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Comparison of Water Relations of Three Cultivated *Pleurotus* Species and *Trichoderma* Green Moulds

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The effect of ionic osmotic potential (ψ_π), and matric potential (ψ_m) in the range of -0.2 to -4.0 Mpa on mycelial growth of three species of *Pleurotus* (*P. florida, P. ostreatus* and *P. sajor-caju*) were determined over a range of temperature (15-30°C) on a 2% malt extract agar medium and compared with the ψ_π effect on growth of two strains of *Trichoderma* green mould. With the ionic solute KCl, optimun ψ_π for growth was -0.2 MPa for *P. florida* and in the range of -0.2 to -0.5 MPa for *P. ostreatus* and *P. sajor-caju*. The growth of all the species decreased significantly below -1.5 MPa, with slight growth at -3.0 MPa and with no growth at -4.0 MPa. Of the species of *Pleurotus, P. florida* grew significantly slower than the other two species. Growth of the species of *Pleurocus* was significantly slower when water potential (ψ_ω) was modified matrically with polyethylene glycol (PEG) 8000 then osmotically with KCl. They were also more sensitive to changes in ψ_m than ψ_π. The optimum ψ_m of the *Pleurotus* was -0.5 ψ_m, with no growth below -3.0 MPa. Of the species of *Pleurotus, P. florida* was most sensitive and *P. sajor-caju* was more tolerent to lowered ψ_π, but *P. sajor-caju* was most sensitive to lowered ψ_m. The growth rate of the *Trichoderma* green mould strains was much faster than that observed for the *Pleurotus* spp. Optimum growth for both strains of *Trichoderma* was in the range of -0.2 to -0.5 MPa. Strain CNU 503 was more tolerant to water stress than strain CNU 501. Both strains were able to grow up to 30% of optimum growth at -4.0 MPa at 25-30°C.

Keywords : green mould, matric potential, osmotic potential, *Pleurotus* spp., *Trichoderma* spp.

Pleurotus species are economically important cultivated mushrooms, particularly in Asia. They are predominantly grown on waste cotton and rice straw in Korea. The production of these mushrooms is dependent on the relationship between nutrient status of the substrates, strain type and the prevailing environmental conditions. The major abiotic factors which determine colonization and rate of decomposition are water potential of the substrate, temperature and gas composition. The total water content is often known but this does not describe the amount of water actually available to the mushroom. This availability is defined by the water potential of the substrate. Water potential (ψ_ω) is predominantly the sum of osmotic (ψ_π), matric (ψ_m), turgor (pressure, ψ_p) potentials (Griffin, 1981) and measured in pascals. Thus, the total water potential and its osmotic and matric components, and interactions with temperature are important factors affecting efficient colonization of the substrate and for efficient fruiting.

Magan, Challen and Elliot (1995) demonstrated that a range of wild type and commercially grown strains of *Agaricus bisporus* and *A. bitorquis* were significantly more sensitive to matric than osmotic stress. Other tropical basidiomycetes have also been demonstrated to be significantly more sensitive to matric than osmotic potentials (Mswaka & Magan, 2000). However, information on the impact of water potential and temperature on growth of *Pleurotus* species are scarce. Furthermore, in recent years *Trichoderma* green moulds have become a severe problem, affecting development of *Pleurotus* and significantly reducing yields. Thus comparisons of their respective water relations may enable a better understanding of the competition between these species.

The objectives of this work were therefore to determine (a) effect of ionic osmotic potential, (b) matric potential on *in vitro* growth of *P. florida, P. ostreatus* and *P. sajor-caju*, and (c) osmotic potential on growth of strains of *Trichoderma* green moulds *in vitro* in relation to temperature.

Materials and Methods

Fungal isolates. One strain each of *Pleurotus florida, P. ostreatus* and *P. sajor-caju* and two strains of the *Trichoderma* green moulds (CNU 501, CNU 503) were used in this study. All strains of *Pleurotus* spp. were obtained from Division of Applied Micro-

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biology, National Institute of Agricultural Science and Technology and strains of the *Trichoderma* were isolated from oyster mushroom cultivation facilities in Korea.

Media and inoculation. The basic medium used in this study was a 2% malt extract agar medium (-0.2 MPa water potential). This was modified osmotically with KCl (Lang, 1967) to 0.5, 1.0, 1.5, 2 0, 2.5, 3.0, 3.5 and 4.0 (-MPa). KCl was added directly to the medium together with 2% technical agar No. 2 (Lab M Ltd.). Media were checked with a Wescor thermocouple psychrometer and a C52 chamber for water potential values > -2 MPa, and a Novasina Humidat IC II (Novasina AG, Zurich, Switzerland) at lower values.

The matric potential of the medium was modified with polyethylene glycol 8000 (PEG 8000) which predominantly generates matric forces (99%) in the medium (Steuter, Mozafar & Goodin, 1981). The amounts required to obtain the same matric potentials as for the osmotic media were calculated as described by Michel and Kaufmann (1973) for each temperature. PEG 8000 does not solidify in the presence of agar, so the medium was covered with a 8.5 cm diameter layer of sterile black polyester cloth and cellophane (P400, Canning Ltd., Bristol) to support the fungal culture.

Media were centrally inoculated with a 4 mm agar discs from the margin of an actively growing culture of each strain. The same osmotic/matric potential treatments were sealed in polyethylene bags and incubated at 15, 20, 25 and 30°C. All experiments were carried out twice with three replicates

Measurement. The diameter (mms) of the mycelium colony was measured daily by making two measurements per colony at right angles to each other. In all cases the linear regression lines of increase in radius against time (in days) was used to obtain the growth rates (mm day^{-1}) under each set of treatment conditions. The growth rates in mm day^{-1} were then compared by analysis of variance and the least significant differences ($P < 0.05$) determined for strain and treatment temperature, ψ_π and ψ_m differences.

Results

Effect of osmotic potential × temperature on growth of *Pleurotus* spp. The effect of different ψ_π (KCl) at four different temperature on growth rate of *P. florida, P. ostreatus* and *P. sajor-caju* is shown in Fig. 1. There was little difference between the response of *P. ostreatus* and *P. sajor-caju*. However, they grew significantly faster than *P. florida*. The optimum ψ_π for growth of *P. florida* was -0.2 MPa, while that of the other species was in the range of -0.2 and -0.5 MPa. Growth rate of the *Pleurotus* spp. decreased significantly below -1.5 MPa, with slight growth occurring at -3.0 MPa and with no growth occurring at -4.0 MPa (the driest conditions tested). Of the three species of *Pleurotus*, *P. florida* was most sensitive to changes in ψ_π than the other two species. Growth rates of the *Pleurotus* spp. were overall about 50% greater at 25-30°C than at 15°C. In all cases, *P. florida* grew consistently slower than the other two species.

Effect of matric potential on growth of *Pleurotus* spp.

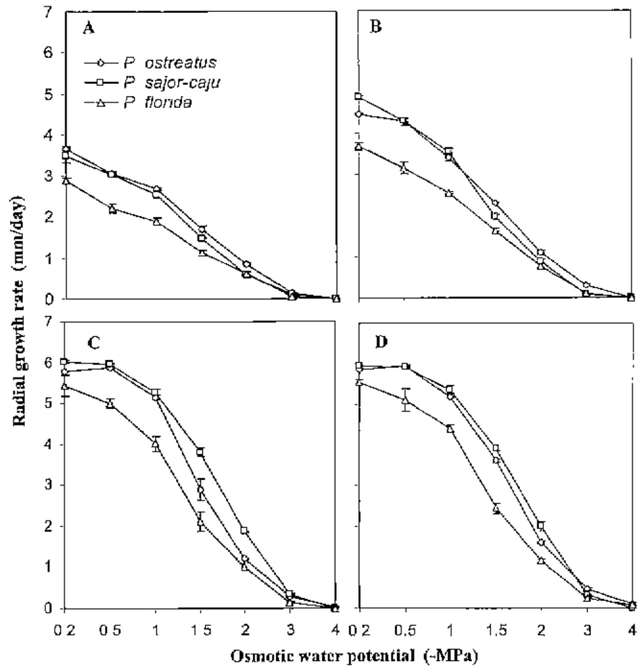

Fig. 1. Effect of osmotic water potential on growth of *Pleurotus ostreatus*, *P. sajor-caju* and *P. florida* on 2% malt extract agar mediated with KCl at 15°C (A), 20°C (B), 25°C (C) and 30°C (D) Vertical bars represent the values of least significant difference at P=0.05.

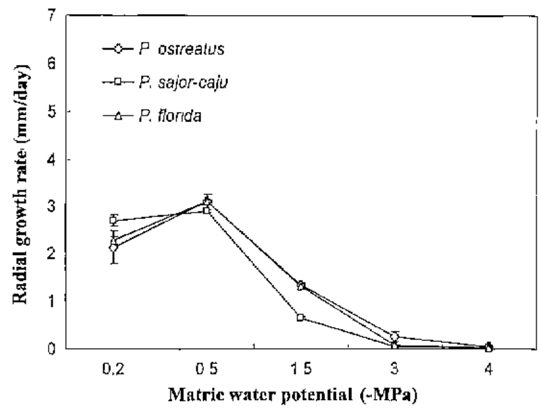

Fig. 2. Effect of matric water potential on growth rate of *Pleurotus ostreatus*, *P. sajor-caju* and *P. florida* on 2% malt extract agar mediated with PEG 8000 at 25°C. Vertical bars represent the values of least significant difference P=0.05.

Fig. 2 shows the effect of ψ_m (PEG8000) on growth rate of the three species of *Pleurotus*. This shows that the sensitivity to matric stress of the *Pleurotus* spp. was different from that observed on osmotically-modified media. The species were generally more sensitive to changes in ψ_m than ψ_π. The optimum ψ_m was -0.5 ψ_m, with growth being significantly less at -1.5 MPa and completely inhibited at -3.0 MPa. *P. florida* and *P. ostreatus* behaved similarly with no significant difference in growth response. *P. sajor-caju* was

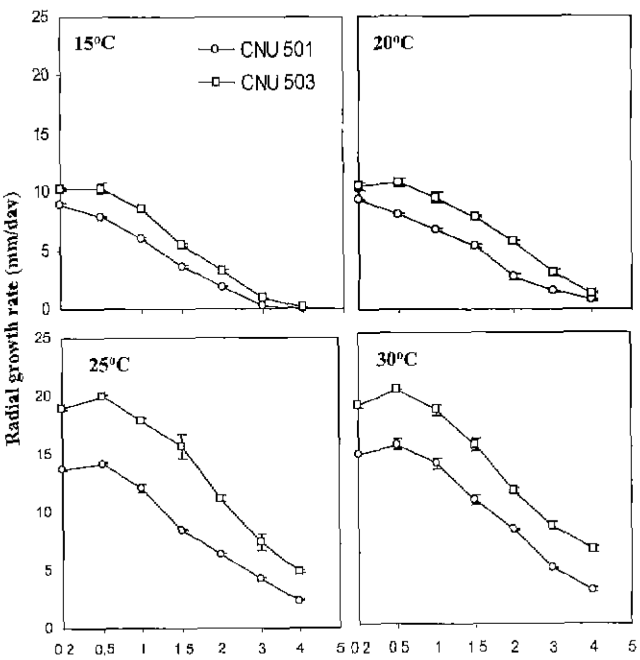

Fig. 3. Effect of osmotic water potential on growth rate of two strains of *Trichoderma* (CNU 501 and CNU 503) on 2% malt extract agar mediated with KCl at 15°C, 20°C, 25°C and 30°C. Vertical bars represent the values of least significant difference P=0.05.

most sensitive to change in ψ_m than the other two species.

Effect of osmotic potential × temperature on growth of *Trichoderma* green moulds. The effect of interaction between osmotic potential and temperature on growth rate of two strains of *Trichoderma* green mould is shown in Fig. 3. This shows that the growth rate of the *Trichoderma* strains was significantly faster than that observed for the *Pleurotus* spp.

The optimum growth responses to ψ_π by the strains were at -0.2/-0.5 MPa with the growth being decreased by >50% at -1.5 MPa. Both strains, however, were able to grow up to 30% of optimum growth at -4.0 MPa (the driest conditions tested) at 25-30°C, in contrast to the *Pleurotus* spp. which were completely inhibited for the growth. Of the strains of *Trichoderma*, strain CNU 501 grew consistently slower and was more sensitive to changes in ψ_π than strain CNU 503.

Discussion

This study has shown that there are some statistically significant differences in the water and temperature relations of strains of both *Pleurotus* and *Trichoderma in vitro* on MEA. There were intra-species differences in growth rate of *Pleurotus*, with *P. florida* growing significantly slower on osmotically modified media than the other two species tested. The knowledge that *Pleurotus* spp. were more sensitive to ψ_m than ψ_π has not previously been demonstrated. Previous studies on wood decay basidiomycetes showed that *Serpula lachrymans* ceased growth at between -3 and -6 MPa on agar (Clarke et al., 1980), while Boddy (1983) found that growth of temperate fungal species ceased growth at between -4.4 and -7.1 MPa ψ_r. On the other hand, *Trichoderma* has been shown to be active particularly in colonizing waste cotton and rice straw substrates and decomposing cellulose *in vitro*. However the water relations of the substrates and the influence which these may have on growth and cellulose decomposition have not been considered. This is particularly important in understanding the possible interaction between groups of fungi competing for the substrates.

The information obtained in this study may be important as water availability in composts is probably determined matrically with a smaller osmotic contribution due to salts. Limited work on the growth rate of *A. bisporus* in both sterile and unsterile compost has suggested linear growth rates of between 5 and 8.6 mm day^{-1} (Straatsma et al., 1989; Straatsma et al., 1991). However, in their studies, water potential was not maintained accurately. Recently, Magan et al. (1995) demonstrated that a range of wild type and commercially grown strains of *Agaricus bisporus* and *A. bitorquis* were significantly more sensitive to matric than osmotic stress. The growth rates of *Agaricus bisporus* strains were found a maximum of up to 6 mm day^{-1}. However, no previous information was available for strains of *Pleurotus*. Extensive studies by Kalberer (1987, 1990) have elucidated the changes in water relations within compost and casing layers, and the harvested fruit bodies of *A. bisporus* but not examined the actual water fluxes in mycelium during spawning or during subsequent flushing. Examination of the partitioning of water in the mycelium colonizing compost, the casing layer and fruit bodies may improve our understanding of the mechanisms of translation to the fruit bodies (Thompson et al., 1985; Eamus & Jennings, 1986).

Studies are now necessary to determine the effect of osmotically and matrically modified compost substrates on growth of strains of *Pleurotus* spp. and *Trichoderma* green moulds. Such information will enable strain specific advice to be given on watering regimes for optimum spawn running, appropriate subsequent cropping and further mushroom disease control.

Acknowledgement

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