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Hybrid Production of Oyster Mushroom *Pleurotus ostreatus* (Jacq: Fries) Kummer

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Abstract: Optimization of industrial mushroom production depends on improving the culture process and breeding new strains with higher yields and productivities. So many works have been done on process improvement, Although few systematic studies of genetic breeding of *Pleurotus ostreatus* strains have been reported. The major aim of hybridization is to combine desirable characteristics from different strains and create variability in the existing germ plasm. In this study, we used a breeding approach to hybrid production from cultivated Oyster mushrooms *Pleurotus ostreatus*. Five strains of *Pleurotus ostreatus* (Jacq: Fries) Kummer were used in this research. Basidiospores were suspended in sterile distilled water and counted with a haemocytometer. After germination, colony of each isolate transferred into the PDA medium. Growth rate and colony type of each isolate was determined and then 17 monokaryons were selected. Consequently screening monokaryons were crossed to each other. Some characteristics such as morphological interaction in the contact zone of mycelium, increasing in growth rate of hybrid, change of colony morphology and the presence of clamp connections between dikaryotic cells used to distinction of monokaryons from dikaryons. We recognized 27 hybrids by these characteristics.

Key words: *Pleurotus ostreatus*, hyphae, compatible and incompatible reactions, monokaryon, dikaryon, clamp connection

INTRODUCTION

The edible mushroom production industry is more important every year for several reasons: mushrooms are an efficient low-fat protein source, they may be cultivated on a wide variety of substrates and they have many industrial and medical applications (Larraya *et al*., 2003; Mohammadi Goltapeh and Pourjam, 2005). So far 25 species of more than 2000 edible fungi are widely accepted for human consumption but only a few of them are cultivated commercially (Bhandal and Mehta, 1993).

Pleurotus ostreatus (Jacq: Fries) kummer is a commercially important edible mushroom commonly known as the oyster mushroom. This fungus is industrially produced as human food and it accounts for nearly a quarter of the world mushroom production. It is also used for the bioconversion of agricultural, industrial and lignocelluloses wastes as a source of enzymes and other chemicals for industrial and medical applications, as an agent for bioremediation and as organic fertilizer (Abdellah *et al*., 2000). On the other hand, the desirable attributes like rapid mycelia growth, high saprophytic colonization ability simple and cheap cultivation techniques and easy post harvest storage have cultivated to popularity of *Pleurotus ostreatus* (Bhandal and Mehta, 1993).

With the constantly growing popularity of edible mushrooms and their tremendously increased use for mushroom products in commercially significant amounts, there is a great incentive for obtaining improved strains. Several methods used for strain improvement in *Pleurotus* including selection, hybridization and gene transformation. However, proper attention has to be paid to the breeding goals in any such efforts (Kaul, 2001). Understanding mushroom breeding systems is a major landmark when commercial breeding programs are being established (Larraya *et al*., 2003). *Pleurotus ostreatus* production is dependent on the life cycle, which alternates between monokaryotic (haploid nucleus) and dikaryotic (dihaploid nucleus) phases. Two compatible monokaryotic hyphae are able to fuse and give rise to a dikaryotic mycelium in which the two parental nuclei

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remain independent (dikaryon, heterokaryon) throughout vegetative growth and fruiting body development. True diploidy occurs only in the basidia, where karyogamy takes place immediately before the meiotic division which produces four uninucleate basidiospores (Larraya *et al*., 2003). Formation of clamp connections is used as presumptive evidence of this sexual compatibility. Clamp connections are formed during the conjugate division of the nuclei in the growing hyphae tip. When a binucleate hyphal tip is ready to divide, a short branch-the camp connection-arises between the two nuclei and begins to form a hook. The presence of clamp connections is therefore generally indicative of the dikaryotic condition. (Petersen and Bermudes, 1992).

MATERIALS AND METHODS

Isolates: Isolates of *Pleurotus ostreatus* (Jacq: Fries) Kummer for this study obtained from field collections (samples) representing geographic regions in west Iran. Sources of isolates included spore prints taken in the field. This study was conducted at Department of Horticulture, University of Tabriz, Iran in 2003-2004.

Single basidiospore isolates: Single basidiospore isolates were obtained from 5 collections. To obtain them, an aqueous suspension was prepared from spore prints deposited in Petri plates. For this propose, spore prints of five collections cut into 1-2 cm^2 pieces and dropped into the sterile water (2 mL). The test tube was carefully shaken to obtain a dense white spore suspension (ssI). Using a hypodermic syringe, sterile water was added to ssI to obtain a concentration of ca. $1/0\times10^3$ spores/mL (ssII). The concentration of spores in spore suspensions was determined by using a hemicytometer and a microscope. Suspensions were spread on PDA (39 g of Potato Dextrose Agar) supplemented with 100 mg L^{-1} of streptomycin. After incubation for 4 days at 24°C in dark, individual germinated basidiospores were removed manually with the aid of a microscope at 65× and transferred into fresh PDA.

Mating studies: Monosporous isolates were used 3 weeks after isolation. Pairs of monosporous isolates were placed 15 mm apart in the center of a Petri dish containing PDA. The plate was then incubated at 24°C in the darkness for about 14 days, until the two mycelia formed a large contact zone. A strip about 2 mm of mycelium was cut off from the contact zone, placed on a new culture plate, allowed to grow for some days and examined under the microscope for the presence of true clamp connections (dikaryons), unfused (false) clamp connections (heterokaryons), or the absence of clamp connections (heterokaryons). Eleven to fifteen monosporous isolates from each of the five basidiomes were paired in all possible combinations.

RESULTS AND DISCUSSION

Mating studies in PDA medium showed that *Pleurotus ostreatus* has a tetra polar mating system (Table 1). Matings of homokaryotic cultures from the other basidiocarps also had a tetrapolar pattern (Esser and Blaich, 1994).

Compatible reactions observed between two compatible homokaryotic mycelia through hyphal fusion, resulting in the formation of a heterokaryotic fertile mycelium. The nuclear division and septum formation in this mycelium occur through clamp connections (Fig. 1). The formation of clamp connections

Table 1: Mating between selected monokaryons

	Parent 1																
Parent 2	III37	I35	II75	V29	IV40	I8	III36	II59	V31	I6	IV12	III26	II9	IV27	I22	III20	V5
III37	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
I35	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-
II75	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	-	-
V29	+	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-
IV40	-	+	-	+	-	-	-	+	-	-	-	-	+	-	-	+	-
I8	-	-	+	-	-	-	+	-	-	-	+	-	+	-	-	-	-
III36	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
II59	+	-	-	-	+	-	--	-	-	+	-	-	-	-	-	-	-
V31	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I6	-	+	+	+	-	-	+	+	-	-	-	-	-	-	-	+	-
IV12	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-
III26	-	+	-	+	-	-	-	-	-	-	+	-	+	-	-	+	-
II9	-	-	-	-	+	+	-	-	-	-	-	+	-	+	-	-	-
IV27	-	-	+	-	-	-	+	-	-	-	+	-	+	-	-	-	-
I22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III20	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-
V5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ Indicates a compatible interaction, secondary mycelium formed, -Indicates an incompatible interaction, no secondary mycelium formed

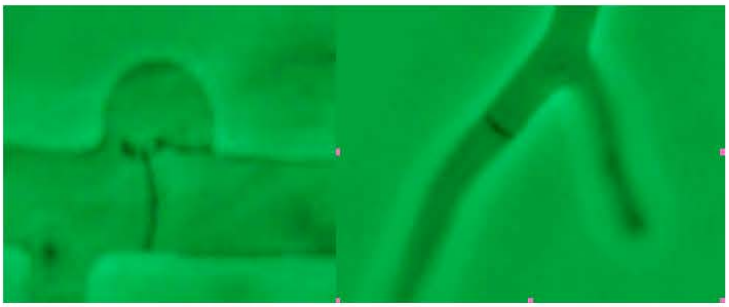

Fig. 1: Clamp connection in IV12×I8 Hybrid, Mycelium of monokaryon of *Pleurotus ostreatus*

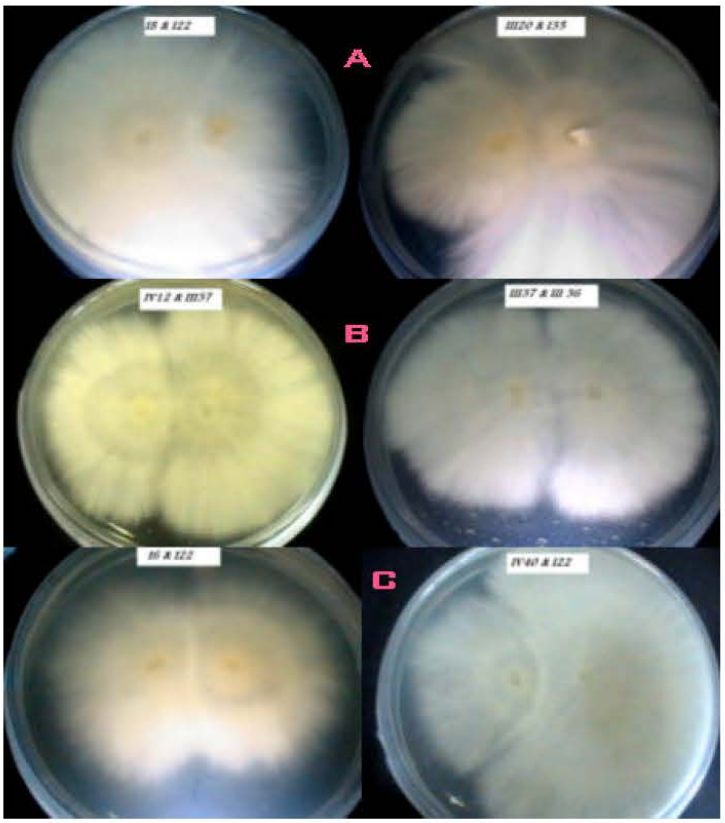

Fig. 2: Incompatible pairings of single spore isolates from *Pleurotus ostreatus* (Jacq: Fries) Kummer. A: Surrounding a mycelium by another mycelium. B: Line of demarcation. C: Barrage formation

by compatible haploid isolate, indicative of dikaryon formation, is a useful *in vitro* tool for basidiomycete systematic (Larsen *et al*., 1992).

In this case, the two types of mycelia intermingle in the zone of contact and show numerous hyphal fusions via anastomosis. Fischer and Bresinsky (1992) showed that compatible reactions of hyphal fusions in *Phellinus torulosus* resulted in the formation of heterokarotic mycelium in the contact zone. Formation of the heterokaryon was restricted to the contact zone. Then the border zone between two mycelia becomes unrecognizable through the time (Esser and Blaich, 1994).

Three types of incompatible reactions were observed:

Inhibition: In pairing of incompatible isolates, one of mycelia grows fast and surrounded another mycelium. In these cultures we cannot observe any clamp connection (Fig. 2A). This phenomenon may be caused by unilateral

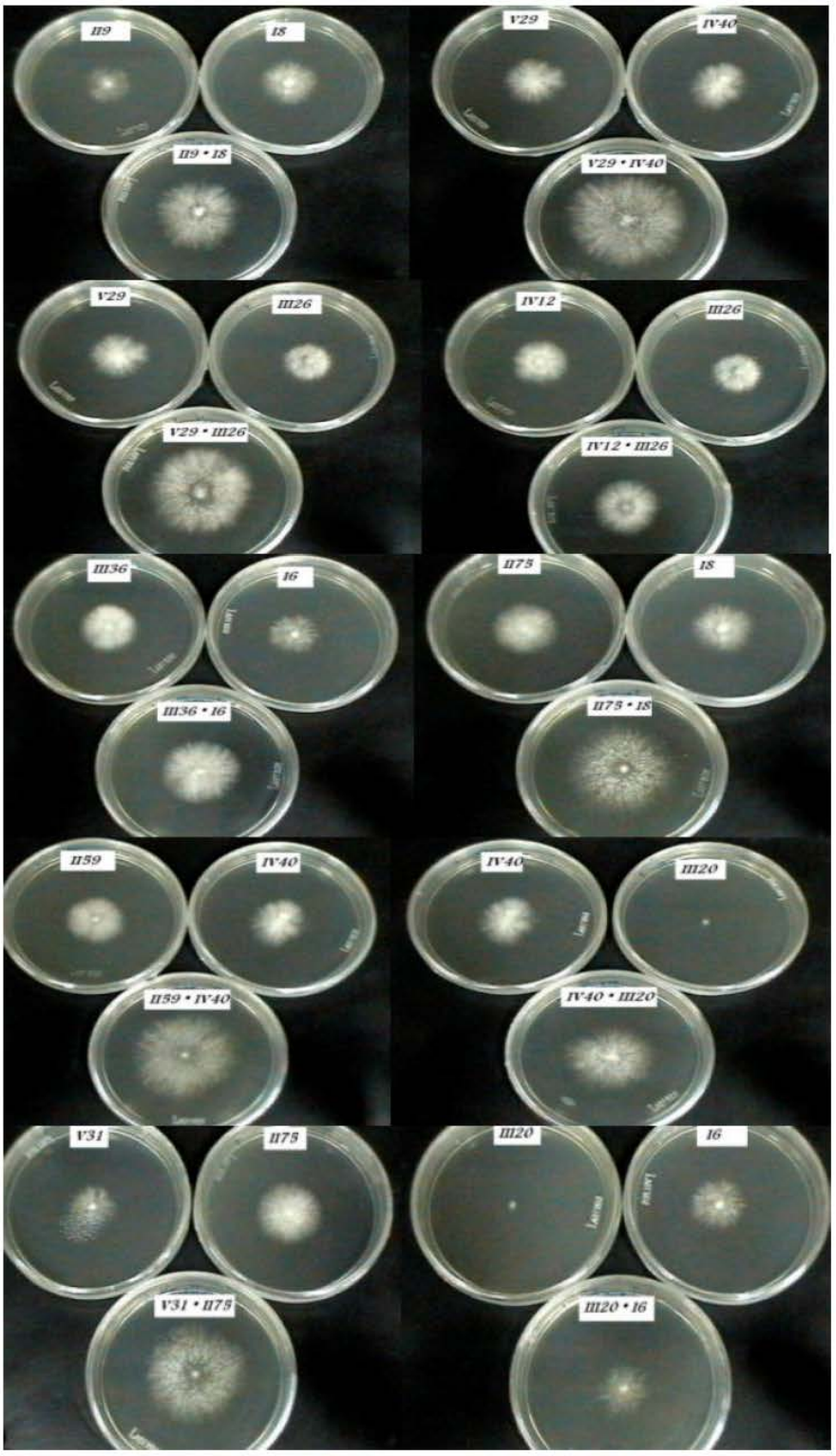

Fig. 3: Countinued

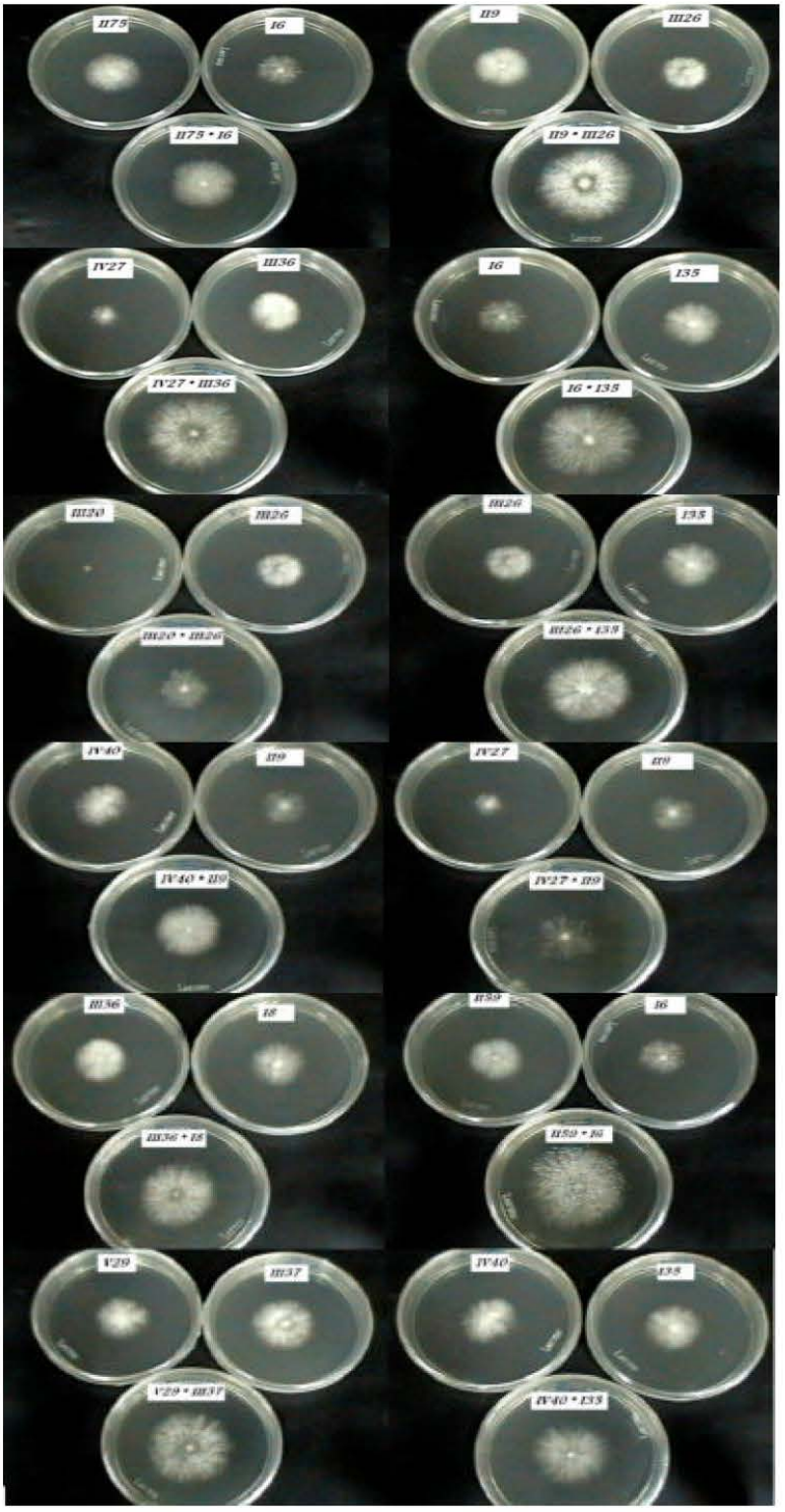

Fig. 3: Countinued

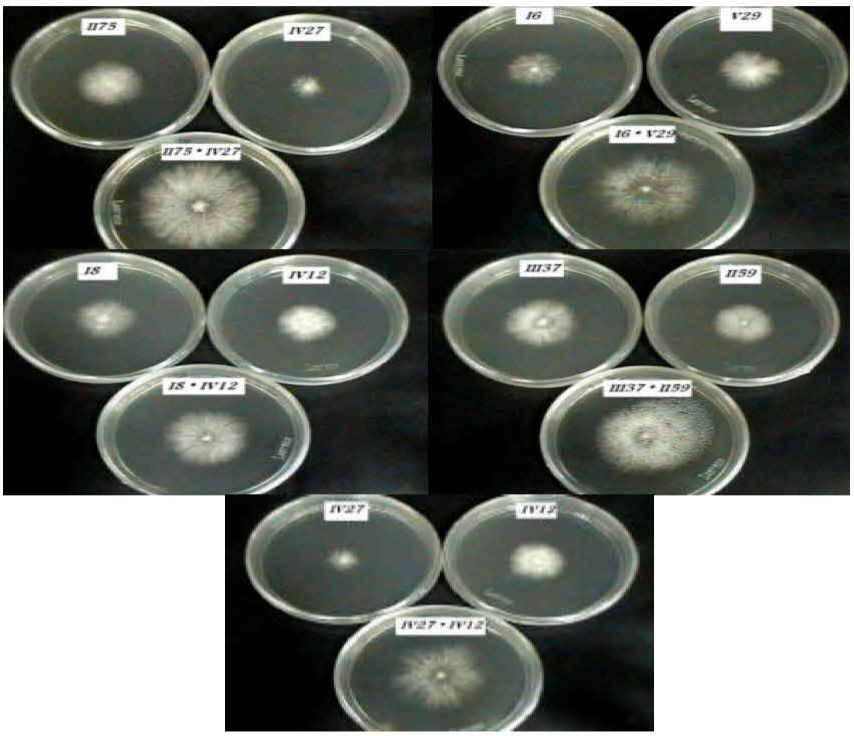

Fig. 3: Pairings and their parental monokaryons of *Pleurotus ostreatus*. Parental monokaryons were shown by two Petri plates on top of each picture and Petri plate on bottom of each picture shows the pairings

or mutual interaction, due to the secretion and diffusion of inhibitory substances (Esser and Blaich, 1994).

Mutual repulsion = Borderline, Demarcation line: In pairing of incompatible isolates in this study, a mutual repulsion and antagonistic reaction is evident which leads to formation of a line of demarcation. There is no analysis of the microscopic structure of these lines (Fig. 2B). It has not been established whether or not there is hyphal fusion allowing cytoplasmic contact and nuclear exchange in demarcation line (Esser and Blaich, 1994).

Mutual intermingling and inhibition = Barrage formation: In some cases the two types of mycelia grow into each other and intermingle, but an antagonistic reaction ensues. In fact the two types of mycelia form lethal fusions (Fig. 2C). In these cultures the clamp connection was not observed Esser and Blaich (1994) showed that nuclear exchange is not inhibited, in all barrages known so far but in most cases two types of mycelia form abnormal and even lethal fusions. The hyphal tips may branch profusely. A clear line of contract appears with increasing age of the culture. Barrages are mainly found in intra specific.

To confirm compatible and incompatible reactions, pieces of agar from the interaction zones were cut out and plated into fresh PDA. Compatible reactions resulted in one colony; incompatible reactions resulted in two or more colonies. Monokaryotic and dikaryotic hyphae were compared by the radial growth, the appearance of colony and the change of colony morphology. A comparison of colony radial growth was made between heterokaryons and homokaryons after one week of incubation. The colony diameter mean for the heterokaryon isolates was 34-75 mm compared to 16-46 mm. It was a significant difference between heterokaryon and homokaryon cultures in colony diameter mean in probability level of $p<0.01$. The correlation coefficient was 0.49. So growth rate could be used to distinguish homokaryon isolates and putative heterokaryon isolates. Eillott (1993) showed dikaryons grow faster than their component monokaryons and there was a high correlation between monokaryotic and dikaryotic growth rates. Moreover, mating compatibility between collections from monokaryons with high radial growth was generally high, with 63% or more of all pairing being compatible. A slightly lower frequency of compatible matinges was observed for crosses made between single-spore isolates with low radial growth.

The observation of colony morphology between dikaryons and monokaryons resulted in a clearly change of colony morphology in pairing. Depending on which isolates were paired, mycelia development varied within

the interaction zone, which ranged from appressed, fluffy to cottony. Also Kay and Vilgalys (1992) showed that mycelia development within the interaction zone in paring among natural (wild) dikaryons from the population survey, ranged from densely to sparsely interwoven, appressed to cottony and narrow to broad. In this study, 82% of pairings derived from compatibility between appressed mycelium of monokaryons. All of crosses between fluffy mycelium of monokaryons were incompatible. So the fluffy mycelium was not considered as a desirable character for hybrid making. This result showed by many investigators (Kligman, 1943). Therefore, it is concluded that fluffy type colonies should be avoided in any strain improvement program.

As this study was made with five strains, it would seem worthwhile to determine whether these methods apply to other particular strains of *Pleurotus* that seem to have breeding potential. Also the specific methods need to be developed in order to understand combining ability between monokaryons and to define more precisely the relationships between parental monokaryons and the dikaryons produced by intercrossing them. These methods were potentially useful to recognize 27 hybrids in this study (Fig. 3).

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