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Short Communication

Multispore Isolation of *Pleurotus* species - A Modified Simple Technique

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The method of multispore isolation of *Pleurotus* was modified to develop a simple and easy technique. For this, mature fruiting bodies of *P. flabellatus* and *P. membranaceus* collected from nature and that of *P. sajor-caju* collected from mushroom house were used. The sporocarp was cleaned with sterile cotton to remove dust particles, if any, adhering to it. The pileus of the sporocrap was separated from the stipe by cutting with a razor blade sterilized with alcohol and taken in a pre-sterilized petriplate to the laboratory and kept inside laminar flow chamber. By holding the pileus with a sterile forceps inside the petriplate, 0.5×0.5 cm size piece of tissue was cut from the central portion of the pileus with a sterile blade. Five fruiting bodies were taken from each *Pleurotus* species and one piece was cut from each fruiting body. The pileus tissue pieces were kept in another sterile petriplate.

Carrot agar (CA) medium was used for the isolation of *Pleurotus*. Other suitable culture media like potato dextrose agar can also be used. CA medium was poured into sterile petriplates of 90 mm diameter @ 15 ml/petriplate. When the medium was solidified, the plates were reversed (upside down) without opening and kept with the lid at the bottom. By slightly lifting the base plate with medium from the lid little D.P.X. mountant was smeared at the center of the inner surface of the lid to an area of approximately 0.5×0.5 cm with the help of a sterile glass rod. The piece of pileus cut and kept in a petriplate was used for multispore isolation of *Pleurotus*. Each piece of pileus was stuck to the inner surface of the lid where D.P.X. mountant was smeared, so that the gills were facing the surface of the culture medium. One piece of pileus tissue only was kept in each plate. After about 5 minutes when the pileus piece had stuck properly, the petriplates were reversed and kept in the normal position with the lid above the base. In this position, the gills were hanging from inside center of the lid facing the surface of culture medium. The petriplates were thus prepared for the *Pleurotus* to self-inoculate the medium with the basidiospores falling from the gills. These plates were incubated at room temperature (26±3°C) for 3-7 days. After 3 days of incubation, fungal growth appeared on the surface of the medium, just below the portion where gills were placed. It was subcultured and maintained.

Table 1 : Success of isolation of *Pleurotus* species in two methods.

Sl. No.	Method of isolation	*Pleurotus* species	*Colony diameter (in mm) Days after inoculation			**No. of plates without microbial contamination (out of 5 plates)	Percentage of success in isolation
			3	5	7		
1.	Tissue culture method using surface sterilized pileus tissue plated on carrot agar medium	*P. sajor-caju*	03.0	14.5	32.0	02	40
		P. flabellatus	–	11.0	34.5	02	40
		P. membranaceus	–	08.7	28.7	03	60
2.	Spore fall petriplate culture method	*P. sajor - caju*	02.8	08.4	34.0	05	100
		P. flabellatus	03.0	09.3	41.0	04	80
		P. membranaceus	03.2	13.0	43.4	05	100

* Mean diameter of colonies without contamination. ** Number of fruiting bodies of each *Pleurotus* species used for isolation : 5 (1 piece from each pileus/plate). – No growth.

Simultaneously, isolations were also carried out by plating surface sterilized tissue taken from the same fruiting body used for the collection of tissue for the new method of isolation. For this, 3×3 mm size pileus tissue from each fruiting body was surface sterilized with 0.1% mercuric chloride solution, washed in three changes of sterile water and plated on CA medium. Since commercial oyster mushroom growers and most of the scientists and students engaged in *Pleurotus* cultivation follow this tissue culture technique for isolation, mother spawn preparation etc., the new technique was compared with this method.

In the new technique of 'spore fall petriplate culture' method of multispore isolation of *Pleurotus* the success of isolation without any contamination was 80-100% (table 1). On the other hand, when the surface sterilized pileus tissue was plated on culture medium success of isolation without microbial contamination was only 40-60%. The rate of microbial contamination was high in this method. About 3 mm diameter (2.8 to 3.2 mm) fungal growth of all the three *Pleurotus* species was observed on 3rd day of incubation in the new method of multispore isolation. The new method of isolation was repeated 20 times using *P. sajor-caju* fruiting bodies and obtained 100% success in isolation without any microbial contamination.

The conventional method of spore culture is carried out by obtaining spore print on sterile paper/petriplate and mixing a mass of spores in sterile water. This spore suspension is used for multispore isolation by mixing 1-2 ml of spore suspension with culture medium (1). Thus there are several steps in this method of isolation whereas the modified method of isolation is very simple and easy to practice even for a beginner in mushroom cultivation. The spore fall petriplate culture method requires lesser time than the conventional methods of isolation. Therefore, large number of samples can be processed in a short period with high rate of success in isolation of *Pleurotus* species.

References

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