J. Mycopathol. Res. **40**(2) : 135–138 (2002)

Developing of temperature tolerant isolates of *Pleurotus sajorcaju*

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Tropical oyster mushroom produces fructification upto 26°C and thus its cultivation becomes restricted in the winter months only. Sixty monosporous lines were developed from three ecotypes of *Pleurotus sajorcaju*, keeping germinated single spores at the elevated temperature (34°C+1). Some of the cultures in the population were with higher possible temperature tolerance. Monosporous lines were compared by measuring cultural characters as radial growth, production of mycelial mass, optimum temperature for growth and maximum temperature tolerance. Selected members were subjected to crossing reactions with ''tester strains'' and classified into respective incompatibility groups. Monosporous lines selected on the basis of expressible cultural characters with reference to temperature tolerance awaits possible crossing among them for chance development of higher temperature tolerant recombined cultivars.

Key words : Temperature tolerant isolates, *Pleurotus sajorcaju*

INTRODUCTION

Single basidiospore on germination produces monokaryotic mycelium and needs to fuse with a compatible partner to produce a fertile dikaryon. *Pleurotus sajorcaju*, a well established tropical oyster mushroom in the plains of India, bears some demerits as temperature tolerance only upto 25° or 26°C (Jandaik and Kapoor, 1975 ; Kaul, 1983). Monosporous population shows a wide variation in temperature sensitivity (Ghosh, 1988) along with other qualitative characters. Selective breeding in *P. ostreatus* (Eger *et al.*, 1976) as well as *Agaricus* sp. (Pahil *et al.*, 1999) to raise temperature tolerant variety has already been demonstrated. Isolation of temperature tolerant monokaryons in *P. sajorcaju* and its selective dikaryotization may help in raising higher temperature tolerant variety suitable for cultivation in the plains of India for a longer period.

MATERIALS AND METHODS

Three ecotypes of *Pleurotus sajorcaju* viz. PS_{1725} PS_{3048} and PS_{3703} were received from ITCC, IARI, New Delhi. Twenty monosporous lines each were developed from the three ecotypes of *P. sajor-caju* by keeping the isolated single basidiospores at the elevated temperature of 34°C (+1). The isolates survived and formed colonies were picked up and maintained in PDA medium at the optimum temperature of 25°C.

Petriplates with PDA medium were inoculated with uniform mycelial disc (4 mm dia.) cut out by cork borer from fresh growth of previously grown mycelial cultures. Mycelial discs were placed at the centre of poured petriplates. Inoculated petriplates were incubated at the fixed temperature of 25°C for a period of 10 days. After incubation period radial growth was measured from four different angles as- i) maximum diameter, ii) at right angle to maximum diameter, iii) minimum diameter and iv) at right angle to minimum diameter. Finally, from the mean diameters the areas of growth were calculated and compared with the wild mother of *P. sajorcaju*. Uniform physical and nutritional conditions were maintained during the experiments.

Sterilized PD-broth media in conical flasks were inoculated with uniform inoculum density and the cultures were incubated at 25°C for 10 days. After the scheduled incubatiopn period mycelial mats were filtered dried and weighed. Net weight of dry mycelial mass was compared with the wild mother culture.

Table 1 : Studies on the comparative mycelial growth pattern in the population of monosporous lines.

Culture No.	Circular growth on semisolid medium		Productin of dry mycelial mass in liquid medium	
	Area covered after 10 days (cm^2)	% area compared to wild mother	Net wt. of dry mycelial mass (mg)	% dry mycelial mass compared to wild mother
A1	36.73	39.75	107.46	47.41
A2	35.24	38.13	98.95	43.65
A3	6.97	7.54	142.22	62.74
A4	27.14	29.37	118.63	52.34
A5	16.83	18.21	149.12	65.78
A6	23.75	25.70	121.16	53.45
A7	35.87	38.82	188.27	83.05
A8	56.72	61.38	169.28	74.68
A9	14.51	15.70	146.02	64.42
A10	24.00	25.97	187.81	82.85
A11	40.81	44.16	153.73	67.82
A12	34.51	37.34	184.84	81.54
A13	36.51	39.51	142.77	62.98
A14	45.94	49.71	198.05	87.37
A15	40.24	43.54	151.24	66.72
PS_{1725}	92.41	—	226.68	—
B1	44.98	50.23	186.28	84.47
B2	21.55	24.07	192.36	87.23
B3	32.35	36.13	150.15	68.08
B4	31.35	35.01	169.37	76.80
B5	6.46	7.21	118.28	53.63
B6	35.55	39.70	175.87	79.75
B7	65.00	72.59	162.48	73.68
B8	44.16	49.32	123.40	55.96
B9	53.43	59.67	166.25	75.39
B10	45.46	50.77	147.98	67.10
B11	25.32	28.28	159.77	72.45
B12	47.27	52.74	136.26	61.79
B13	14.45	16.14	172.62	78.27
B14	18.09	20.20	124.88	56.63
B15	50.24	56.11	161.21	73.10
PS_{3048}	89.54	—	220.53	—
C1	33.37	39.30	139.26	59.86
C2	29.11	34.29	160.05	68.80
C3	2.66	3.13	95.92	41.23
C4	11.33	13.34	135.39	58.20
C5	28.07	33.06	169.90	73.04
C6	24.09	28.37	170.02	73.09
C7	29.98	35.31	182.64	78.51
C8	43.22	50.91	118.95	51.13
C9	78.81	92.83	205.44	88.31
C10	52.78	62.17	189.96	81.66
C11	15.06	17.74	129.65	55.73
C12	42.99	50.64	168.24	72.32
C13	73.10	86.10	210.16	90.34
C14	38.24	45.04	188.62	81.08
C15	34.40	40.52	191.59	82.36
PS_{3703}	84.90	—	232.62	—

Table 2 : Comparative temperature sensitivity among the monosporous lines.

Culture No.	Net area of growth (cm^2)				
	25°C	30°C	34°C	36°C	38°C
A1	25.18	23.30	8.95	0.76	—
A2	21.73	22.65	3.08	—	—
A3	5.68	1.75	1.13	—	—
A4	18.57	11.72	2.06	—	—
A5	15.74	13.90	7.88	0.55	—
A6	16.23	15.00	5.11	1.20	—
A7	37.22	31.96	26.83	0.59	—
A8	30.81	29.66	23.25	5.90	—
A9	8.28	6.30	3.19	0.86	—
A10	22.87	13.45	3.26	0.96	—
A11	23.40	18.85	14.15	1.51	—
A12	29.70	30.24	19.87	4.86	—
A13	27.95	25.33	0.92	—	—
A14	44.08	38.84	10.19	5.61	—
A15	33.27	28.18	1.72	—	—
PS_{1725}	52.25	50.03	30.81	2.95	—
B1	33.10	9.67	3.79	—	—
B2	15.96	15.60	3.53	—	—
B3	18.94	13.85	1.87	—	—
B4	18.65	21.10	16.22	2.81	—
B5	4.95	4.90	2.00	—	—
B6	24.89	24.66	10.62	2.99	—
B7	49.12	44.35	8.81	2.87	—
B8	34.50	32.69	20.15	1.84	—
B9	39.87	38.29	15.90	3.33	—
B10	32.13	31.64	3.98	0.75	—
B11	16.60	15.39	2.90	—	—
B12	32.93	29.81	9.95	2.26	—
B13	8.86	7.44	4.90	—	—
B14	11.05	5.28	3.80	—	—
B15	30.79	29.08	0.90	—	—
PS_{3048}	62.99	51.27	42.66	3.12	—
C1	22.46	6.60	0.78	—	—
C2	29.53	24.03	3.88	—	—
C3	2.69	2.10	1.77	—	—
C4	5.81	4.90	2.36	—	—
C5	4.56	2.19	1.62	—	—
C6	20.17	6.39	0.86	—	—
C7	21.23	17.86	9.34	2.39	—
C8	28.14	28.09	3.03	—	—
C9	59.89	56.67	25.16	4.75	—
C10	34.95	35.12	13.58	2.21	—
C11	8.98	5.22	2.54	—	—
C12	32.09	32.45	28.80	3.19	—
C13	55.25	49.98	25.67	6.05	—
C14	22.92	23.16	5.27	2.19	—
C15	23.64	22.10	2.80	—	—
PS_{3703}	66.05	61.43	39.98	4.05	—

Table 3 : Tester strains with incompatibility factors.

I (A_1 B_1)	II (A_1 B_2)	III (A_2 B_1)	IV (A_2 B_2)
12	1	4	10
14	2	9	11
	3	13	
	6		
	7		

Petriplates with PDA medium were inoculated with freshly grown mycelial disc of monosporous lines separately. Inoculated plates were kept for incubation to the prefixed temperatures from 25°-38°C (25°C, 30°C, 34°C, 36°C and 38°C) for a period of 5 days. Net areas of radial growth were measured and compared among monosporous lines as well as with wild mother culture. Relative temperature sensitivity as well as maximum temperature tolerance were recorded.

Monosporous lines were subjected to crossing reactions using the "Tester strain" (Ghosh, 1988) following the standard method as advanced by Eger (1974). Positive crossing reactions with 'incompatibility bridge' were confirmed by microscopic examination of hyphal anastomoses at the meeting zone followed by marking clamp connections in the new growth of secondary mycelium.

Table 4 : Classification of monosporous lines into incompatibility classes.

I (A_1 B_1)	II (A_1 B_2)	III (A_2 B_1)	IV (A_2 B_2)
A_{12}	A_1	A_4	A_5
A_{14}	A_2	A_8	A_{10}
B_1	A_3	A_9	A_{11}
B_5	A_6	A_{13}	A_{15}
B_9	A_7	B_2	B_6
B_{11}	B_4	B_3	B_{10}
B_{13}	C_3	B_7	C_2
B_{15}	C_{10}	B_8	C_7
C_4		B_{12}	C_{12}
C_5		B_{14}	C_{13}
C_6		C_1	C_{15}
C_8		C_{11}	C_{18}
C_9			
C_{14}			

Table 5 : Selected monosporus lines as recombining parents.

Factors of selection	Culture No. with incompatibility factors
Cultures with higher growth	B_7 (A_2B_1)
	C_9 (A_2B_1)
	C_{13} (A_2B_2)
Cultures with high temperature tolerance	A_8 (A_2B_1)
	A_{12} (A_1B_1)
	A_{14} (A_1B_1)
	C_9 (A_1B_1)
	C_{13} (A_2B_2)

RESULTS

Comparative growth behaviour–Growth behaviour of monosporous lines on semisolid medium showed (Table 1) a tremendrous variation having a minimum of 2.66 cm^2 to as high as 78.81 cm^2 area of growth. Percentage area of growth compared to wild mother culture showed that most of the cultures could not cross the 50% mark. In this respect cultures as B_7, B_{20}, C_9 and C_{13} with more than 65% growth are of special mention.

Regarding growth in liquid culture (Table 1) and production of mycelial mass, percentage values ranges from 41.23% to 90.34% compared to the wild mother. Most of the cultures got restricted within 50-80% mark. The picture is somewhat different from radial growth behaviour, because some cultures produced much aerial mycelia.

Temperature sensitivity — It is aparent that some monosporous lines were very much temperature sensitive that were already eliminated during selective isolation at the elevated temperature of 34°C. Hence, the population is more or less tolerant at least upto 34°C. Further, a few of the members managed to withstand higher temperature upto 36°C and beyond which no one could survive. Considering temperature sensitivity isolates as A_8, A_{12}, A_{14}, B_{19}, C_9, C_{13} and C_{19} are labelled as more temperature tolerant members.

Determination of incompatibility groups – P. *sajorcaju* is a tetrapolar heterothallic species. Based on mating responses with the "Tester strains" already developed, members of monosporous population were classified into four distinct incompatibility groups. Also, incompatibility factors were determined. Some of the members responded erratically and were kept aside.

DISCUSSION

Natural population of monosporous lines in *Pleurotus sajorcaju* showed a wide variation in respect of growth pattern as well as temperature sensitivity. It is an indicative of the fact that monosporous population carries a natural variation in cultural properties which is further widened when different ecological variants are considered. Tetrapolar heterothallism in *Pleurotus sajorcaju* is an added advantage which keeps open huge scope of accumulting desired traits available in the population.

Monosporous lines are generally slow growing in nature as they bear an inherent dificiency which may be reinstated only after dikaryotozation. Within our limited population of fortyfive monosporous lines B_7, (A_2B_1), C_9 (A_2B_1) and C_{13} (A_2B_2) are considered with fast growing ability. Regarding temperature sensitivity A_8 (A_2B_2), A_{12} (A_1B_1), A_{14} (A_1B_1) and C_9 (A_1B_1), C_{13} (A_2B_2) have given special status for their higher temperature tolerance. Although, such many desired characters do not express in homokaryons or may not express in recombined isolates after crossing reactions. Still, such elevation of desired traits particularly the temperature tolerant isolates may help selecting suitable recombining parents. Recombination among the selected monosporous lines may lead to raise higher temperature tolerant recombined isolate(s) suitable for the tropical environment.

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(Accepted for publication July 14 2002)