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Effect of Cadmium on the Axenic Growth and Phosphatase Activity of Ectomycorrhizal Fungi, *Amanita muscaria* and *Laccaria ohiensis*

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Abstract: ***The mycorrhizal fungi are of importance in view of interest in the reclamation of toxic metals to polluted sites and influence on plant growth and productivity. Although there appears to be a wide diversity in responses between different plant and fungal symbionts, the amelioration of metal phytotoxicity by mycorrhizal fungi has been widely demonstrated. Ectomycorrhizal fungi inoculated forest trees improve their growth and survival in metal polluted soils than non-mycorrhizal trees. Severe contamination by pollutants such as heavy metals can result in widespread seedling mortality, delay of several decades in revegetation schemes. Metal tolerant strains of fungi have been developed in some laboratories by repeated sub-culturing in metal contaminated medium. The tolerance of ectomycorrhizal fungi growing in vitro towards mine metals, Al, Fe, Cu, Zn, Ni, Cd, Cr, Pb and Hg is more important in order to assess their potential for the establishment of ectomycorrhizae in metal contaminated sites. In the present study, the effect of different concentrations of cadmium on the growth and phosphatase activity of two ectomycorrhizal fungi, Amanita muscaria and Laccaria ohiensis were investigated.***

Keywords: ectomycorrhizal fungi, cadmium, phosphatase activity, phytotoxicity

1. Introduction

In modern days, various types of activities, including agriculture, industry and transportation, produce a large amount of wastes and new types of pollutants. Soil, air and water have traditionally been used as sites for the disposal of all these wastes. Pollution of the biosphere with toxic metals due to man-made activities pose a major environmental and human health problem. New technologies are needed to address the problems of contaminants, especially those that are neither volatile nor mobile in soil solutions. One emerging technology, phytoremediation, is attracting more attention and is offering a way to reclaim many of our urban sites lost due to heavy metal contamination. The mycorrhizal fungi are considered as an important tool in afforestation and rehabilitation of degraded lands especially in mine spoils, eroded sites and polluted wastelands (Raman and Sambandan, 1998).

2. Materials and Methods

Isolation

The cultures of *Amanita muscaria* and *Laccaria ohiensis* were isolated from sporocarps growing in *Pinus patula* plantations at Kodaikanal, Tamil Nadu, India. The sporocarps were brought to the laboratory and surface was sterilized with 30% hydrogen peroxide and the inner sterile tissue was maintained on the Modified Melin Norkrans (MMN) nutrient medium (Marx, 1969; Raman and Mohankumar, 1988).

Concentrations of Cd

Eight mm plugs of fungal colonies were transferred to 250 mL Erlenmeyer flasks containing 100 mL of Palmer Hacskaylo medium (pH 4.5) containing 5 g glucose, 0.5 g NH_4Cl, 0.5 g KH_2PO_4, 5 µg biotin, 1 mg thiamine HCl and micronutrient solution 2 mL/L. The medium was amended with various concentrations of Cd (0.5, 1.0, 1.5, 2.0 and 2.5 µM) in the form of $CdSO_4$ incubated at 25±1°C for 45 days.

Harvests

Five replicates of the treatments were harvested at 15th, 30th and 45th-day intervals. For each harvest, mycelial dry weight, intracellular and extracellular enzyme activity namely acid and alkaline phosphatase activity were estimated.

Dry weight of the mycelium

The mycelial mat in each flask was transferred to a pre-washed, pre-dried and pre-weighed filter paper and washed thoroughly with distilled water to make it free from any trace of adherent medium and dried in an oven at 80°C for 24 h with a constant weight.

Estimation of acid and alkaline phosphatase

Acid and alkaline phosphatase activity of the mycelium was estimated by following the method of Rokicka (1992). Fungal colonies were grounded in 0.1 M acetate buffer (pH 5.0) containing 0.01 MEDTA, 0.01% Triton X 100 and 2.5% PVP. Crude enzyme extracts were obtained after centrifugation at 4°C. The incubation medium of acid phosphatase contained 250 µL of 0.1 M acetate buffer (pH 4.8), 50 µL of 0.015 M *p*-nitrophenyl phosphate disodium salt and 50 µL of enzyme solution. The reaction was stopped after 30 min by adding 2.5 mL of 0.2 M Na_2CO_3. The specific enzyme activity was measured in a Beckman DU-40 spectrophotometer at 410 nm and expressed as µmoles *p*-nitrophenol released/g dry wt/30 min.

3. Results

Biomass

The results of the mycelial dry weight are presented in Table 1. The mycelial growth decreased in all the concentrations of Cd with increasing day intervals except in 0.5 µM when compared with control. Changes in the dry matter content is a prime indicator to the tolerance of fungi. *L. ohiensis* yielded more mycelia in all concentrations of Cd when compared with *A. muscaria.*

Table 1: Effect of different concentrations of Cd on the biomass of *A. muscaria* and *L. ohiensis* grown at different day intervals

Concentrations of Cd (µM)	Dry weight of the mycelium (g)					
	A. muscaria			*L. ohiensis*		
	15 d	30 d	45 d	15 d	30 d	45 d
Control	0.64	1.26	1.49	0.77	1.31	1.50
0.5	0.75	1.33	1.52	0.81	1.38	1.54
1	0.38	0.95	1.29	0.72	1.30	1.50
1.5	0.29	0.82	1.32	0.40	1.23	1.41
2	0.24	0.59	1.21	0.38	0.74	1.03
2.5	0.18	0.37	1.03	0.20	0.51	0.95

Intracellular and extracellular acid phosphatase

The effect of different concentrations of Cd on intracellular and extracellular acid phosphatase activity of *A. muscaria* and *L. ohiensis* is presented in Tables 2 and 3. The intracellular acid phosphatase activity increased in lower concentration of Cd and it decreased in higher concentrations and the enzyme activity decreased with increasing day intervals. Whereas the extracellular acid phosphatase increased in the fungus grown in all the concentrations of Cd except in higher concentration (2.5 µM) and with increasing day intervals, the activity was also increased. In *A. muscaria*, the maximum intracellular and extracellular acid phosphatase activity was noticed in 1.0 µM concentration of Cd on 15th and 45th day, respectively, and the minimum activity was observed in 2.5 µM on 45th day. In *L. ohiensis*, 1.5 µM concentration of Cd showed the maximum intracellular activity on 15th day and the minimum activity was observed in 2.5 µM on 45th day and the maximum extracellular activity was noticed in 1.0 µM concentration of Cd on 45th day and the minimum activity was observed in 2.5 µM on 15th day. In comparison, both intracellular and extracellular acid phosphatase activity of *L. ohiensis* was higher than *A. muscaria*.

Table 2: Effect of different concentrations of Cd on the intracellular acid phosphatase of *A. muscaria* and *L. ohiensis* at different day intervals

Concentrations of Cd (µM)	Intracellular acid phosphatase activity (µmoles PNP released/mg protein/min)					
	A. muscaria			*L. ohiensis*		
	15 d	30 d	45 d	15 d	30 d	45 d
Control	315	217	139	230	250	156
0.5	439	342	320	447	360	342
1	538	390	340	490	385	351
1.5	485	360	275	560	405	287
2	413	238	140	425	325	170
2.5	215	199	95	220	210	110

Table 3: Effect of different concentrations of Cd on the extracellular acid phosphatase of *A. muscaria* and *L. ohiensis* at different day intervals

Concentrations of Cd (µM)	Extracellular acid phosphatase activity (µmoles PNP released/mg protein/min)					
	A. muscaria			*L. ohiensis*		
	15 d	30 d	45 d	15 d	30 d	45 d
Control	487	562	609	513	592	660
0.5	645	760	895	640	630	790
1.0	610	690	975	690	795	921
1.5	572	618	951	594	710	843
2.0	536	640	845	570	660	802
2.5	270	418	490	300	443	510

Intracellular and extracellular alkaline phosphatase

The effect of different concentrations of Cd on intracellular and extracellular alkaline phosphatase activity of *A. muscaria* and *L. ohiensis* is presented in Tables 4 and 5. The intracellular alkaline phosphatase activity increased in lower concentrations of Cd and decreased in higher concentrations in all the day intervals. Whereas the extracellular alkaline phosphatase activity was increased with increasing concentration of Cd in all the day intervals except 0.5 µM concentration of Cd on 30th day. In *A. muscaria*, the intracellular activity increased up to 1.0 µM concentrations of Cd when compared with control over that concentration it was decreased. Significantly increased extracellular activity was found in 2.5 µM of Cd on 15th day and lesser activity was found in control. In *L. ohiensis*, the intracellular activity increased up to 1.5 µM concentration of Cd when compared to control. Significant increase of extracellular activity was found in 2.5 µM of Cd on 15th day and lesser activity was found in control as like as *A. muscaria*. In comparison, both intracellular and extracellular alkaline phosphatase activity of *L. ohiensis* was higher than *A. muscaria*.

Table 4: Effect of different concentrations of Cd on the intracellular alkaline phosphatase of *A. muscaria* and *L. ohiensis* at different day intervals

Concentrations of Cd (µM)	Intracellular alkaline phosphatase activity (µmoles PNP released/mg protein/min)					
	A. muscaria			*L. ohiensis*		
	15 d	30 d	45 d	15 d	30 d	45 d
Control	87	67	63	90	75	69
0.5	93	73	72	99	77	75
1.0	118	105	94	110	90	82
1.5	97	84	73	121	118	108
2.0	85	78	59	89	80	63
2.5	72	60	38	75	66	45

Table 5: Effect of different concentrations of Cd on the extracellular alkaline phosphatase of *A. muscaria* and *L. ohiensis* at different day intervals

Concentrations of Cd (µM)	Extracellular alkaline phosphatase activity (µmoles PNP released/mg protein/min)					
	A. muscaria			*L. ohiensis*		
	15 d	30 d	45 d	15 d	30 d	45 d
Control	31	42	48	35	44	51
0.5	37	39	58	39	47	60
1.0	45	49	64	48	51	68
1.5	49	56	69	55	61	74
2.0	53	57	72	61	68	79
2.5	55	60	73	65	70	75

4. Discussion

In the present study, the ectomycorrhizal fungi of *A. muscaria* and *L. ohiensis* showed decreased growth in all the concentrations of Cd except 0.5 µM when compared with control in all the day intervals. This is in support of the observation made by Xiang (1995) who observed that the mycelial growth of *A. muscaria* was strongly inhibited by higher concentration of Cu and also Raman et al. (2002) reported that the increased dry matter production of *Laccaria laccata* and *Suillus bovinus* in 0.5 mM of Cr. Jones and Meuhlchen (1994) also observed that the growth studies on agar and liquid culture of *L. laccata* proved sensitive to Cu and Al but not to Zn. Changes in dry matter accumulation and metal content of tissues are indicators of the different tolerance of an organism to metallic cations (Ernst et al., 1992).

Also in this study, intracellular acid and alkaline phosphatase activity of *A. muscaria* and *L. ohiensis* grown in Cd amended medium enhanced in 15th day and declined in 30th and 45th day when compared with control. In contrast, extracellular acid and alkaline phosphatase activity of *A. muscaria* and *L. ohiensis* were increased in all the concentration of Cd on 45th day when compared with 15th- and 30th-day intervals. High acid phosphatase activity and tolerance to high concentrations of Cu and Ni were reported in *L. laccata* (Periasamy and Raman, 1995). Whereas in *A. muscaria*, the activity of acid phosphatase declined as Mn concentration was higher (Xiang, 1995).

5. Conclusion

Both the fungi have maximum phosphatase activity and tolerance to high concentration of Cd. These fungi are potential in the usage of reclamation of polluted soils.

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