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A Positive Selection for Pyrimidine Auxotrophic Mutants from Basidiospores of *Pleurotus sajor-caju* Using 5'-Fluoro-orotic acid

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5'-Fluoro-orotic acid를 이용한 여름느타리버섯의 pyrimidine 영양요구성 균주의 positive 선발

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ABSTRACT: Pyrimidine auxotrophic basidiospores of *Pleurotus sajor-caju* were selected using positive selection method. Wild type basidiospores could not grow on minimal medium containing the pyrimidine analog 5'-fluoro-orotic acid (5'-FOA) whereas pyrimidine auxotrophs grew normally. After treatment of basidiospores with ultraviolet light, a total of 13 pyrimidine auxotrophic basidiospores were isolated among 24 5'-FOA resistant mutants. These mutants require the pyrimidine such as uracil, cytosine, thymine. Mating type group and growth rate of their mutants were determined.

KEYWORDS: 5'-Fluoro-orotic acid, Pyrimidine auxotrophic mutant, U.V mutagenesis, *Pleurotus sajor-caju*, Basidiomycotina

Transformation, the uptake and expression of foreign DNA by cells, had been accomplished in studies of various organisms involving several species of basidiomycetes (Noel and Laberere, 1995). Transformation provides a molecular tool for the study of gene structure and function, the regulation of their expression, and the genetic engineering of the species which have a economic importance.

The edible mushroom *P. sajor-caju* is suitable species for the summer cultivation in Korea. However, it had not been well studied for the classical genetics and transformation system of the species (Go *et al.*, 1984). The transformation system in *P. ostreatus* had been developed using hygromycin resistant selection marker (Peng *et al.*, 1992). This system was heterologous transformation system. For the development of homologous transformation system, it was necessary to isolate auxotrophic mutant host and gene to complement the auxotrophic marker.

Recently the orotidine-5'-monophosphate decarboxylase gene and pyrimidine auxotrophic mutant had been used for efficient transformation in several filamentous fungi, but negative selection of pyrimidine auxotrophic mutants using replica plate method was time-consuming and tedious process (Ballance *et al.*, 1983; Goosen *et al.*, 1987).

We selected positively pyrimidine auxotrophic mutants using the pyrimidine analog

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5'-fluoro-orotic acid (5'-FOA) and determined the mating type and growth rate of the mutants. This selection method was first reported in *Saccharomyces cerevisiae* and the method had been successfully applied to other fungi but first to basidiomycetes in this study (Boeke *et al.*, 1984).

Materials and Methods

Strains and Media

P. sajor-caju MGL2084 stocked at National Institute of Agricultural Science and Technology was used in this experiment. Basidiospores were collected from the mature fruit body as described by Yoo *et al.* (1985).

Minimal medium (MMM) described by Raper *et al.* (1972) and complete medium (MCM) which was minimal medium supplemented with 0.2% yeast extract and 0.2% bacto peptone were used to culture the basidiospores. 5'-FOA selection medium was MMM supplemented with 1.5 mg/m*l* of 5'-FOA and 50 μg/m*l* of uracil.

Isolation of pyrimidine auxotrophic mutants

Basidiospores suspension at a concentration of 10^7 spores/m*l* were treated with U.V light as described by Yoo *et al.* (1985). About 10^8 spores were mutagenized by U.V light for an exposure time of 5~20% survival rates and then plated on 5'-FOA selection media. After 20 days of incubation at 25°C, colonies which had grown on 5'-FOA selection media were inoculated again on the same media for 2nd screening. The resulting 5'-FOA resistant colonies were tested for their pyrimidine requirement.

Determination of mating type and mycelial growth rate

Fifteen wild type monokaryons and 6 pyrimidine auxotrophic mutants were mated by inoculating in 3 cm distance between two colonies on the MCM plate. Mating was determined by formation of clamp connections between mating strains.

The pyrimidine auxotrophs were inoculated on the MCM plate supplemented with 50 μg/m*l* of uracil and were incubated at 27°C for 11 days and the mycelial growth rate was measured.

Results and Discussion

Survival ratio after U.V irradiation

Fig. 1 showed effect of U.V light on the survival ratio of basidiospores of *P. sajor-caju*. At UV light exposure time between 80 and 120 sec, the survival ratio was rapidly decreased from 65% to 15%. These results were in general agreement with the survival ratio in other basidiospores (Yoo *et al.*, 1985, Lee *et al.*, 1986). Basidiospores of *P. sajor-caju* were more sensitive than those of *Flammulina velutipes*, but more resistant than those of *Lentinus edodes* and *Pleurotus cornucopiae*.

It was reported that the most efficient in-

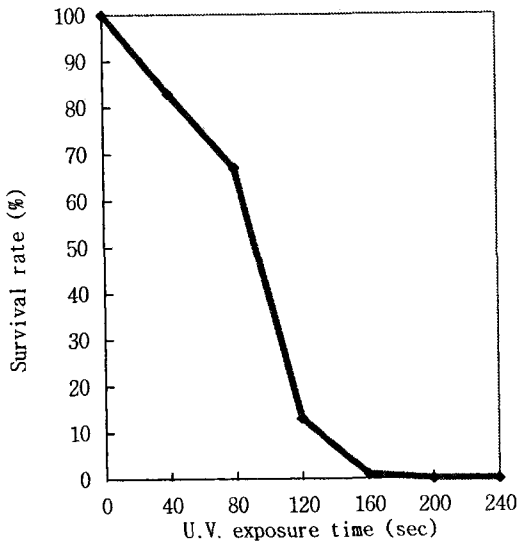

Fig. 1. Effect of ultraviolet light on the survival of basidiospores of *P. sajor-caju*.

duction of auxotrphic mutants by U.V light in *P. cornucopiae* occured at 1~0.4% survival rate (Lee *et al.*, 1986). In this experiment, U.V exposure time used was around 120 seconds.

Isolation of pyrimidine auxotrophic mutants

Mutants resistant to 5'-FOA were isolated by plating mutagenized basidiospores on 5'-FOA selection media. The screening of pyrimidine auxotrophs with 5'-FOA had been successfully applied to isolate either orotidine monophosphate decarboxylase (OMP decarboxylase) or orotate pyrophosphoribosyl transferase (OPRTase) mutant from several fungi such as *Saccharomyces cerevisiae* and *Phycomyces blaskesleeanus* (Boeke *et al.*, 1984; Campuzano *et al.*, 1993).

About 10^8 spores were mutagenized and then 5'-FOA resistant colonies as many as 24 basidiospores were selected. After transfer to minimal medium and minimal medium supplemented with pyrimidine, thirteen out of 24 5'-FOA resistant colonies were shown to be true pyrimidine auxotrophs (Table 1). Fifty-four percent of 5'-FOA resistant mutants was turned out to be pyrimidine auxotrophes. This result was higher than *Phycomyces blakesleeanus*. In *P. blakesleeanus*, the recycling of the mutagenized spores through rich medium plus uracil was necessary to complete a full vegetative growth cycle but not necessary in this study (Campuzano *et al.*, 1993).

Table 1. Identification of genetic markers in 5'-fluoro-orotic acid resistant strains of *P. sajor-caju*

Mutant	MM	MCM.	MM+ Ura	MM+ Thy	MM+ Cyt	MM+ Thd
PRY707	+++	+++	+++	N.D.	N.D.	N.D.
PYR708	+++	+++	+++	N.D.	N.D.	N.D.
PYR7016	+++	+++	+++	N.D.	N.D.	N.D.
PYR7033	++	+++	+++	N.D.	N.D.	N.D.
PYR7034	++	+++	+++	N.D.	N.D.	N.D.
PYR7029	+	+++	+++	N.D.	N.D.	N.D.
PYR7023	+	+	+++	N.D.	N.D.	N.D.
PYR7024	+	++	++	N.D.	N.D.	N.D.
PYR7026	+	+	++	N.D.	N.D.	N.D.
PYR7027	+	+	++	N.D.	N.D.	N.D.
PYR7032	+	+	++	N.D.	N.D.	N.D.
PYR7012	-	++	+++	+++	+++	+++
PYR7028	-	+	++++	+++	+++	+++
PYR705	-	+	+++	++	++	++
PYR7020	-	+	+++	++	++	++
PYR7011	-	+	++	++	++	++
PYR701	-	-	+++	++	++	++
PYR709	-	-	+++	++	++	+
PYR7015	-	-	+++	++	+++	++
PYR7017	-	-	+++	+	++	+
PYR7019	-	-	+++	+	++	++
PYR703	-	-	+++	+	+	+
PYR704	-	+	+++	+	+	+
PYR7031	-	++	++++	+	++	+

MM (Mushroom minimal medium), MCM (Mushroom complete medium), Ura (Uracil), Thy (Thymine), Thd (Thymidine), N.D. (not determined)

Determination of mating type

The mating system of *P. sajor-caju* was controlled by two incompatibility factors of tetrapolar heterothallism (Roxon and Jong, 1977). Fifteen wild type monokaryons were divided with four mating type groups (Table 2). Group I could be mated with group IV, and group II could be mated with group III. Frequence of mating type was different and this

Table 2. Determination of mating type groups in wild type monokaryons and pyrimidine mutants of *P. sajor-caju*

Mating type / Strain	I	II	III	IV
Wild type	17	9	14	8
	13	20		2
	18	21		
	16	5		
	10			
	19			
	6			
	7			
Pyr-mutant	PYR701	PYR7012	PYR7015	
	PYR703	PYR7028		
	PYR7019			

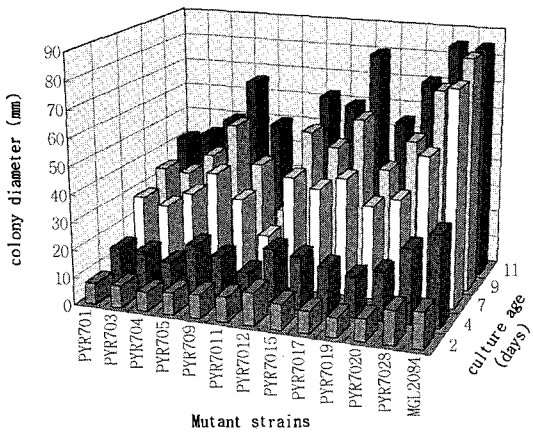

Fig. 2. Growth rate of pyrimidine mutant and wild strains of *P. sajor-caju*.

result was reported in Go *et al.* (1986). Six mutants were divided with 3 groups. Group I was also most frequent, and group IV among six mutants was not present.

Mycelial growth rate of pyrimidine auxotrophic mutants

Auxotrophic mutants strains showed different growth rate (Fig. 2). Some strains grew very slowly. Transformation host was necessary for fast growth rate. The PYR 7028 was considered with optimal host for gene transformation.

적 요

여름느타리버섯의 담자포자에 자외선을 조사하여 5'-fluoro-orotic acid(FOA) 선발배지에서 pyrimidine 영양요구성 균주를 직접 선발하였다. 24개의 5'-FOA 저항성 균주중 13개의 pyrimidine 영양요구성 균주를 선발할 수 있었으며, 이들의 mating type group과 성장속도를 측정하여 형질전환을 위한 최적의 DNA수용체를 선발할 수 있었다.

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