an opening switch. A thin copper fuse is used as the opening switch to interrupt large current in short time. **Figure 8(a)** shows typical circuit current and output voltage waveforms at 12 kV charging voltage. The 8 cm-length fuse and the 15 μH-inductance secondary energy storage inductor are used. The current starts to flow after closing the switch GS with LC oscillation. The circuit current is interrupted after fuse melting phase within 50 ns. The output voltage increases rapidly and has a 120 kV maximum voltage. This output voltage corresponds to 10 times amplification. The high voltage pulse is produced by the total circuit inductance and rapid current interruption produces a high-voltage pulse expressed as

$$v = V_0 - \frac{1}{C}\int i dt - L\frac{di}{dt} \approx -L\frac{di}{dt}, \tag{1}$$

where *i* means the circuit current. The output voltage waveforms for various charging voltages are shown in **Figure 8(b)**. The peak voltage increases from 80 to 130 kV with increasing charging voltage from 10 to 16 kV. These values correspond to 8.0 and 8.1 of voltage amplification factors.

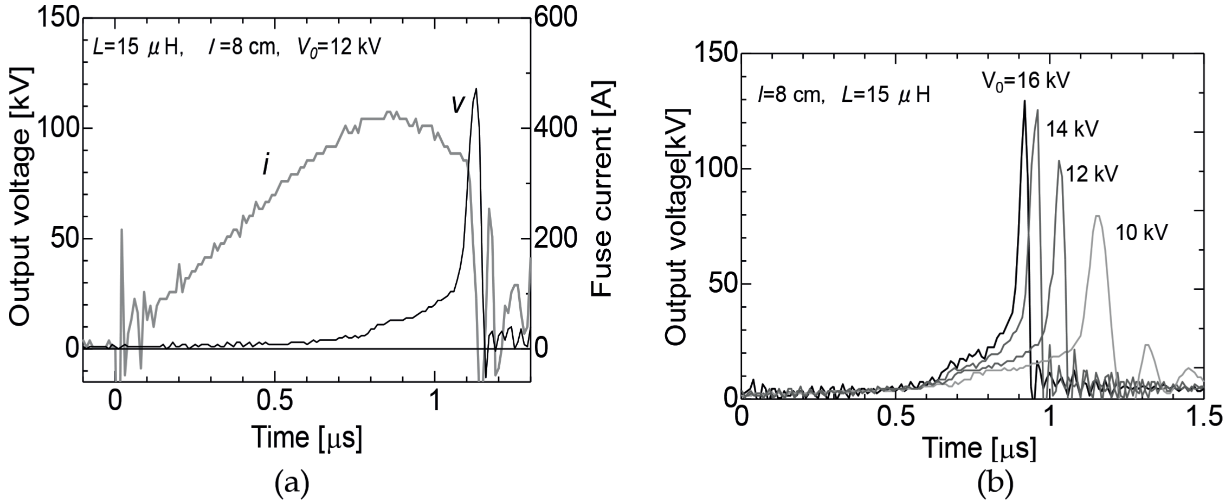

Figure 8. Typical waveforms of (a) circuit current though the fuse and output voltage at 12 kV charging voltage and (b) output voltage for various charging voltages [8].

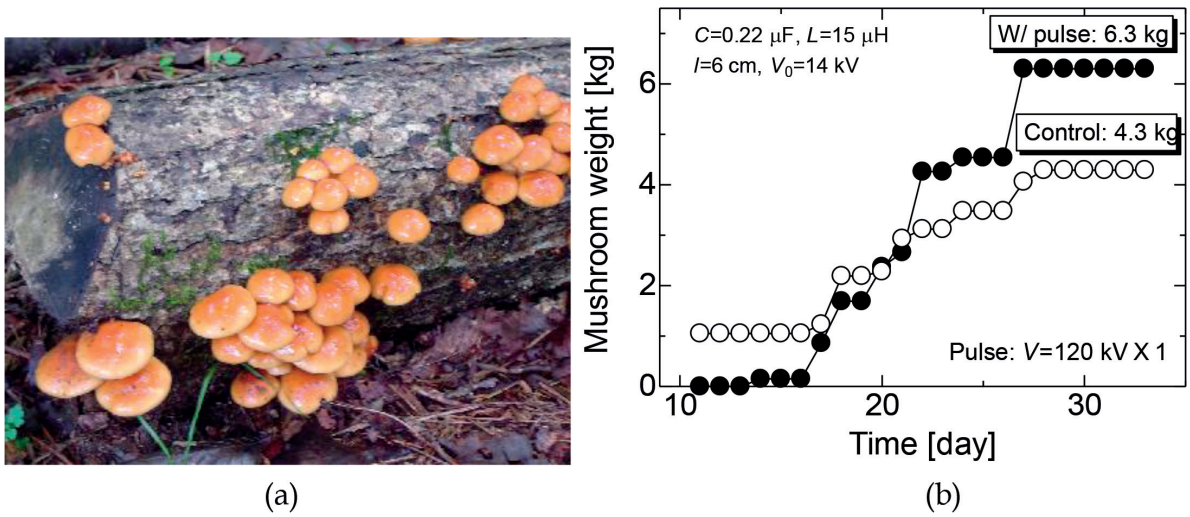

Figure 9. The cultured *P. microspora*; (a) photograph of fruiting-bodies and (b) its total weight yield as a function of days from the stimulation of 120 kV applied voltage [8].

Figure 9(a) shows the total weight of *P. microspora* mushroom cropped by 15 logs as a function of days from the high-voltage stimulation [8]. The logs of applying voltage group are stimulated with the pulsed voltage of 120 kV. The 15 logs of the control group are not stimulated. **Figure 9(b)** shows the photograph of cultured *P. microspora*. The *P. microspora* start to appear about 2 weeks after the stimulation and stop to appear at day 26. The yield of *P. microspora* is improved with the pulse voltage stimulation. The total weight of the cropped *P. microspora* with the high-voltage stimulation is 6.3 kg. This value is 1.5 times larger than 4.3 kg total weight under condition without the stimulation.

Figure 10(a) and **(b)** shows photograph and equivalent circuit of a compact pulsed power generator based on combining IES with Marx circuit to reduce the primary charging voltage [14]. After charging up the four primary energy storage capacitors, the gap switches GS are

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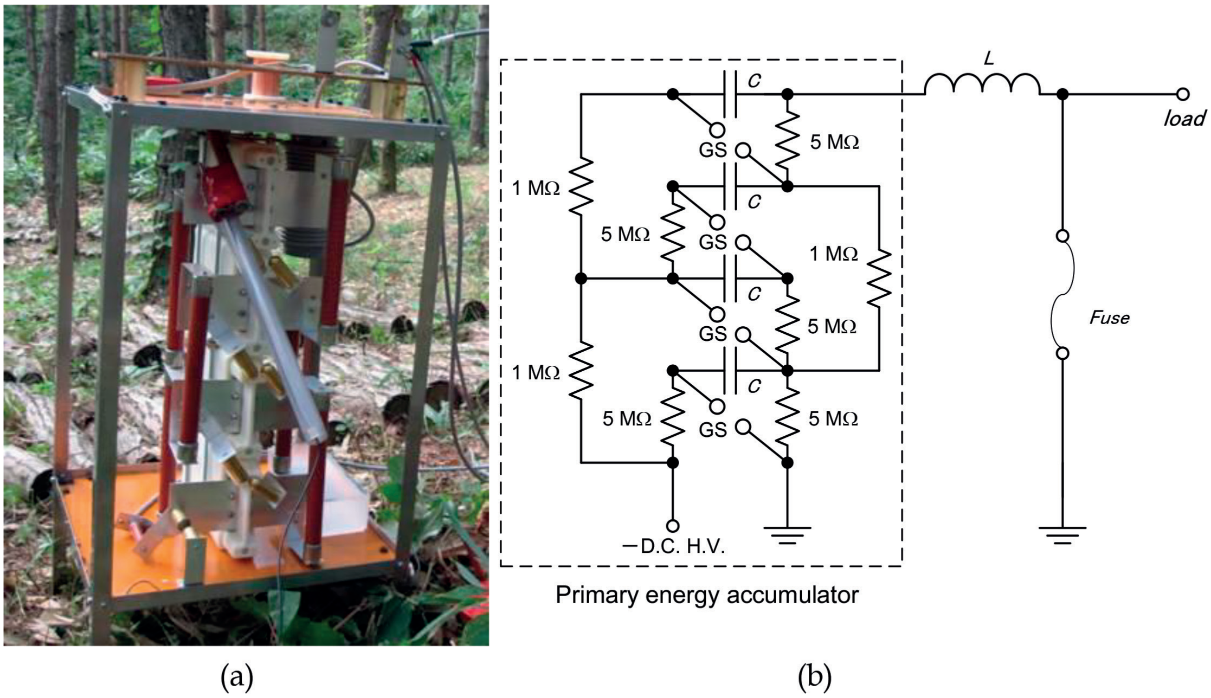

Figure 10. Marx-IES pulsed power generator with fuse opening switch; (a) photograph and (b) its circuit with fuse opening switch. (*C*: Primary energy storage capacitor, *L*: Secondary energy storage inductor) [14].

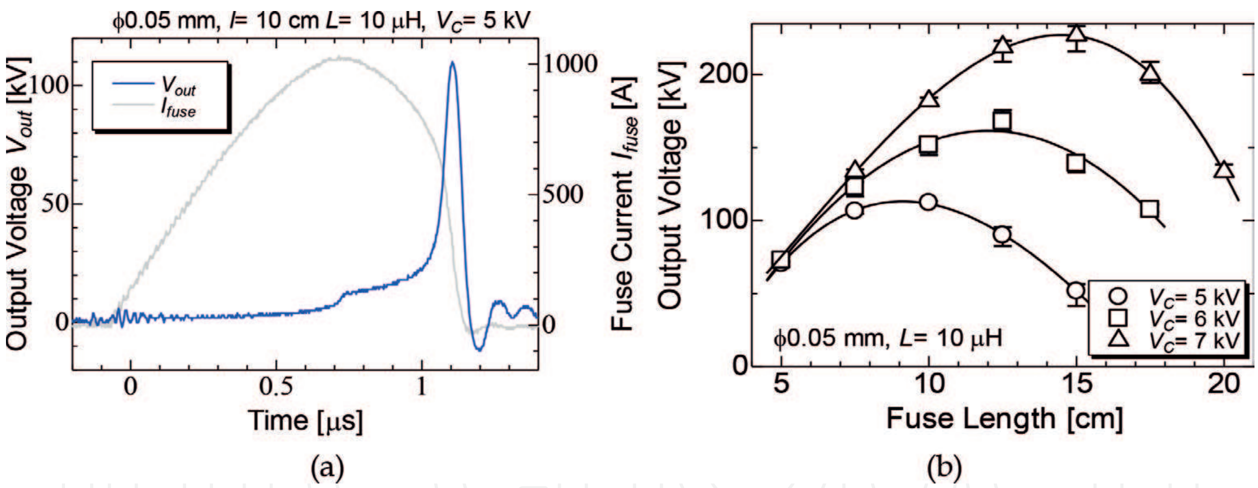

Figure 11. Typical waveforms of (a) circuit current though the fuse and output voltage at 5 kV charging voltage and (b) output voltage as a function of fuse length for various charging voltages of the primary energy storage capacitor [14].

triggered externally. The closing switch GS changes the connection of the capacitors from parallel to series. As a result, the voltage is multiplied from V_C to 4 V_C in same manner to the Marx generator. **Figure 11(a)** and **(b)** shows typical waveforms of the circuit current and output voltage at 5 kV charging voltage and peak voltage as a function of fuse length for various charging voltages of the primary energy storage capacitor, respectively. The circuit current starts to flow after closing the switch GS with LC oscillation. The circuit current is interrupted after fuse melting phase. The output voltage increases rapidly and has a peak voltage of 110 kV. This peak voltage corresponds to 22 amplification defined as the ratio of

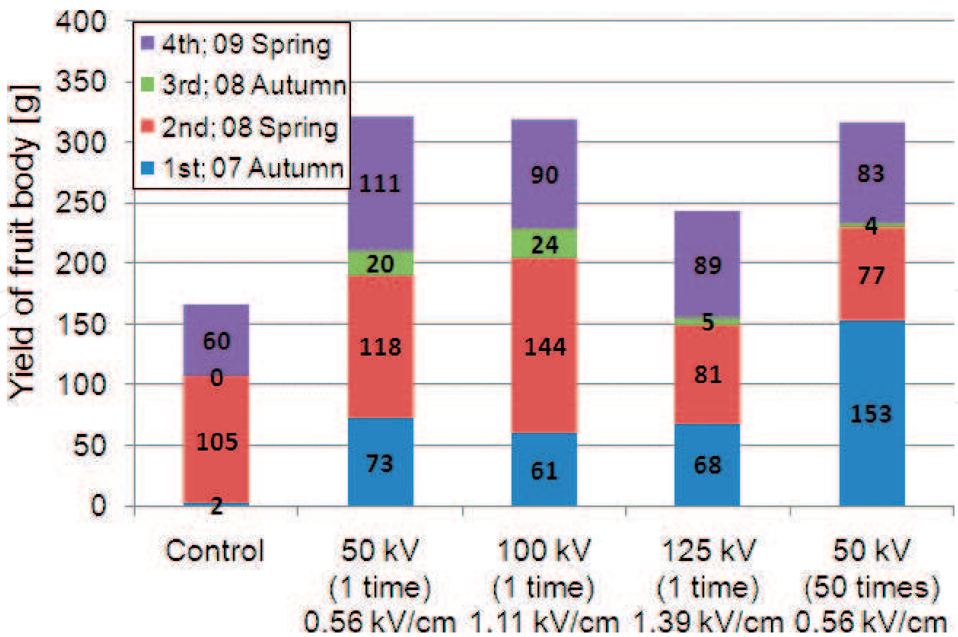

Figure 12. Total weight of cultured *L. edodes* for various electrical stimulation conditions. The total yield are 167, 322, 319, 243 and 317 g for control, 50 kV-1 time, 100 kV-1 time, 125 kV-1 time and 50 kV-50 times, respectively [3].

the maximum output voltage to the charging voltage. The peak voltage increases from 110 to 230 kV with increasing the charging voltage from 5 to 7 kV. These values correspond to 22 and 33 of voltage amplification, respectively.

Figure 12 shows the *L. edodes* yield for different applying voltages. One group is cultured without high-voltage stimulation (control group). Three groups are stimulated by a single high-voltage pulse (one time application) at three different amplitudes: 50, 90 and 125 kV. The last group is stimulated 50 times with a 50 kV pulsed voltage. The yield of the fruit body is evaluated as the total weight harvested during four seasons. It includes the crops from all 15 logs, appropriately averaged without statistical analysis. The yield of the control group was only 2 g in the first harvesting season, autumn of 2007, because the *L. edodes* species used in the present experiment mainly fruits in the spring. In this case, the 30 g weight of fruit bodies is harvested from only one log. Therefore, the standard deviation is 7.5 g, which is larger than the 2 g average weight. This result indicates that the mushroom species employed in the experiment usually does not develop fruit bodies. However, the yield from the first season increased from 2 to 73 g when a 50 kV pulsed voltage is applied. The yield increased from 73 to 153 g when the number of pulses increased from 1 to 50. In this case, the standard deviation is determined to be 73.0 g, which is lower than the 153 g average weight. This result indicates that the mushrooms develop fruit bodies as the result of applying high voltages. The total harvested weight over four seasons is 167 g in the control group. The yield increases to 322 and 319 g when pulsed voltages of 50 and 100 kV are applied, respectively. However, the yield decreases to 243 g at 125 kV voltage applying. This result indicates that optimum voltage amplitude exists and is estimated in range from 50 to 100 kV/m.

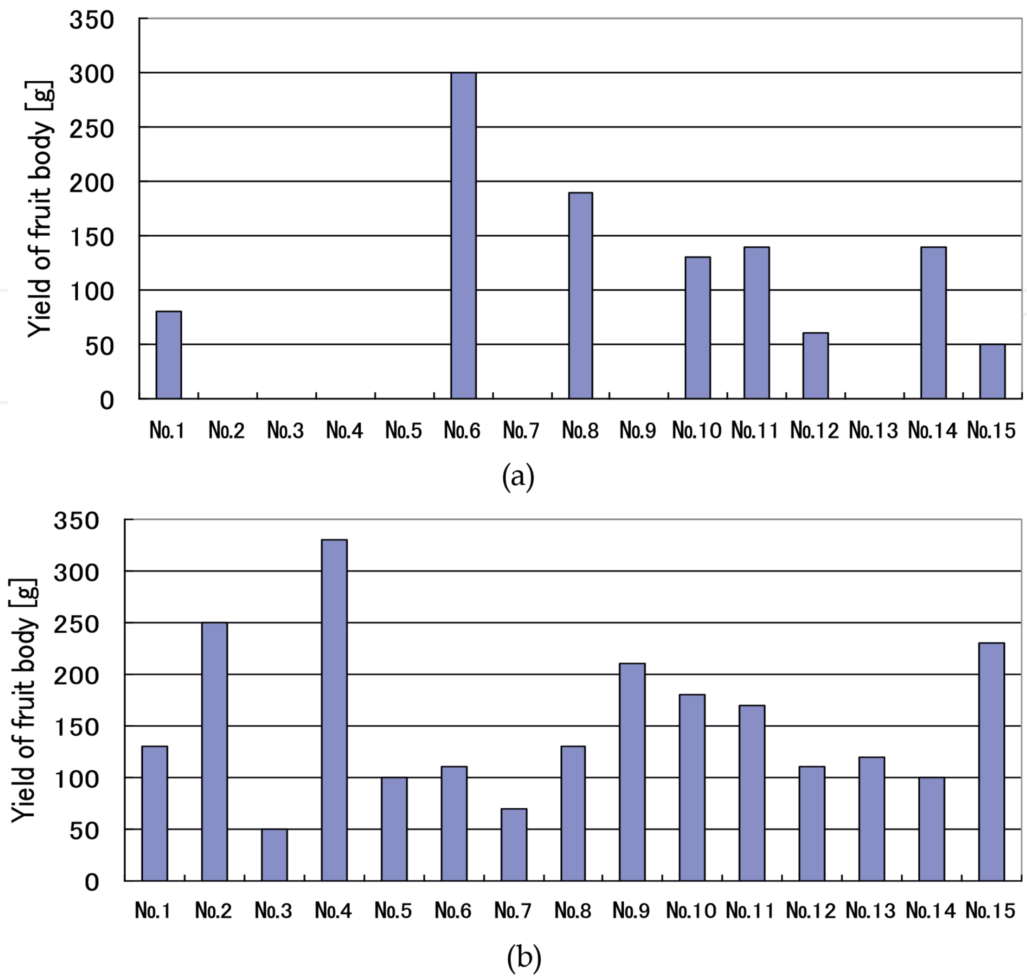

Figure 13. Difference in the yield of fruit bodies of *L. edodes* based on the number of 50 kV applied voltage treatments received. No. 1–15 indicates labels for each cultivation log. (a) One-pulse stimulation; (b) 50-pulse stimulation [3].

Figure 13 shows the weights of *L. edodes* harvested from each log at two different numbers of pulse voltage stimulation. The applied voltage was 50 kV in all cases. The total weight from the logs after 50-pulse stimulation was 2.29 kg (=153 g × 15), as shown in **Figure 5**, which is larger than the 1.09 kg (=73 g × 15) harvested after a one-pulse stimulation. The maximum value of the harvested fruiting body from one log after a one-pulse stimulation was 300 g, which is similar to the 320 g obtained after 50-pulse stimulations. Although there were no logs observed without fruiting body formation for 50-pulse stimulation, after a one-pulse stimulation, seven logs contained no fruiting bodies. The average yield for one log was approximately 73 g (=1090/15) after a one-pulse stimulation. Only 6 logs showed a yield larger than the 73 g average value, whereas 14 logs showed a yield larger than 73 g in the case of 50-pulse stimulation. This result indicates that on particular logs, use of the pulsed voltage decreased the deviation in the mushroom formation. The standard deviations are 27 and 19 g at one- and 50-pulse stimulations, respectively.

Figure 14 shows the time history of the amount of mushrooms cultured under various stimulation conditions in the spring of 2009. The yield is normalized by the total crop weight for one harvesting season and is evaluated as an aggregate of all crops. The total crop weights

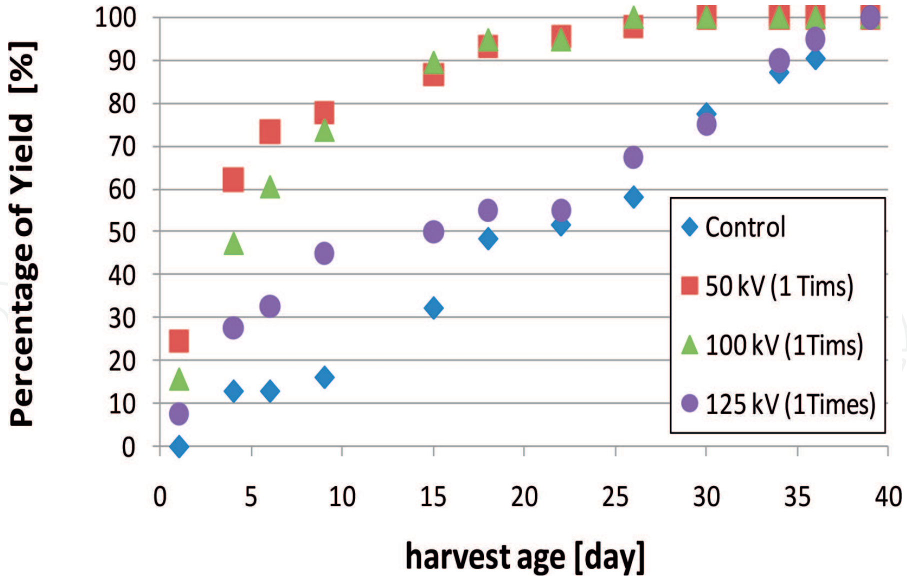

Figure 14. Time-history of the total amount of harvested fruit bodies for various stimulation voltages [3].

were 60, 111, 90 and 89 g in the control, 50, 100 and 125 kV stimulation groups, respectively. Compared with the control group, the total yield increased when applying a voltage of 50 and 100 kV. The harvested weight for 15 days after the first crop (day 18) was approximately 50% of the total in the control group. However, the crop weight during this period increased to 86% of the total when applying voltages of 50 and 100 kV. This result indicates that the mushrooms can be harvested in fewer days by applying high voltage as electrical stimulation.

Figure 15 shows the crop weight of *L. decaste* stimulated with three different voltage amplitudes: 50, 90 and 130 kV. The yield of the fruiting body at the first flash in substrate cultivation

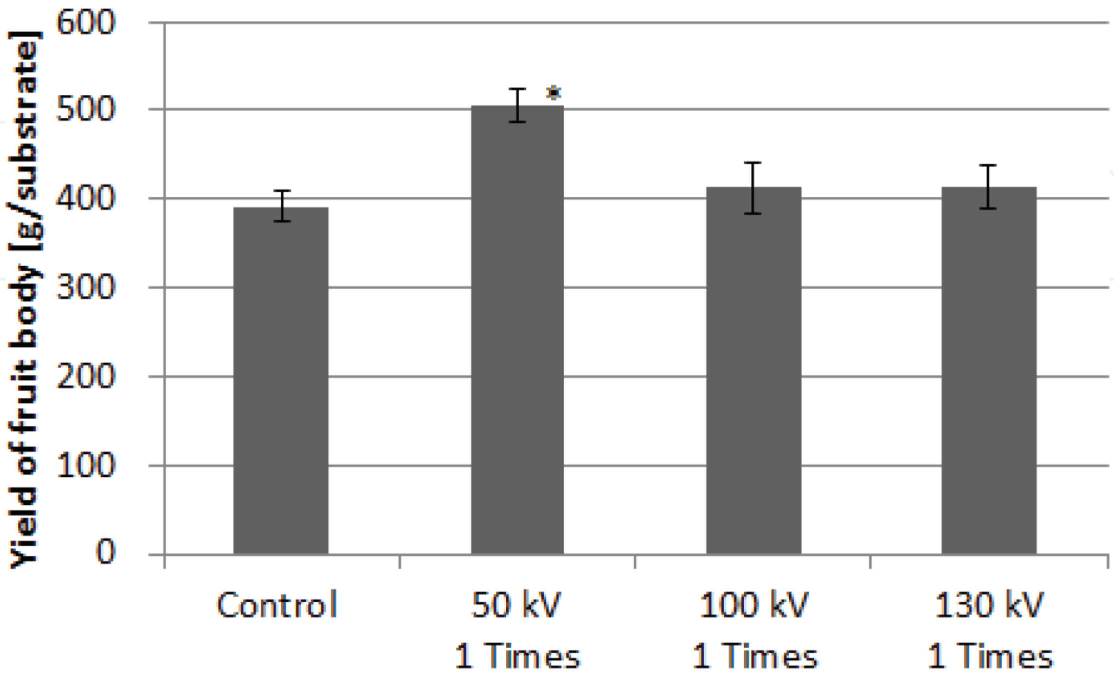

Figure 15. Yield of *Lyophyllum decastes* fruit bodies for various stimulation conditions. Vertical bars indicate the standard errors of the mean (number of samples; $n = 20$). Asterisks indicate the significant differences at $p < 0.05$ (*) [3].

Figure 16. Typical photographs of the cultured *L. decastes* without (left) and with (right) electrical stimulation [3].

was used. The average yield was obtained using the total weight harvested from 20 substrate beds. The average yield of the control group is approximately 392 (±17) g/substrate. The average yield increased to 505 (±19) g/substrate by applying a voltage of 50 kV. The yield was 1.3 times larger than that of the control group with statistical significance of $p < 0.05$. The applied voltage of 100 kV corresponds to 3.57 kV/cm in an averaged electric field. **Figure 16** shows photographs of cultured *L. decastes* taken the same day. The *L. decastes* in the stimulation group grew faster than those in the control group.

6. Morphological changes after electrical stimulation

It is very difficult to reveal how electric stimulation affects fruiting body induction in mushroom species. Because molecular mechanisms for fruiting body induction in mushroom species have not still been well understood yet. Therefore, we focused on morphological changes after electrical stimulation.

Figure 17(a) and **(b)** shows images of *L. edodes* hyphae before (a, red) and after (b, blue) application of electric pulses. **Figure 17(c)** shows a superimposed image of (a) and (b) with purple (red + blue) indicating that hyphae retained the same position before and after applying the pulsed electric fields. Red and blue colored hyphae in **Figure 17(c)** show displaced hyphae. Displacement can be explained by the slightly negative charge of mushroom hyphae. When an electrical field E is applied, hyphae will thus be subjected to a Coulomb force f ($f = qE$; q means total charge of the hypha) from the electrical field. As a result, the hyphae are accelerated towards the positive electrode according to the equation $f = ma$, where m and a mean mass of the hypha and acceleration of the hypha, respectively. The application of electric pulses, resulting in hyphal displacement and sometimes damage, can be considered as a form of physical stress. Other physical stresses such as scrapping of surface hyphae (Kinkaki) have been known to induce fruiting body formation in several mushrooms, suggesting that electric pulses that induce fruiting body formation act through a similar mechanism. **Figure 17(d, e)** shows scanning electron microscope (SEM) images of hyphae before and after applying an electrical pulse of 10 kV between wire electrodes with a gap length of 9 cm. It was observed in the SEM image that after

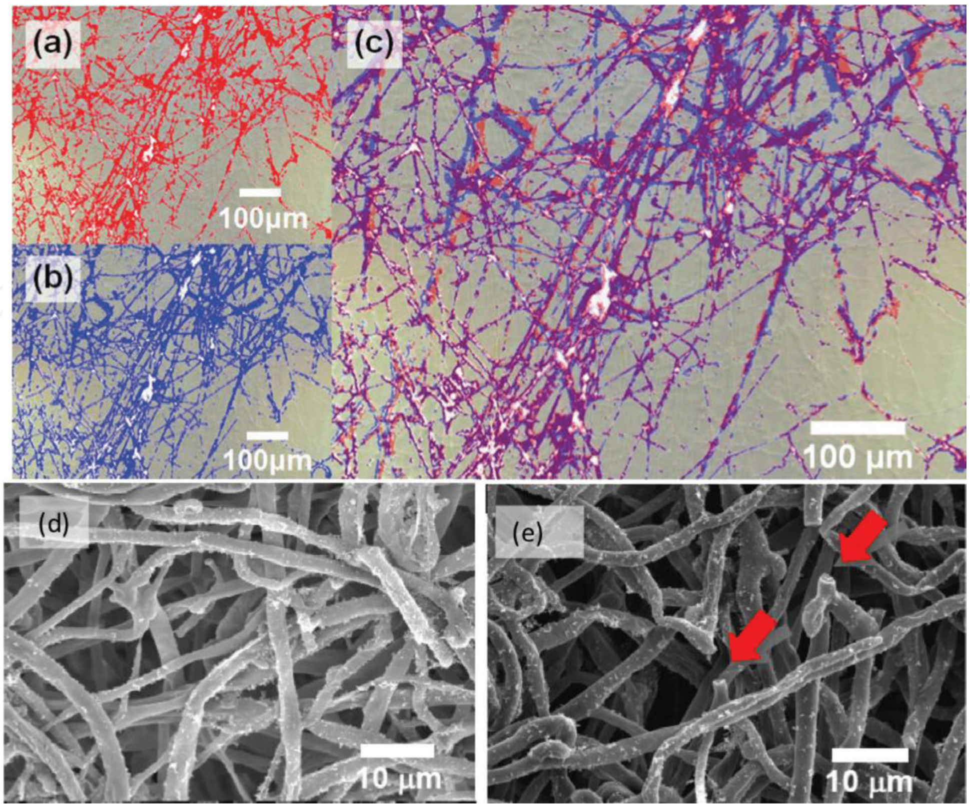

Figure 17. Microscopic images of *Lentinula edodes* hypha (a) before and (b) after applying 5 kV/cm pulse electric field with pulse width of 100 ns and 500 times of repetition. (c) Superimposed image of two images (a) and (b). (d) and (e): SEM images of *L. edodes* hyphae before (d) and after (e) applying 10 kV pulse voltages. White bar indicates 100 μm in (a), (b), (c) and 10 μm in (d) and (e).

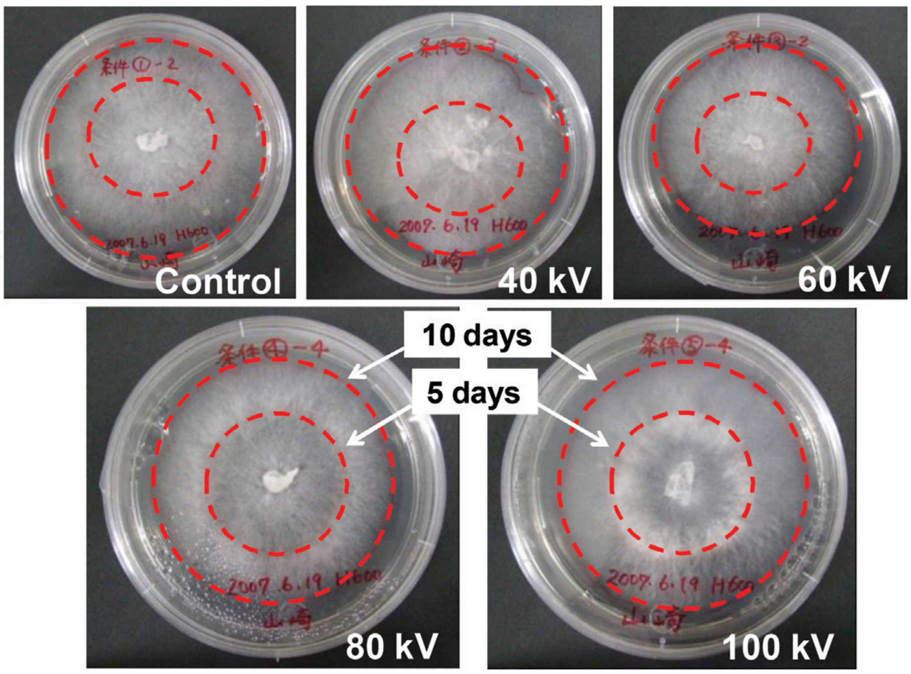

Figure 18. Influence of the pulsed voltage stimulation on hypha growth in agar medium cultivation. The diameter of the petri dishes is 10 cm in the all cases. The inner and outer dotted circles indicate growth positions of hyphae at 5- and 10-days cultivation, respectively [3].

some hyphae were broken by the electric pulse (**Figure 17(e)** arrow). This suggests that the electric pulse will be a similar stimulation as scratching mycelia on the surface of the saw-dust media for mushroom production. Furthermore, it would be possible that new hyphae will be generated after electric pulse stimulation and Kinkaki. Hydrophobin, which is involved in hyphal structure and architecture in fungi [15, 16], would be involved in new hyphae generation after pulse stimulation.

Figure 18 shows typical photographs 10 days after cultivation at various amplitudes of the applied voltage. The pulsed voltage was applied after 5 days of cultivation of *L. edodes* hyphae. The tip positions of the hyphae after 5 days of cultivation were marked by the inner dotted circles. The hyphae grew from the inner to the outer circle positions after 5 days cultivation from the pulse voltage stimulation. From the microscopic observation, the growth direction of the hyphae changed perpendicular to the surface of the agar medium between the inner and the outer dotted circles as the result of applying a high voltage.

7. Conclusions

High-voltage electrical stimulation on fruiting body formation in cultivating mushrooms was described. The compact high-voltage pulsed power supplies were developed for the electrical stimulation to promote fruiting body formation on cultivation bed-logs and sawdust substrate (bed-block). The promotion effects of high-voltage stimulation of sawdust-based substrate of *L. decastes* and natural logs hosting *L. edodes*, *P. microspora* and *H. lateritium* were confirmed through the evaluation using a developed compact pulsed power generator. The fruiting body formation of mushrooms increases 1.3–2.0 times in terms of the total weight. The accumulated yield of *L. edodes* for four cultivation seasons was improved from 160 to 320 g by applying voltages of 50 or 100 kV. However, the yield was decreased from 320 to 240 g upon increasing the applied voltage from 100 to 130 kV. The yield of the other types of mushrooms show tendencies similar to those of *L. edodes* when voltage was applied. An optimal voltage was confirmed for efficient fruiting body induction.

Securing profitability of the electrical stimulation is important for the widespread to the mushroom famers. The pulse voltage stimulation systems for improvement of mushroom yield have been developed and sold by some companies. Typical price of the stimulation system is around 5000 USD. The increment of *L. edodes* yield is around 155 g/(1-log, 2-year) at 50 kV. The price of the *L. edodes* is around 20 USD/1-kg at natural-log cultivation in Japan. If the mushroom farmer uses 1612 logs, the initial cost of 5000 USD can be recovered with increment of the mushroom yield.

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