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# ACTIVITY AND PROPERTIES OF ARGINASE IN MYCELIAL CULTURES OF SOME MEDICINAL MUSHROOMS

НАНАГЮЛЯН С. Г., НИКОЯН А. А., ГАСПАРЯН А. А. АКТИВНОСТЬ И СВОЙСТВА АРГИНАЗЫ В МИЦЕЛИАЛЬНЫХ КУЛЬТУРАХ НЕКОТОРЫХ ЛЕЧЕБНЫХ ГРИБОВ

In many countries of the world, edible mushrooms are used not only as foodstuffs, but also as a valuable source for getting substances having medical-preventive value (Minasbekyan et al., 2003). For most plants and animals ammonia is a cellular poison and should be cleared, which takes place through the biosynthesis of urea. Higher fungi accumulate significant amount of urea in the mycelium and fruiting bodies (Ewaze, Al-Naama, 1989; Wagemaker et al., 2005). Unlike animals (where urea is a waste and withdraws from the orgamism) the mushroom urea is a source of reserve nitrogen and in higher plants it is asparagine and glutamine that have this function (Bekker, 1988). But the considerable quantity of urea in fruit bodies influences both flavouring qualities, and normal growth and development of fungi. Arginase [EC 3.5.3.1; L-arginine aminohydrolase] — the enzyme of ornithine cycle is a trimetric binuclear manganese metalloenzyme that catalyzes the hydrolysis of the amino acid L-arginine to L-ornithine and urea (Di Costanzo et al., 2005). Arginine is a precursor for the biosynthesis of polyamines, agmatine, proline, nitric oxide (Chen et al., 2004; Durante et al., 2007).

The aim of our research was to determine arginase activity and investigate some properties of arginase in several species of medicinal mushrooms.

## Material and methods

F u n g a l s t r a i n s a n d g r o w t h c o n d i t i o n s. Arginase activity has been studied in mycelial strains of the following fungi: *Ganoderma lucidum* (M. A. Curtis: Fr.) P. Karst., *Lentinula edodes* (Berk.) Pegler, *Stereum hirsutum* (Willd.: Fr.) Gray, *Trametes versicolor* (L.: Fr.) Pilát, *Fomitopsis pinicola* (Schwein.: Fr.) P. Karst., *Fomes fomentarius* (L.: Fr.) Fr., and *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. Mycelia from each species was obtained by tissue culture method from basidiocarps and maintained on PDA medium at 4 °C (Dudka et al., 1982). Cultures were obtained and maintained in the Culture Collection Laboratory of Experimental Mycology, Department of Botany, Yerevan State University. For inoculation the mycelial disks (7 mm diameter) from 7 days old cultures were transferred onto potato dextrose agar (PDA). Fungal strains were grown in 9 cm Petri dishes on PDA (pH 4.8) in the dark at 26—28 °C for 7 days.

E n z y m e e x t r a c t i o n a n d a s s a y s. The collected mycelium (1g) was grounded with ice and homogenized at 4 °C with 10 ml of 0.05 M Na, K phosphate buffer — pH 9.0 or distilled water — pH 7.0 in a Potter-Elvejem Homogenizator (Braun, Melsungen, Germa-

ny). Arginase activity was determined by the method of Ratner and Pappas (Ratner, Pappas, 1949). The assay mixtures contained 0.5 ml enzyme preparation, 50 mmol L-arginine, 5 mmol $MnCl_2$, 0.04 M glycine-HCl buffer (pH 9.5), in a final volume of 2.5 ml. Reactions were carried out at 37 °C for 60 min and stopped by the addition of 1 ml of 20 % $HClO_4$. The mixtures were centrifuged at 4 °C at 5000 g for 15 min. Urea in the samples was determined by the method of Moore and Kaufmann (Moore, Kaufman, 1970). Arginase activity was expressed as mmol of urea formed per 1 g wet tissue. The following ions: $Cd^{2+}$, $Co^{2+}$, $Ni^{2+}$ were added in the quantity of 5 mmol and 10 mmol to the incubation mixture. The metals were added as salts: $NiCl_2$, $CdCl_2$, and $CoCl_2$. The percentage of inhibition of the arginase activity by each metal was calculated by comparison with control (absence of metal). To investigate the function of L-arginine as a fungal arginase inducer, 0.5, 1 and 1.5 g/L L-arginine were added to the growth medium. All assays were performed in triplicate.

## Results and discussion

Higher arginase activity has been detected in the strains of *Fomitopsis pinicola* (147.8 ± ± 7.52 mmol/g), *Ganoderma lucidum* (30 ± 9.53 mmol/g), *Pleurotus ostreatus* (10.26 ± ± 2.4 mmol/g), *Fomes fomentarius* (9.45 ± 1.2 mmol/g), *Trametes versicolor* (7.02 ± ± 1.2 mmol/g). Strains of *Lentinula edodes* and *Stereum hirsutum* have slightly arginase activity. As a result of the conducted research strains of the *Ganoderma lucidum* and *Fomitopsis pinicola* with highest arginase activity have been selected.

A common feature of all arginases thus far studied, whether of eukaryotic or prokaryotic origin, is a requirement of divalent cations for activity (Ash, 2004). The impact of metal ions ($Mn^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Ni^{2+}$) on arginase activity was investigated. It is known that $Mn^{2+}$ ions are the part of the structure of the active center of the arginase, thus they serve as a major positive effectors of the arginase activity. Researches of some authors studying the role of metals on the arginase activity in human liver showed that not only $Mn^{2+}$ ions are essential in linkage of enzyme with a substratum but also $Co^{2+}$ and $Ni^{2+}$ ions have a great impact on this process (Carvajal, 1995). Thus in fungus *Ganoderma lucidum* the presence of ions $Mn^{2+}$ (5 mmol) in the incubation mixture leads to arginase activity increase at 40.7 %, and in fungus *Fomitopsis pinicola* at 13.2 %. Obviously, in the case of fungus *F. pinicola* the presence of $Mn^{2+}$ ions in the incubation mixture leads to slight changes in activity.

It should be noted that the most powerful inhibitor of arginase activity of both fungi is $Cd^{2+}$. At the concentrations of $Cd^{2+}$ 5 mmol and 10 mmol arginase activity of *Ganoderma lucidum* falls to 81 and 91.7 %, respectively.

Studies on the effects of $Ni^{2+}$ on the arginase activity showed that the fungus *G. lucidum*, with concentrations of 5 mmol and 10 mmol $Ni^{2+}$ indicated falling activity up to 38.5 and 69 %, respectively. In the concentrations of 5 mmol and 10 mmol $Co^{2+}$ ions have the same effect on arginase activity, reducing it to 32.7 %. Arginase activity of *Fomitopsis pinicola* is maximally inhibited by $Cd^{2+}$ ions (5 mmol and 10 mmol) to 97.5 %. Unlike the fungus *Ganoderma lucidum* the inhibitory effect of $Ni^{2+}$ ions at the arginase activity in *Fomitopsis pinicola* is more pronounced. At the concentrations of 5 mmol activity is reduced to 92.3 %, while at 10 mmol — to 96.2 %. $Co^{2+}$ ions also have inhibitory effect, which in comparison

**Arginase activity of *Ganoderma lucidum* as a function of L-arginine**

| L-arginine concentration, g/L | Arginase activity, mmol/g |
|---|---|
| 0 | 30 ± 9.53 |
| 0.5 | 76 ± 5.56 |
| 1.0 | 97 ± 6.55 |
| 1.5 | 90 ± 3.60 |

with $Cd^{2+}$ and $Ni^{2+}$ is less pronounced, albeit constitutes a fairly high percentage — 48.3 % (5 mmol) and 56.2 % (10 mmol).

Our study indicated L-arginine as an inducer of arginase activity of *G. lucidum* and *Fomitopsis pinicola*. Significant induction was noted in the strain of *Ganoderma lucidum*. The relation between L-arginine concentration in the medium and arginase activity is presented in the table.

To conclude our research we would like to note that out of all mycelial cultures studied by us the strains of *G. lucidum* and *Fomitopsis pinicola* showed high arginase activity. The investigation of the effect of some metals on the arginase activity has shown that, excluding the $Mn^{2+}$ ions, all investigated metals have inhibitor effects. Addition of L-arginine into the medium has brought to enzyme induction. Studies of arginase activities and properties of other perspective medicinal mushrooms strains are currently in progress and will be continued.

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## РЕЗЮМЕ


Аргиназа [КФ 3.5.3.1; L-аргинин аминогидролаза] — фермент орнитинового цикла, который катализирует гидролиз аргинина на мочевину и орнитин. Аргиназа играет ключевую роль в процессе формирования мочевины. Были исследованы аргиназная активность и некоторые ее

свойства. Наши результаты показали высокую аргиназную активность культур *Ganoderma lucidum* и *Fomitopsis pinicola*. Металлы $Cd^{2+}$, $Co^{2+}$, $Ni^{2+}$ ингибировали аргиназную активность обоих грибов. Добавление L-аргинина в среду роста привело к индукции фермента.

Ключевые слова: аргиназа, лечебные грибы, дереворазрушающие грибы, тяжелые металлы, индукция аргиназы.

## SUMMARY

Arginase [EC 3.5.3.1; L-arginine aminohydrolase] — the enzyme of ornithine cycle which catalyzes hydrolysis of arginine to urea and ornithine. Arginase plays a key role in the formation of urea. Arginase activity and some properties in mycelial cultures of several medicinal mushrooms have been investigated. Our results have shown higher arginase activity in the strains of *Ganoderma lucidum* and *Fomitopsis pinicola*. The metal ions $Cd^{2+}$, $Co^{2+}$, $Ni^{2+}$ have inhibitory effect on arginase activity of both fungi. Addition of L-arginine into the medium has brought to enzyme induction.

Key words: arginase, medicinal mushrooms, wood-decaying fungi, heavy metals, the induction of arginase.