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Lokesh Taram
Department of Plant Pathology, 2 Indira Gandhi Krishi Vishwavidyalaya, College of Agriculture, Raipur, Chhattisgarh, India

Ashok Kumar Koshariya
Department of Plant Pathology, 2 Indira Gandhi Krishi Vishwavidyalaya, College of Agriculture, Raipur, Chhattisgarh, India

Indra Kumar
Department of Plant Pathology, 2 Indira Gandhi Krishi Vishwavidyalaya, College of Agriculture, Raipur, Chhattisgarh, India

Sachin Dwivedi
Department of Plant Molecular Biology and Biotechnology, Indira Gandhi Krishi Vishwavidyalaya, Krishak Nagar, Raipur, CG, India

Dr. CS Shukla
Department of Plant Pathology, 2 Indira Gandhi Krishi Vishwavidyalaya, College of Agriculture, Raipur, Chhattisgarh, India

HK Singh
Department of Plant Pathology, 2 Indira Gandhi Krishi Vishwavidyalaya, College of Agriculture, Raipur, Chhattisgarh, India

Dr. AS Kotasthene
Department of Plant Pathology, 2 Indira Gandhi Krishi Vishwavidyalaya, College of Agriculture, Raipur, Chhattisgarh, India

Corresponding Author:
Lokesh Taram
Department of Plant Pathology, 2 Indira Gandhi Krishi Vishwavidyalaya, College of Agriculture, Raipur, Chhattisgarh, India

A technique to derive dikaryons through intra-specific compatible mating-pairs of *Pleurotus florida*

Lokesh Taram, Ashok Kumar Koshariya, Indra Kumar, Sachin Dwivedi, Dr. CS Shukla, HK Singh and Dr. AS Kotasthene

Abstract
Mushrooms are macroscopic fruiting bodies of fleshy fungi, belonging to the class Basidiomycetes or Ascomycetes. Mushrooms are an important source of nutrition particularly in the cereal based diet of the vegetarian people. Oyster mushroom is considered as first in quality followed by button, shiitake, straw mushroom and black ear mushroom. From fresh fruiting bodies of *Pleurotus florida* 31 single basidiospore were derived and evaluate for mating type status of monokaryotic isolates by two point culture technique given by Kothasthane 2003. All monokaryotic isolates derived from one species were inoculated in the same slant (2-3cm. apart) in a culture tube and were observed for the formation of tuft. Total of 961 possible pairs of *P. florida* were made between the isolates. In the present investigation one pairs of isolate which formed a thick tuft at the region of confluence were identified as compatible are P flor 10 and P flor 28 the derivatives of *Pleurotus florida* and were tentatively given the mating type status as AxBx for P flor 10 and AyBy for P flor 28. Among this one pair we took total 15 replication for different quality parameters like number of flushes in one bag flush size and yield.

Keywords: Heteroallelism, fleshy fungi, Strain, single basidiospore, isolation, *Pleurotus florida*

Introduction
Production of Mushroom is dependent on the quality of the spawn which is used to inoculate the substrate. To produce high quality spawn, breeding programs for strains resistant to certain diseases and able to form high-quality fruit bodies under standard growth conditions are necessary. Single basidiospores of *Pleurotus* are sterile in nature and can form dikaryotic mushroom only after mating with the other compatible isolate (Brown and Casselton, 2001) [1]. For a successful mating between monokaryons, A and B have to be of different allelic specificity (x and y). Only the dikaryon is fertile and able to form mushrooms under the right environmental conditions. Most mushroom species possess two mating-type loci that control their breeding. However, it improves the breeding program when the breeder is able to quickly identify compatible strains in a given set of progeny. Therefore, the mating-type genes themselves are the most important markers for breeding program in mushroom strain improvement. Since different mating-type alleles are present, but their function is obliterated in dikaryon formation through mating mycelia, this is called a secondary homothallic species (In ascomycetes, pseudo homothallic species) (Poggeler, 2001) [8]. Breeding is dependent on rarely formed, unicellular, and haploid basidiospores and hence the dependence on mating, karyogamy and meiosis prevails (Sonnenberg, 2000) [12], so breeding programs nevertheless use the mating-type genes. Vanaderies was the first to detect bifactorial heteroallelism in *P. ostreatus* by the analysis of clamp connection (Vanaderies, 1993) [13]. Bifactorial heteroallelism has also been reported in *P. sajor-caju* (Roxon *et al*., 1977; Singh, 1983) [9]. *P. flabellatus*. Inter-strain/intra-species hybridization always involves isolation of single spores and intermating of the single spore isolates (May and Royse 1982) [6].

Materials and Methods
Experimental site
The research experiments was conducted in the Plant Molecular Biology and Biotechnology College of Agriculture, Raipur and Mushroom Research Laboratory, Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.)

Source of material
Mature, healthy and fresh mushroom bodies of *P. florida* (P flor) was procured from Mushroom Research Laboratory, Department of Plant Pathology, College of Agriculture, Raipur, India and used for isolation of single basidiospores.

Single basidiospore isolation
Single basidiospore isolation from *Pleurotus florida* were isolated by using technique of Kotasthane *et al*., (2010) [3].

Mating procedure for the identification of compatible combination
To test for a mycelial interaction, single spore cultures of *Pleurotus florida* were multiplied on PDA medium at 26ºC. Isolates were paired against each other in every possible combination in the test tube by two point inoculation technique (Kotasthane, 2003) [3]. The inoculated plates and tubes were incubated at 27 ºC. The combination were observed regularly till one month for the formation of barrage. Mating types of the single basidiospore cultures were designated as AxBx and AyBy, respectively.

Isolation of the dikaryotic mycelium
In the compatible interaction, some well prominent zone forming compatible isolates as P flor10 × P flor 28, were selected. These selected crosses were inoculated in the petriplates and incubated to get well grown contact zone. After 7 days, dikaryotic mycelium was picked up from the different places of contact zone using fine inoculation needle and transferred on PDA slants and incubated at 27 ± 1°c. After getting full growth, all the dikaryotic cultures were transferred into the petriplates containing 20 ml PDA medium and incubated 27 ± 1 ºC for 7 days.

Statistical analysis
Completely Randomized Design was employed for analysis of some experimental data. The critical difference (C.D.) was calculated at five per cent probability level.

Results and Discussion
Collection and isolation of single basidiospores of *Pleurotus florida*
In this experiment total thirty one single basidiospores isolates weres derived from the fruiting bodies of *Pleurotus florida* (Table 1). These basidiospore of *Pleurotus florida* were designated as P flor for *Pleurotus florida.*

Table 1: Designation and number of monokaryotic isolates derived from *Pleurotus florida*

Monokaryotic isolates derived from *Pleurotus florida*
P. flor 1, P. flor 2, P. flor 3, P. flor 4, P. flor 7, P. flor 8, P. flor 9, P. flor 10, P. flor 11, P. flor 13, P. flor 18, P. flor 19, P. flor 20, P. flor 21, P. flor 22, P. flor 24, P. flor 25, P. flor 28, P. flor 29, P. flor 32, P. flor 34, P. flor 35, P. flor 36, P. flor 38, P. flor 39, P. flor 40, P. flor 41, P. flor 42, P. flor 44, P. flor 49, P. flor 50
Total 31

Identification of mating types and compatibility of isolate
All monokaryotic isolates derived from fruit body of *P. florida* were inoculated in the slants (2-3 cm. apart) in a culture tube and observed for the formation of tuft. Similarly small blocks of fungus was cut from the periphery in petridish and incubated at 25 ± 2 °c for 7-15 days. Total of 961 possible pairs of *P. florida* were made between the isolates. Out of which only one pair of isolate formed a thick tuft at the region of confluence were identified as compatible P flor 10 and P flor 28 the derivatives of *Pleurotus florida* and were tentatively given the mating type status as AxBx for P flor 10 and AyBy for P flor 28. Where one is fast growing isolate and one is slow growing isolate and remaining isolates not showing compatibility with this both isolates (P flor 10) AxBx, and (P flor 10) 28 AyBy (Table 2).

Table 2: Single basidiospore isolates (derived from *Pleurotus florida*) showing non compatibility with any one of the parents

Strain	**Non compatible single basidiospore**
Pleurotus florida	P flor 1, P flor 2, P flor 3, P flor 4, P flor 7, P flor 8, P flor 9, P flor 11, P flor 13, P flor 18, P flor 19, P flor 20, P flor 21, P flor 22, P flor 24, P flor 25, P flor 29, P flor 31, P flor 34, P flor 32, P flor 35, P flor 38, P flor 39, P flor 40, P flor 41, P flor 42, P flor 44, P flor 49, P flor 50.

The present findings are close to the results obtained by Kotasthane *et al.* (2011) [4]. The Out of 83 monokaryotic isolates tested against both Ps56 and Ps13, only 28 derivatives of *Pleurotus sajor-caju* were compatible with the Ps56 but not with the Ps13. Mating type of AyBy, opposite to that of Ps56 (AxBx) was assigned to 28 isolates. Gupta (2011) were selected Four compatible mating pairs derivatives of *Pleurotus sajor-caju* and *H. ulmaris* (Ps13 ×Ps56, Ps13 × Ps47, Ps13 × Ps35 and H6 × H23) for dikaryon isolation from prominent tuft in the confluence region of the component isolates.
Total fifteen strains were isolated and grown on PDA medium and evaluated for cultural characteristics (Colony character and radial growth). There was no significant differences observed among the cultural and morphological characters of all the dikaryons derived from P flor (10 × 28), because they showed similar type of colony character and pattern. Radial growth of different strains of P flor (10 × 28) on potato dextrose agar medium was studied and the data are given in table-3.
The radial growth in different strains of P flor (10 × 28) at completion of growth of any one strains were studied. It is clearly evident from the table that strains of P flor (10 × 28)3 *P. florida* gave significantly more (90.00 mm) radial growth followed by P flor (10 × 28) 2 (89.55 mm), (10 × 28)1 (89.00 mm), (10 × 28)4 and at par statistically. However it was significantly less (67.55 mm) recorded in strain P flor (10 × 28)4, P flor (10 × 28) 10 (67.55 mm) and P flor (10 × 28) 13 as compared to other strains at par with each other (fig 4.1). The radial growth in other strains of P flor (10 × 28) 9, P flor (10 × 28) 8, 14, 15, 12 (83.55, 82.55, 80.55, 80.44 mm) respectively and did not differ significantly with each other, similarly no significant differences was noticed in strains of 9, (84.00 mm) 5 (87.55 mm), 2 (89.55 mm) and 1(89.00 mm) (plate 3).
The present findings are similar to Gupta (2011) and Pandey (2016), and they made 34 dikaryons derived from the contact zone of three crosses by Gupta (2011). Similar type of studies also carried out Pandey (2016) and isolated 30 dikaryons of *P. flabellatus* from the contact zone of two crosses.

Table 3: Cultural characteristics and radial growth of different strains of P flor (10 × 28) derived from *Pleurotus Florida*

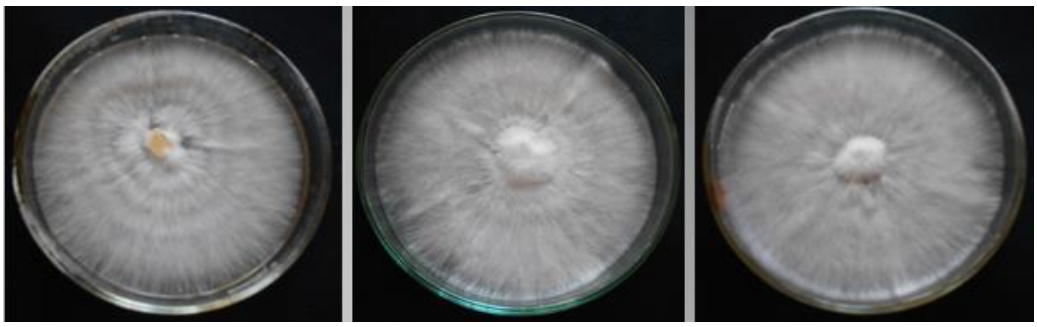
S. no.	Strains	Colours	Colony characters	Radial growth (mm)*
1	P flor(10 × 28) 1	White	Flat and with two concentric ring, margin even	89.00
2	P flor (10 × 28) 2	Absolutely white	Raised in centre ,flat at margin	89.55
3	P flor (10 × 28) 3	Milky white	Raised in centre and margin even	90.00
4	P flor (10 × 28) 4	Cottony white	Flat mycelial growth, with concentric ring, margin even	67.55
5	P flor (10 × 28) 5	Cottony white	Flat and with two concentric ring, margin even	87.55
6	P flor (10 × 28) 6	Cottony white	Flat and with two concentric ring, margin even	79.00
7	P flor (10 × 28) 7	Absolutely white	Thin white cottony growth flat at margin	80.55
8	P flor (10 × 28) 8	Cottony white	Thin white flat growth with concentric ring	83.55
9	P flor (10 × 28) 9	Absolutely white	Absolutely white with concentric ring, margin even	84.00
10	P flor (10 × 28) 10	White	Raised fulfy growth	67.55
11	P flor (10 × 28) 11	Absolutely white	Cottony growth ,flat at margin, margin even	76.00
12	P flor (10 × 28) 12	Absolutely white	Cottony growth ,flat at margin, margin even	80.44
13	P flor (10 × 28) 13	Absolutely white	Concentric ring, margin even	73.00
14	P flor (10 × 28) 14	Milky white	Flat even margin with concentric ring	82.55
15	P flor (10 × 28) 15	Cottony white	Raised growth in centre with concentric ring	80.55
	CD (P= 0.05%) SE(m)±			5.83 2.02

* (Average of three replication)

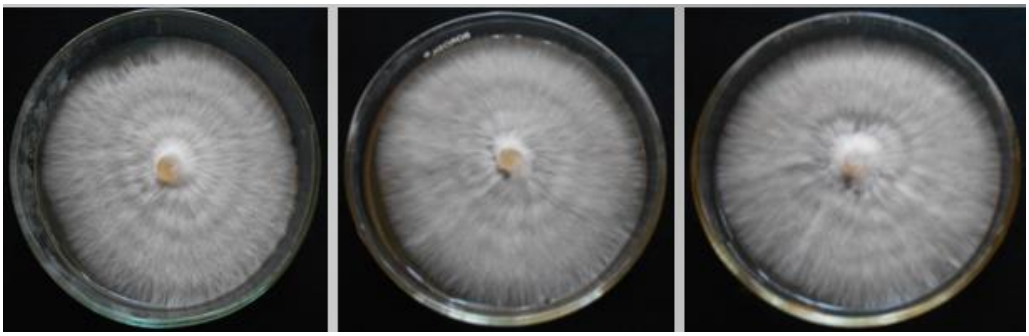
Magnitude view of the barrage formation in the region of confluence of *Pleurotus florida*

Two point inoculation technique in plate indicating the compatible mating pairs as confirmed by The barrage formation in the region of confluence.

Plate 1.1: Two point inoculation technique in plate for the identification of compatible mating pairs (As indicating by the formation of barrage)



P flor (10 × 28) 1 **P flor (10 × 28) 2** **P flor (10 × 28) 3**



P flor (10 × 28) 4 **P flor (10 × 28) 5** **P flor (10 × 28) 6**

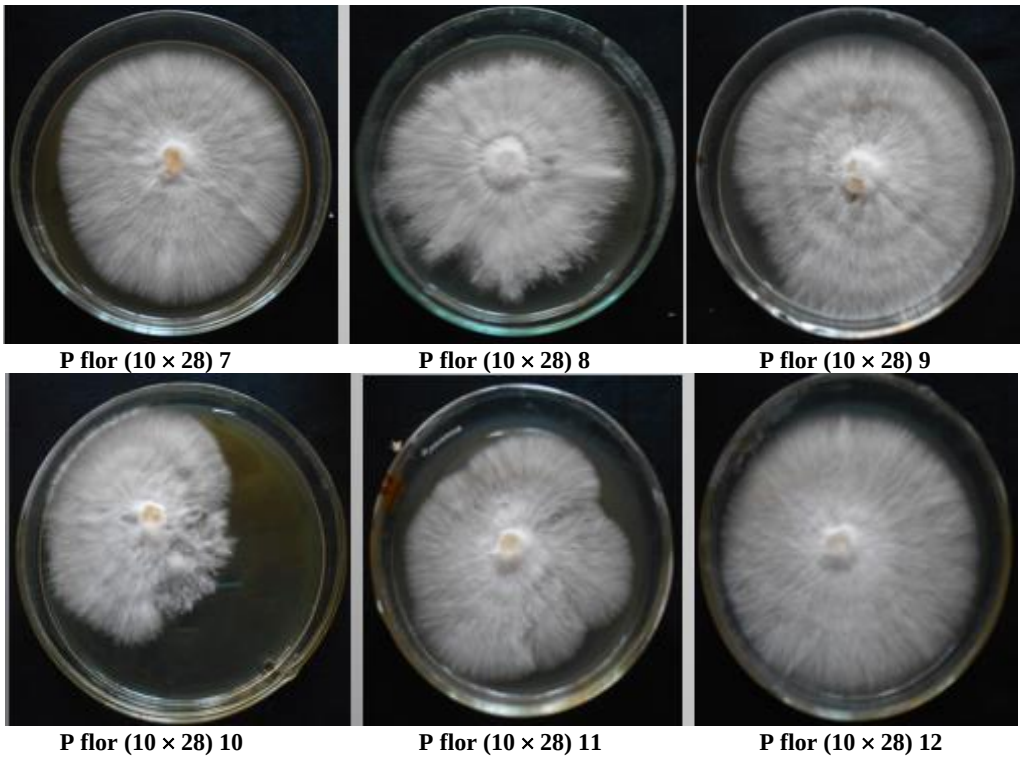


Plate 2.1: Cultural characteristics of strains of P flor (10 × 28) of *P. florida*

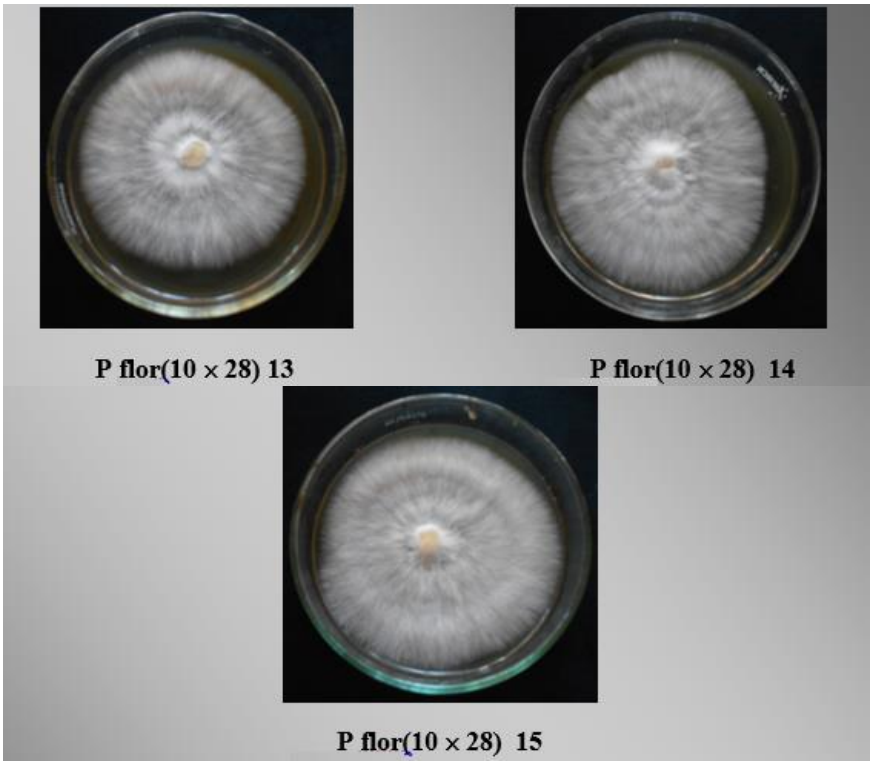


Plate 2.2: Cultural characteristic of 15 strains of P flor (10 × 28) of *Pleurotus florida*

Conclusions

1) The growth appears on Potato Dextrose Agar medium within 3-4 days and has several advantages. 1) Spore prints are taken from a small piece of the healthy mushroom fruiting body without surface sterilization. 2) The germinated and well proliferated spores are clearly discernible on the water agar surface under the dissecting stereo-binocular microscope and then can be directly lifted and transferred to the PDA slants. The technique of basidiospore isolation is direct, simple rapid and the chances of recovery of true product of meiosis are 100%.
2) Total thirty one single basidiospores were derived by single spore isolation.
3) One compatible mating pairs was selected (P flor 10 × P flor 28) and paired in petridishes for final confirmation for mating types and isolation of dikaryons. A total of 15 dikaryons (P flor 10 × P flor 28) from the tuft at the region of confluence were isolated.
4) There was no significant differences among the cultural morphology of all the dikaryons derived from P flor (10 × 28), because they showed similar type of colony character, pattern and radial growth. Radial growth of

different strains of P flor (10 × 28) on potato dextrose agar medium was studied and the data are given in table-3. (Plat 2.1 and 2.2).

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