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Strain improvement in oyster mushroom (*Pleurotus* sp.) through hybridization

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Abstract
Pleurotus is one of the most important and widely use mushroom in the world it contributed about 25% of the world total mushroom production. It can utilize agricultural waste and converted into a highly nutritious food. Now a days for strain improvement in mushrooms several modified breedings techniques such as selection and hybridization by the process of Protoplast fusion, use of chemical mutagenesis, use of resistance markers, have been employed with new findings of high yielding, more nutritious, disease resistance and more biological efficiency.

Keywords: *Pleurotus* sp., hybridization, strain improvement, monokaryotic. dikaryotization

1. Introduction
Mushroom contribute as a part of nutritious diet since ancient ages, they were used as a food even before man understood use of other micro organism. Undoubtedly mushrooms were one of man's earliest foods, and they were often considered an exotic and luxurious food. *Pleurotus* can be grown in different agricultural waste to different agro- climatic condition, moreover, difficulty in composting as a preliminary step for button and straw mushroom is not required for oyster mushroom cultivation. All this flexible nature of oyster mushroom gives a main reason for contributing 25% of the world total mushroom production, International Society for mushroom sciences (2016). In India, Bano and Srivastava (1962) first standardized the cultivation of *Pleurotus flabellatus* and first domesticated species of *Pleurotus* was *P. ostreatus*. Later in the year 1974, *P. sajor-caju* was mostly cultivated when Jandaik and Kapoor first reported its cultivation on banana pseudo stem chopped with paddy straw.
In the year 1904 Blakeslee was the first person to find the sexuality of the fungi *Mucorales* through the mycelium mating from the single spores. Later in the year 1918-20 the mechanism of sexuality in basidiomycetes was discovered. Now a days for strain improvement in mushrooms several modified breedings techniques such as selection and hybridization by the process of Protoplast fusion, use of chemical mutagenesis, use of resistance markers, have been employed with new findings of high yielding, more nutritious, disease resistance and more biological efficiency. For developing good strains of *Pleurotus*, several breeding strategy like hyphal anastomosis in *P. sajor caju* has been widely used (Jandaik, 1997) [12]. Protoplast fusion is another intresting process which is now been frequently implied for strain improvement. These methods are used especially when conventional method cannot be achieved. As conventional hybridization, protoplast fusion can be performed intraspecifically (Kiguchi and Yanagi 1985) [17], interspecifically, intergenerically and even interheterogenerically (Eguchi and Higaki 1995) [7]. Dikaryotization of selective strains is another very important tool in strain improvement for bringing genetic recombination and developing somatic hybrids which has been used by several workers to develop new strains of *Pleurotus* with the findings of fast colonizing ability which lead to early flushing of the fruit body with good shape , size, low mortality rate of the bud, good color of the pelius and the high protein content (Bahukhandi and Sharma, 2002, Rosanina *et al.*, 2007, Kumara and Edirimanna, 2009, Jaswal *et al.*, 2013) [2, 21, 18, 14].

2. Hybridization Process of *Pleurotus.*
2.1. Isolation of single spore for strain improvement of *Pleurotus*.
The existing literature indicates that various techniques such as spore prints, dilution and growth test method can be implied in order to get single spore.

2.1.1 Spore Print method for isolation of single spore

The best described method for single spore isolation through spore print has been given by Petersen and Ridly (1996) [20] in which the spore prints were prepared by cutting a small piece (about 2.5 ×2.5 cm) of a fresh mushroom cap and sticking it gills-down on the sterilized lid of a Petri dish with paraffin or sticky tape which was then put on top of a sterilized beaker lined with 1×1 cm of sterilized filter papers. The spores were then allowed to discharge onto the pieces of paper for 3-10h before the lid was replaced with a new sterilized one. The beaker having spore prints on pieces of paper were than kept in a refrigerator until single basidiospore isolations were formed. Single spore isolates which are monokaryons (n) and without clamp connections (clamps) on their hyphae are used for hybridization.

2.1.2 Serial dilution method for isolation of single spore

The dilution method demonstrated by Bahukandi and Sharma (2002) [2] from prepared spore suspension by taking small loop of spore from the spore print and suspended in sterile distilled water. From this suspension further dilution up to 10^{-4} was made, where the spore concentration was as low as up to 4-5 spores when seen under low power microscope (10x). Therefore, from this solution a loop of spores were streaked on plain agar in petriplates.

Another serial dilution method for the isolation of single spore was done by Jaswal *et al*., (2013) [14] in which spore coated paper (5mm X 5mm) was cut and aseptically transferred into 10 ml saline solution. Serial dilution was made to get approximately 500 spores per ml using haemocytometer. 0.1 ml of spore suspension was spread on PDA agar plate and incubated at 25±1 °C for germination. After 3-4 days of incubation, single spore isolates (monokaryons) appeared as small colony heads which were carefully transferred on to the PDA slants and incubated at 25+1 °C for 7-10 days. These monospore cultures, confirmed as monokaryons, with no clamp connections as seen under the microscope at 45x and were maintained on PDA slants at 4 °C.

2.2 Conformation of monokayotic mycelium

Bahukhandi *et al.,* (2002) [2] reported that the single spore isolates obtained in the three species of *Pleurotus* viz. (*P. sajor-caju* ,*P. sapidus* and *P. comucopiae*) had some peculiar features, by which they were differentiated from dikaryotic mycelium, (i) The single spore isolates were having slower growth and in some cases the growth was limited to inoculum. (ii) Absence of clamps. (iii) They did not produce fruiting bodies, during cultivation. Although some primordial formation was seen in some monokaryons but they were abortive and did not grow further. Some of the isolates were not able to colonize the substrate. Jaswal *et al*., (2013) [14] reported that when monospore of *Pleurotus* are incubated in 25^0C for 7-10 days these monosporus cultures confirmed as monokaryons, with no clamp connections when seen under microscope at 45x.

2.3 Dikaryotization of mycelium

Peng *et al.,* (2001) [19] while working with the king oyster mushroom he selected seven monokaryons from *P. seryngii* strain Holland 150 and cross with the strain ATCC 36947. They got totally 92 hybrid dikaryons with normal mycelial growth which was selected for primary fruit test. Yield, biological efficiency, shape, texture and taste of the fruit bodies were recorded. Sharma (2002) [2] subjected fifty seven *Pleurotus* hybrids to confirm dikaryotic nature of mycelium. Among them thirty five *Pleurotus* hybrids were able to fructify. Further observation was recorded for good character of fruit yield, total number of fruit bodies and of early spawn running which was completed between 26-65 days of inoculation. Above that six hybrids showed early spawn running comparing to parent.

Bahukhandi *et al,*. (2002) [2] described four type of reaction during the mating process between two monokaryons of *Pleurotus* which was seen in the petriplates. First reaction mating occurred and fertile progeny was produced. In this process, the hyphae developing from both the oppositely placed inocula were anastomosed and showed clear difference in appearance between individual parents and mating hyphae. When tested for fertility, they were found to be fertile. The non-mated pairs belonging to remaining three categories have some special morphological characters. In first category, the hyphae of the monosporous pairs were intermingled and gave an idea that these have mated, but after testing they did not produce fruit bodies and were found sterile. Most of the non-mated pairs belonged to this category. In the second category, the hyphae of both the paired mono spores grew but did not mate and remain separated. This reaction of the non mated isolates was neutral. In the third category, a zone of inhibition was developed between paired isolates and their growth occurred in different direction to each other. This reaction was termed as inhibitory and the number of pair in this category was lowest among all the three crosses. Jaswal *et al.,* (2013) [14] reported that when inter strain crosses between two monokaryons of *P. florida* PAU-5 and *P. sajor caju* PAU-3 by putting 4 mm diameter mycelium colony of both strain on sterilized PDA. The plates were incubated at 25±1°C for 4-5 days. Depending on their compatibility, the dikaryons were picked, where the mycelium of the two showed dense aerial growth at the junction. This cottony fluffy growth indicated the putative dikaryon formation which were examined microscopically (45X) for presence of clamp connections to confirm their dikaryotic nature.

3. Hybridization of *Pleurotus*

Earlier work to produce the high yield producing strain of the *Pleurotus* sp. was first performed in *P. ostreatus*. Heterokaryons were developed by Eugenio and Anderson (1968) [8] and Eger *et al.,* (1976) [6]. Peng *et al.,* (2001) [19] observed that while growing three strain of the king oyster mushroom i.e., ATCC 36047, ATCC 90212 and Holland 150, Strain ATCC 36047 had high commercial value with medium to large fruit bodies, fine texture and long shelf life but it produce many warts on caps surface during the high humidity. Holland 150 produced early large fruit bodies with a soft and fine texture and with short shelf life. For a goal of obtaining high productivity, long shelf life with good texture and other good quality, the crosses were made between the monokaryon of strain ATCC 3647 and Holland 150 and by screening of biological efficiency, yield efficiency, fruit shape, texture, taste of fruit body and days for fruiting they selected four highly productive strains. The hybrid B122 have large fruit bodies with a small cap, equal to fusiform stripe, a medium soft and fine textured. The hybrid B012 produced fan shaped cap, stout fruit bodies with a prominent eccentric or lateral stripe but it produce warts during high humid condition. Hybrid B030 produced slender fruit bodies, round cap with medium size. Hybrid B011 showed stout fruit bodies with a

large cap and a white and solid stipe.

Sharma (2002) [2] observed that the change of morphology of hybrid cultures compared to parents when he performed the inter-specific hybrid by mating of *P. sajor-caju* and *P. cornucopiae*. The shape and size of fruit body was similar to *P. sajor-caju* with white color resembling *P. cornucopiae*. They had categorized hybrid dikaryons into nine groups based on size, shape, color, yield etc. From each group, one type sample having high yield, early fruiting and light color was selected for further studies. Kaur (2007) [16] reported that when intra-species crossing of *P. florida* was made new crosses with funnel shaped fruit bodies, lateral fruit bodies with wavy margins and gray colour fruit bodies was obtained. Five *Pleurotus* hybrid dikaryons were developed by among monokaryons of *P. florida* PAU-5, Out of which PFJ 11 out yielded the parent while average weight of fruit bodies was higher in PFJ 13 (9.9 g) as compared to parent (9.6 g). Spawn run was recorded faster in PFJ 11 (39 days) and PFJ14 (41 days) with respect to that of the parent (48 days).

4. Hybridization efficiency in *Pleurotus.*

Hybridization efficiency varied both in intra and inter species which was proven by Sawashe and Sawant (2005) [22] who reported that the hybridization efficiency depends upon the compatibility between randomly selected progeny of monospore cultures, while doing the inter species hybridization between *P. florida* and *P. ostreatus*. He got the new hybrid with hybridization efficiency of 12.5%. Intra species hybridization was conducted by Gharehaghaji *et al.*, (2007) [9] where they had made 289 crosses between monokaryons of different strains of *P. ostreatus* to develop 27 hybrid dikaryons with hybridization efficiency of 9.3 per cent. Shnyreva and Shtaer (2006) [23] isolated monokaryons and dikaryons based on presence and absence of clamp connections for mono-mono and di-mono matings of *P. pulmonarius* and *P. ostreatus*. Hernandez and Salmones (2008) [11] had isolated 20 monokaryons from each strain of *P. ostreatus*, which showed the varied performance in both physical and chemical attributes of mushroom.

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