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Fermentation for the Production of Mushrooms

Shitole Prajwal[1], Dhwani Upadhyay[2], Indrani Bhattacharya[2], Anjali Thakur[2], Prasad Andhare[3]*
[1]Student, M.Sc Microbiology, Parul Institute of Applied Science, Parul University, Post Limda, Waghodiya, Gujarat
[2] Assistant Professor, Parul Institute of Applied Science, Parul University, Post Limda, Waghodiya , Gujarat
[3] Assistant Professor, Biological Sciences, PDPIAS, Charotar University of Science and Technology, Changa, Anand, Gujarat.
*Corresponding Author: Dr. Prasad Andhare;
E-Mail: prasadandhare.as@charusat.ac.in

ABSTRACT
The accumulation of agronomic waste has led to various environmental problems, such as air and soil pollution, as well as the proliferation of insects and diseases. In order to address this issue, researchers have investigated the potential use of these waste materials as substrates for cultivating Pleurotus mushrooms, which are a cost-effective and nutrient-rich crop. The objective of this study was to evaluate the suitability of maize stubble and rice straw as substrates for the in vitro development of oyster mushrooms (Pleurotus ostreatus Jacq.), as well as the productivity of four isolated strains in terms of biological efficiency, production rate, earliness, and daily productive capacity. The strains PO/A01, PO/A02, PO/A03, and PO/A04 were cultivated in Potato-Dextrose-Agar medium until complete colonization. The experiment, which employed a completely randomized design with six replications, involved the disinfection, inoculation, bagging, incubation, and induction of fructification of the corn stubble and rice straw. After three harvests, the fruitful season concluded. The experiment followed a totally randomized design with a 4 2 factorial layout and 8 replications. When compared to rice straw, the P. ostreatus strains inoculated in corn stubble exhibited a biological efficiency of 93.93 percent and a production rate of 2.07 percent, representing increases of 30 percent and 50 percent, respectively. The strains PO/A03 and PO/A04 demonstrated higher biological efficiency and organic matter loss. The PO/A02 strain displayed a more opportunistic behavior, with a harvest time of approximately 10 days. The findings of this study suggest that the isolated strains of P. ostreatus enable the effective utilization of maize stubble and rice straw, while also contributing to the management of agronomic waste.
Keywords: Solid-State fermentation, fermented mushrooms, edible mushrooms.

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INTRODUCTION

Pleurotus ostreatus, P. eryngii, P. djamor, P. citrinopileatus, and P. pulmonarius are edible mushrooms that possess notable nutritional, medicinal, and pharmacological properties (1). These species are recognized for their rapid mycelial development and enzymatic capabilities, making them efficient decomposers of lignocellulosic materials. Consequently, they are able to effectively colonize and convert various agricultural wastes such as rice straw, corn, barley, wheat, coconut husks, and banana leaves into protein (2-4). Pleurotus species exhibit adaptability to temperatures ranging from 10 to 28 °C and humidity levels between 50 to 75 percent, in addition to their resistance to xenobiotics (7,8).

Pleurotus ostreatus, commonly known as oyster mushroom, is one of the most extensively cultivated edible mushroom species worldwide, accounting for 19% of global production, second only to Lentinula edodes (Shiitake) with 22% (10). P. ostreatus is not only a nutrient-rich food but also possesses antioxidant, anti-mutagenic, and hepatoprotective properties (1). Moreover, it can be produced using cost-effective technologies and offers a high economic profit margin (11).

Agricultural activities generate a significant amount of agronomic waste annually, predominantly consisting of lignocellulosic biomass (12). Inadequate waste management practices can lead to environmental issues such as greenhouse gas emissions, insect proliferation, and nitrogen immobilization in the soil (13). However, research indicates that efficient management of agricultural waste promotes microbial activity, carbon sequestration, and reduction of soil erosion (14).

MATERIALS AND METHODS
Area for experimentation:
PO/A01, PO/A02, PO/A03, and PO/A04, four strains of Pleurotus ostreatus (Jacq.) isolated from Lima and Cusco, Peru, were employed. The strains were isolated, identified, and maintained.
Under in vitro conditions, pure colonies of P. ostreatus strains were grown in Potato-Dextrose-Agar (PDA) medium for one week at 25 2 °C. The culture medium was made out of 500 mL L1 of a 250 g potato infusion, 6.5 g L1 of agar, and 18 g L1 of dextrose, pH adjusted to 6.5. It was sterilized in an autoclave at 121° C and 103 kPa for 20 minutes. In this phase, the strains' mycelial development was monitored until the nutritional medium was completely colonized.
The spawn preparation was done in accordance with [12] with a few changes. The initial inoculum was made by introducing P. ostreatus colonies grown in vitro to wheat grains until they were completely colonized. By transferring the main inoculum to wheat grains, the secondary inoculum (spawn) was created. Using a 5% rate of inoculation of the wet substrate, the quantity of secondary inoculum was estimated. For one week, the inoculum was incubated at 25 2 °C in complete darkness. Pre-cooked wheat grains were combined with 3 g kg1 CaCO3 and 13 g kg1 CaSO4.2H2O. They were then sterilized in an autoclave for 20 minutes at 121° C and 103 kPa before being utilized to make the inoculum.
The disinfected substrates were inoculated at a rate of 5% and dispersed in polypropylene bags (14 cm 20 cm 2 mm) containing the equivalent of 1 kilogram's dry mass without nitrogen supplementation. The bags were incubated in absolute darkness for two weeks at 22 1 °C and 81 3 percent relative humidity (relative humidity). The bags were moved to a fructification chamber at 20 2 °C and 85 % RH after complete mycelial colonization of the substrate. To encourage the mushrooms to develop, vertical incisions of 2 cm were made on the surface of the bags in this chamber. Between the third and sixth days following inoculation, the primordia arrived at the collecting station.
The experiment was carried out in vitro using a fully randomized design (CRD) with six replicates for each strain of P. ostreatus. A Petri plate containing PDA medium was infected with a 0.5 cm2 section of mycelium as the experimental unit. The experiment was carried out under productive circumstances utilizing a CRD with a 4 2 factorial design employing four strains of P. ostreatus and two substrates (corn stubble and rice straw), with eight repetitions. A 14 cm x 20 cm x 2 mm polypropylene bag containing the equivalent of 1 kilogramme of dry substrate infected with P. ostreatus served as the experimental unit.
The variables studied were: A) mycelial growth in vitro (mm day1), which was determined by the ratio of the radius of a Petri dish (40 mm) to the days it took the P. ostreatus mycelium to colonise the PDA medium; and B) mycelial growth in the substrate (cm day1), which was determined by the ratio of the radius of each experimental unit (bag) to the time it took the P. ostreatus mycelium This variable refers to the authors' mathematical design for assessing mycelial growth and reducing package handling during the incubation period.
RESULT AND DISCUSSION
The study identified four strains of P. ostreatus and observed significant variations in their mycelial growth when tested in vitro. Among the strains, PO/A02 exhibited the slowest growth rate, with an average diameter increase of 3.48 mm per day. On the other hand, strains PO/A04 and PO/A03 demonstrated faster growth rates, with diameters of 4.08 mm and 3.91 mm, respectively.
In the context of edible mushroom production, rapid mycelial colonization has been found to reduce contamination losses and shorten the incubation time and productive period. In vitro evaluation of mycelial growth can be achieved by manipulating temperature, humidity, and nutritional media. This allows for the assessment of the vigor and growth speed of isolated strains. The different stages of mycelial growth, which involve the consumption of soluble sugars during absorption, cell division, and mycelial development, can be distinguished. This results in mycelial growth rates ranging from 4 to 10 mm per day in the substrate and 13 to 30 mm per day in the culture medium. However, our findings indicate mycelial growth rates of less than 5 mm per day, suggesting that these responses may vary depending on the assessment approach employed [15, 16].
The study also investigated the impact of lignocellulosic substrate characteristics on various variables, including biological efficiency, production rate, and earliness of isolated P. ostreatus strains. Additionally, the variables of mycelial growth in the substrate, daily productive capacity, organic matter loss of the isolated strains, and substrate types (corn stubble and rice straw) were found to be independent of each other, as they did not exhibit significant interactions.
Mycelial growth can be influenced by the chemical content, plant structure, origin, and particle size of the agronomic wastes used as substrate after inoculation. When combined with environmental management and substrate treatment, these factors determine the productive performance of mushrooms [17].

Given that the lignocellulolytic potential of this species is associated with its enzyme production [9], further research is needed, particularly in the field of molecular biology. This includes investigating the synthesis of enzymes such as laccase, manganese peroxidase, and lignin peroxidase, as well as studying their gene expression.

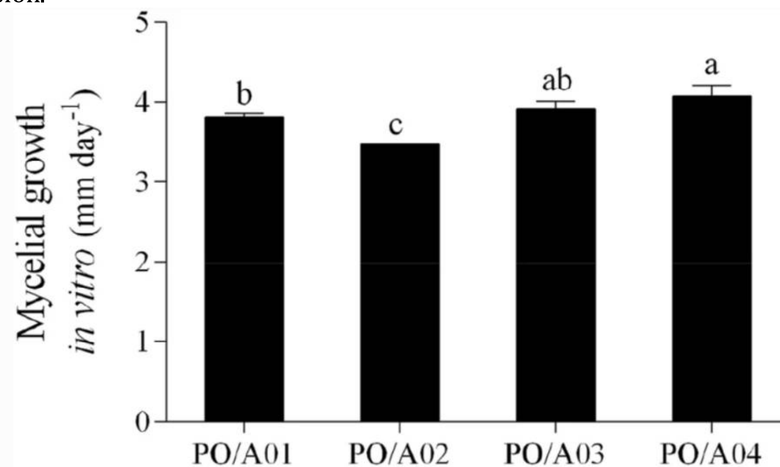

Fig 1: The four strains of P. ostreatus identified revealed substantial variations in mycelial growth when tested in vitro

CONCLUSION

In conclusion, corn stubble and rice straw agronomic wastes are recommended for P. ostreatus cultivation. Corn stubble promotes stronger mycelial development, higher biological efficiency, shorter harvest times, improved earliness, and increased output and daily productive capacity compared to rice straw. The strains PO/A03 and PO/A04 exhibited greater biological efficiency, production rate, and daily productive capacity compared to the other strains. They also showed the potential to enhance organic material loss in the substrates. Under in vitro conditions, the strain PO/A02 produced mushrooms earlier, with longer productive periods each year and a higher production rate. The strains displayed different mycelial growth patterns in the substrate compared to their behavior in the PDA media, indicating that a strain may grow slower in the media but faster in the substrate.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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