Successful outdoor cultivation of a photosensitive wild strain of edible *Pleurotus ostreatus* (Fr.) Kummel (Oyster mushroom) from the Western Ghats region of Goa

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Abstract

There is considerable interest in identifying wild tropical strains of oyster mushrooms useful for cultivation, strain improvement and systematic breeding programme. During routine surveys to identify such strains from Goa's western ghats an interesting wild form of *Pleurotus ostreatus* was found growing on logs of wild *Mangiferra indica* L a novel habitat for the species reported for the first time. After obtaining and screening several pure isolates, a fertile strain BA-50/GUOMS was selected for cultivation under natural outdoor conditions. Spawn prepared on wheat grains was produced within 11 days and was used to ramify sterilized paddy straw as substrate using standard polybag technique. Spawn run was initially slow and took 52 days. Mushrooms fruitbodies identical to the original wild type were produced in eight flushes at intervals of 8 to 13 days for each flush over a period of four months. These had pleasant odour, better shelf life, tougher texture, resistance to insects, fungi and bacteria, exhibited photosensitivity by producing brownish pigment in direct sunlight and responded well to outdoor conditions for fruit body initiation with ambient temperature ranging from 23-35 ^{o}C.

Introduction

Pleurotus spp., commonly known as oyster mushrooms , are well known primary decomposers of wood and other biodegradable vegetable residues (Zadrazil and Kurtzman, 1982). Genus *Pleurotus* has several cultivated species that are becoming increasingly economically important as a food source (Moore and Chiu, 2001). Chang and Miles (2004) recognize 50 *Pleurotus* species as economically important. The widely cultivated species-oyster mushroom - *Pleurotus ostreatus* (Fr.) Kummel occupies 14% of the global market and ranks third in world trade. It tolerates a temperature of 7-37 ^{o} C with an optimal range of 26-28 ^{o} C. Various *Pleurotus* species have been intensively studied in many different parts of the world. These have high gastronomic value, can efficiently colonize and decompose a wide variety of lignocellulosic residues, require shorter cultivation time when compared to other edible mushrooms and demand few environmental controls. *Pleurotus* fruiting bodies are not very often attacked by diseases and pests and they can be cultivated in a simple and cheap way (Jwanny et al., 1995; Patrabansh and Madan, 1997).Due to relative ease of cultivation and use of wide range of agrowastes as substrate, oyster mushrooms offer a promise to rural areas in India for income generation. However rural people find indoor cultivation difficult. They may prefer strains which are easy to cultivate under outdoor conditions. Therefore rational explorations of natural genetic stocks of *P. ostreatus* is necessary. The CABI mycological database (http://wwww.indexfungorum.org) lists 765 unrevised records for Genus *Pleurotus*. Among these for the edible oyster mushroom *Pleurotus ostreatus* (Fr.) Kummel, one subspecies, seven forms, four subforms and 11 varieties have been reported (Table 1). The white *florida* form has been commercialized. Underexplored tropical areas are likely to reveal many interesting and potentially cultivable forms. In the natural

environment these forms are well adapted to local environmental and ecophysiological conditions-such as weak or intense direct sunlight which may be responsible for variable pigmentation, wind conditions, low and high humidity, drying-wetting cycles, grazing by insects and other animals. Such adaptation is useful when these strains are brought into pure culture and fruiting is induced under outdoor conditions. Although for artificial, commercial purposes, hybrid strains of *P. ostreatus* are preferred and cultivation is done indoors-scanty attention has been paid to development of strains for outdoor conditions which are preferred in rural areas because less management is required at production and harvesting stages. The present work was therefore aimed at trial cultivation of a wild form of *P. ostreatus* under outdoor conditions and gather necessary data for further trials.

Table 1

Wild forms and varieties of oyster mushroom *Pleurotus ostreatus* (Jacq.) P. Kumm. (1871)

Pleurotus ostreatus subsp. *ostreatus* (Jacq.) P. Kumm. (1871)
Pleurotus ostreatus f. *carpathicus* (Fr.) Pilát (1935)
Pleurotus ostreatus f. *florida* Cetto (1987)
Pleurotus ostreatus f. *ostreatus* (Jacq.) P. Kumm. (1871)
Pleurotus ostreatus f. *peregrinus* (Hazsl.) Pilát (1935)
Pleurotus ostreatus f. *polonicus* F. Teodorowicz (1937)
Pleurotus ostreatus f. *subalutaceus* Malençon & Bertault (1975)
Pleurotus ostreatus f. *suberis* (Pat.) Malençon & Bertault (1975)
Pleurotus ostreatus subf. *glandulosus* (Bull.) Pilát (1935)
Pleurotus ostreatus subf. *hirsutus* Pilát (1935)
Pleurotus ostreatus subf. *ostreatus* (Jacq.) P. Kumm. (1871)
Pleurotus ostreatus subf. *typicus* Pilát (1935)
Pleurotus ostreatus var. *appalachiensis* O. Hilber (1993)
Pleurotus ostreatus var. *flavocinereus* (Pers.) Sacc. (1887)
Pleurotus ostreatus var. *flavovirens* (V. Brig.) Sacc. (1887)
Pleurotus ostreatus var. *glandulosus* (Bull.) Fr. (1874)
Pleurotus ostreatus var. *magnificus* Peck (1913)
Pleurotus ostreatus var. *melanodon* (V. Brig.) Sacc. (1887)
Pleurotus ostreatus var. *nigricans* (Tratt.) Sacc. & Traverso (1911)
Pleurotus ostreatus var. *nudipes* Boud. (1897);
Pleurotus ostreatus var. *ostreatus* (Jacq.) P. Kumm. (1871)
Pleurotus ostreatus var. *praecox* E. Ludw. (2001)
Pleurotus ostreatus var. *stipitatus* Scalia (1900)

Source:- http://indexfungorum.org/Names/Names.asp

Materials and methods

Collection and identification of fruit bodies: During regular surveys in western ghats foothills in Sanguem taluka of Goa, wild *Pleurotus* fruitbodies were identified on logs of *Mangifera indica L*. Their morphological characters were noted in the field. The specimens were handpicked, put in clean labeled polythene bags and brought to the laboratory. Standard published (Singer, 1986) as well as electronic taxonomic keys (Trial field key to the Pleurotoid species in the Pacific Northwest, Prepared for the Pacific Northwest Key Council, http://www.svims.ca/council/Pleuro.htm) were used to identify the specimens.

Preparation of spore prints: Overhead transparencies were cleaned with 70% ethanol and cut into square pieces. The fruit bodies were placed on the dry surface with the gills facing downwards and left over night for spore deposition. Spore print colour was noted. The pieces containing the spore print were allowed to dry and then wrapped in butter paper, labeled and stored in a large envelope in a dry place with addition of naphthalene crystals as insect repellant.

Preparation of herbarium material: Specimens were wrapped in butter paper and dried in a Stockly dehydrator at 70^{o}C for several days till bone dry. Dry specimens were transferred to labeled envelopes and then kept in the cupboard with paradichlorobenzene as an acaricide.

Context tissue culture: Healthy fruitbodies were surface sterilized by wiping with a cotton swab soaked in 70% ethanol. A flame sterilized needle was used expose the sterile pileus context. Incisions were made in the pileus context tissue and flame sterilized forceps were used to pick up pieces of tissue and place these explants on PDA medium containing streptomycin and penicillin (1.25mg/L each). to inhibit bacterial growth. Plates were incubated at 25^{o}C +/- 2^{o}C and observed for growth of mycelium and were observed for growth daily. Well grown colonies were subcultured on fresh PDA devoid of antibiotics and growth was monitored regularly. 2 replicates were maintained on PDA slants and 2 more were preserved in sterile 10% glycerol in sterile cryovials for long term preservation. The strains were deposited in Goa University fungus culture collection (GFCC).

Selection of potentially fertile strain for master spawn production:-Colonies were characterized on basis of growth rate, texture, margin, homogeneity and ability to form mycelial strands and cords indicating potential for rapid ramification of natural lignocellulosic substrates. Strain BA-50 subsequently redesignated as GUOMS (Goa University Oyster Mushroom Strain) showing rapid growth rate, stable morphology and ability to form profuse aerial mycelium and distinct ropy strands was selected for making master spawn.

Preparation of master spawn: 50g of wheat grains were cleaned and soaked in water for about an hour. This was followed by boiling in water for about 20 minutes till the grains were soft but intact and starch was not released. The grains were weighed and to it 4% (w/w) of calcium carbonate was added. Grains were equally divided and put in two identical conical flasks. Flasks were sealed using tight cotton plugs and were wrapped in paper and autoclaved. After an interval of 24 hours flasks were autoclaved for the second time to eliminate any chances of contamination. These were then inoculated with 10-12 plugs from 4 days old culture of GUOMS and were incubated at ambient conditions. After two days the flasks were shaken thoroughly to enhance colonization of grains.

Completion of growth was confirmed by noting uniform, dense and compact colonization.

Multiplication of spawn: The master spawn was used for multiplication in glass bottles. For this purpose empty saline bottles were used. These were washed thoroughly and allowed to dry in the oven. 500g of wheat grains were weighed and soaked in water for about one hour, then boiled for about 20 minutes till grains were soft. The boiled wheat grains were weighed again. Gypsum at the rate of 2% and 4% (w/w) calcium carbonate was added. The bottles were autoclaved twice as was done for master spawn. The master spawn from one of the inoculum flaks was divided into three parts and used for inoculating the spawn bottles. An uninoculated control was maintained to rule out experimental errors. Bottles were shaken after two days and then incubated till spawn run was complete.

Substrate preparation: Sun dried paddy straw- (Annapurna variety) was cut to 3-5 cm length. 2 kg of this was soaked under weight overnight (upto 12 hours) in chlorine free water. Excess water was squeezed out. Previous estimates had shown that a kilogram of dry paddy straw with this treatment retains double the weight of moisture. The wet paddy straw was tied into bundles wrapped in several layers of news paper and autoclaved at 15psi for 20 minutes.

Inoculation of substrate and spawn run: After autoclaving, the paddy straw bundles were opened and allowed to cool by spreading the straw on a clean surface under the fan. Three Polyethylene bags designated B-1, B-2, B-3 of 45 X 30 cms dimension were used to fill the sterilized straw. A layer of compressed straw was followed by a layer of grain spawn removed under sterile conditions from the bottles. About 100 g of grain spawn was used per bag. In this manner substrate and grain spawn were put in alternate layers till the bags were full and made compact. Each bag contained about 1200 gms of soaked straw corresponding to 380-415 g of dry paddy straw. The mouths of the bags were tied and spawn run was carried out in the dark at 25 ^{o}C. Ramification was monitored every day.

Outdoor cultivation conditions:-The well ventilated cultivation gallery faced east west direction and received direct sunlight for 2-3 hours and diffuse sunlight for about 8-10 hours with daily average minimum temperature ranging between-20-25 ^{o}C and maximum temperature 26-35 ^{o}C and relative humidity 70-85 %.

Opening of spawned bags: When spawn run as evidenced by uniform ramification and compaction of substrate was complete, the bags were slit open and rolled down, kept in trays containing water to create a humid environment opened and transferred to an open ventilated gallery outdoors for fruit body initiation. Bags were lightly irrigated twice daily using a domestic atomizer.

Photodocumentation and harvesting of fruit bodies: Bags were observed daily for pin head appearance. Photographs were taken using a Sony Cybershot SL-3 digital camera daily till mushrooms were ready for harvesting. Fruitbodies were plucked using forceps to ensure that they were removed right from the base. Details such as the total number of clusters, total fruit bodies, no of tiers in each cluster, etc. were recorded. Fruit bodies were arranged in increasing order of size and obverse and reverse photographs were taken.

Photosensitivity tests:- This was done after harvesting of first three flushes by orienting the clusters of primordia in B -1 and B -3 originally developing in shade towards direct

sunlight for 2-3 hours a day and observing the effect of solar radiation on pileal pigmentation of the maturing fruitbodies.

Morphometry of fruit bodies: All harvested specimens were subjected to morphometric studies such as size, dimensions of pileus and stipe and fresh weight.

Marketability index:-Owing to fibrous and unpalatable nature of the stipe, oyster mushrooms with larger, fleshy pilei and without stipes or with smaller stipes are preferred. This affords to use a marketability index (MI) which was computed from calculating the ratio of pileus diameter to stipe length and multiplying the value by 100. A larger value for MI indicates good marketability whereas a smaller value indicates unacceptable product.

Morphological analysis of harvested specimens:- This was done by studying the standard taxonomic characters to compare the cultivated form with wild type characteristics of fruitbodies and to record deviations if any.

Assessment of shelf life:- This was done under ambient conditions by estimating moisture loss, change in smell, colour, shape, texture for upto 4 days after harvesting the specimens.

Sensory evaluation and assessment score:-This was done using a modified technique described by Prabhu dessai et al. (1991) which uses scores for colour (white 10-Black 1) , texture (firem, crisp 10, leathery 1), taste and flavour (Excellent 10, poor 1). The appreciation percentage was calculated using the formula-

Appreciation percentage=(score obtained X 100) /maximum attainable score (30). A panel of five independent volunteers was used and the mean scores were obtained.

Biological efficiency (BE) :- This was obtained for each bag by using the equation-the total biomass from all the flushes X 100/ dry weight of the substrate (Prabhudessai et al., 1991).

Relationship of BE to spawn ratio (BESR) :-This is a derived quantity which gives a measure of vigour, genetic and biochemical efficiency of the spawn contributing to bioconversion of the substrate and mass transfer of cellulosic material to mushrooms. The value was obtained by (BE X 100) / spawn ratio. A higher BESR would indicate and validate the choice of strain and optimal spawn ratio.

Results

Wild *Pleurotus ostreatus* (Fr.) Kummel

About 30 wild specimens were collected and diagnosed**.** The fresh spore print colour was white. Except for the bluish grey tint of the pileus these matched with collection of *P. ostreatus* reported from Agumbe, Karnataka (Sathe & Kulkarni, 1980). However this is first report from Goa on wild native *Magnifera indica* L. as a host. It was observed that good spore prints were obtained within half an hour of exposure. Spore deposition continued for almost 36 hours after harvesting. Spores had the tendency to clump with each other to form small clusters. Although the fruit bodies were kept in closed trays, it was noticed that spores were expelled upto 3 cm away from the fruit body.

Cultivated *Pleurotus ostreatus* (Fr.) Kummel

(Plate I)

Habit: Pleurotoid. *Habitat:* Cespitose; imbricate, on paddy straw.

Pileus: 0.5-11.6 cm in diameter; yellowish with a grayish tinge towards the margin ranging from flabelliform, infundibulliform to petalloid; margin entire plane; context 0.4-1.0, flesh confluent with the stipe,; surface smooth; an epicutis of thin walled, repent, pale yellow with grayish – beige tinge; 6-9µm broad hyphae; tramal hyphae thin walled, hyaline, 6-13µm broad. *Lamellae*: decurrent ;unequal with 2-4 sets of lamellulae; white; fleshy; moderately placed; upto 2.5 mm broad; lanceolate; margin entire; trama homoiomerous, irregular (interwoven) hyphae thin walled, hyaline, 2-12µm broad; surface euhymenial; subhymenium cellular, 4-8 µm wide; pleurocystidia absent; edge sterile; cheilocystidia clavate, hyaline, thin walled, 20-38 x 5-7.5 µm. *Basidia:* 18-34 x 5-7.5µm, tetrasporic, narrowly clavate to cylindrical , hyaline *Basidiospores:* grey in mass; 8.5-13 x 3-5.5 (9 x4) µm; cylindrical (Q = 2.2); non porate; apicule lateral; wall hyaline; inamyloid, weakly cyanophilic; thin smooth. *Stipe:* present in most of the specimens, 0.5-3.0 x 0.5-1.5cm. *Hyphal system:* monomitic with thin walled, generative hyphae, all septa with clamp connections.

Specimens examined:-GUOM-1 to GUOM-45 representing the largest mature specimens from a harvested collection of 596 healthy specimens.

Remarks:-This mycocultivar showed some variation in pileal colour and shape as compared to parental wild type (Plate I). The parental wild type showed conchiform shape whereas the cultivated form produced flabelliform, infundibulliform to petalloid fruitbodies. It was distinctly phototropic and photosensitive. Pileal colour was observed to be influenced by intensity and duration of natural light. Stipe length was restricted in presence of sunlight.

Strain characteristics:- The stain selected for cultivation BA-50 (GUOMS) showed rapid growth, cottony, wooly texture and formed a rhizoid margin interlaced with thick mycelial strands (Plate I) .

Spawn characteristics:-Master spawn took 8 days and the spawn multiplied in the bottles took 12 days for full growth. The colour was uniformly white, vigour was good and the growth was uniform and dense (Plate I) .

Spawn ratio:- This was maintained at 8-8.5 % on wet substrate weight basis and 25% of dry substrate weight basis.

Indoor spawn run:- Indoor spawn run under laboratory conditions did not help fruiting for more than seven weeks. It was only after the bags were opened and exposed to outdoor conditions that fruiting was initiated.

Outdoor fruiting:-The first flush appeared after 59 days of spawn run. Subsequent flushes were obtained at intervals of 8 to 13 days. It was found that the mushrooms required warm temperature, sunlight and frequent irrigation for fruiting to occur.

Photosensitivity test:-After harvesting of first three flushes it was noticed that a slight exposure to direct sunlight was producing brownish pigmentation in some fruitbodies in B 1 and B 3 whereas those which were shielded didn't show such pigmentation. When developing primordia were deliberately exposed to direct sunlight for 2-3 hours it was noticed that the mature fruitbodies had produced brown pileal pigmentation. This phenomenon was reproduced in more than four flushes. It was also observed that orientation of the bags towards light reduced the length of stipes. This confirmed that the strain was photosensitive as well as positively phototropic. .

Mushroom harvest analysis:-Although the production was done in replicate polybags designated as B-1, B-2 and B-3 , only B-1 and B-3 produced 13-14 flushes over a period

of 120 days from the first flush. Fruitbodies preferred to emerge as clusters as compared to solitary emergence (Plate I) . B-2 could produce first flush after 93 days and only 4 flushes over next five weeks but yielded 178 fruitbodies with 185 g biomass. Population analysis of entire harvest showed that fruitbodies appeared in a single or maximum 12 clusters with largest cluster producing 21 fruitbodies. The pileal width ranged from 0.5 cm to 11 cm. Single fruitbody biomass at harvest ranged from 0.2 to 20 g. B-1 produced 41 clusters of fruitbodies and 38 single fruitbodies accounting for 203 specimens with a biomass of 205 g whereas B 3 produced 48 clusters and 37 single fruitbodies accounting for 215 specimens weighing 173 g.

Marketability index (MI) :- The production showed a range of 138-414 for all fruitbodies with a mean of 226. For B-1 the MI ranged from 162-414. For B-2 it was 155-216 and for B 3 MI was 138-279. Higher MI scores were seen during first four flushes.

Shelf life:-After 4 days under room temperature the freshly harvested fruitbodies lost upto 80% of moisture, changed the colour from grey to yellowish grey, became wrinkled and shrunk but maintained an appealing odour without indicating any contamination by bacteria or fungi.

Sensory evaluation and assessment score:- The mean score for colour of fruitbody was six, texture got a score of 10, taste and flavour a score of five and the mean overall sensory assessment score was 21 yielding an appreciation percentage of 70.

Biological efficiency:-Biological efficiency (BE) ranged from 51.5 % for B-1, 46. 25% for B- 2 and 43.25% for B-3 with a mean BE of 47 %.

Relationship of BE to spawn ratio (BESR):-On dry weight basis, the BESR was found to be 188.

Discussion

Pleurotus ostreatus (Fr.) Kummel on wild local mango logs, *Mangifera indica* L. was reported for the first time in Goa and from the entire western ghats region. Earlier Sathe and Kukarni (1980) had reported it from Agumbe, Karnataka without indicating the host. The local species prefers high altitude (>500 m) and seems well acclimatized to humidity variation and light. Chang and Miles had identified three strains of P. ostreatus- the high temperature strain fruiting at 25-30 ^{o}C, the medium temperature strains fruiting at 16-22 ^{o}C and the low temperature strain fruiting at 12-15 ^{o}C. The western ghats species seems to be a high temperature one. This was also confirmed 'ex situ' by growing the pure cultures at 25-27 ^{o}C and inducing fruiting under outdoor conditions. The strain appears to be a high temperature lignicolus fruiting physiotype. The mean maximum temperature throughout the outdoor cultivation period was 27 ^{o}C. The fertile strain selection was governed by ability to differentiate to form mycelial cords. In commercial *Agaricus bisporus* (button mushroom) cultivation this has been found to be an useful trait. It gives an idea of the ability of the strain to grow rapidly and ramify the substrate. This was also confirmed by noting the 'ropiness' or "strandiness" of the grain spawn. Under identical temperature on sterile wheat grain commercial strains of *P. ostreatus* takes upto 20 days for full colonization. However this period was less for the GUOMS. The short spawn production period can be matched with a prolonged substrate colonization phase- a lag of upto 52 days as compared to just 10-15 days for the commercial strains. The extended lag

period can be explained as a genetic trait. The strain may be well adapted to moist woody substrate. Since paddy straw used for cultivation contains cellulose and hemicellulose, the mainly laccase producing *P.ostreatus* strain may show slow induction of the cellulolytic enzymes. Studies on the enzymes secreted by the basidiomycete fungus *P. ostreatus* have shown that the concerted action of laccase and aryl-alcohol oxidase, produces significant reduction in the molecular mass of soluble lignosulphonates (Marzullo et al 1995). Chakravarthy and Sarkar (1982) had reported *M. indica* as a suitable substrate for *P. sajor- caju* cultivation. Indoor cultivation of *P. sajor-caju* on logs of 15 common trees in Kerala had been attempted (Suharban & Nair, 1991). M. indica logs were found to yield maximum crop after two years of spawning. The commercial strains of *Pleurotus* have been always cultivated under secure, indoor conditions. The spawn run is normally indoors under dark or low light conditions. However GUOMS preferred outdoor conditions for initiation of the primordia. Within seven days of shifting from indoor spawn run stage the first primordia appeared. The first three flushes were heavy confirming similar observations for commercial strains. But despite near exhaustion of the limited substrate and heavy chances of grazing by insects, nematodes and contamination by microorganisms, 5 -12 flushes were produced over 120 days. This is possible only if the substrate is fully colonized by mycelium at high density and has intrinsic capacity of pathogen resistance. Use of ordinary tap water for light irrigation did not cause deterioration of the mycelia. Nematicidal activity has been reported in *Pleurotus* species. But in the present study it was not evaluated. The phototropic and photosensitive nature of GUOMS showed that stipe size and pileal colour could be regulated by manipulation of cultivation conditions. Both pileal colour and stipe size therefore can not be taken as stable taxonomic characters. It is possible to obtain a higher marketability index by calibrating the light flux. An uniform light regime may produce very small stipes and almost uniform fruitbodies. The shelf life of the crop obtained in this study was better than commercial strains which without refrigeration deteriorate in 48 hours. The crop had a natural dessication property. A score of 70 for appreciation percentage makes the GUOMS comparable to a score 57 obtained for popular commercial species such as *Pleurotus sajor-caju* (Prabhudessai et al, 1991). However, the lower biological efficiency of GUOMS at 47 % has to be compared with values obtained for *P. ostreatus,* 42 % (Savalgi and Savalgi, 1991), *P. florida,* 51 % (Kothandarman et al, 1991), 56 % (Mathew et al, 1991) 64 % (Chander Rao, 1991) and 73 % (Pandey and Tewari 1991) and *P. sajor-caju* 50 % (Savalgi and Savalgi, 1991) and 77-97% (Daniel et al. 1991). All these studies had used paddy straw as substrate for cultivation. Considering relatively poor attention paid to survey of local wild type genetic stocks of *P. ostreatus* and their cultivation potential the results obtained with GUOMS hold the promise that it would be possible to develop a pathogen resistant, high yielding commercial strain amenable to cultivation under outdoor conditions in the western ghats. Outdoor cultivation on *M. indica* logs may also be feasible. Trials of GUOMS have been planned in this direction on different substrates to standardize the technology.

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Plate I ***P.ostreatus GUOMS various stages of cultivation***