Scientia Horticulturae 125 (2010) 95–102

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effects of temperature and hydrogen peroxide on mycelial growth of eight *Pleurotus* strains

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ARTICLE INFO

Article history:
Received 28 July 2009
Received in revised form 21 March 2010
Accepted 22 March 2010

Keywords:
Chemical sterilization
Hydrogen peroxide
Mycelial growth
Oyster mushroom
Pleurotus

ABSTRACT

Effective use of hydrogen peroxide as a chemical sterilant in mushroom production and selection of cultivable mushroom strains for tropical conditions require knowledge of the genetic diversity in the tolerance of the strains to hydrogen peroxide and to high temperatures. Therefore, three experiments were conducted to examine the sensitivity of *Pleurotus* mycelium to temperature and hydrogen peroxide. In Experiment 1, eight *Pleurotus* strains, which included two *Pleurotus sajor-caju* strains, three *Pleurotus ostreatus* strains, *Pleurotus salmoneo stramineus*, *Pleurotus cornicopae* and *Pleurotus eryngii* were cultured aseptically on agar at 25, 30 and or 35 °C. In Experiment 2, the eight strains were cultured aseptically on agar at six hydrogen peroxide concentrations (0–0.1%, v/v) at 27 °C. In Experiment 3, *P. sajor-caju* strain 1, a fast growing strain, was cultured non-asceptically at six hydrogen peroxide concentrations (0–0.1%, v/v) at 27 °C. In Experiment 1, mycelial growth was maximal at 25–30 °C, whereas a temperature of 35 °C was detrimental to mycelial growth except in one strain. At the highest temperature tested (35 °C), the relative mycelial growth rate (% of maximum) ranged from 6 to 91%, indicating marked differences in tolerance of the strains to high temperature. In Experiment 2, the mycelial growth rate in all strains increased when hydrogen peroxide was increased from 0 to 0.001% (v/v), and then decreased with further increments in hydrogen peroxide concentration. The strains differed markedly in sensitivity to hydrogen peroxide. The hydrogen peroxide concentration associated with 50% reduction in maximum mycelial growth rate due to toxicity (EC_{50}) ranged from 0.009 to 0.045% (v/v). It was noted that *P. sajor-caju* strain 1 which was the most tolerant strain to high temperature was also the most tolerant to high hydrogen peroxide concentration. In Experiment 3, involving non-aseptic culture of *P. sajor-caju* strain 1, bacterial growth was observed at concentrations ≤0.016%, whilst the upper hydrogen peroxide concentration limit for fungal growth was 0.025% (v/v). The highest hydrogen peroxide concentrations 0.016% (v/v) and 0.025% (v/v) in which bacteria and fungi, respectively, were observed to grow were within the concentration range 0.009–0.028% (v/v) that was found in Experiment 2 to cause a 50% reduction in mycelia growth in six of the eight *Pleurotus* strains tested. Use of hydrogen peroxide as a chemical sterilant in conjunction with strains highly tolerant of its toxicity offers a very cheap method of producing spawn as well as the mushrooms, and opens up opportunities for poor rural people.

1. Introduction

Oyster mushrooms are a diverse group of saprotrophic fungi species that fall under the genus *Pleurotus* (Kong, 2004). All of the oyster mushroom species are edible and cultivated world wide; being popular for their nutritional and health benefits (Mizuno et al., 1995). There is a conscious effort by many governments of developing countries to promote oyster mushroom production in rural communities as one of the tools for poverty alleviation and diversification of agricultural production. The major challenge for poor rural communities who wish to grow mushrooms is keeping out contamination by other microorganism in the culture media and production substrates. In the tropics, another added problem is the high temperatures through out the year, which range between 27 and 35 °C during the day, and thus necessitate selection for tolerance to high temperatures in the cultivable mushroom species (Gaitán-Hernández and Salmones, 2008).

The cultivation of oyster mushrooms consists of four general steps wiz; production of pure mycelium culture on agar, making of spawn, spawns run and fructification (Stamets and Chilton, 1983; Chang and Miles, 1989). The first three steps involve the transfer

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doi:10.1016/j.scienta.2010.03.006

Table 1
The eight *Pleurotus* strains that were used in the study.

Names of *Pleurotus* strains
P. cornicopae var citrinopiliae
P. Eryngii
P. sajor-cajor strain 1
P. sajor-caju strain 2
P. ostreatus var columbinus
P. ostreatus strain 1
P. ostreatus strain 2
P. salmoneo stramineus

of mycelium from one substrate to another. This transfer process exposes the growth medium to contamination by other microorganism, which leads to poor growth of the mycelium resulting in reduced mushroom yields. Contamination is traditionally contained by autoclaving the growth media for subcultures and spawn, pasteurisation of the substrate for spawn run and transferring the mycelium under aseptic conditions in laminar flow hood. The traditional facilities for prevention of contamination are too expensive for adoption by rural people in developing countries who might want to grow oyster mushrooms. An alternative technique developed by Wayne (1999) replaces the use of the expensive autoclave and laminar flow hood with chemical sterilization of the growth media and substrates using hydrogen peroxide. The technology offers a very cheap method of producing spawn as well as the mushrooms, and opens up opportunities for poor rural people.

Hydrogen peroxide is active against a wide range of microorganisms, including bacteria, yeasts, fungi, viruses, and spores (Rutala et al., 2008; Block, 2001) depending on the concentration and time of exposure. For example, a 0.5% accelerated hydrogen peroxide demonstrated bactericidal and virucidal activity in 1 min and mycobactericidal and fungicidal activity in 5 min (Omidbakhsh and Sattar, 2006). Similar differential activity of hydrogen peroxide concentration against spores of bacteria and fungi compared to live fungal mycelium is exploited in its use as a chemical sterilant in mushroom production. At suitable concentrations, hydrogen peroxide stops germination of fungal and bacterial spores whilst existing mycelium may be able to grow (Wayne, 1999). Thus, enabling the handling and inoculation of mushroom cultures and spawn in the open air (Wayne, 1999). Currently, there is limited information on genetic variation in the tolerance of oyster mushrooms to hydrogen peroxide. The aim of the present study was to examine variation among oyster mushroom strains in temperature requirements for mycelial growth and tolerance to hydrogen peroxide.

2. Materials and methods

Three experiments were conducted. The first and second experiments tested the effects of temperature and hydrogen concentration, respectively, on mycelial growth of eight oyster mushroom strains (Table 1) on potato dextrose agar (PDA). The third experiment tested the efficacy of hydrogen peroxide in preventing contamination in PDA-cultured mycelium of *Pleurotus sajor-caju* strain 1. The strains were obtained from Agricultural Research Council (South Africa), and represented the most commonly cultivated *Pleurotus* species.

2.1. Experiment 1: effect of temperature on mycelial growth

Solidified PDA plates were prepared in 90 mm plastic Petri dishes under sterile conditions. Outside the bottom of each of the Petri dishes, four radial lines (axis) were marked perpendicular to each other from the centre, and labeled r1, r2, r3 and r4 (Fig. 1). Eight *Pleurotus* strains (Table 1) were then inoculated on the sterile PDA in the Petri dishes by transferring in a laminar flow hood a small block (ca. 2 mm^2) of mycelium from an actively growing pure culture to the centre of the PDA at the point where the radial lines intercepted (Fig. 1). The eight test *Pleurotus* strains were inoculated separately in the Petri dishes, and incubated at 25, 30 or 35 °C in 10 replicates. The mycelium grew largely in concentric circles, and it was the radial growth of the mycelium that was measured daily for 5 days along each of the radial lines (axes r1–r4) in each of the Petri dishes. The four measurements were then averaged to obtain a mean radial growth per Petri dish.

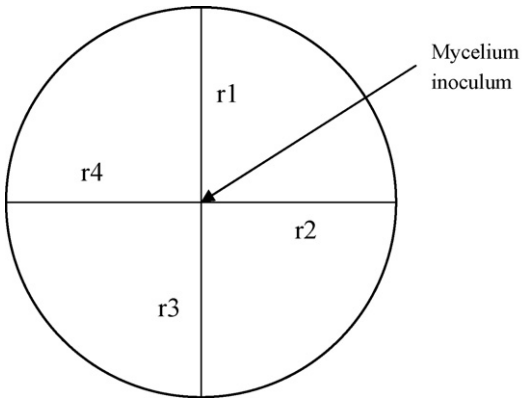

Fig. 1. Illustration of the four axes along which the mycelial growth was measured.

2.2. Experiment 2: effect of hydrogen peroxide on mycelium growth

Six hydrogen peroxide concentration treatments wiz; 0, 0.001, 0.0032, 0.01, 0.0316 and 0.1% (v/v) were established in PDA. The hydrogen peroxide treatment interval was logarithmic, and the treatment levels were established using the equation; $ar^{n-1} = b$, where a is the lowest concentration (0.001%) above zero (control), b is the highest concentration, n is the number of treatments (5) within the concentration range a–b, and r is a constant (obtained by solving the equation $0.001r^4 = 0.01$). The hydrogen peroxide was added to autoclaved PDA as per concentration treatments when the PDA had cooled to about 40 °C, and vigorously mixed by stirring with a sterile glass rod, poured into 90 mm plastic Petri dishes and then left to solidify. The same eight test *Pleurotus* strains used in Experiment 1 were then inoculation separately in the Petri dishes in 10 replicates for each hydrogen peroxide concentration treatment and incubated at 27 °C.

Radial mycelial growth of each *Pleurotus* strain was measured daily for 5 days as described in Experiment 1. Daily growth rates were computed and averaged for the 5-day growth period for each replicate.

2.3. Experiment 3: effect of hydrogen peroxide on contaminants

Six hydrogen peroxide concentration treatments (0, 0.01, 0.025, 0.040, 0.063 and 0.1%, v/v) were established in sterilized PDA in Petri dishes as described for Experiment 2. Again, the hydrogen peroxide concentration treatment levels were established using equation $ar^{n-1} = b$, but the lowest concentration level (a) above zero was changed to 0.01, which closely matched the hydrogen peroxide EC_{50} value (0.009%) for the least tolerant strain (*Pleurotus cornicopae var citrinopiliae*) to hydrogen peroxide toxicity. Hence, the hydrogen peroxide concentration treatments differed from those of Experiment 2. The PDA was inoculated with *P. sajor-caju* strain 1 in open air in 10 replicates for each of the hydrogen peroxide concentration treatment, and incubated at 27 °C.