MULTIPLICATION AND PRODUCTION OF OYSTER MUSHROOM ON LABORATORY SCALE ON DIFFERENT SUBSTRATES

ALMI H.
Laboratory of Mycology, Biotechnology and Microbial Activity, SNV, University of Mentouri **Constantine, 25000 Algeria**

LAOUFI O.
Laboratory of Mycology, Biotechnology and Microbial Activity, SNV, University of Mentouri **Constantine, 25000 Algeria**

BOULMAREKA A.
Laboratory of Mycology, Biotechnology and Microbial Activity, SNV, University of Mentouri **Constantine, 25000 Algeria**

OUFROUKH A.
Algerian Institute of Agriculture Research ***Constantine, 25000 Algeria***

KACEM CHAOUCH N.
Laboratory of Mycology, Biotechnology and Microbial Activity, SNV, University of Mentouri **Constantine, 25000 Algeria**

DEHIMAT L.
Laboratory of Mycology, Biotechnology and Microbial Activity, SNV, University of Mentouri **Constantine, 25000 Algeria**

ABSTRACT

The present work was conducted in the laboratory of mycology of biotechnology and microbial activity. The mycelium was obtained following a culture on two agar media, one based on potato (Potato Dextrose Agar) and the other based on the Sound of rice (Son Dextrose Agra). The growth of the *Pleurotus sp* mycelium was satiating on the two media used and covered the petri dishes for a maximum of ten days at an incubation temperature of 20° C. The edible mushroom fungus isolated on agar medium (PDA or SDA) was tested on a mother culture with as substrates: maize grains, barley grains and wheat grains, supplemented with glucose, $CaSO_4$ and $CaCO_3$. The results obtained showed an upward growth rate in the various substrates mentioned in the previous order (maize grains, barley grains and wheat grains). Sawdust substrates based on sawdust and wheat straw supplemented with $CaSO_4$ and $CaCO_3$ gave encouraging fruiting results with an first incubation at a temperature of 20 ° C, and second incubation at a temperature of 20° C, a humidity of 90 % and a photoperiod of 10 h / 24 h.

Keywords: *Pleurotus sp.*, wheat grains, barley grains, sawdust, wheat straw.

INTRODUCTION

Pleurotus species, commonly known as oyster mushrooms, are saprophyte fungi cultivated worldwide especially in South East Asia, India, Europe and Africa (Mandeel and *al.*, 2005). Oyster mushrooms is the third largest (Obodai and *al.*, 2003) commercially produced mushroom in the world.

From a classification point of view, the genus *Pleurotus* are characterize by a complex taxonomic structure and includes about 30 species. However, traditional taxonomic identification of some closely related species or twin-species with similar morphology based on macro and micromorphological characteristics is not always unambiguou (Anastasia and *al.*, 2017). In general, , oyster mushrooms of the genus *Pleurotus* are classed in the Phylum *Basidiomycota*, Subphylum *Agaricomycotina*, Class *Agaricomycetes*, Order *Agaricales*, Family *Pleurotaceae*.

The fungi of the gender *Pleurotus* are edible mushrooms with high nutritional value, easy growing regarding substrate and with good development under rustic conditions (Schmidt et *al.*, 2003). It is easily cultivated in a great variety of agricultural residues, such as straws, grass, sawdust, coconut husk, corncob, sugarcane bagasse, and others of organic nature (Donini et *al.*, 2005; Donini et *al.*, 2009). This excellent development is due to the production of some lignocellulosic enzymes that allow the easy degradation of the lignin and cellulose of the wood, as well as other plant substrates used for this particular cultivation (Capelari, 1996).

Oysters are naturally founds on rotten wood material. The growing and consumption interest of oyster mushroom is increasing largely due to its taste, medicinal (antitumor, antioxidant and hypolipidemic activities) and nutritional properties (Garcha et *al.*, 1993; Wasser, S.P. and Weis, A.L., 1999; Reshetnikov et *al.*, 2001; Zaidman et *al.*, 2005; Ooi, 2008). In addition, the spent substrates from mushroom cultivation can also potentially used as an animal feed supplement, possibly providing additional animal feed resources (Obodai et *al.*, 2003).
The objective of this work was to multiplicate and produce the *Pleurotus* mushroom on laboratory scale on different substrates

METHODOLOGY

Isolation and obtaining the pure culture

- **Preparation of agar media:** the isolation is carried out on PDA medium (cooking filtrate of 200 g potato in 1 L of demineralized water + 20 g agar + 20 g Dextrose, increased to 1 L) or SDA (cooking filtrate of 18 g of rice in 1 L Demineralized water + 18 g agar + 18 g Dextrose, increased to 1 L). The two prepared media are heated on plate for 15 min for homogenization and dissolution of the ingredients. Finally, they are distributed in clean bottles and sterilized at 120° C during 20 min.
- **Isolation and incubation:** after cooling, the PDA and SDA were poured into Petri dishes and used for *Pleurotus* inoculation. To obtain pure culture a small piece of hat of the mushroom, face hymeneal was placed on the Petri dishes contained sterilized PDA or SDA media under aseptic condition. It was then kept for 7-10 days in an incubator at 20°C for sufficient growth. This pure culture was used for the entire experiment.

Mother culture

- **Preparation of the seedling substrate:** three substrates were used for the preparation of the mother culture: 1) Corn Kernels, 2) Wheat Kernels, 3) Barley Kernels. First, the substrates in question were soaked separately in distilled water for 24 hours, cooked for 30 minutes in a water bath and then drained in a colander. Subsequently, it was distributed in vials (9 X 16.8 cm) at the rate of 250 g per bottle. The latter were enriched with a mixture of $CaSO_4$ (2 g), Glucose (2 g) and $CaCO_3$ (1 mL) before being closed by a screwed lid and sterilizing at 120 ° C. for 20 min.
- **Inoculation and incubation:** cut piece of pure culture was placed aseptically through the hole of the mother culture bottle and again the bottle were plugged with cotton. It was placed into the growth chamber at 25°C in dark place.

Fruiting culture

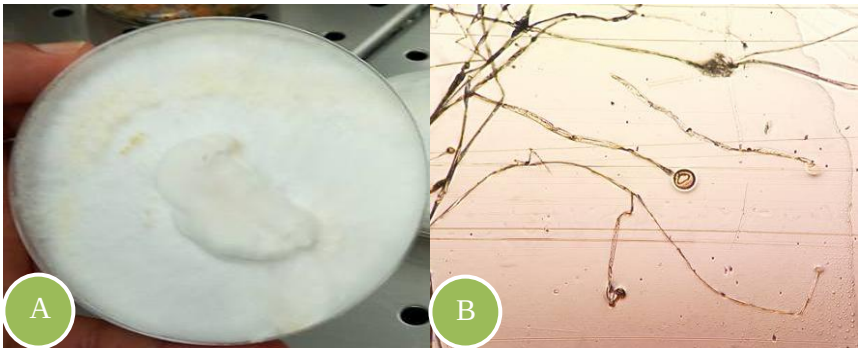
- **Preparation of the fruiting substrate:** Two substrates of culture were for the fruiting of Pleurotus: Sawdust and Wheat Straw. First, the substrates were soaked in boiling tap water overnight to moisten them and make them soft so that the colonization of the hyphae of oyster mushrooms was favorable. Subsequently, the substrates were

drained and added 2 g of $CaSO_4$ and 2 g of glucose. Finally, the sterilization was carried out at 120 ° C. for 20 min.

- **Inoculation and incubation:** the inoculation was carried out in a stage (wheat straw / sawdust of the wood and then mycelium of mother culture), at a rate of 3% of mother culture with respect to the mass of substrate. The bags are then hermetically sealed and incubated in the dark for three weeks at a temperature between 18 and 20 ° C. After this first incubation step, the bags were placed in a second incubation at a temperature of 20 ° C., a humidity of 90% and under a white light (10 h / 24h) until fruiting of mushrooms.

RESULTS

Isolation and obtaining the pure culture: PDA is the simplest and the most popular medium for growing mycelia of most cultivated mushrooms (Chang, 1999). *Plorotus sp* was successfully grown on PDA and in SDA for 240 h after inoculation. The oyster completely covered the petri dishes in 7 days and its color and appearance looks like pure white cotton (Fig. 1).



Mother culture: the growth rate of the mycelium on the different substrates (Fig.2.) used shows that growth is greater on the barley grains and begins on the third day of incubation, followed by maize seeds (fifth day) and at the end Those of wheat (sixth day). The growth rate becomes maximal after 21 days of growth.

Fig. 2. Aspect of mycelium on barley (A), corn (B) and wheat grain (C).

Fruiting culture: after incubation in the dark at a temperature of about 20 ° C, the *Pleurotus* mycelium covered all the substance in which it was inoculated (sawdust or wheat straw) (Fig.3). The mycelium had the same appearance (cotton) as when developing on mother culture.

Fig.3. Appearance of wheat straw mycelium (A) and sawdust (B) after the first incubation phase.

The development of the mycelium in the second incubation stages was more demanding in terms of condition. The maintenance of a temperature of 20 ° C, a humidity of 90% and a photoperiod of 10h / 24h, allowed a good fruiting of the fungus (Fig.4). The foot of the Pleurote was off-centered and the hat was very fleshy and convex towards the base of the foot. The hats had a gray to dark brown beige color that grew on top of each other like the tiles of a roof and had white strips to the base of the foot.

Pleurotus growth and vigor, lineage was cultivated for 240 hours (10 days) at 20ºC. After this period, an white cotton carpet was covered the petridishes surface, it's matches the mycelium of *Pleurotus sp*.. The appearance of yellow, blue, green or grey mycelia form on the surface, is traducing as a fungal contaminants (Oei et van Nieuwenhuijzen, 2005). Of the same, a creamy, shiny growth often indicates bacterial contamination (Oei et van Nieuwenhuijzen, 2005). The best growth that we obtained was favored by good condition of thermal incubation (20 ° C). Indeed, Edible mushrooms cultivated between 15-20ºC present better quality and durability than those at 25ºC (Eger et *al.*, 1976; Przybylowicz & Donoghue, 1990).

In addition, the multiplication of *Pleurotus sp* mycelium on different substrates (Corn Kernels, Wheat Kernels and Barley Kernels): mother culture; allowed us to obtain bottles completely covered with a white mycelium. The analysis of the grains of the different mycelium allowed us to detect an attachment of *Pleurotus sp* over the entire surface of the seed. The premolar tests that we obtained allowed us to observe that the addition of a fragment of the mycelium from the Petri dish gives a less rapid development in comparison with the use of a fresh tissue of *Pleurotus sp.*

The moisture content of the substrates was varying from 60% to 70%. Significantly the highest run rate was recorded at 70% moisture level (Sarker et *al.*, 2007).
In addition, $CaSO_4$ and $CaCO_3$ act as buffer. Besides, the Ca2t obtained from gypsum ($CaSO_4$) and chalk ($CaCO_3$) neutralize oxalic acid produced during mycelia growth (Dawit,

1998). The addition of glucose to the substrate promotes the development of mycelium during the first growth hours, since cellulose degradation requires time.

The fruiting stage, is too delicate a step in terms of incubation conditions. , especially in terms of the moisture factor. Good control of the humidity during growth of *Pleurotus sp* is very important, seen that, the proper moisture content value encourages the growth, while higher or lower ones had a negative effect on growth (Oei et van Nieuwenhuijzen, 2005). It is interesting keep the humidity high (80 - 90%) by spraying water several times per day (Oei et van Nieuwenhuijzen, 2005). However, no water should be sprayed directly onto mushrooms that are ready for picking; their shelf life will decrease drastically if they become too wet (Dawit, 1998).

CONCLUSION

Our work was carried out during the period from February 2017 to April 2017. The results obtained are encouraging because they demonstrate that the cultivation of local strains of edible fungi is possible in the region of Constantine -Algeria-. The work we have done enabled us to find that humidity, aeration, temperature, and contaminations are most important factors to control during Pleurote cultivation. Also, the good development of mycelium on different cellulosic substrates is an encouraging factor in the valorization of cellulosic waste. After harvesting the sporophore, the depleted substrate could be recycled as food for small livestock or as compost for gardens.

REFERENCES

Anastasia A. Shnyreva, Elena Y. Kozhevnikova, Artem V. Barkov, Alla V. (2017) Shnyreva. Solid-State Cultivation of Edible Oyster Mushrooms, Pleurotus spp. under Laboratory Conditions. Advances in Microbiology, 7, 125-136.

CAPELARI, M. (1996) Atividade biodegradadora e cultivo de três espécies comestíveis de basidiomicetos: Pleurotus sp. e Agrocybe perfecta (Rick) Sing. 154f. Tese (Doutorado em Botânica) - Instituto de Biociências, Universidade de São Paulo, São Paulo, 1996.

Chang S-T. (1999) World Production of Cultivated Edible and Medicinal Mushrooms in 1997 with Emphasis on< i> Lentinus edodes</i>(Berk.) Sing, in China. International Journal of Medicinal Mushrooms,1(4):291-300.

Dawit A. (1998) Mushroom Cultivation: A practical approach. Addis Ababa:Birhannaselam printing enterprise.

DONINI, L.P.; BERNARDI, E.; MINOTTO, E.; NASCIMENTO, J.S. (2009) Cultivation of shimeji on elephant grass substrate supplemented with different kinds of bran. Scientia Agraria, v.10, n.1, p.67- 74.

DONINI, L.P.; BERNARDI, E.; MINOTTO, E.; NASCIMENTO, J.S. (2005) Desenvolvimento in vitro de Pleurotus sp. sob a influência de diferentes substratos e dextrose. Arquivos do Instituto Biológico, v.72, n.3, p.331-338.

EGER, G.; EDEN, G.; WISSING, E. (1976) Pleurotus ostreatus, breeding potential of a new cultivated mushroom. Theoretical and Applied Genetics, v.47, p.155-163.

Garcha H, Khanna P, Soni G. (1993) Nutritional importance of mushrooms. In: Chang S, Buswell J, Chiu S, editors. Mushroom biology and mushroom products Chinese University. Hong Kong: Chinese University Press.

Mandeel Q, Al-Laith A, Mohamed S. (2005) Cultivation of oyster mushrooms (Pleurotus spp.) on various lignocellulosic wastes. World Journal of Microbiology and Biotechnology;21 (4):601-7.

Obodai M, Cleland-Okine J, Vowotor K. (2003) Comparative study on the growth and yield of Pleurotus ostreatus mushroom on different lignocellulosic by-products. Journal of Industrial Microbiology and Biotechnology; 30(3):146-9.

Oei P, van Nieuwenhuijzen B. (2005) Small-scale mushroom cultivation: oyster, shiitake and wood ear mushrooms. Wageningen, The Netherlands: Digigrafi.

Ooi, V. (2008) Antitumor and Immunomodulatory Activities of Mushroom Polysaccharides. In: Cheung, P., Ed., Mushrooms as Functional Foods, Wiley & Sons Inc., Hoboken, 147-198. https://doi.org/10.1002/9780470367285.ch5.

PRZYBYLOWICZ, P.; DONOGHUE, J. (1990) Shiitake growes handbook. 2.ed. New York: Hunt Publishing Co. 217p.

Reshetnikov, S.V., Wasser, S.P. and Tan, K.K. (2001) Higher Basidiomycota as a Source of Antitumor and Immunostimulating Polysaccharides (Review). International Journal of Medicinal Mushrooms , 3, 361-394. https://doi.org/10.1615/IntJMedMushr.v3.i4.80.

Sarker N, Hossain M, Sultana N, Mian I, IJ SK, Ruhul Amin S. (2007) Effect of Different pH Levels on the Growth and Yield of P. ostreatus (Jacquin ex Fr.) Kummer. Bangladesh Journal of Mushroom. 1(1):57-67.

SCHMIDT, P.; WECHSLER, F.S.; NASCIMENTO, J.S.; JUNIOR, F.M.V. (2003) Tratamento do feno de braquiária pelo fungo Pleurotus ostratus. Revista Brasileira de Zootecnia;v.32, n.6, p.1866- 1871.

Wasser, S.P. and Weis, A.L. (1999) Therapeutic Effects of Substances Occurring in Higher Basidiomycetes Mushrooms: A Modern Perspective. Critical Reviews in Immunology, 19, 65-96.

Zaidman, B.Z., Yassin, M., Mahajna, J. and Wasser, S.P. (2005) Medicinal Mushrooms Modulator of Molecular Targets as Cancer Therapeutics. Applied Microbiology and Biotechnology, 67, 453-468. https://doi.org/10.1007/s00253-004-1787-z.