THE KOREAN JOURNAL OF MYCOLOGY
Vol. 22, No 2, p117-121 June 1994

Printed in S. KOREA

Variations in Mitochondrial DNA of *Pleurotus sajor-caju*

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여름느타리 버섯류의 미토콘드리아 DNA 비교

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ABSTRACT: Five strains of *Pleurotus sajor-caju* were collected from some countries including India and Papua New Guinea. Four strains produced brown fruitbody but the other strain from Papua New Guinea produced white fruitbody. Monokaryons obtained from both strains producing brown fruitbody and white fruitbody were mated each other. They showed different mating types such as brown strain of $A_1A_2B_1B_2$ and white strain of $A_3A_4B_3B_4$. DNAs were isolated from mycelia of five strains of *P. sajor-caju*. Mitochondrial DNA was seperated from nuclear DNA by bisbenzimide-CsCl ultracentrifugation. Digestion of the fungal mitochondrial DNA with Eco RI restriction endonuclease yielded from ten to fourteen fragments. Two strains of the five strains showed different restriction pattern of mitochondrial DNA from the other three strains. Summation of the fragment sizes gave a mitochondrial DNA size of about 60 to 65kb.

KEYWORDS: *Pleurotus sajor-caju*, mating type, mitochondrial DNA, restriction enzyme pattern

Introduction

P. sajor-caju is regarded as tropical mushroom by it's characteristics producing fruitbody at about 20-25℃. As the species has not been found in Korean wild flora until now, the strains introduced from foreign countries including India have been cultivated in the farm at summer season. The strain introduced from India showed brown cap color but the strain from Papua New Guinea showed white color of fruitbody.

As *P. sajor-caju* formed primordia in the petridish, this species is thought as a good material for genetic studies of edible mushroom(Go, 1984).

The objective of this research was to distinguish *P. sajor-caju* strains by fruitbody color, inter-strain mating and mitochondrial DNA band pattern digested with restriction enzyme.

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Table 1. *P. sajor-caju* strains used in this experiment

Strains	Origin	Fruitbody color
ASI 2070	India	Brown
ASI 2085	Hong Kong	"
ASI 2096	Thailand	"
ASI 2108	England	"
ASI 2139	Papua New Guinea (Wild species)	white

Materials and Methods

Strains and their culture

All strains used in this study(Table 1) were preserved on Potato Dextrose Agar at 4℃. Homo-

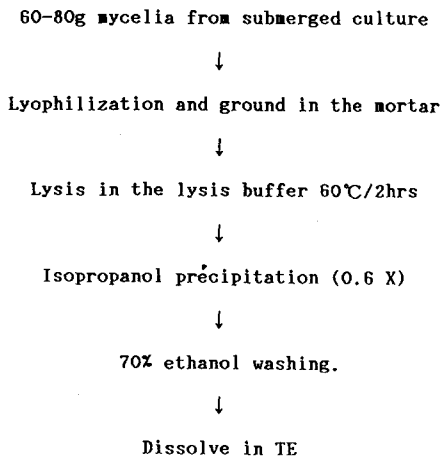

Fig. 1. Process of DNA isolation from *P. sajor-caju* mycelia.

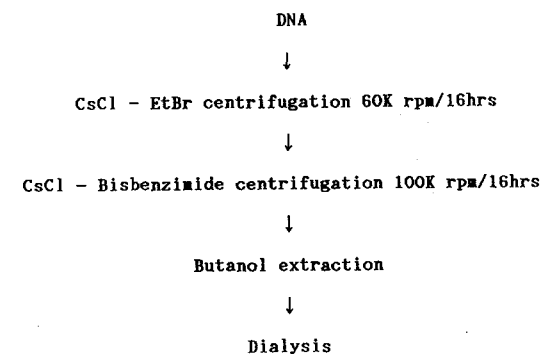

Fig. 2. Mitochondrial DNA isolation of *P. sajor-caju*.

genized mycelia were inoculated in a liquid Mushroom Complete Media and grown for 5 days at the orbital shaker of 28℃. Fruitbody was produced by the methods of Go *et al*(1984).

DNA extraction

DNA was isolated by the method of Fig. 1. Lysis buffer was contained 100 mM Tris HCl(pH 8.3), 50 mM EDTA, 500 mM NaCl and 1% SDS. Protein of isolated DNA was separated by equilibrium centrifugation in CsCl(for 1 hr at 22,000 g) and upper phase was easily removed. Purification of DNA was done by CsCl-Ethidium bromide density gradient centrifugation. After removal of EtBr and CsCl, mitochondrial DNA was separated by CsCl-Bisbenzimide centrifugation as described in Fig. 2(Hudspeth, 1980). DNA bands in CsCl-Bisbenzimide were distinguished under the U.V. illumination and collected by side puncture. Bisbenzimide was removed by extracting with TES(0.1 M NaCl, 10 mM Tris·HCl, pH 8.0, 1 mM EDTA) saturated butanol. The CsCl solution was then dialyzed with TE pH 8.0(10 mM Tris-HCl, 1 mM Na·EDTA)(Fig. 1, Fig. 2).

Restriction Enzyme Analysis

Restriction endonucleases of Eco R1, Bgl II(New England Biolabs) and Xba I(Pharmacia) was used according to manufacturer's description. Restricted or unrestricted DNA were separated electrophoretically in 0.7% agarose gels that contained 80 mM Tris, 89 mM boric acid and 4.3 mM EDTA. Gels were stained with ethidium bromide and visualized with a UV transilluminator.

Results and Discussion

Inter-strain mating

P. sajor-caju produced whitish fruitbody in strain of Papua New Guinea and brownish fruitbody in strains collected from several countries including India. Monokaryons were prepared by single spore isolation from spore print of the fruitbody.

Monokaryons of four mating types such as A_1B_1, A_1B_2, A_2B_1 and A_2B_2 were selected from the brown strain. Those four each mating type monokaryons were mated with four different mating type monokaryons from white strain. All of them produced clamp connections on the mycelia and fruitbody on the rice-straw media.

As mating type of the brown strain was reported as $A_1A_2B_1B_2$(Peberdy, 1993), mating type of the white strain was presumed as $A_3A_4B_3B_4$.

Mitochondrial DNA isolation

Total DNA from five isolates of *P. sajor-caju* was extracted by SDS treatment from lyophilized mycelia and purified by ultracentrifugation. DNA showed red band by naked eye after EtBr-CsCl ultracentrifugation. Red band was transfered to new tube by side puncture and decolorized by

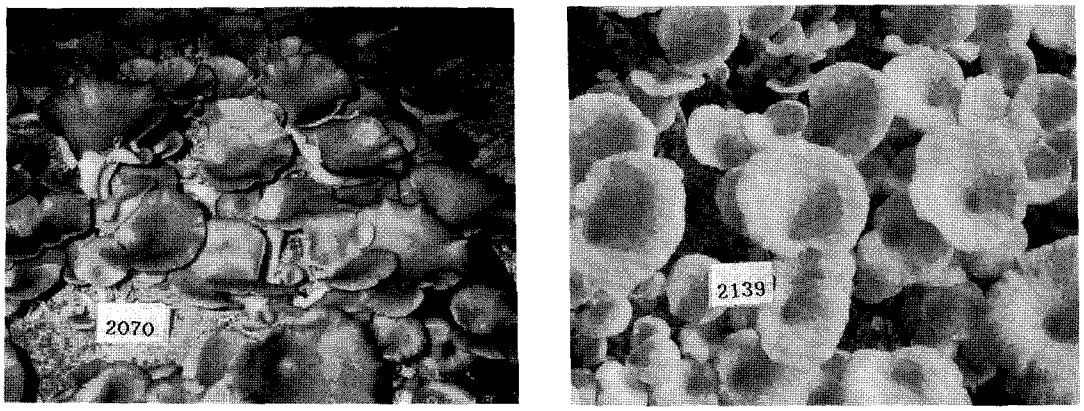

Fig. 3. Fruitbodies from strain ASI 2070 and 2139 of *P. sajor-caju.*

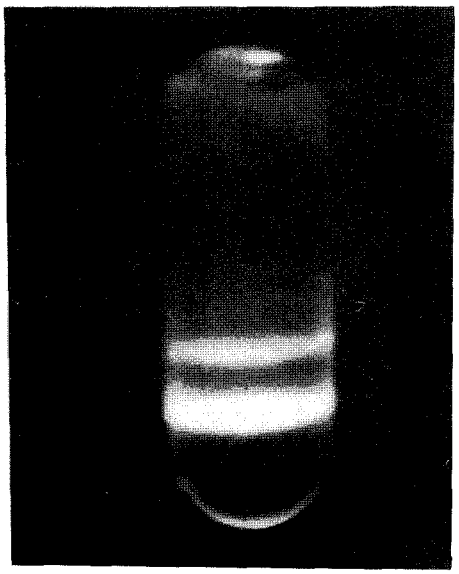

Fig. 4. DNA separation by bisbenzimide-CsCl gradients.

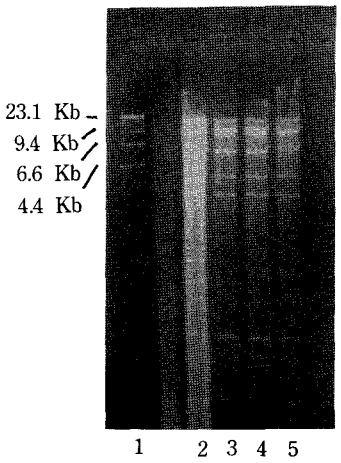

Fig. 5. Eco RI digests of *P. sajor-caju* mitochondrial DNA.
Lane 1: Lambda Hind III, Lane 2: 2085, Lane 3: 2070, Lane 4: 2096, Lane 5: 2139

several butanol extractions. DNA containing CsCl was dialized with TE buffer to remove CsCl. The DNA solution was further fractionated on CsCl-bisbenzimide density gradients. Any band was not observed by naked eye but two major bands containing DNA were detected under UV illumination (Fig. 4). Bisbenzimide and CsCl were removed by butanol extraction and dialysis with TE buffer, respectively.

Dense lower band contained high molecular weight which showed complicated smearing DNA pattern in agarose gel when digested with restriction enzyme and was presumed to be nuclear DNA. Upper band from CsCl-bisbenzimide gradients contained high molecular weight DNA but showed several band patterns in agarose gel after restriction endonuclease digestion. This DNA was presumed to be mitochondrial DNA.

Restriction digestion of mitochondrial DNA

Fig. 4 compares Eco RI digests of mitochondrial DNA from different geographical strain of *P. sajor-caju.* The digest patterns show a basic similarity in all strains, but with a number of differences. Among strains producing brown fruitbody, strain

Table 2. Determination of fragment sizes(in kbps) after treatment of mitochondrial DNA of *P. sajor-caju* with restiction enzyme

Fragment	2070, 2096, 2108	2085	2139		
	EcoRI	EcoRI	EcoRI	Bgl II	Xba I
1	16.0	16.0	16.0	13.5	18.0
2	12.0	12.0	12.0	12.0	11.0
3	8.5	8.5	8.5	9.0	6.0
4	8.0	8.0	8.0	8.0	5.5
5			6.0	6.5	5.3
6	5.7	5.7	5.7	4.2	5.2
7	4.0	4.0	4.0	4.0	4.1
8	3.5	3.5	3.5	3.0	3.2
9		1.8		2.8	2.8
10		1.5		2.2	1.5
11	0.9	0.9	0.9		1.3
12	0.8	0.8			0.7
13	0.7	0.7			
14	0.6	0.6	0.6		
Total	60.7	64.0	65.2	65.2	64.6

ASI 2085 has additional two bands of 1.5 kb and 1.8 kb compared to 2070, and 2096 strains. Whereas, ASI 2139 strain producing white fruitbody shows similar pattern to the other strains of brown fruitbody except for the additional presence of about 6 kb and absence of 0.8 and 0.7 kb of DNA fragments(Fig. 5). The molecular weights of the mitochondrial DNAs, calculated as the sum of the molecular weights of the Eco RI fragments, were in the range of 60-65 kb(Table 2).

Variations in the size of mitochondrial DNA occur in Basidiomycetes. *Agaricus bitorquis* mitochondrial genome is the largest yet described for fungi(approx. 176 kbp) and the *A. brunnescens* genome is only as 56% as large(approx. 98 kbp).

The size of *Coprinus stercorarius*(91.1 kbp) is approximately twice as large as that of *C. cinereus* (43.3 kbp). The size of *Schizophyllum commune* mitochondrial genome is ranged from 50.3 to 52.2 kbp.

Mitochondrial DNA of *P. sajor-caju* is estimated to be approximately 60 kbp in its size. Three strains of the five strains showed the same restriction pattern but the other two strains showed variability in a few bands.

Mitochondrial DNA of four strains of *A. brunnescens* lacked any variability, but mitochondrial DNA from 10 isolates of the closely related *A. bitorquis* had abundunt RFLPs(Hintz, 1985).

Specht *et al* found that the variability of the mitochondrial DNA was high in four *S. commune* isolates and Economou et al found 1.23 kbp insertions induced restriction polymorphism in mitochondrial DNA of *C. cinereus.*

Although the variability of the mitochondrial DNA is not high enough to distinguish every individual, it is effective for the studies of population and genetics. The observed variability of *P. sajor-caju* mitochondrial DNA will provide valuable marker for mitochondrial genetic inheritance.

적 요

여름느타리버섯 *Pleurotus sajor-caju* 균주를 인디아, 파푸아뉴기니아를 포함한 5개국으로부터 수집하였다. 파푸아뉴기니아 균주는 흰색 자실체를 형성하나 나머지 4개 지역 균주는 갈색 자실체를 형성하였다. 갈색 자실체와 백색 자실체로부터 각각 1핵 균주를 얻어 서로 교배하였다. 그들은 다른 교배형을 나타냈으며 갈색종은 $A_1A_2B_1B_2$이었고 백색종은 $A_3A_4B_3B_4$이었다.

여름느타리버섯 5개 균주의 균사체에서 DNA를 분리하였으며, 미토콘드리아 DNA는 bisbenzimide-CsCl 초원심 분리에 의해 핵 DNA와 분리되었다. 5개 균주중 2개 균주의 미토콘드리아 DNA는 Eco RI 제한효소 패턴이 다르게 나타났다. 분리된 각 band의 절편 크기를 합산한 미토콘드리아 DNA 크기는 60-65 kb로 추정되었다.

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