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ARGINASE AND ORNITHINE CARBAMOYL TRANSFERASE ACTIVITY AND UREA ACCUMULATION DYNAMICS IN DIFFERENT PHASES OF *PLEUROTUS OSTREATUS* (JACQ.:FR.)KUMM. FUNGI FRUCTIFICATION

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Two enzymes of the ornithine cycle (ornitine carbamoyl transferase and arginase) activities and urea accumulation dynamics of being wood decaying and food product fungi *Pleurotus ostreatus* (Jacq.:Fr.)Kumm. during aging and developing different phases were investigated. Concomitant to fungi development and growth, arginase activity and positive dynamics of urea are connected for providing the corresponding level of osmotic pressure in hymenium.

Keywords: *Pleurotus ostreatus*, pure culture, fruit body, arginase, ornithine carbamoyl transferase.

Introduction. It is well known that NH_3, urea or uric acid are the final products of protein degradation in living organisms. Urea has a special role in fungi metabolism, and, as it is inert from chemical aspect, it may function as a type of nitrogen storage [1]. Fungi have two main mechanisms of urea formation: ornithine cycle and degradation of nucleic acids [2], though, the first mechanism is the main way of urea formation. The uric acid in mushrooms is amply accumulated in mycelium, and in the case of hymenomycetous fungi at the maturation phase of fruit body – mainly in hymenium [3].

The results of R. B. Moore and N. J. Kaufman investigations have revealed significant changes in *Coprinus cinereus* fungi enzyme systems, including the ornithine cycle and different phases of aging [4]. Ornithine carbamoyl transferase (OCT) activity is noticeably changed at fruit body formation phase, particularly in fungus cap. But there is no urease enzyme here. It means that the role of nitrogen metabolism enzymes here is to accumulate urea and, possibly, other nitrogen metabolites in fungus hymenium. It promotes water accumulation in the cells of hymenium plates and, as a result, the cells swell and the fungi cap sizes increase. Consequently the spore dispers ion process becomes simplified.

The high intake of accumulated urea in the fruit bodies of edible fungus has a negative effect on their flavoring properties. In this respect the investigation of special properties of the enzymes systems, that take place in metabolic processes, is very important for optimization of conditions of fungi industrial cultivation.

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In the present study two enzymes of the ornithine cycle (OCT and arginase) activities and urea accumulation dynamics of wood decaying and food product fungi *Pleurotus ostreatus*(Jacq.:Fr.)Kumm. in different phases of fruit bodies formation were investigated.

Materials and Methods. Pure cultures *Pleurotus ostreatus* were obtained from different strains isolated from carpophores or grain mycelium of the mushroom growing in different regions of Armenia. Fresh fruit bodies were found on wood of deciduous (beech, oak) trees. Beer-wort agar (pH 4–5) was used as a solid media. The 8-day cultures of xylotrophic fungi of *Pleurotus ostreatus* were included in experiments.

Fig. 1. Pure culture of *Pleurotus ostreatus*.

Fig. 2. Spawn for cultivation.

We have used the fungus 20% water homogenate at different phases of the growth: mycelium, primordia of fruit body, non-formatted and formatted fruit body (Fig. 1–3). The fungus growth has been realized by commonly used mycological method [5].

Fig. 3. Strain bag with primordia and fruit bodies of *Pleurotus ostreatus*.

The homogenization is realized in cold conditions in Potter-Elvejame glass homogenizer using the Teflon pounder (5×30 *s*). The arginase activity was determined by S. Ratner and A. Papas method [6] and was expressed in urea. The urea was determined by R.A. Archibald method [7]. OCT activity was determined by the method of citrulline arsenolysis [8]. The activity of enzymes has been investigated in fungus mycelium, fruit body primordia and at II (non-formatted), IV, VI and IX days of their growth (formatted fruit body).

Results and Discussions. At the preliminary phases of fungus development the rapid growth of *Pleurotus ostreatus* arginase activity was revealed during the investigations. According to the data in Table 1, the activity of arginase, which is expressed by the least level in mycelium culture (69 μM/g), rapidly increases in fruit body primordia (720 μM/g), and more expressed on the second day of primordia growth in non-formatted fruit bodies (1044 μM/g).

Table 1

The activity of arginase and OCT in Pleurotus ostreatus mycelium, primordia of fruit bodies and non-formatted fruit bodies (n = 5)

Phases of fungus development	Arginase, μM urea/g	OCT, μM NH_3/g
micelium	69 ± 3.6	3.9 ± 0.15
embryos of fruit bodies	720 ± 32.3	4.2 ± 0.09
non-formatted fruit bodies	1044 ± 80.5	4.5 ± 0.12

Table 2

The activity of arginase and OCT in Pleurotus ostreatus non-formatted fruit bodies growth at different phases (n = 5)

Days of growth	Fruit body and its parts	Arginase, μM urea/g	OCT, μM NH_3/g
IV	whole fruit body	1250 ± 81.2	5.9 ± 0.5
	fungus cap	1845 ± 87.5	3.2 ± 0.18
	stipe	338 ± 18.5	10.1 ± 0.85
VI	whole fruit body	1057 ± 68.2	6.5 ± 0.75
	fungus cap	1800 ± 102.3	3.5 ± 0.35
	stipe	198 ± 13.7	11.4 ± 2.6
IX	whole fruit body	988 ± 25.3	3.4 ± 0.17
	fungus cap	1711 ± 95.6	3.2 ± 0.21
	stipe	74 ± 16.3	3.7 ± 0.22

According to the data in Tab. 2, during the growth of formatted fruit body arginase activity at all the phases of investigation is expressed in the fungus cap and very low in the stipe. That's why the arginase activity in the whole fruit body is lower in the fungus cap comparing with the activity of mentioned enzyme.

As for OCT, opposite to arginase, the activity of which has noticeably positive dynamics at preliminary phases of development, the activity of OCT during three phases is almost kept at the same level: with a slight increase parallel to development: 3,9–4,5 (μM NH_3/g) (Tab. 1).

OCT activity in the formatted fruit body on the IV and VI days of growth increases, but more rapid growth increase is revealed in the stipe, opposite to arginase, the activity of which is more expressed in fungus cap at all the phases of its growth (Tab. 2).

We have also studied urea dynamics at different phases of fungus growth and development. The data in Tab. 3 show, that there is no accumulation of urea during the phase of mycelium formation and at the preliminary phases of fruit body formation (primordia of fruit body, non-formatted fruit body), while at the same phases more rapid increase of arginase activity, nearly 15 times more, is revealed (Tab. 1). We can assume that the formatted urea is actively included in metabolic processes.

Table 3

The urea dynamics at different phases of growth and development of Pleurotus ostreatus (μM urea/g, n = 5)

Mycelium	Fruit body							
	Primordia	Non-formatted	Formatted					
			IV day		VI day		IX day	
9.0	8.1	8.1	cap 27.2	stipe 9.8	cap 61.0	stipe 14.0	cap 85.5	stipe 22.5

The content of urea in the fungus tissues of formatted fruit body on IV, VI and IX days is rapidly increased opposite to the preliminary three phases (mycelium, primordia, non-formatted fruit body), reaching 85.5 *μM*/g in fungus cap and 22.5 *μM*/g in the stipe. Summing up the data on the positive dynamics of arginase activity and urea accumulation concomitant to fungus development and growth, we can assume that it is connected with the necessity to provide the corresponding level of osmotic pressure in hymenium, which will create favorable conditions for sporulation.

According to literature data, OCT is able to function regardless of the first enzyme of ornithine cycle – carbamoyl phosphate synthesis [9], therefore, we cannot confirm the availability of urea synthesis in ornithine cycle in our research object without carbamoyl phosphate synthesis activity detection in *Pleurotus ostreatus* fungus. However, based on literature data on ornithine cycle availability in fungi, as well as high arginase activity, observable in our object, and the positive dynamics of urea concomitant to growth, we can assume the availability of ornithine cycle in *Pleurotus ostreatus* fungus.

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