Available online at www.sciencedirect.com
ScienceDirect
Nuclear Science and Techniques 19 (2008) 13–16
NUCLEAR SCIENCE AND TECHNIQUES

Mutation induction of *Pleurotus ferulae* by low-energy N^+ ion implantation and characters of the selected mutant

CHEN Henglei[1,2] WAN Honggui[1] ZHANG Jun[2] ZENG Xianxian[2,*]

[1] *College of Life Science and Pharmacy Engineering, Nanjing University of Technology, Nanjing 210009, China*

[2] *Ion Beam Bio-engineering Center, Xinjiang University, Urumchi 830008, China*

Abstract In order to obtain *Pleurotus ferulae* with high temperature tolerance, mycelium mono-cells of wild type strain ACK was treated by nitrogen ion (5~30 keV, 1.5×10^{15}~1.5×10^{16} cm^{-2}) implantation, and mutant CGMCC1762 was selected through auxotrophy screening method, which was Lys, VB6 auxotrophy stress with high temperature. We found that during riper period the surface layer mycelium of the mutant was not aging neither grew tegument even above 30°C. The mycelium endurable temperature of the mutant was increased 7°C compared with that of the wild type strain. The fruiting bodies growth temperature of the mutant was 16~20°C in daytime and was 6~12°C at night. The highest growth temperature of fruiting bodies of the mutant was increased by 5°C than that of original strain. Through three generation investigation, we found that the mutant CGMCC1762 was stable with high temperature tolerance.

Key words *Pleurotus ferulae*, N^+ beam, High temperature tolerance

CLC numbers $Q319^+.33$, $Q345^+.1$

1 Introduction

As a new mutagenic method, low-energy ion beam implantation has been increasingly used in many research areas since the first report by a Chinese group[1-3]. The mechanism including energy absorption, mass deposition, and charge exchange has been proposed[4-6] by accumulated evidences in breeding[7-12], gene transfer[13], and cell modification.

Pleurotus ferulae is a low-temperature type edible fungus. Its mycelium grows best at 23~25°C, but stops growing at 35°C. Its bud forms at 0~13°C, and its fruiting bodies grow at 10~15°C in daytime and 5~8°C at night. Therefore, *Pleurotus ferulae* cannot be planted in all seasons. Higher temperatures would prevent the bud formation and fruiting body growth, with decreased mushroom production or even no harvest at all. For actualizing year-round production of *Pleurotus ferulae*, one must breed new strains of *Pleurotus ferulae* with high temperature tolerance.

In this study, the mycelium mono-cells of wild type strain ACK were treated by nitrogen ions to induce mutations, and the auxotrophy screening methods were adopted to produce CGMCC1762 with high temperature tolerance.

2 Materials and methods

2.1 *Pleurotus ferulae* and culture conditions

The strain ACK from Ion Beam Bio-engineering Center of Xinjiang University was cultured for 7 or 8 days in PDA medium of 20 $g\cdot L^{-1}$ glucose, 200 $g\cdot L^{-1}$ potato extract and 20 $g\cdot L^{-1}$ agar powder. It was transferred into a 150 mL flask containing 30 mL sterilized water and scattered in a rotary shaker (200 $r\cdot min^{-1}$) for 3 h. Finally, a mono-cell solution containing about 10^7 mycelium cells per milliliter was obtained after a filtration (double filter paper and

Supported by the National Developing and Reform Committee Project of China [2004]2077

* Corresponding author. *E-mail address:* xian@xj.cninfo.net

Received date: 2007-10-16

single degreased cotton).

The basic plate medium of 10 $g \cdot L^{-1}$ corn starch, 20 $g \cdot L^{-1}$ glucose, 2.0 $g \cdot L^{-1}$ KH_2PO_4 and 1.5 $g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$ was solidified with 18-20 $g \cdot L^{-1}$of agar powder at pH 7.0. The plate solidification agent used in this study was the same.

The complex plate medium containing 15 $g \cdot L^{-1}$ wheat powder, 4.0 $g \cdot L^{-1}$ yeast extract, 2.0 $g \cdot L^{-1}$ KH_2PO_4, 1.5 $g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$ and 30 $g \cdot L^{-1}$ bran extract was solidified.

The screen plate medium containing 10 $g \cdot L^{-1}$ corn starch, 20 $g \cdot L^{-1}$ glucose, 2.0 $g \cdot L^{-1}$ KH_2PO_4 and 1.5 $g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$ was added separately with 18 kinds of 0.2 mL 100 $\mu g \cdot g^{-1}$ amino acids and VB1, VB2 and VB6 vitamin solution on the surface layer. The plate was solidified.

The main fermentation medium for ACK and mutant consisted 10 $g \cdot L^{-1}$ wheat powder, 5.0 $g \cdot L^{-1}$ sucrose, 2.0 $g \cdot L^{-1}$ yeast extract, 2.0 $g \cdot L^{-1}$ KH_2PO_4, 1.5 $g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$ and 15 $g \cdot L^{-1}$ bran extract. It was in pH 7.2.

The solid medium for ACK and mutant consisted of 292 $g \cdot L^{-1}$ cottonseed putamina, 32 $g \cdot L^{-1}$ corn powder, 60 $g \cdot L^{-1}$ bran powder, 4.0 $g \cdot L^{-1}$ plaster, 12 $g \cdot L^{-1}$ lime and 600 $g \cdot L^{-1}$ water. The medium was sterilized at 1.22 $N \cdot m^{-2}$ for 3 h.

The mediums were sterilized at 1.22 $N \cdot m^{-2}$ for 30 min. The reagents were of microbiological or analytical grade.

2.2 Ion implantation

The ion implantation was performed on the LCD-1000 multifunctional ion implanter (Southwest Institute of Physics, Chinese Academy of Sciences). A 0.2 mL mycelium mono-cell solution with 100 dilution was added with 20% degrease milk as protecting reagent, and was spread on a sterile plates. It was implanted with 5~15 keV nitrogen ions to 1.5×10^{15}~1.5×10^{16} cm^{-2}. As a control, mycelium mono-cell solution without ion implantation was placed in the same target chamber.

2.3 Screening method

The sample was washed with 2 mL sterile water, and smeared on each complex plate medium with 0.2 mL sterile water. After incubation at 30°C for 10~12 d, the colonies were transferred correspondingly to the basic and complex plate medium. After incubation at 30°C for 6~7 d, the colonies, which grew normally in the complex medium but could not grow in the basic plate medium, were transferred to the screening plate medium and incubated at 30°C for 6~7 d, and the mutant were screened. Then, with shake-flask cultivation and mycelium growth at 30°C, and different conditions (Table 1) for fruiting body growth in mushroom bag, characteristics of the mutant were studied. Finally, through generations passed on experiment, the heredity stability of mutants was ascertained.

Table 1 Managed parameters of *Pleurotus ferulae*'s fruiting bodies outgrowth

Growth period	Temperature(°C) (Mutant/CK)		Illumination / lx	Humidity / %	Concentration of CO_2 / $\mu g \cdot g^{-1}$
	Night	Day			
Bud urged period	6/5	16/10	500	70	500
Granule period	6/5	16/10	500	80	500
Formation period	12/8	20/15	600	90	475
Outspread period	12/8	18/13	800	95	450
Autumn	12/8	18/13	1000	85	450

3 Results and discussion

3.1 Parameters determination of the ion implantation

It is well recognized that nitrogen ion has higher mutation frequency and wider mutation spectra and the mutation frequency and screen efficiency are closely related to energy and dose of the ions.

A survival rate curve (Fig.1) was obtained by implanting the mycelium mono-cell of *Pleurotus ferulae* to 1.2×10^{16} cm^{-2} of N^+ ions in different energies. At lower energies, the survival rate decreased

slowly, whereas it decreased sharply at 10 keV and higher energies. In order to obtain the highest positive mutation, 15 keV was chosen as the optimum energy based on the theory in Ref.[14].

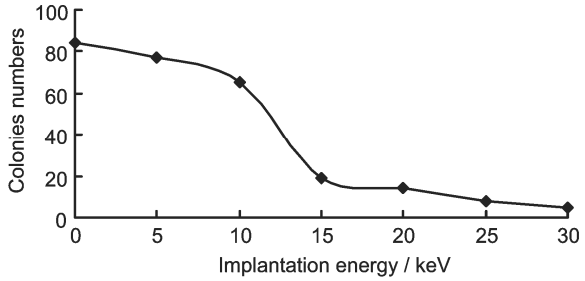

Fig.1 Livability curve of implanting mycelium mono-cell of *Pleurotus ferulae* to 1.2×10^{16} cm^{-2} of N^+ ions in different energies.

Fig.2 is the survival rate of the mycelium mono-cell of *Pleurotus ferulae* implanted with different doses of 15 keV N^+ ions. The survival rate was not in a log-linear model. Instead, it decreased first with dose. From 5.0×10^{15}~1.2×10^{16} cm^{-2}, it increased, and it decreased again after 1.2×10^{16} cm^{-2}. These indicate that the dose range of 5.0×10^{15}~1.5×10^{16} cm^{-2} has a higher peak of survival, hence an abnormal mechanism of radiation damages induced by low energy ion beam implantation.

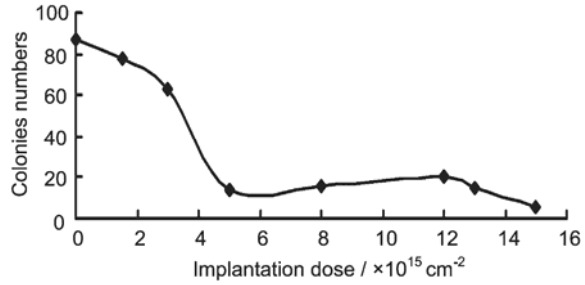

Fig.2 Livability curve of implanting mycelium mono-cell of *Pleurotus ferulae* to different doses with 15 keV N^+ ions.

3.2 Characters of mutant CGMCC1762

From the mycelium incubation at 30°C in basic, complex and screening plate medium, we found, as shown in Fig.3, that the mycelium of mutant CGMCC1762 could grow normally in complex plate medium, but could not grow in basic plate medium. And the CGMCC1762 could grow normally only in screening plate medium added with 0.2 mL 100 $\mu g\cdot g^{-1}$ Lys and VB6, but could not grow normally in any screening plate medium added with amino acids or vitamins.

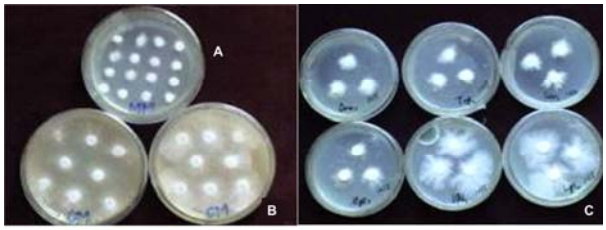

Fig.3 Comparison of mutant CGMCC1762 incubated in basic, complex and screening plate medium. Mutant CGMCC1762 incubated in basic plate medium (A), Mutant CGMCC1762 incubated in complex plate medium (B) and Mutant CGMCC1762 incubated in screening plate medium (C).

In the fermentation experiment in shake-flask at 30℃, as shown in Fig.4, the ferment fluid of mutant CGMCC1762 was limpidity and the size of mycelium ball was in homogeneous distribution. The ferment fluid of mutant CGMCC1762 was orange in color. But the ferment fluid of original strain ACK, in khaki color, was ropy and the mycelium could not form ball.

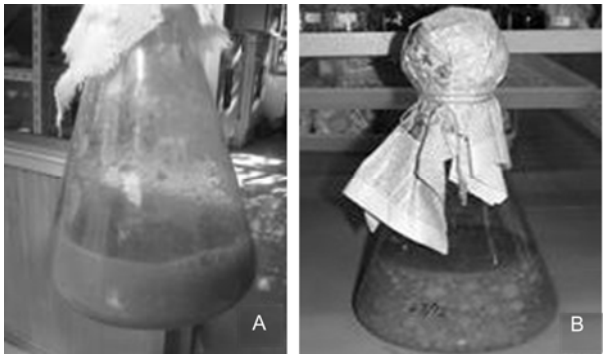

Fig.4 Comparison between mutant CGMCC1762 and original strain ACK incubated in shake-flask. Fermentation production of original strain ACK (A) and fermentation production of mutant CGMCC1762 (B).

From the mycelium growth experiment above 30°C, we found no aging of the surface layer mycelium of mushroom bag of mutant CGMCC1762, whereas the surface layer mycelium of mushroom bag of original strain ACK aged. The mutant CGMC-C1762 grew fruiting bodies normally into round mushrooms, with an average mushroom being 165 g for mutant CGMCC1762 incubated in 1 kg mushroom bag, whereas the mycelium of original strain ACK could not transform fruiting bodies, with most of the mushroom bags being infected by bacteria at the top (Fig.5).

A B C

Fig.5 Mycelium and fruiting bodies growth comparison between mutant CGMCC1762 and wild type strain ACK. Mushroom bag of wild type strain ACK (A), Mushroom bag of mutant CGMCC1762 (B) and Fruiting body of mutant CGMC-C1762 (C).

4 Conclusion

The main points of this work include the energy and dose used, mutant CGMCC1762 selected through Lys and VB6 auxotrophy screening method. It proved once again that ion beam implantation has higher mutation frequency. From fermentation experiment in shake-flask at 30°C, the ferment fluid of mutant CGMCC1762 is limpidity and size distribution of the mycelium ball is homogeneous. The ferment fluid of mutant CGMCC1762 is orange in color. During riper period, the surface layer mycelium of the mutant does not age or grow tegument even above 30°C, at least 7°C higher than the wild type strain. The fruiting bodies of the mutant grow at 16~20°C in daytime and 6~12°C at night, with an increase of 5°C in the highest growth temperature of fruiting bodies in comparison to that of original strain. The investigation of three generation shows that the mutant CGMCC1762 is stable with high temperature tolerance, and is possible for all year-round production of the mashroom under natural condition.

Acknowledgement

The authors wish to thank Prof. Wu Zuyin for access to study edible fungi *Pleurotus ferulae*, Prof. Mao Peihong for continuous support and helpful discussions. The technical assistance of Mr. Zhao Kaizhi with the experiments and the valuable directions and suggestions of Prof. Wan Honggui and Zhang Jun in the preparation of the manuscript are gratefully acknowledged.

References

1 Yu Z L, Wang X D. J Anhui Agric Sci, 1989, **28:** 12-16.

2 Yu Z L, Qiu L J, Huo Y P, *et al.* J. Anhui Agric Col, 1991, **18:** 251-257.

3 Shao C L, Yu Z L. J. Anhui Agric Univ, 1994, **21:** 349-352.

4 Yu Z L. On ion beam applied to bio-tech. Hefei: Anhui scientific and technical press, 1998, 77-105.

5 Yu Z L, Huo Y P. J Anhui Agric Univ, 1994, **21:** 221-225.

6 Yu Z L. IEEE Trans Plasma Sci, 2000, **28:** 128-132.

7 Yu Z L, Deng J G, He J J, *et al.* Nucl Instrum Methods Phys Res, 1991, **B59/60:** 705～708.

8 Wu L J, Gerhard R P, Xu A. Proc Natl Acad Sci, 1999, **96:** 4959-4964.

9 Wu L F, Yu Z L. Radiat Environ Biophys, 2001, **40:** 53-57.

10 Yuan C L, Wang J, Shang Y. Food Technol Biotechnol, 2002, **40:** 311-315.

11 Yu L, Yu Z L. High Technol Letter, 2002, **12:** 41-46.

12 Wu M, Li S, Yao J. World J Microbiol Biotechnol, 2005, **2l:** 1045-1049.

13 Wang H, Gao X, Guo J H. Plasma Sci Tech, 2002, **4:** 1591-1596.

14 Li S C, Wu M, Yao J M. Plasma Sci Tech, 2005, **7:** 2697-2700.