

EAPPC 2010 / BEAMS2010

(3rd Euro-Asian pulsed power conference
and 18th International Conference on
High Power Particle Beams),
Jeju, Korea, October 10-14, 2010



ThA1-2 Effects of Pulse Voltage Stimulation on Fruit Body Formation in *Lentinula Edodes* Cultivation

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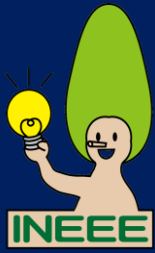
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³ Iwate Biotechnology Research Center, Kitakami, Iwate 024-0003, Japan

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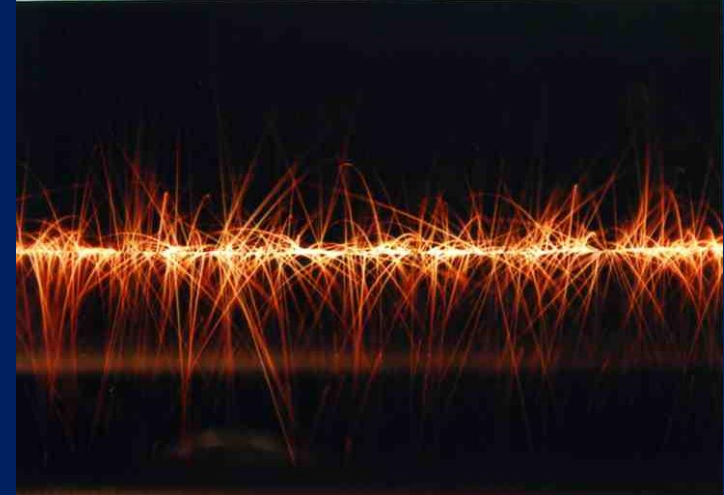
⁵ Nagane Co. Ltd., Hirono-cho, Kunohe-gun, Iwate 028-7906, Japan



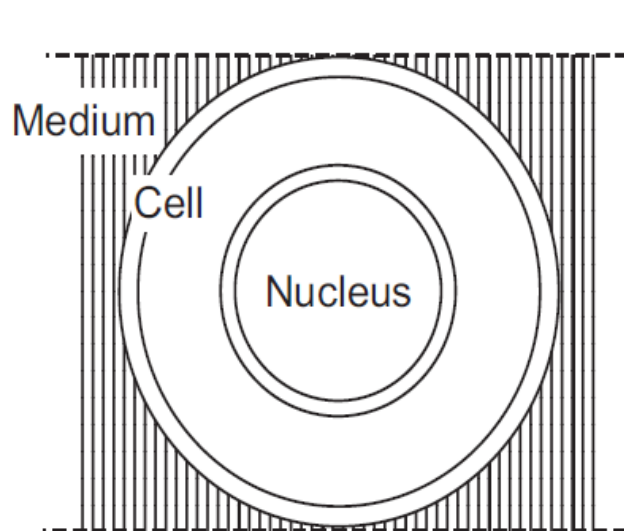
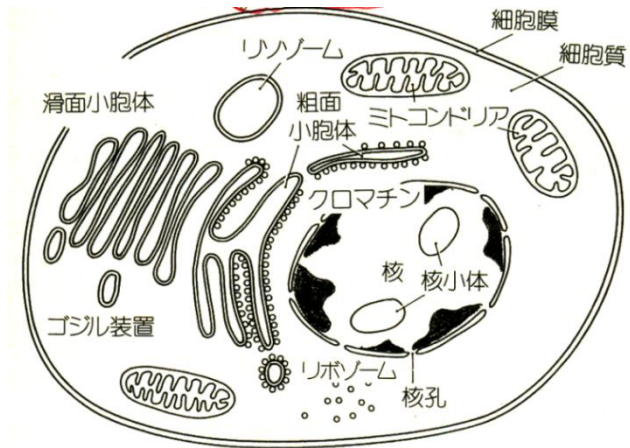
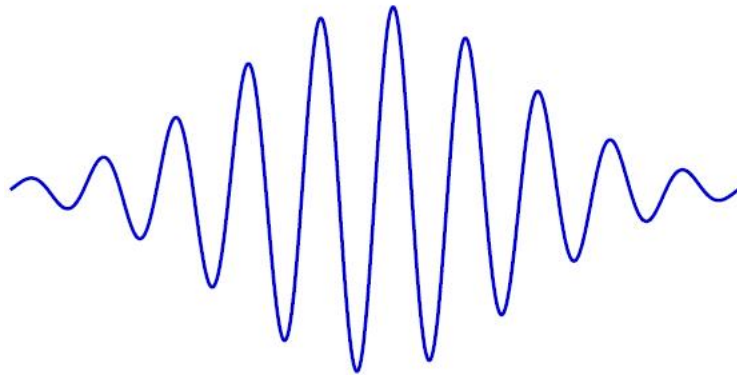
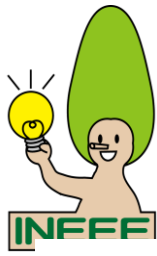
Outline



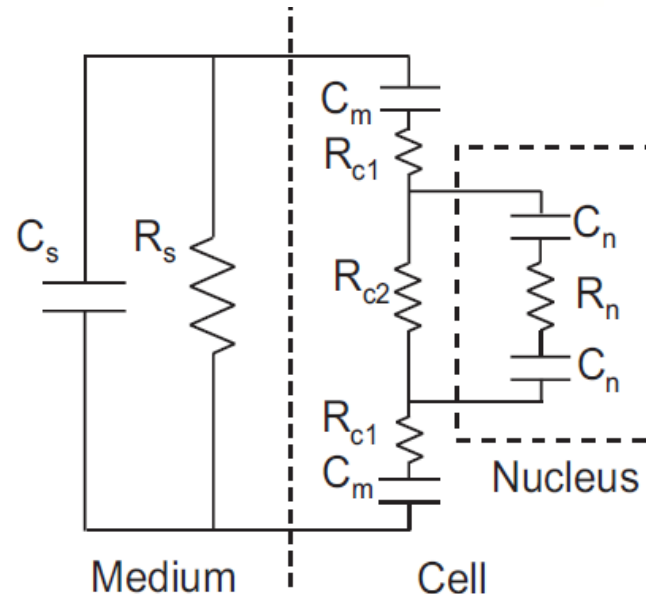
1. Backgrounds
2. IES pulse generator
Design of generator
Output voltage
3. Experimental results
Improvement of **yield**
Hypha activity; **Hyd2, Lcc1**
4. Conclusion



Biological effects of intense AC electric field



Simplified cell model



Equivalent circuit of cell

membrane

cytoplasm

$$C$$

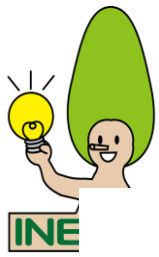
$$\frac{1}{\omega C} \sim \frac{1}{\omega \epsilon}$$

$$R$$

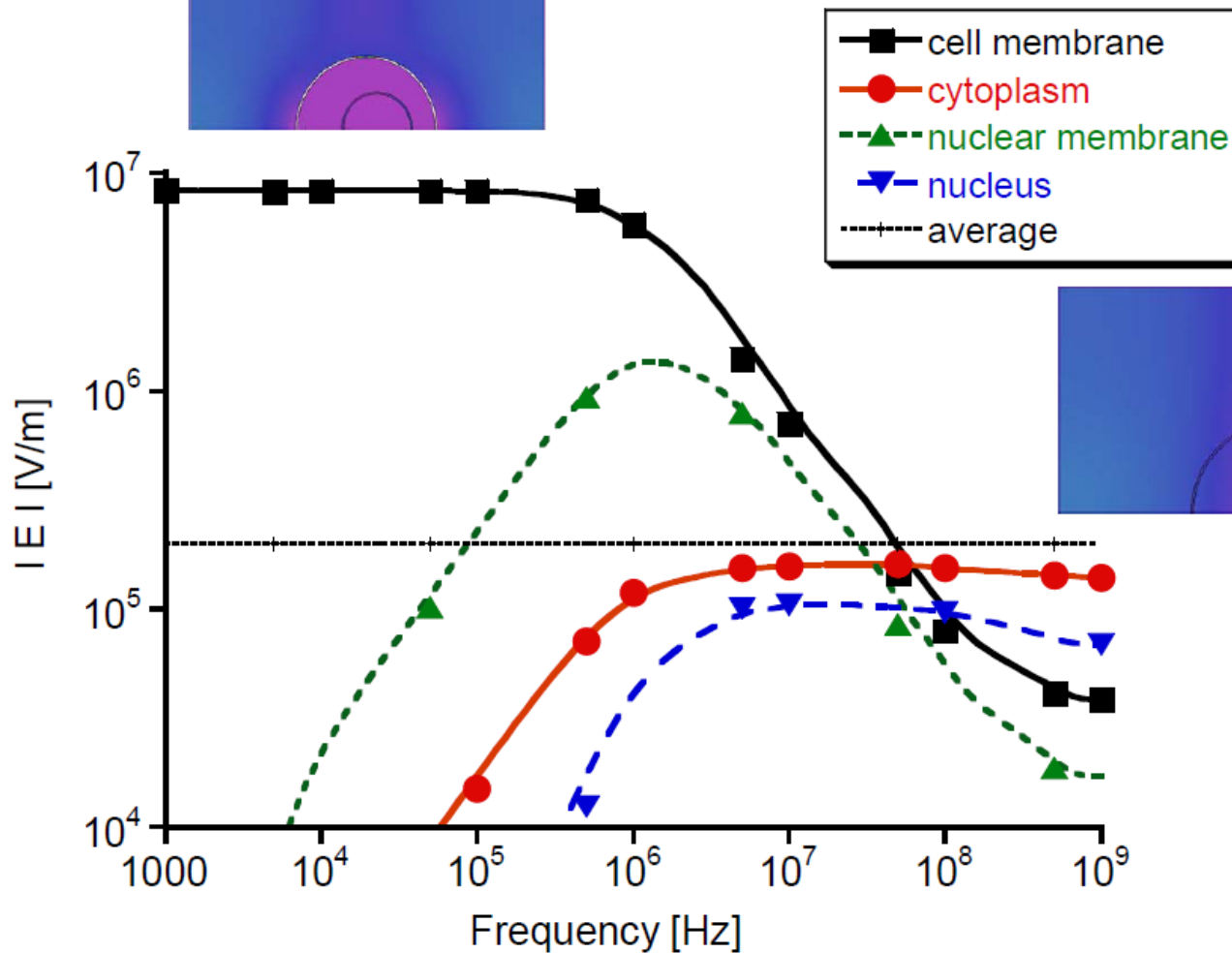
$$R \sim \frac{1}{\sigma}$$



Electric field distribution under AC field

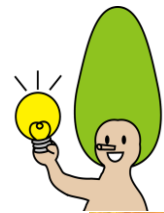


Electric field on cell membrane causes pore formation on it.
(electroporation)

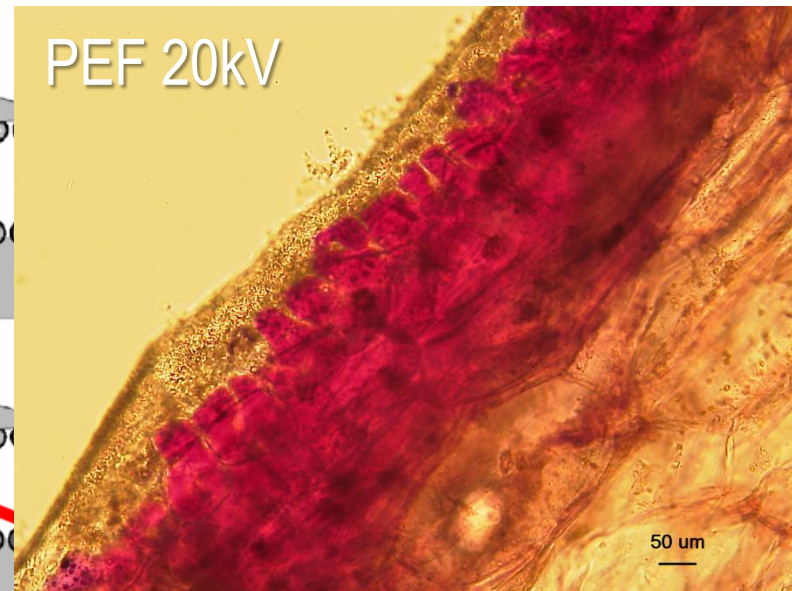
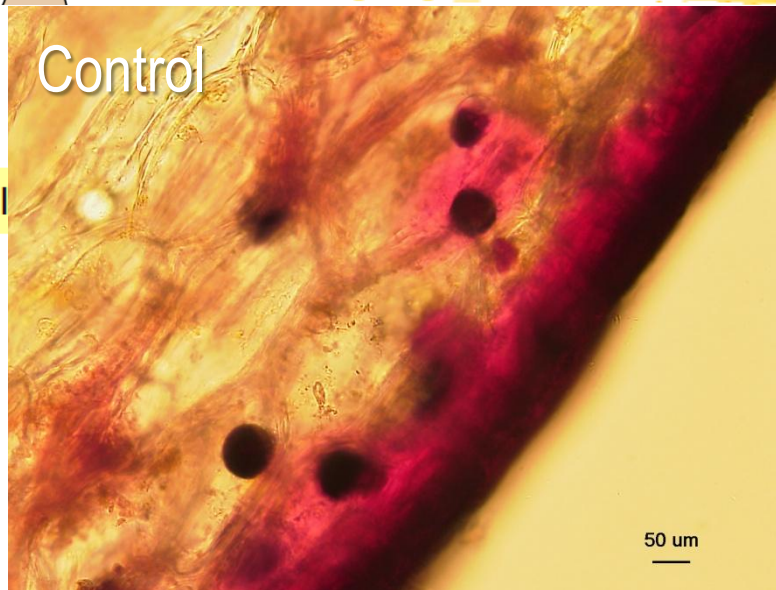


Intracellular electric fields can be inexperienced stresses for biological cells.





Biological effect at low frequency (<1MHz)



Pul

KS

if

:

酸化ストレスとは？

体内では、酸化ストレスの原因物質の 90 %がミトコンドリアで発生する。ミトコンドリアのエネルギー生産では、以下のような反応がおき、その過程でさまざまな活性酸素が発生する。その活性酸素が DNA やタンパク質を攻撃し、傷害することで、細胞のはたらきが低下し、老化へとつながると考えられている。

ミトコンドリア内のエネルギー生産過程で、活性酸素が生まれる反応



スーパーオキシド

過酸化水素

ヒドロキシルラジカル

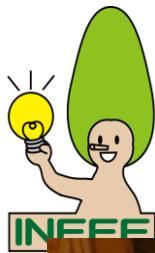
活性酸素

ラジカスカベンジャー
(フリーラジカル除去物質)

- ・ SOD
- ・カタラーゼ
- ・グルタチオン
- ・チオレドキシン など

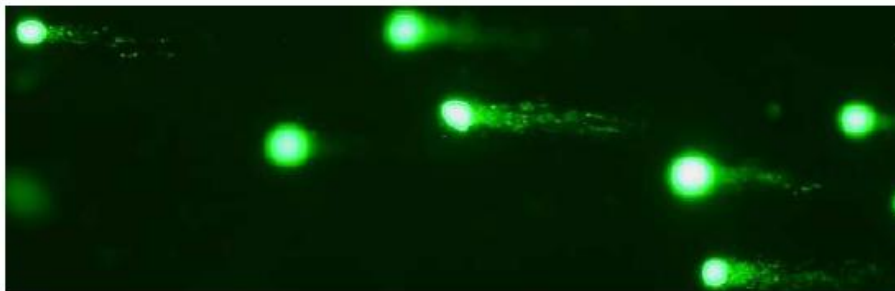
DNA やタン
パク質を傷害

老化



Biological effect at high frequency (>1MHz)

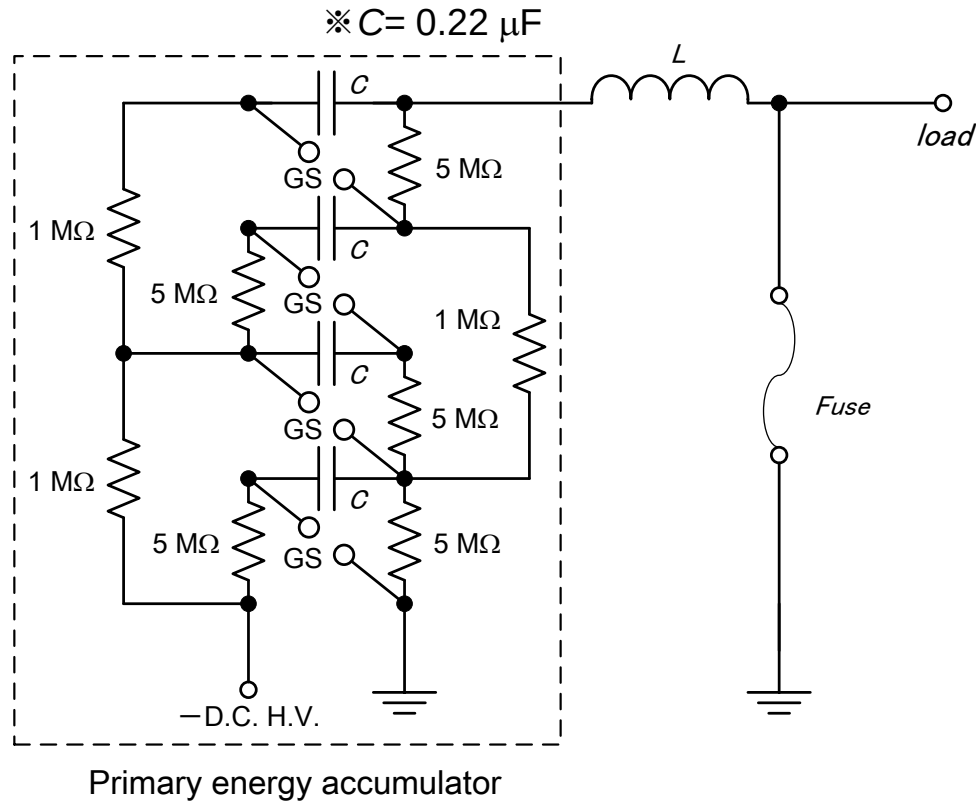
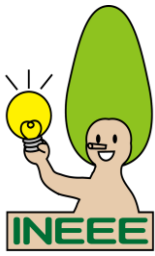
PEF germination



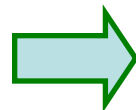
(3) Exposed to BRFF (1.5 kV/cm, 100 MHz, 100 μ s)



Marx IES pulsed power generator



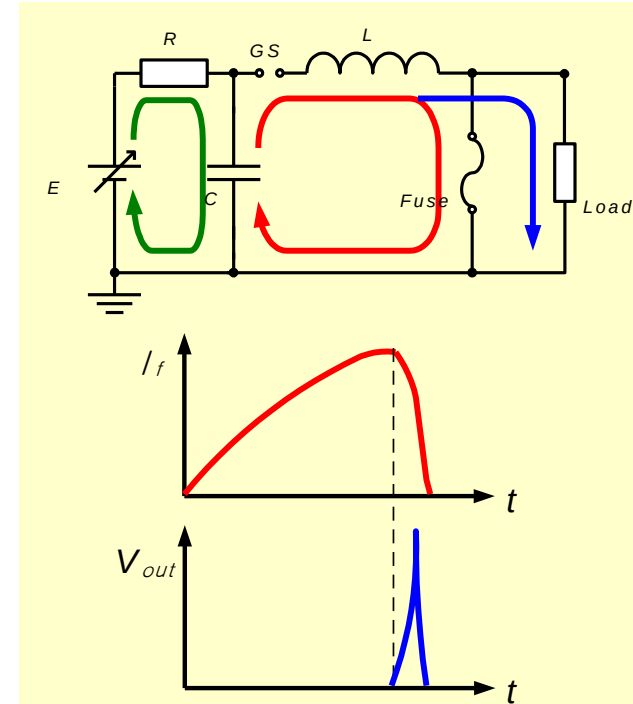
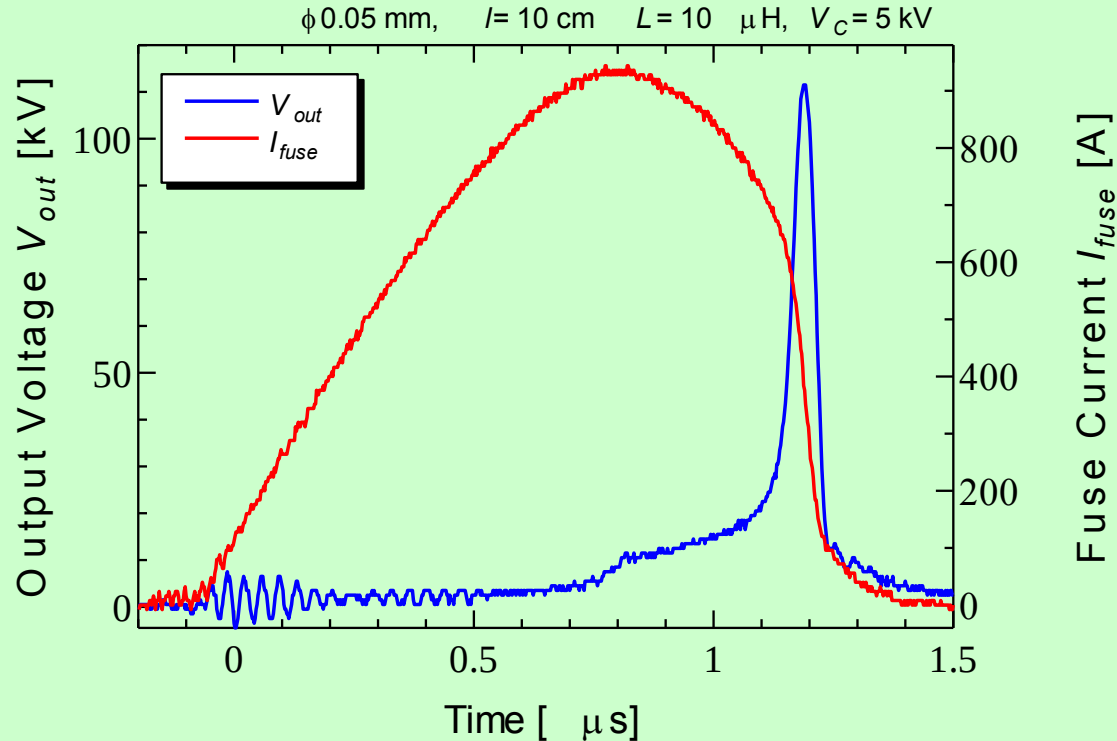
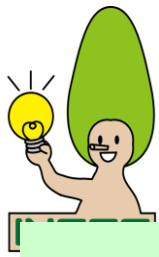
Marx circuit with
Inductive energy
storage system



High output voltage (100 kV) with
low charging voltage (5 kV).



Marx-IES pulsed power generator



Fuse length • • 10 cm

Inductance • • 10 μH

Charging voltage • • 5 kV

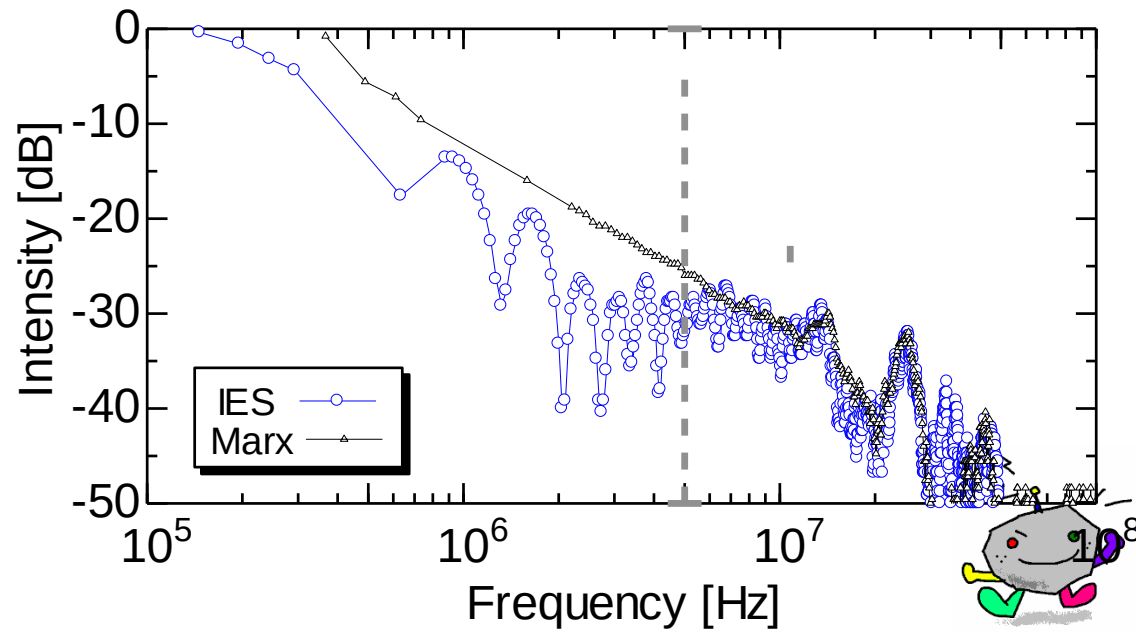
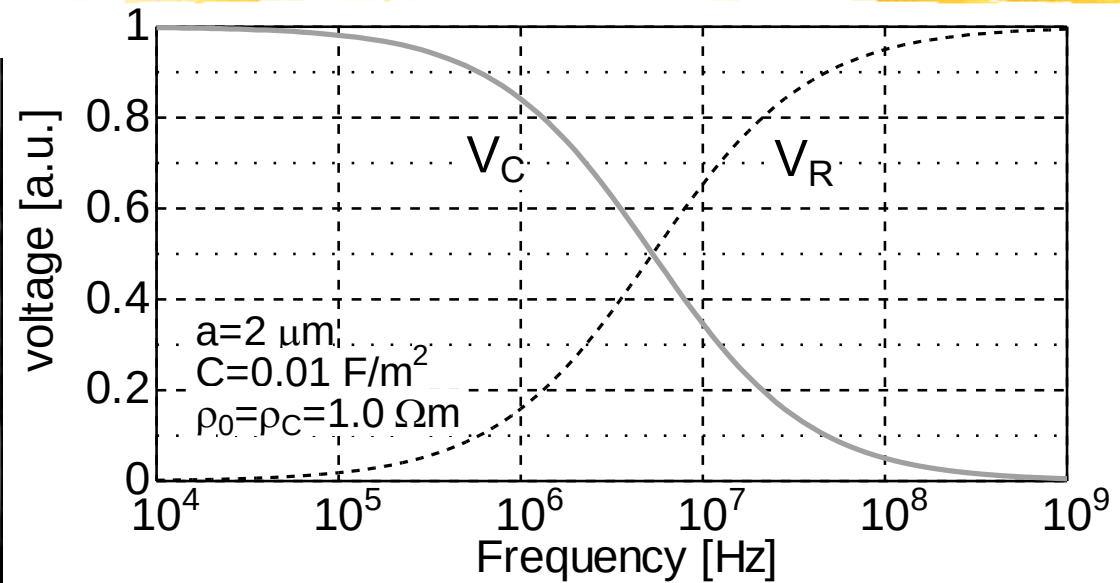
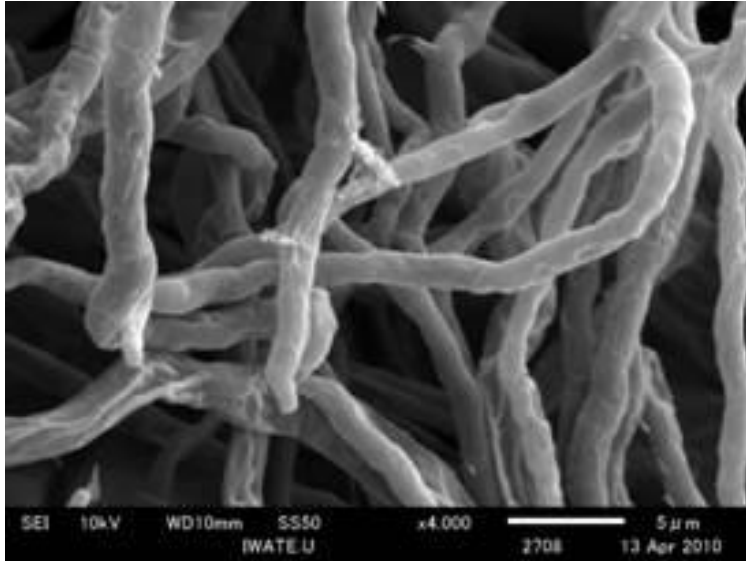
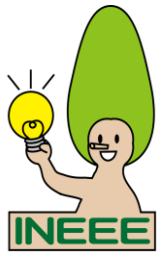
Output voltage • • 110 kV

Amplified ratio • • 22

Pulse width • • 50 ns



FFT analysis



Electrical stimulation using pulsed power generator

Experimental

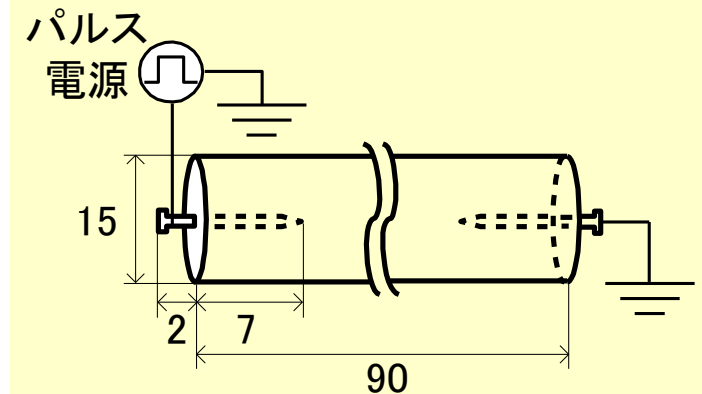
- Field: Sotoyama Forest park, Morioka
- Mushroom : *Lentinula edodes*,
Pholiota nameko, *Naematoloma
sublateritium*

Logs

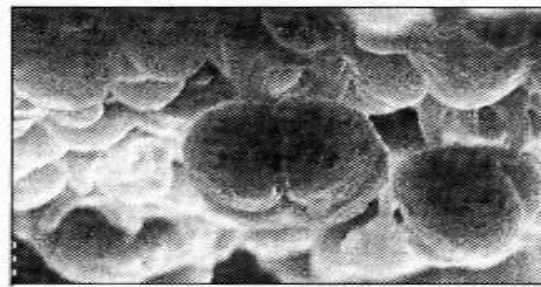
- Length: 90 cm
- Species: *Quercus mongolica* var. *grosseserrata*

Samples

- No.1 : control, 15 logs
- No.2 : 50kV × 1, 15 logs
- No.3 : 90kV × 1, 15 logs
- No.4 : 125kV × 1, 15 logs
- No.5 : 50kV × 50, 15 logs

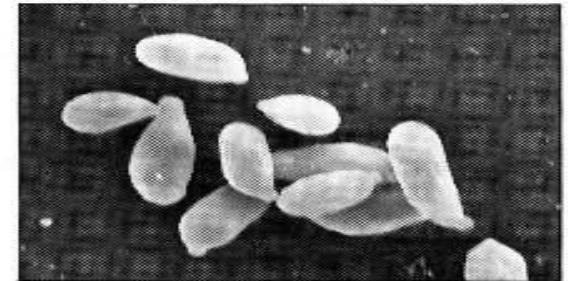


【Life cycle of Basidiomycete】

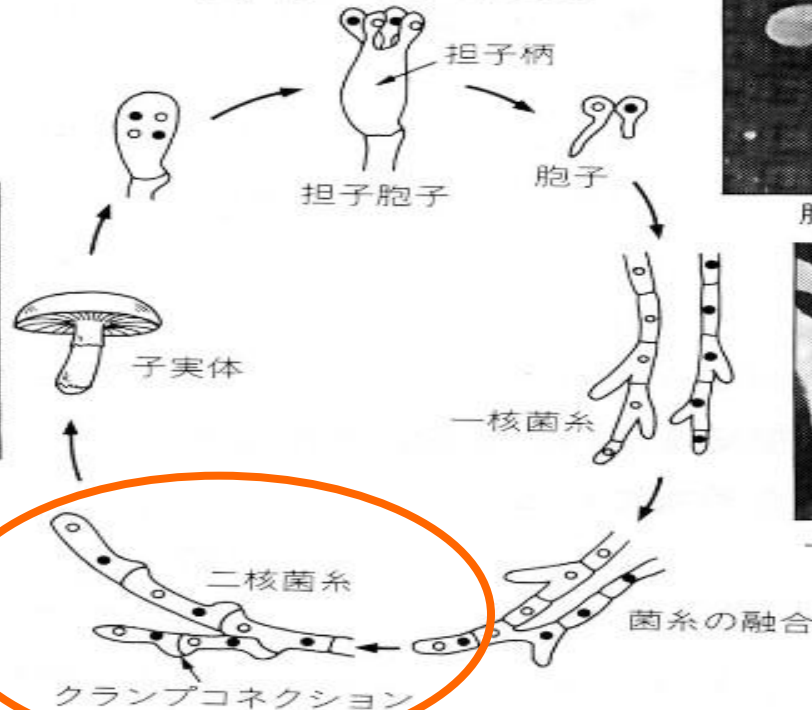


担子柄と4個の外生孢子

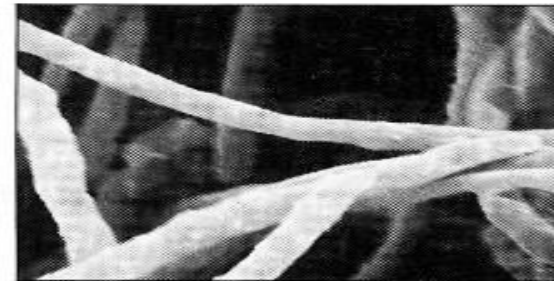
spore



孢子(雌雄の区別がある)



子実体(菌糸のかたまり)



一核菌糸(融合して受精をおこなう)

hypha



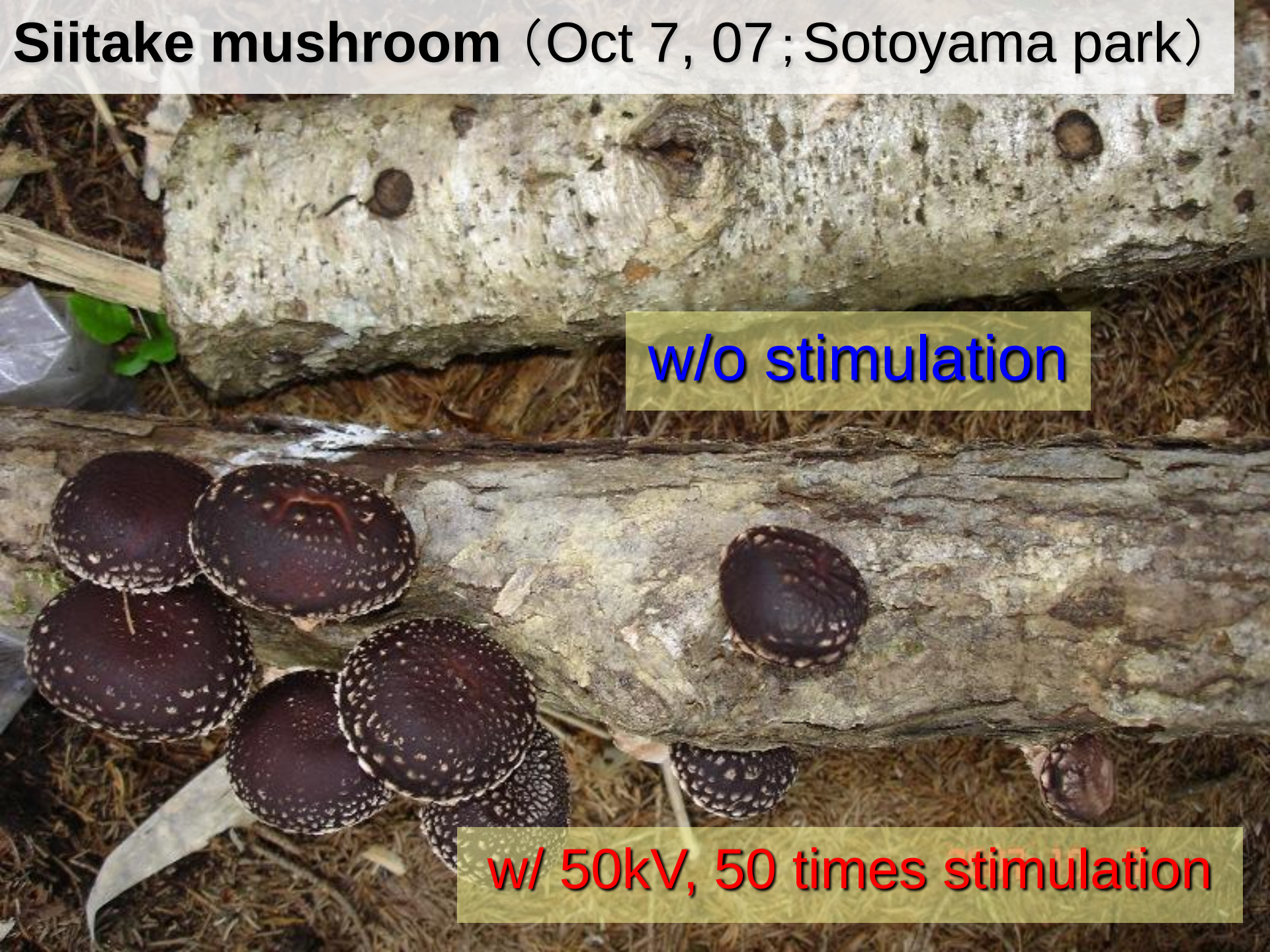
二核菌糸(クランプコネクションがある)

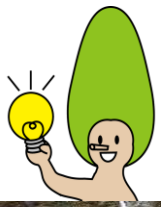
PFE : stimulation?

Siitake mushroom (Oct 7, 07; Sotoyama park)

w/o stimulation

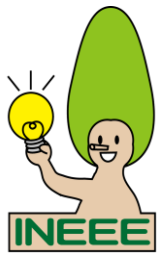
w/ 50kV, 50 times stimulation



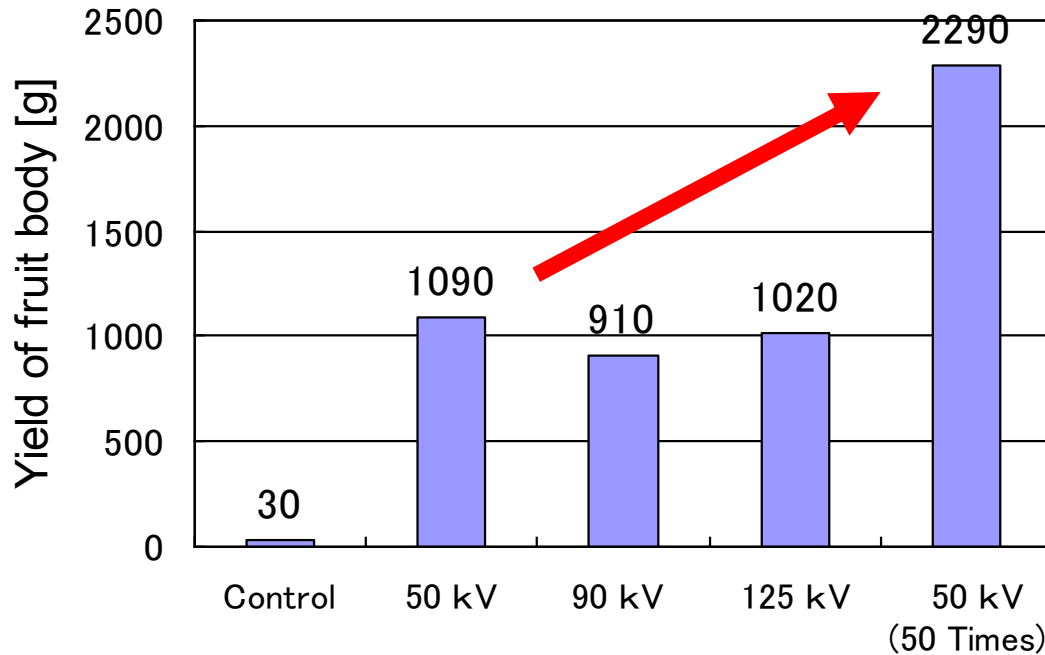


Stimulations by Water, shock and PEF





Lentinula edodes (Shiitake mushroom)

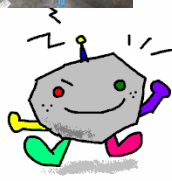


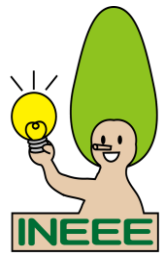
w/o stimulation



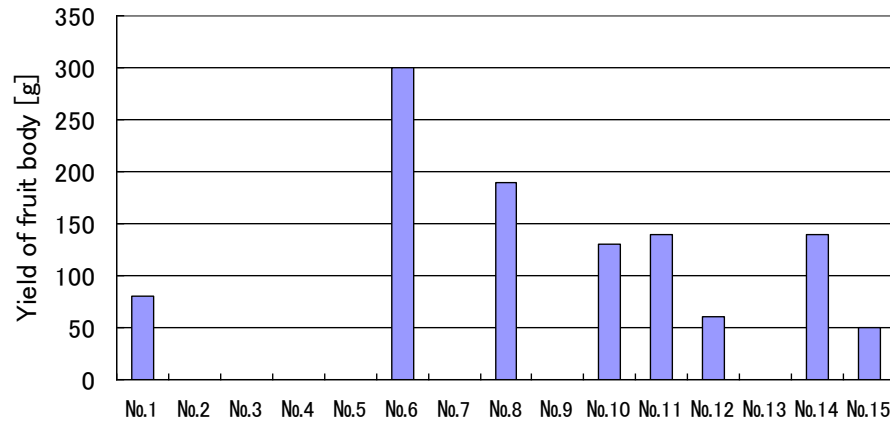
100 kV

Mushroom crops increase about **2.1 times** by applying 50 kV, 50 times pulse voltage

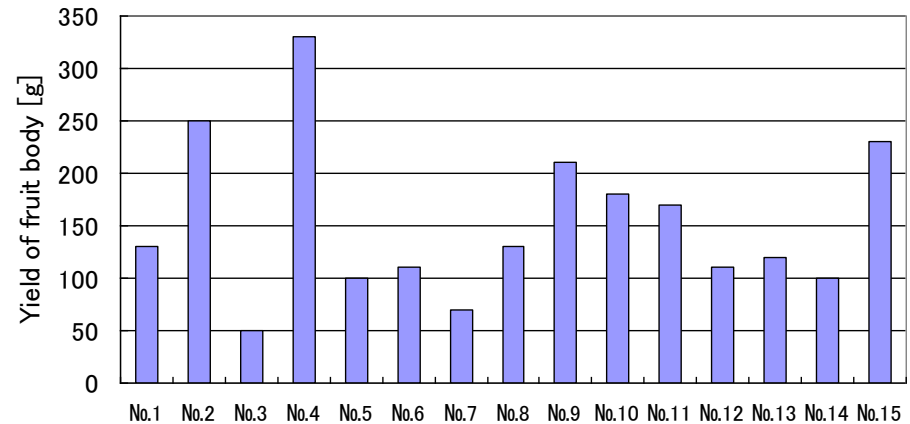




Total cropped weight for each log



50 kV × 1 time

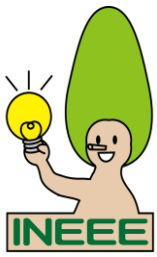


50 kV × 50 times

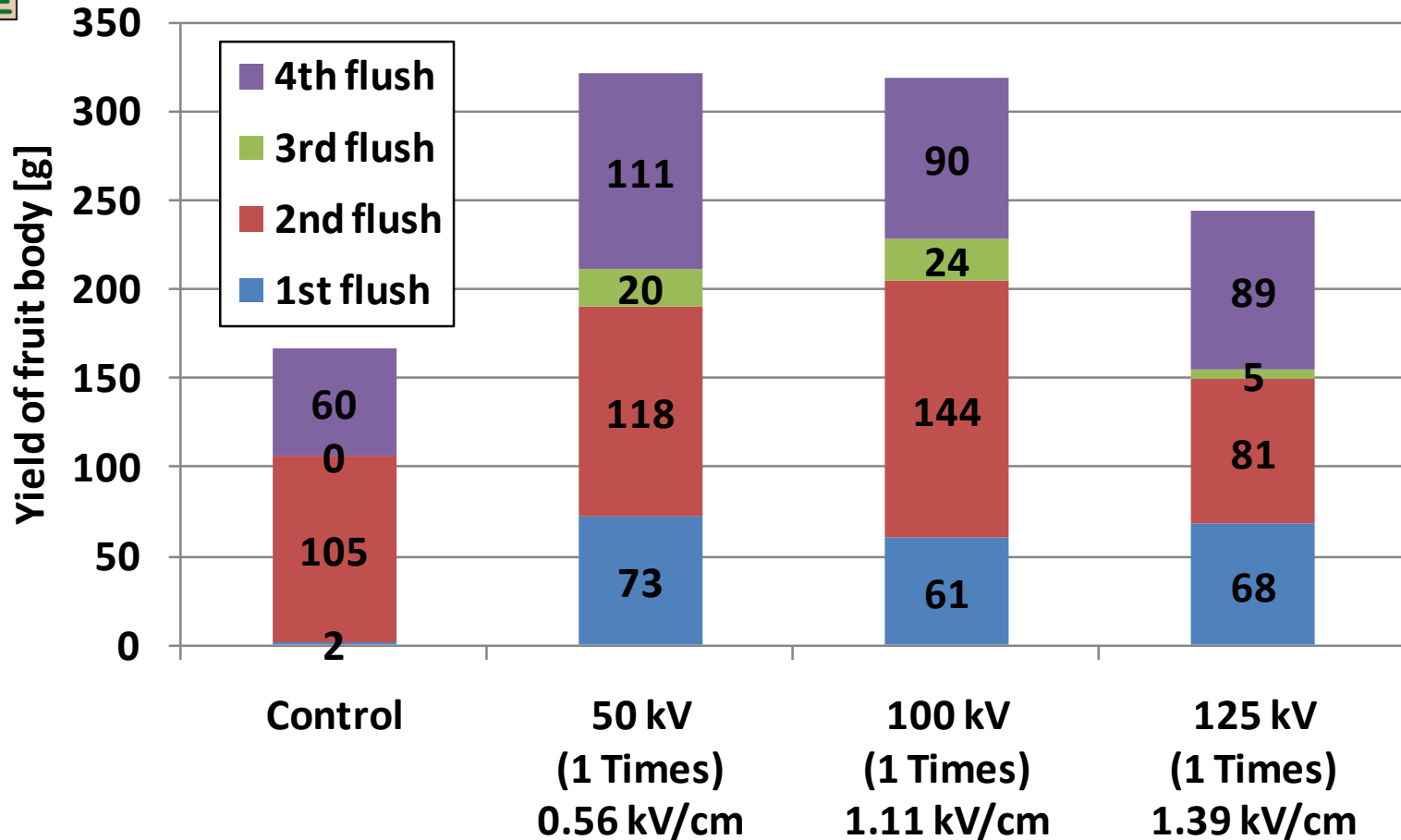
50 kV × 1 time: No mushroom was cropped from 7 / 15 logs

50 kV × 50 times: Mushroom was cropped from all logs





History of total cropped weight

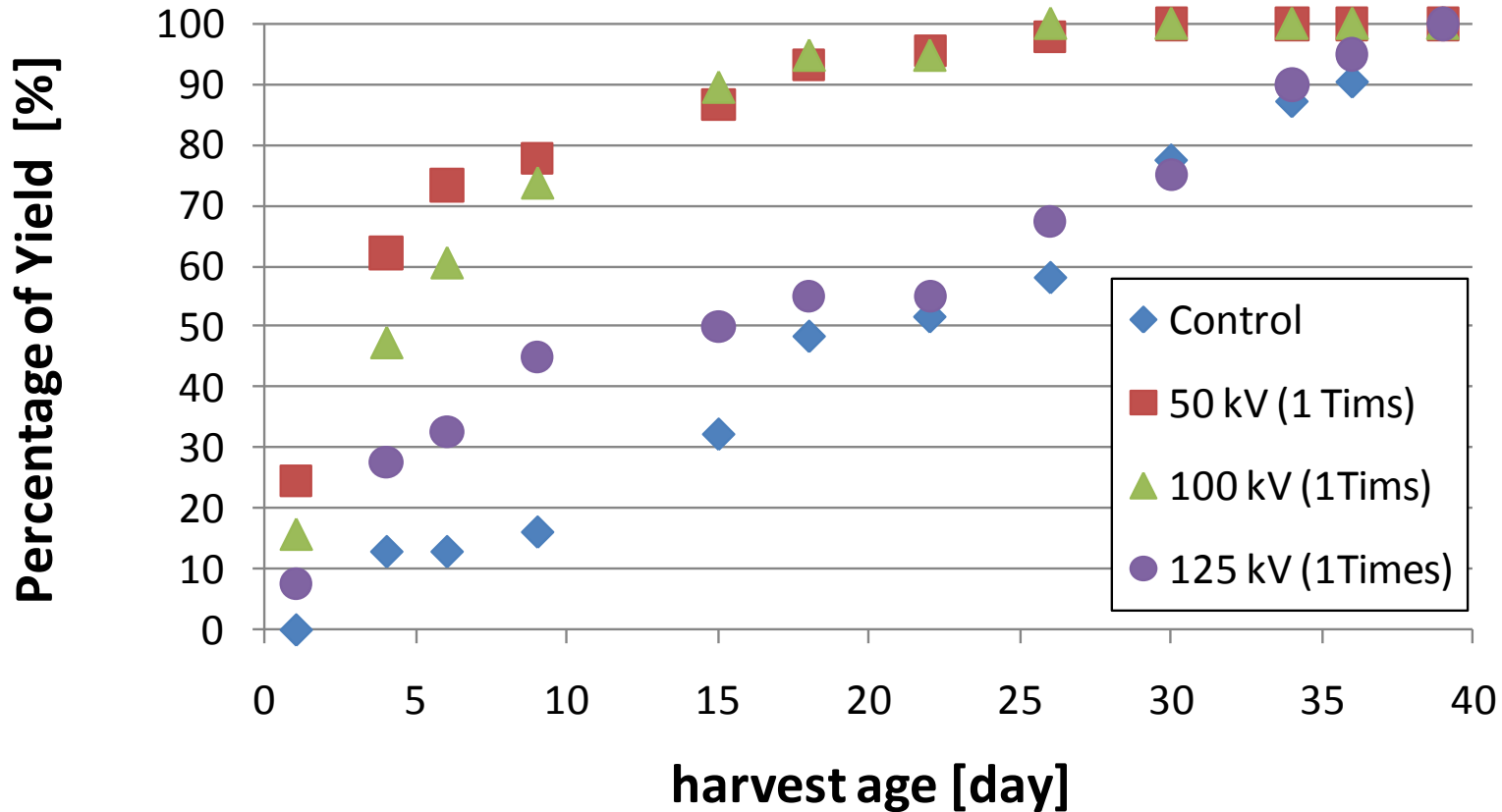
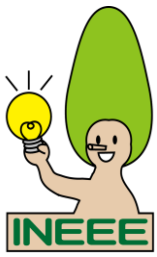


Yield: 2.2 times increase with stimulation 50 kV, 50 times

weight for a fruit body: 34.1 g for control, 69.9 g for 100 kV



History of total cropped rate

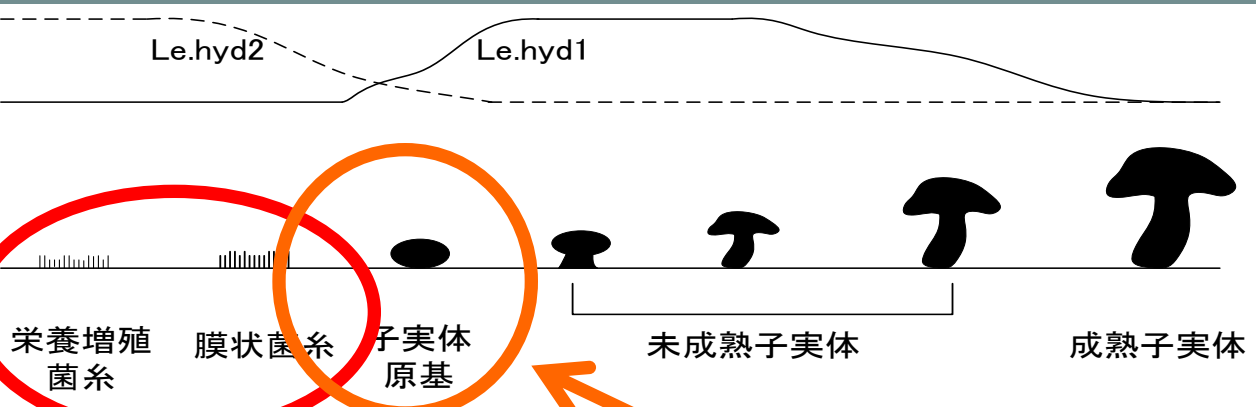


Yield rate: **Improve** with stimulation 50 kV and 100 kV

Cropped weight at 15 day: 50% for control, 90% for 50 and 100 kV



Hydrohobin analysis



sample	hyd2
A-2	12.1
A-3	12.9
A-7	5.31
B-1	5
B-2	6.87
B-3	13.1

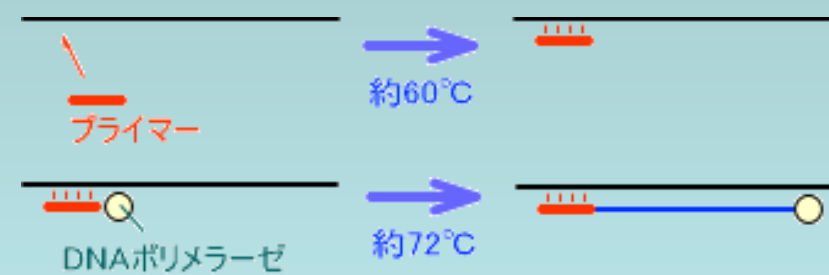
Le.hyd2

Lcc1

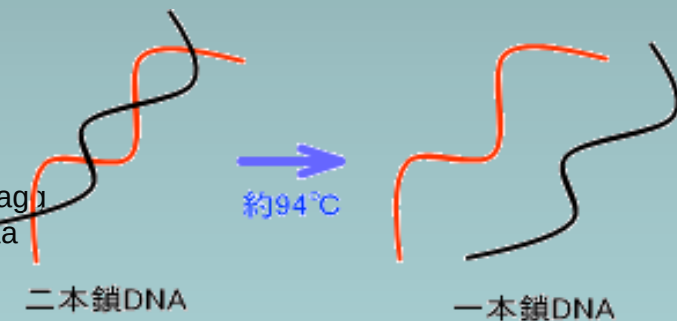
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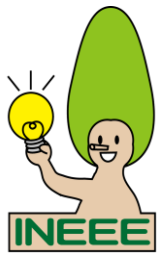
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121 agcgggtag cagctcagc agtgcagc ttccagtcac ttccagtcac ttccagtcac
181 ttccagtcac ttccagtcac ttccagtcac ttccagtcac ttccagtcac ttccagtcac
241 ttccagtcac ttccagtcac ttccagtcac ttccagtcac ttccagtcac ttccagtcac
301 aagtttatct atcccgatatt ttccgggtgt gtgctaacga tggccacagc tctgcgcagg
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541 ttgggggaaa gaaagtcagc ctgtgtgctt tgatcagtcg acggatgttg agctgtagaa
601 tgaactttct tcgcgattgt tgtctatacg aaaatgtttc tatataacca aaaaaaaaaa
661 tagatgttgg gtatgtcttc tagtagaacg cactgtgggt tctgtgatga tgtgtcaggc
721 ctgaacatac aaacctca
    
```

DNA pattern of Le.hyd2

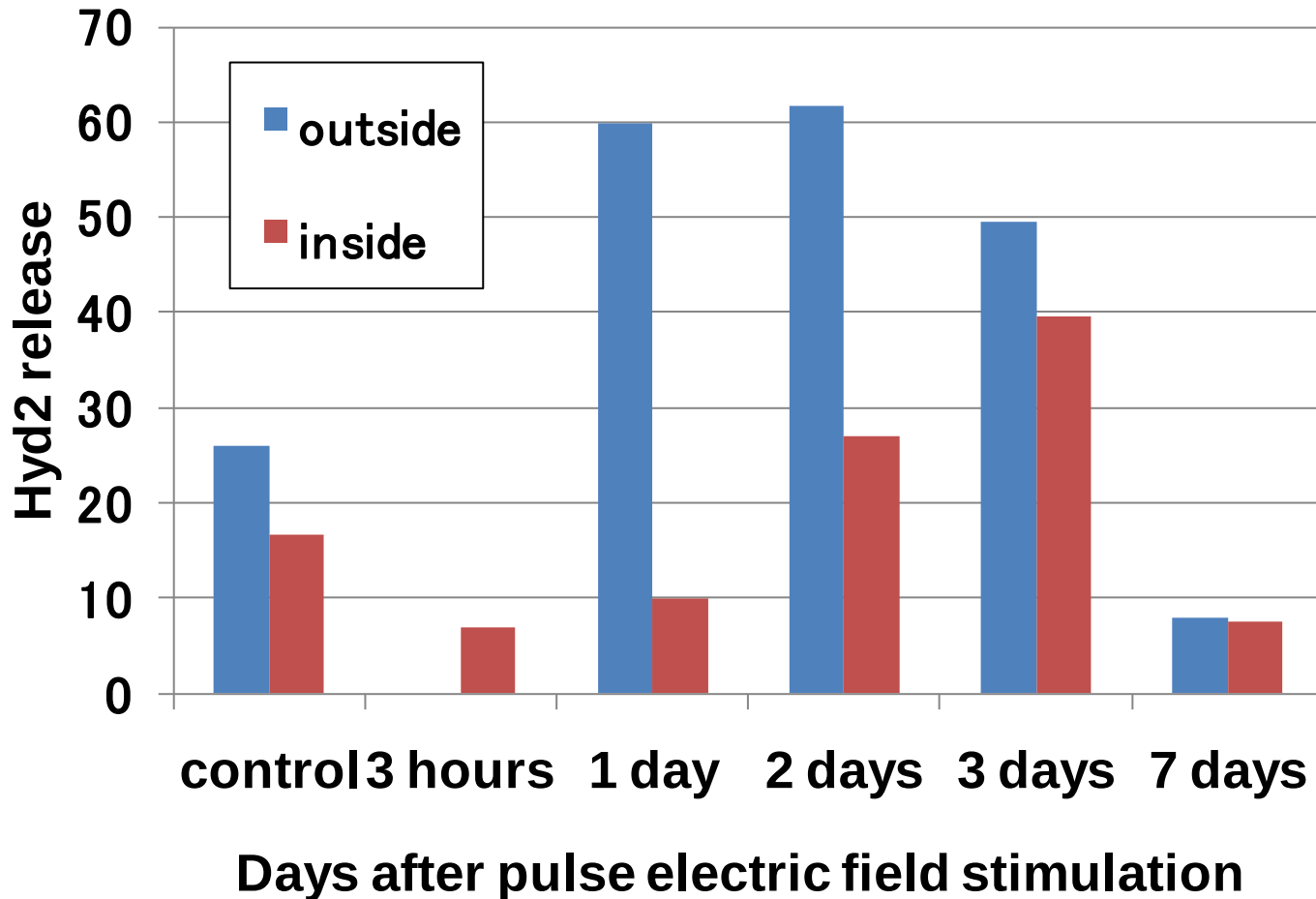


PCR (Polymerase Chain Reaction)

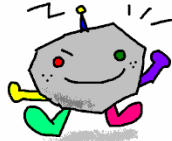


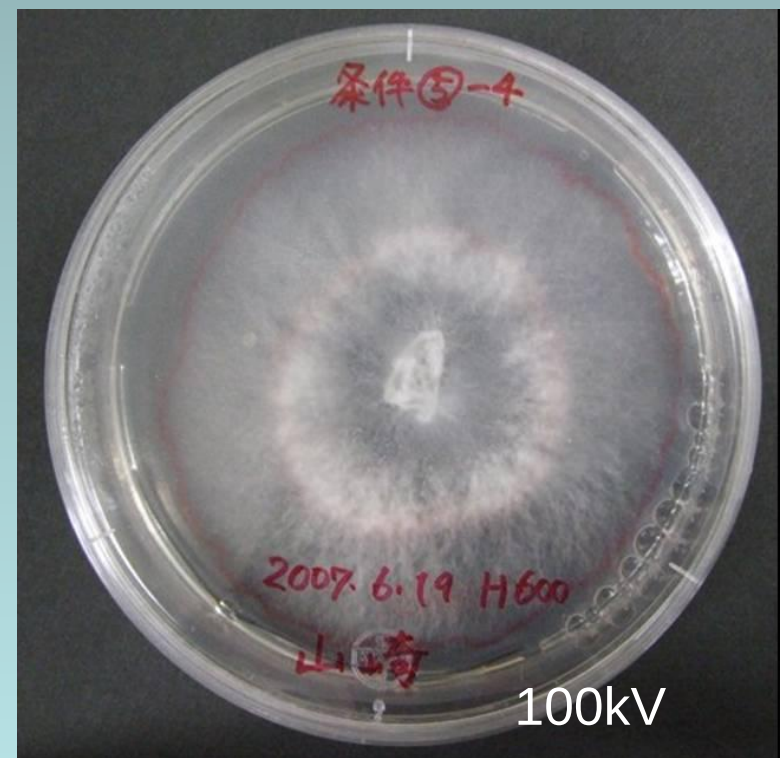
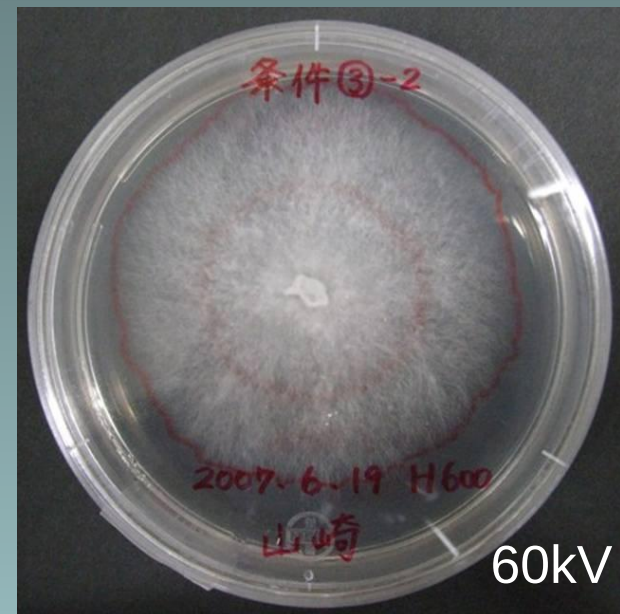
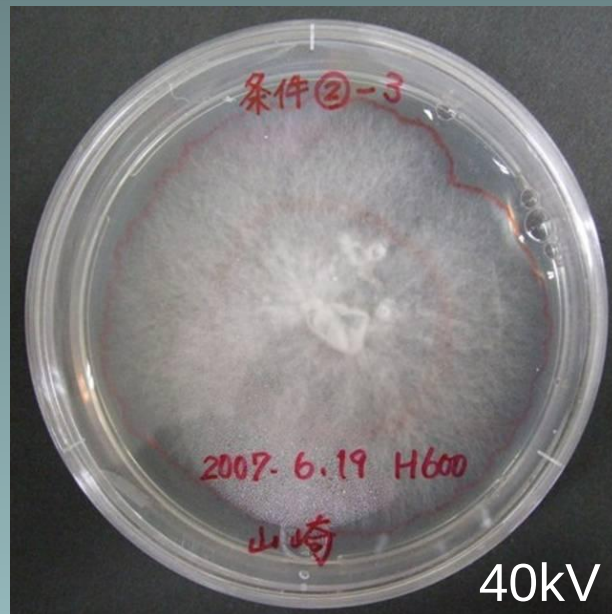
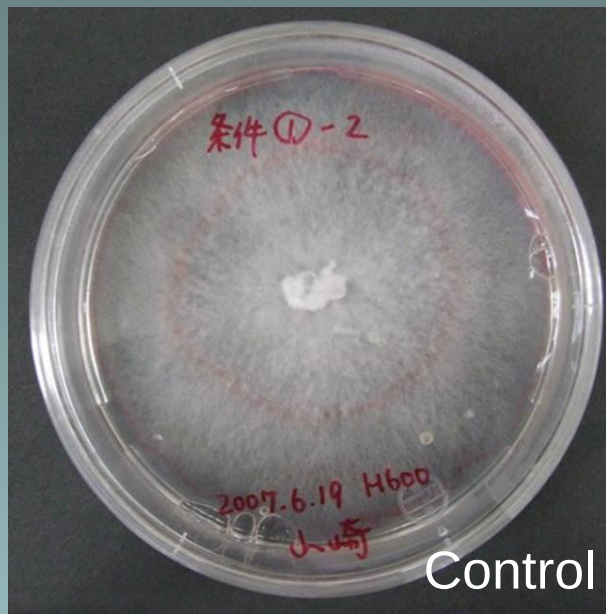


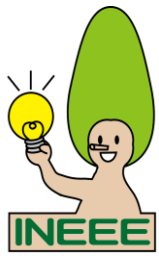
Hydrohobin2 release



- ✦ Hyd2 of PEF sample: **2.3 times larger** than that of control
- ✦ Early stage: **Hyd2 decrease after that increase**



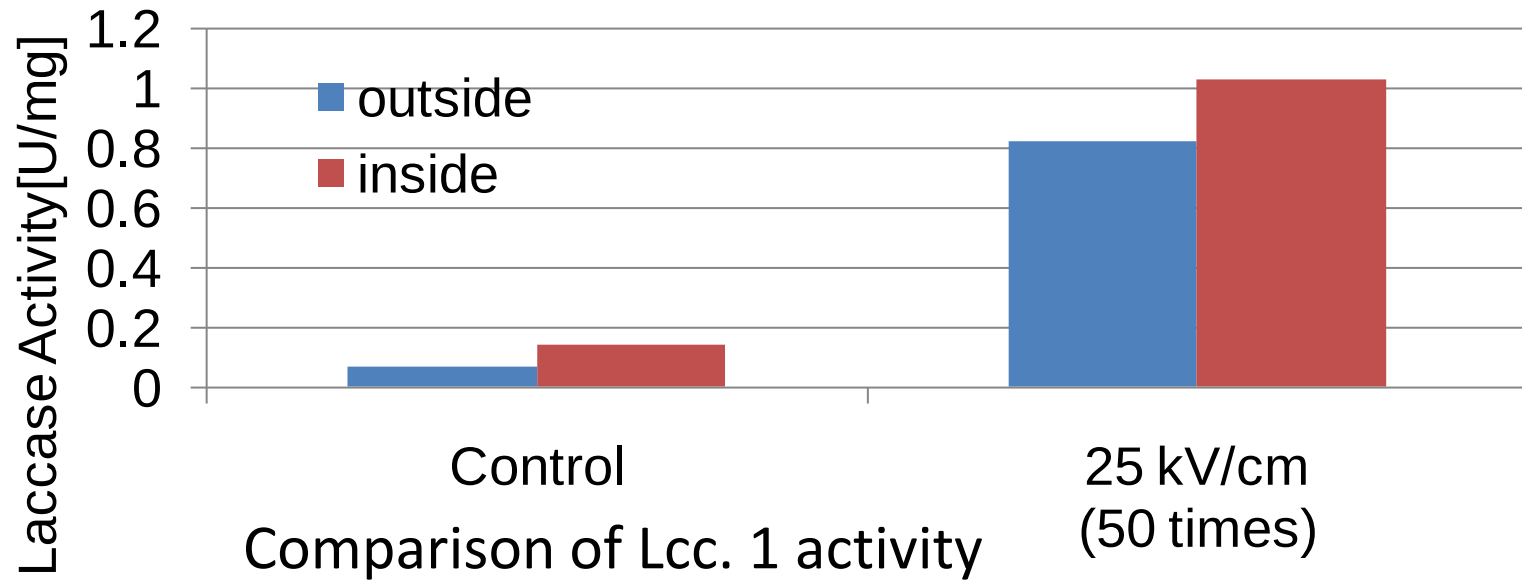




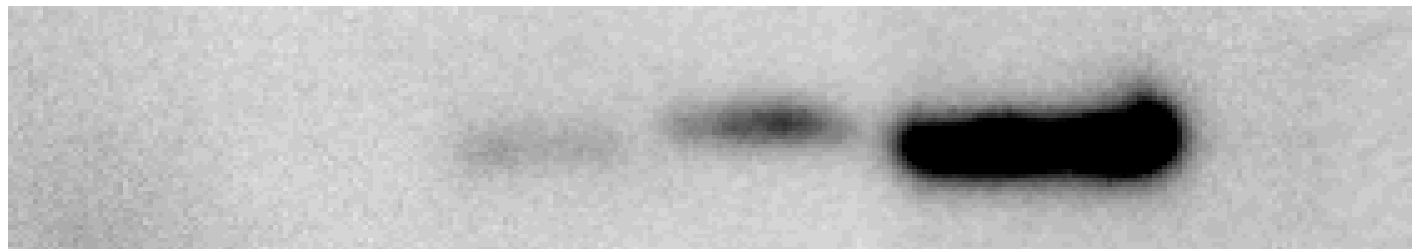
Lcc.1 activity at 28days from stimulation



day 28



Lcc.1
(Western blotting)



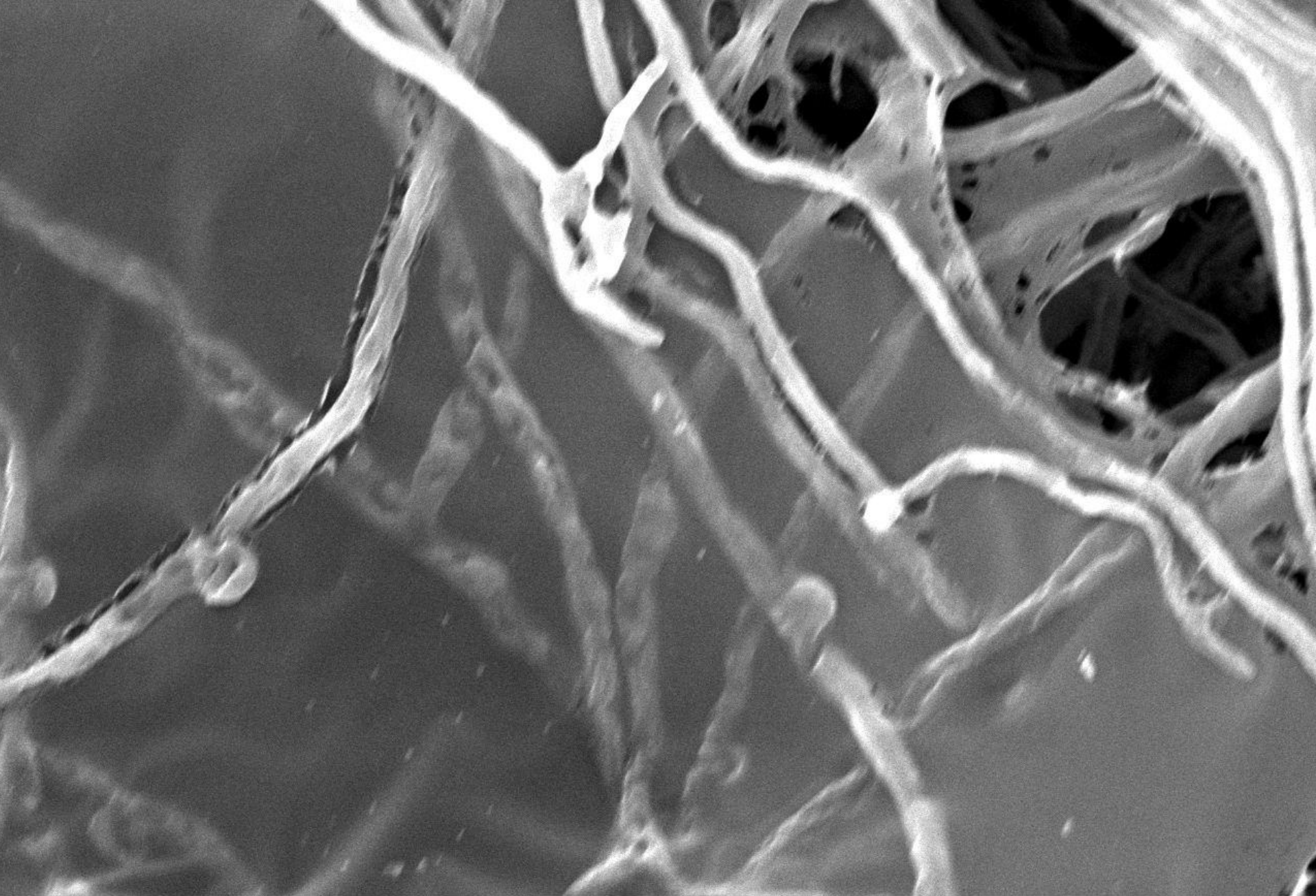
Control
outside

Control
inside

25kV/cm
outside

25kV/cm
inside





SEI 10kV

WD16mm

SS40

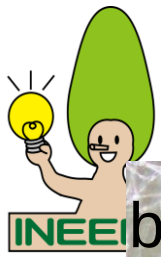
x2,000

10 μ m

IWATE.U

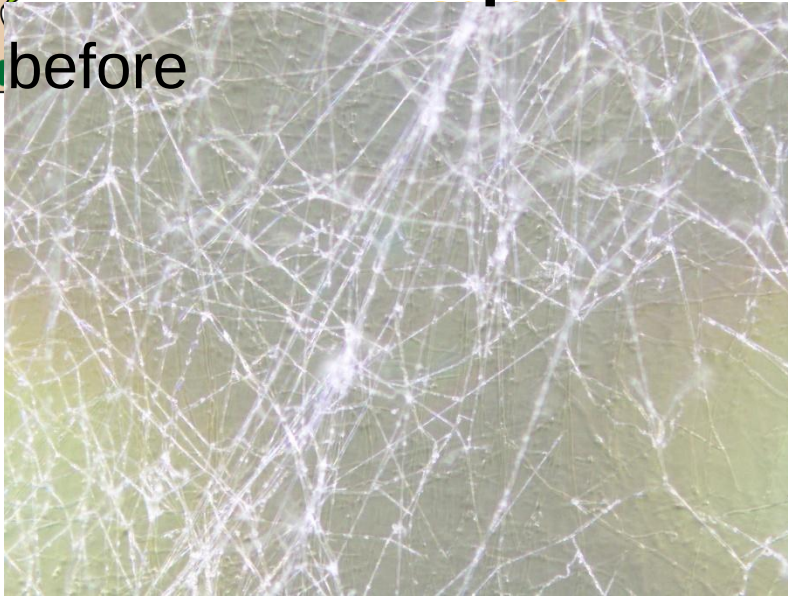
2708

10 May 2010

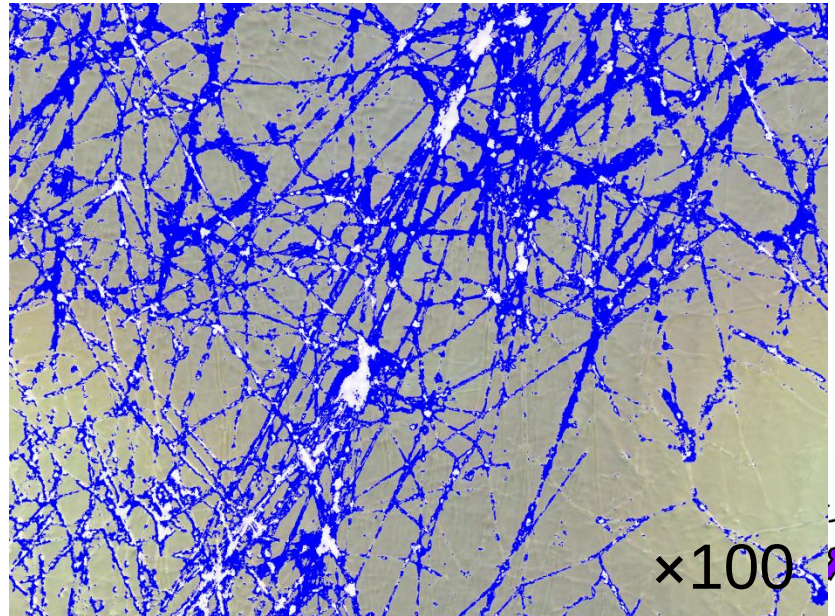
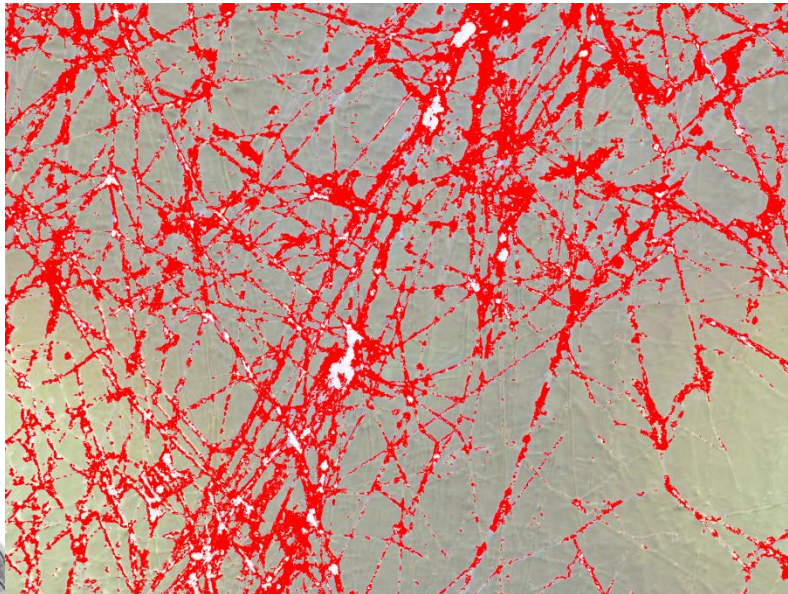
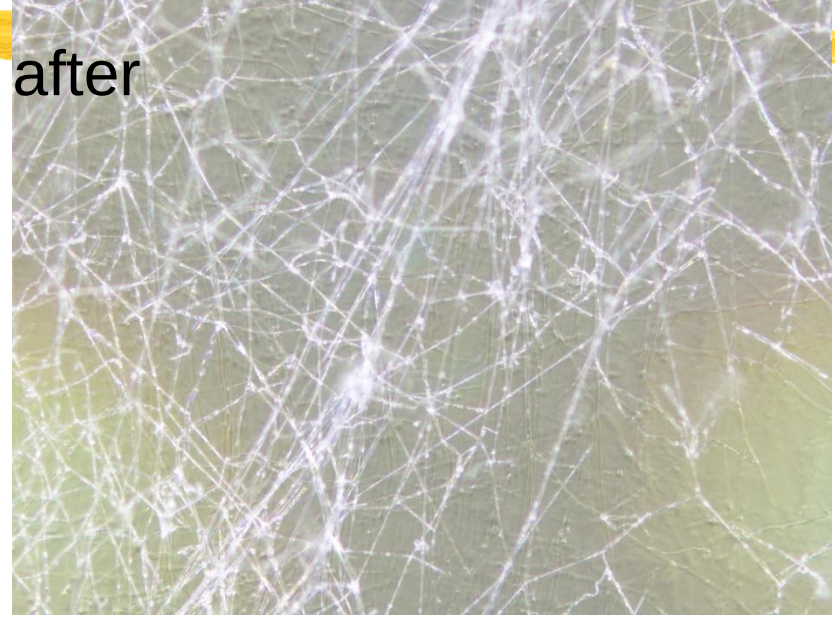


Microscopic observation

before

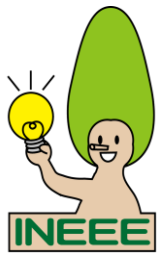


after

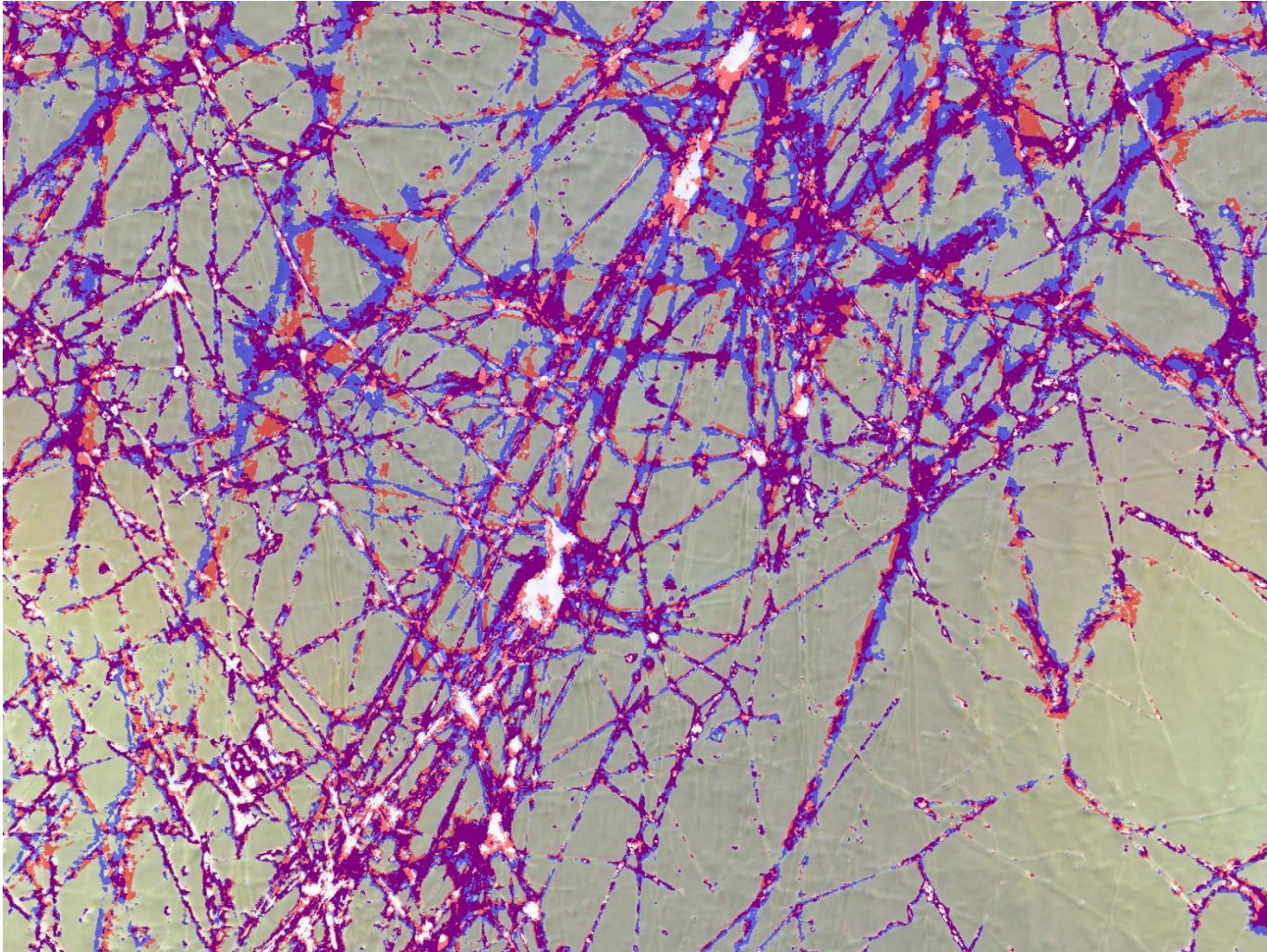


×100



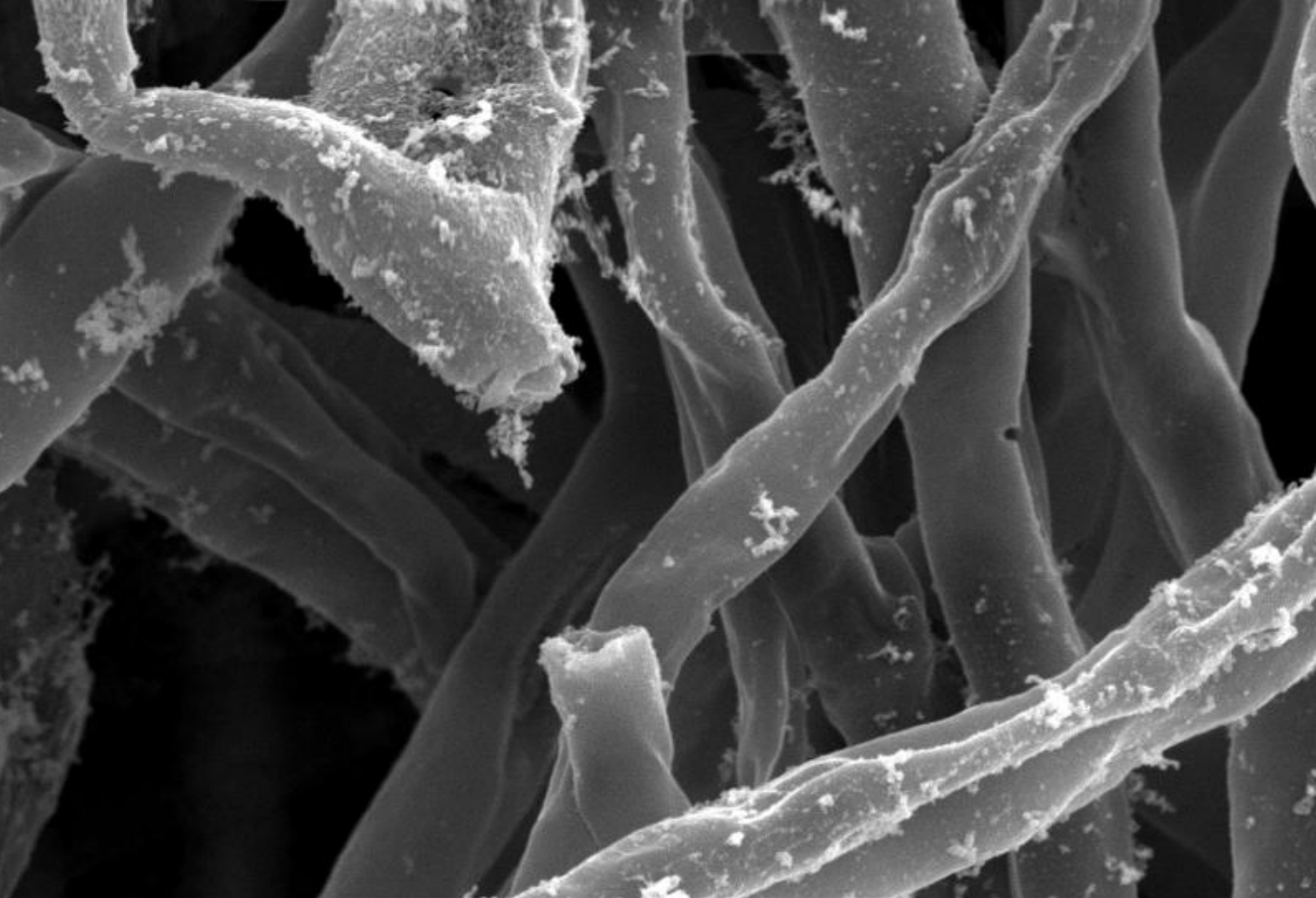


Microscopic observation



Blue and red parts: hyphae moved





SEI

10kV

WD10mm

SS40

x6,000



2 μ m

IWATE.U

2708

16 Apr 2010



L. Edodes:
2.2 times



P. Nameko: **1.3 times**



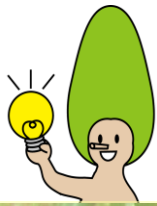
Naematoloma
1.6 times



Ganoderma lucidum
1.7 times



Lyophyllum decastes:
1.3 times



Ganoderma luchidum (靈芝)



w/o stimulation



w/ 100kV stimulation



靈芝: 1USD/1g!!



09年（平成21年）6月19日（金曜日）

の希少キノコ

た食感が特徴で、カルシウム
ラルが豊富に含まれる。繊細
、湿度管理を重ねて菌の培養
力したもので、新たな健康食品
も注目されそうだ。

土が商品化

アギタケ栽培に成功

白色で肉厚なアギタケは、アワビのような名を「三陸あわびたけ」と名付けた。

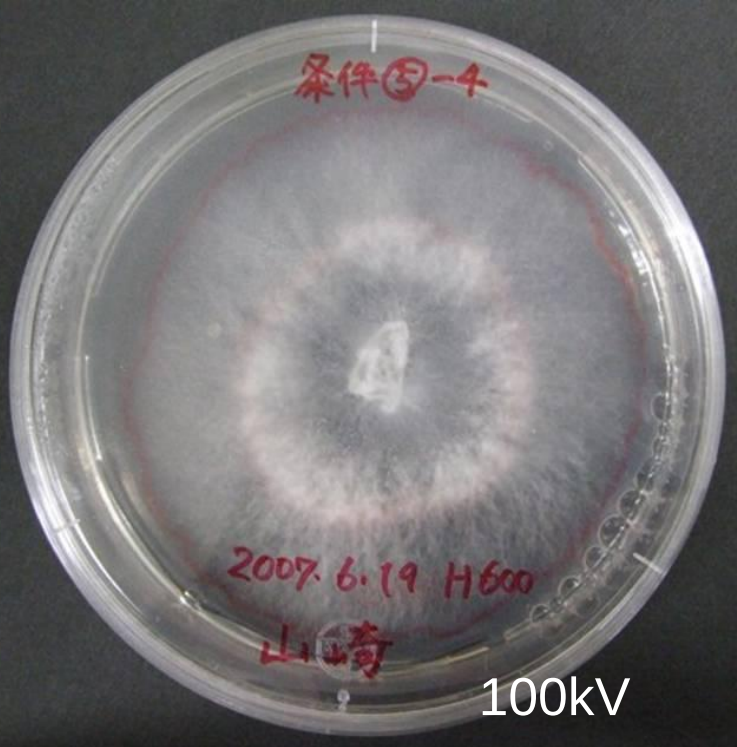
菌糸がある。日本でアギタケは、セリ科の薬用植物阿魏に発生での栽培に取り組んだ。同商店ではカルシウムするキノコ。ほかのキノコ。

が豊富なアワビの貝殻ノコに比べ、免疫力を旧中野中の校舎約500平方メートルで培養に挑戦。4〜5カ月のサイ

ミネラルたっぷり 新たな健康食品に

多糖体β-グルカンが多くの含まれる。中国に駐在している同商店の社員が、3年ほど前に中国・新疆ウイグル自治区の砂漠地帯に自生するアギタケを持ち帰り、洋野町の菌床から1個だけ採れる。かさの直径は15センチほどで約150グラム。4〜5カ月のサイ

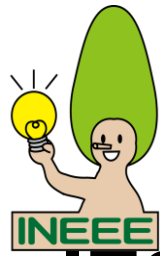
乾燥地域の気候に合うように、温度、湿度管理や風向き、光の調整など工夫を重ね、三陸あわびたけの開発にこぎ着けた。





**Namezosan
(Kantobosai Co.)**





Conclusion

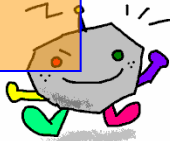
IES Pulsed power generator



Single IES : **110 kV** at **14 kV** charging
Marx IES : **110 kV** at **5 kV** charging
(**220 kV** at **7 kV** charging)

Pulse voltage stimulation effect

1. *L. edodes* : **2.1 times** increase by **50 times** stimulation
2. *P. nameko*, *N. sublateritium* : **1.6 ~ 1.8 times** increase by **90 kV** stimulation
3. **Hyd2, Lcc1 release**: Increasing by PEF stimulation



Thank you for



kind attention