The effect of citrates and sulfates of different metals on the biomass composition of medicinal mushroom *Trametes versicolor* (L.) Lloyd.

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A comparative study has been carried out on the impact of citrate and sulfate of zinc and manganese on the biomass composition of mycelium of medicinal mushroom *Trametes versicolor* 353 cultivated in a liquid media. It was demonstrated that sulfates and citrates of studied metals have different effects on the mycelial growth and some biochemical parameters. Sulfates and citrates of these metals influence to different degrees on the amount of some amino acids in the mycelium of *T. versicolor* 353. Also citrates of both metals decrease amount of cis-linoleic acid (C18:2) relative to the control medium and medium with sulfate of appropriate metals.

Key words: Trametes versicolor, citrate, sulfate, zinc, manganese, amino acids, fatty acids

АЛЬ-МААЛІ Г.А., БІСЬКО Н.А., ОСТАПЧУК А.М. (2016). **Вплив цитратів и сульфатів різних металів на склад біомаси лікарського гриба *Trametes versicolor* (L.) Lloyd.** *Чорноморськ. бот. ж.,* **12** (1): 64-71. doi:10.14255/2308-9628/16.121/6.

В статті наведені дані дослідження впливу цитратів та сульфатів марганцю та цинку на біохімічний склад біомаси цінного лікарського гриба *Trametes versicolor* 353, що зростав на рідкому живильному середовищі в умовах глибинної культури. Результати експерименту свідчать про те, що цитрати та сульфати цих металів впливають різним чином на склад біомаси *T. versicolor* 353. Встановлено, що цитрати та сульфати обох досліджених металів у різній мірі змінюють концентрацію певних амінокислот в міцелії. Наявність цитрату цинку чи марганцю у живильному середовищі зменшувало вміст цис-лінолевої кислоти в біомасі *T. versicolor* 353, порівняно з сульфатами відповідних металів та контрольним середовищем без досліджуваних елементів.

Ключові слова: Trametes versicolor, цитрат, сульфат, цинк, марганець, амінокислоти, жирні кислоти

АЛЬ-МААЛИ Г.А., БИСЬКО Н.А., ОСТАПЧУК А.Н. (2016). **Влияние цитратов и сульфатов различных металлов на состав биомассы лекарственного гриба *Trametes versicolor* (L.) Lloyd.** *Черноморск. бот. ж.*, **12** (1): 64-71. doi:10.14255/2308-9628/16.121/6.

В статье приведены результаты сравнительного исследования влияния цитратов и сульфатов марганца и цинка на биохимический состав биомассы ценного лекарственного гриба *Trametes versicolor* 353, выращенного на жидкой питательной среде в условиях глубинной культуры. В ходе эксперимента было показано, что цитраты и сульфаты исследованных металлов имеют различное влияние на состав биомассы *T. versicolor* 353. Установлено, что цитраты и сульфаты обоих металлов в разной степени воздействовали на содержание ряда аминокислот в мицелии *T. versicolor* 353. Наличие цитрата цинка или марганца в среде уменьшало содержание цис-линолевой кислоты в биомассе *T. versicolor* 353 в сравнении с сульфатами соответствующих металлов и контрольной средой, не содержащей исследованный элемент.

Ключевые слова: Trametes versicolor, цитрат, сульфат, цинк, марганец, аминокислоты, жирные кислоты

Introduction

Higher basidiomycetes of the genus *Trametes* have numerous medicinal properties including antitumor, antibiotic, hepatoprotective and anti-virus properties [CAI…, 2010; MAEHARA…, 2012; PATEL, 2012]. The long history of use of these fungi in traditional oriental medicine is to treat inflammation of the upper respiratory tract, urinary system and digestive channel. It confirms their safety for human health. There are known products of medical purpose of genus of *Trametes* consisting of purified polysaccharide fractions and proteins [STANDISH…, 2008; ZONG…, 2012; KUAN…, 2013]. Moreover, *T. versicolor* has been used as an excellent source for lignocellulose degrading enzymes, such as laccase and Mn-peroxidase.

Modern industrial cultivation of medicinal xylotrophic basidiomycetes is directed at optimization of the process of their cultivation to increase the yield of biomass and biologically active substances. Growing the mycelium of fungus on synthetic liquid nutrient media gives the opportunity to change and modify the mineral composition of the culture substance, and thereby, affecting the biomass growth and synthesis of biologically active substances. A number of authors noted that some trace elements, including zinc and manganese, have a positive effect on biosynthesis of intracellular and extracellular polysaccharides [ZOU, 2005; XIAO…, 2006; ZHI-LING, 2009], gene expression of laccase [SODEN, DOBSEN 2001; VASINA…, 2015] and amino acids composition [ZOU, 2005] in some medicinal mushrooms. It should be noted, the special role of zinc in many key metabolic pathways including synthesis of amino acids, metabolism of RNA and DNA, signal transduction, and gene expression. Zinc is the only metal which appears in all enzyme classes [BROADLEY…, 2007]. Manganese is an important metal for mushrooms physiology, being absolutely necessary for metabolism and the antioxidant system. The classes of enzymes that have manganese cofactors are very broad, and include oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases.

As a rule, inorganic metal salts are used in the culture medium for growth of fungi mycelium. However, aforementioned inorganic salts have several disadvantages, amongst which should be mentioned their low chemical purity and lower bioavailability as compared to the organic metallic compounds. In this sense, the most prospective are the salts of carboxylic acids, including metal citrates, which are allowed for use in the food industry.

Previously, we studied the effect of citrate and sulfate of different metals on mycelial growth of *T. versicolor* 353 [AL-MAALI, 2015]. The results obtained indicate the concentration of 1 mg/L of manganese and zinc (citrate form) was optimal for mycelial biomass synthesis relative to the control medium without investigated metals. The increased the biomass was 28.9 % (medium with manganese citrate) and 36.7 % (medium with zinc citrate).

The aim of our research was to study the influence of citrate and sulfate of manganese and zinc on the biomass composition of medicinal mushroom *Trametes versicolor*.

Materials and Methods

Strain and cultural conditions.

The studied strain of *Trametes versicolor* 353 was obtained from the Culture Collection of Mushrooms (IBK) from M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kyiv [BUCHALO…, 2011]. The selection of the strain was based on biotechnological investigations of Antonenko [ANTONENKO, 2013].

Zinc citrate and manganese citrate was obtained from Institute nanobiotechnologies and resource conservation of Ukraine, Kyiv.

In this study we used glucose-peptone-yeast extract medium (GPY) with a following composition of (g/L): glucose – 25; peptone – 3; yeast extract – 3; K_2HPO_4 – 1; KH_2PO_4 – 1; $MgSO_4 \cdot 7H_2O$ – 0,25; distilled water – 1000 ml; pH 6,5 (control medium). Mn^{2+} or Zn^{2+}

(sulfare form or citrate form) were added to control medium in concetration 1mg/L. Mycelium was grown in a submerged culture on a rotary shaker (120 rpm) at 26 °C in 250 ml Erlenmeyer flasks, containing 50 ml of liquid media. Inoculum obtained under similar conditions in 5 days. We used 5 ml of inoculum for inoculation flasks with liquid medium.

Determination of dry weight and content of total nitrogen, crude protein, lipids, total carbohydrates and ash.

The biomass was harvested after 9 days of cultivation in the liquid medium, filtered, washed, dried to a constant weight at 105 °C and weighted. Total nitrogen content (N_{total}) in the mycelium determined by Kjeldahl method, crude protein content was determined as N_{total} x 6.25 [AOAC, 1995]. The ash was obtained by the standard method [AOAC, 1995]. Lipids were extracted from undried mycelium by a modified method of Bligh and Dyer [MANIRAKIZA…, 2001]. Amount of total carbohydrates was calculated, using the following formula:

$$M_c = M_b - (M_{cp} + M_l + M_a)$$

Where M_c – weight of total carbohydrates, g/l; M_b – weight of biomass; M_{cp}– weight of crude protein, g/l; M_l – weight of lipids, g/l; M_a– weight of ash, g/l.

Amino Acid Detection Method

Amino acid composition was analyzed by high-performance liquid chromatography after derivatization with 9-fluorenylmethyloxycarbonyl chloride and o-phthalic anhydride.

Sample preparation: 0.1 g of the mycelium was placed in vial and 2 mL of 6N HCl were added. Hydrolysis was carried out for 24 hours at 110°C. 0.5 mL of hydrolyzate obtained from centrifugation was evaporated and washed by distilled water 3 times. After evaporation, the extract was dissolved with 0.5 mL of distilled water and filtrated with 0.2 μm regenerated cellulose filter membrane. Obtainment of fluorescent derivative was carried out by an automatic programed procedure before the samples were inserted in chromatography column.

The conditions for detection of amino acids were as follows: high-performance liquid chromatograph Agilent 1200 (Agilent technologies, USA); chromatography column: Zorbax AAA, 150 mm × 4,6 mm × 3 μm; Mobile phase A: 40 mM Na_2HPO_4 pH 7.8; B – acetonitrile:methanol:water (45:45:10, v/v/v); temperature of column thermostat is 40°C. Detection of derivatized amino acids was implemented using fluorescence detector. Identification of amino acids was performed by comparing the retention times with a mix of standard amino acids (Agilent 5061-3334) [HENDERSON…, 2000; JAMBOR, MOLNAR-PERL, 2009A; JAMBOR, MOLNAR-PERL, 2009B].

Fatty Acids Detection Method

The methyl ethers of fatty acids were obtained by a standard method [CHRISTIE, 1989]. The methyl ethers of fatty acids were determined by gas chromatography-mass spectrometry (GC/MS) Agilent 6890N/5973 inert. chromatography column: HP-5MS, 30m × 0.25 mm × 0.25 μm.

Chromatographic conditions: the carrier gas was helium at a flow rate of 1 mL/min. The injector was kept at 250 °C. The temperature gradient was 150–250°C, at the rate of 40C/min.

Mass spectrum conditions: ion source: electron ionization (EI); electric energy: 70 eV; chromatogram was obtained by SCAN mode in the range of 40–700 m / z. The identification of the components of the studied samples was performed using the library of mass spectra NIST 02 and standard mix of methyl esters of fatty acids (Supelco, USA). Amount of each fatty acid was calculated as a percentage of total fatty acids.

Statistical analysis

Values are mean of three independent experiments done in triplicate and are expressed as mean ± errors. Data were statistically analyzed by t test using OriginPro 8.5.1, Origin-Lab Corportion, USA. Differences between means at 5 % ($p < 0,05$) level were considered to be significant.

Results and Discussion

Biochemical composition of biomass.

Analysis of the main components of the biomass showed the following changes. Zinc and manganese in both forms (sulfate and citrate) equally affected the content of crude protein, carbohydrate and ash in the mycelium. In these trials, carbohydrate content in mycelium was reduced by approximately 4 % relative to the control medium without zinc or manganese respectively (table 1). The percentage of ash in *T. versicolor* 353 mycelium on all media with zinc or manganese increased relative to the control medium by about one-third (table 1). Also in all trials, we detected slight increment of crude protein (table 1). It is likely, this effect is due to the action of metals ions.

At the same time, the presence of manganese citrate (Mn^{2+} 1 mg / L) on the GPY medium increased the total content of lipids in the mycelium (approximately twice relative to control medium without manganese and relative to medium with manganese sulfate). While the both forms of zinc (citrate and sulfate) increased the total content of lipids in the mycelium of *T. versicolor* 353 (table 1).

So, in the case of lipids our results demonstrate, that biological activity of manganese citrate is higher than activity of manganese sulfate.

Amino acids content.

We detected 16 amino acids on the *T. versicolor* 353 biomass (fig. 1, 2).

Amino acids composition of the mycelium depends on the occurrence of zinc or manganese (sulfate or citrate form) in the medium. It is necessary to note, that citrate and sulfate form of investigated metals modified amino acid composition of the mycelium differently.

Table 1

The influence of citrate and sulfate of zinc and manganese on the biomass production and biochemical parameters of *T. versicolor* 353 on GPY medium

Таблиця 1

Вплив цитратів та сульфатів цинку та марганцю на синтез біомаси *T. versicolor* 353 та її біохімічні склад на ГПД середовищі

Parameters	Control medium	Zinc citrate	Zinc sulfate	Manganese citrate	Manganese sulfate
Biomass, g/l	4,77 ±0,21	6,52±0,03	5,79±0,23	6,15±0,23	5,05±0,32
Increment of biomass, %	0,00	36,69	21,38	28,93	5,87
Crude protein, % biomass	17,56±0,31	19,00±0,45	18,37±0,22	18,69±0,32	19,00±0,27
Total carbohydrates, % biomass	75,02	70,55	71,15	71,09	71,82
Total lipids, % biomass	1,29±0,11	2,31±0,23	2,18±0,018	2,32±0,18	1,13±0,09
Ash, % biomass	6,13±0,20	8,14±0,36	8,30±0,41	7,90±0,34	8,05±0,29

Table 2

The influence of citrate and sulfate of zinc and manganese on the amino acids content in mycelium of *T. versicolor* 353 on GPY medium

Таблиця 2

Вплив цитратів та сульфатів цинку та марганцю амінокислотний склад міцелію *T. versicolor* 353 на ГПД середовищі

Fatty acids	Control medium	Zinc citrate	Zinc sulfate	Manganese citrate	Manganese sulfate
Pentadecanoic acid (15:0)	0,97±0,24	0,79±0,14	0,73±0,20	1,0±0,24	1,95±0,28
Palmitoleic acid (C16:1)	1,32±0,23	0,47±0,07	0,39±0,03	1,73±0,26	1,8±0,32
Palmitic acid (C16:0)	22,01±1,45	20,39±1,97	20,65±1,21	19,09±1,48	19,04±2,03
Margaric acid (C17:0)	0,54±0,17	0,38±0,11	0,36±0,13	0,34±0,09	0,35±0,13
cis- Linoleic acid (C18:2)	67,27±2,56	57,99±2,1	63,79±2,44	61,64±1,7	64,15±2,02
Oleic acid (C18:1)	3,36±0,87	8,76±1,01	6,74±0,79	7,11±0,49	6,33±0,56
trans- Linoleic acid (C18:2).	0,66±0,41	5,57±0,74	3,73±0,54	4,05±0,48	3,56±0,51
Stearic acid (C18:0)	1,74±0,10	1,03±0,09	1,12±0,23	0,68±0,18	0,76±0,10

In the trial with citrate and sulfate of zinc both compounds influenced to different degrees on the amount of L-serine, L-glycine and L-lysine. Thus, the quantity of L-serine in mycelium on GPY-zinc sulfate medium was increased by 47.9 % and on GPY-zinc citrate medium by 55.8 % relative to the control medium (fig.1). The quantity of L-glycine in mycelium was increased by 23.5 % (GPY-zinc citrate medium) and by 13.2 % (GPY-zinc sulfate medium) relative to the control medium (fig. 1). Also concentration of L-lysine in mycelium of *T. versicolor* 353 was reduced by 19.4 % on GPY-zinc citrate medium and 12.4 % GPY-zinc sulfate medium in relationship to the control medium (Fig. 1). In the same time, citrate and sulfate forms equally decreased the amount of L-aspartic acid, L-histidine, L-threonine, L-arginine, L-tyrosine, L-leucine respective to the control medium (fig. 1). And the amount of L-proline and L-methionine was equally grown up in both cases (zinc sulfate and zinc citrate).

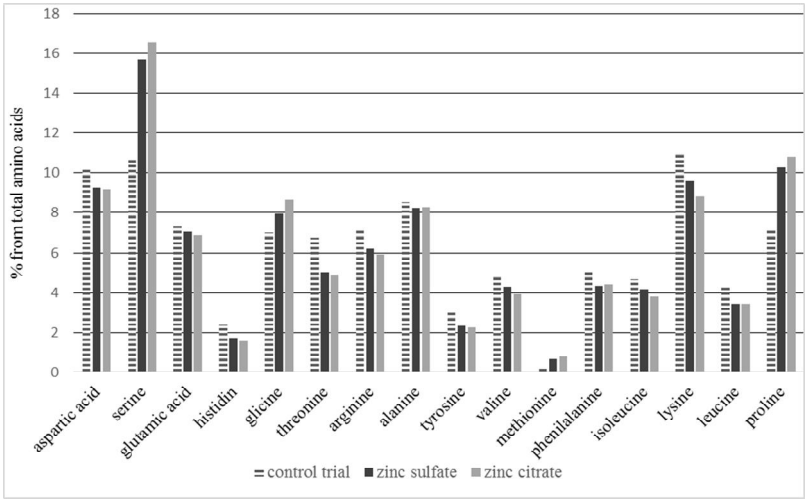

Fig. 1. Amino acids composition of *T. versicolor 353* on GPY medium with zinc sulfate or zinc citrate.

Рис. 1. Амінокислотний склад біомаси *T. versicolor 353* на ГПД середовищі з сульфатом цинку або цитратом цинку.

In the trial with citrate and sulfate of manganese both compounds influenced to different degrees on the amount of L-serine, L-lysine, L-proline. Thus manganese sulfate and manganese citrate increased the quantity of L-serine in mycelium of *T. versicolor* 353 by 57.3 % and 69.6 % respectively relative to the control medium (fig. 2). The quantity of L-proline in mycelium was increased by 41.9 % (GPY- manganese citrate medium) and by 32.3 % (GPY- manganese sulfate medium) relative to the control medium (fig. 2). In the same time, concentration of L-lysine was reduced by 22.4 % (GPY- manganese citrate medium) and 16.8 % (GPY- manganese sulfate medium) in relationship to the control medium. Only manganese sulfate have the positive effect on the amount of L-methionine (fig. 2). Thus, the content of L- methionine in the mycelium on GPY- manganese sulfate medium was increased 5.4 times as opposed to zinc citrate, which did not affect the amount of L- methionine. In the same time, both forms of manganese have the same effect on the amount of L-glycine, L-histidine, L-threonine, L-arginine, L-tyrosine, L-leucine respective to the control medium (fig. 2).

But the amount of total essential amino acids in the mycelium was decreased on all trial by 5-8 % respective to the control medium.

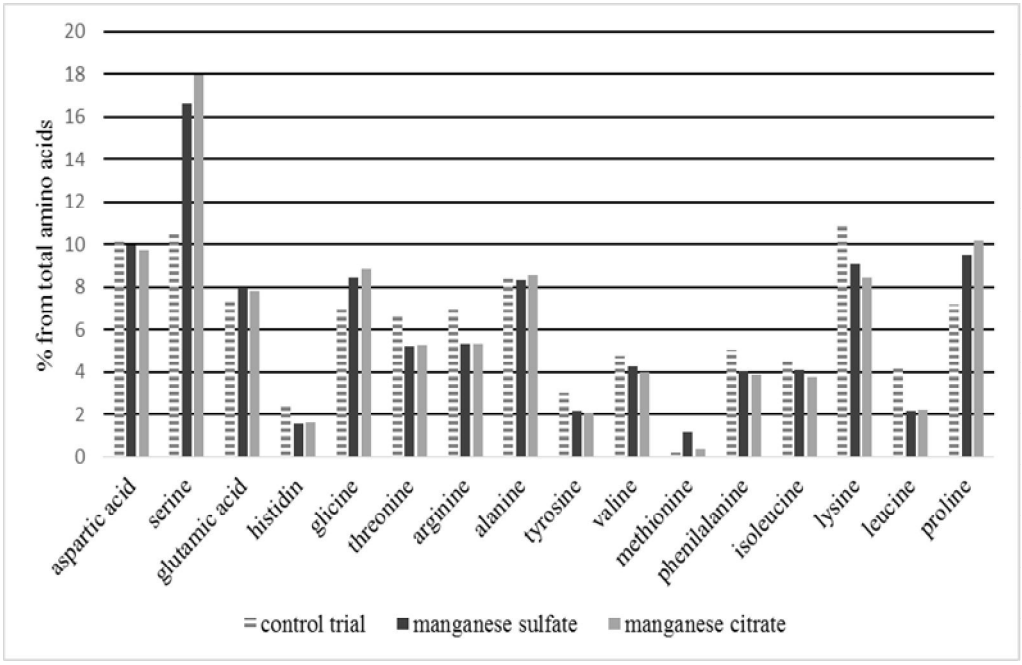

Figure 2. Amino acids composition of *T. versicolor 353* on GPY medium with manganese sulfate or manganese citrate.

Рис. 2. Амінокислотний склад біомаси *T. versicolor 353* на ГПД середовищі з сульфатом марганцю або цитратом марганцю.

Fatty acids content.

We detected 9 fatty acids on the *T. versicolor* 353 biomass: myristic acid (C14:0), pentadecanoic acid (15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), margaric acid (C17:0), stearic acid (C18:0), oleic acid (C18:1), cis- and trans- form of linoleic acid (C18:2).

Both forms of manganese and zinc increased the amount of oleic acid (C18:1) and trans-linoleic acid (C18:2) and decreased the amount of stearic acid (C18:0). In the same time, in both cases, as with zinc citrate and manganese sulfate, we observed significant reduction of quantity of cis-linoleic acid (C18:2) relative to the control medium and medium with sulfate of manganese or zinc respectively. But the total sum of unsaturated fatty acids wasn't changed (table 2).

So, the presence of manganese citrate, zinc citrate or zinc sulfate in media promotes to increase the content of total lipids approximately twice and modifies the fatty acids composition. In that time, manganese sulfate has no effect on the amount of total lipids, but modifies the fatty acids composition, too.

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Conclusion

Our results demonstrate, that biological activity of manganese citrate, in the case of accumulation of lipids, is higher than activity of manganese sulfate. Sulfates and citrates of investigated metals influence to different degrees on the amount of some amino acids in the mycelium of *T. versicolor* 353. Both citrates of zinc and manganese, more efficiently increases the quantity of L-serine and more substantially reduces the amount of L-lysine in mycelium relative to sulfate of these metals. Also citrates of both metals decrease amount of

cis-linoleic acid (C18:2) relative to the control medium and media with sulfate of appropriate metals.

Thus, our investigations show that sulfates and citrates of studied metals have different effects on some biochemical parameters of biomass *T. versicolor* 353.

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