Հայաստանի Գիտությունների Ազգային Ակադեմիա
Национальная Академия Наук Армении
National Academy of Sciences of Armenia

Հայաստանի Կենսաբանական Հանդես
Биологический Журнал Армении
Biological Journal of Armenia

• *Փորձարարական և տեսական հոդվածներ* •*Экспериментальные и теоретические статьи*•
•*Experimental and theoretical articles*•

Biolog. Journal of Armenia, 1 (68), 2016

MODIFICATION OF GROWTH CONDITIONS BY MM-WAVES OF WOOD-DECAYING MUSHROOM'S CULTURES

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Basidial macromycetes are not only value food, but can be used as source of such biological active compounds as the genistein, β -glucans, glioxal–oxidase et al. In this work we used different frequencies of extremely high frequency of electromagnetic irradiation (EHF EMI) with the aim of obtaining mushroom cultures with increased fermentative activity by the modulation of its growth conditions during growth on the peptone media. We investigated the influence of the non-thermal extremely high frequency electromagnetic waves in the interval of 45-53 GHz on β-glucosidase activities of two species of wood-decaying mushroom.

In this study we examined the most popular edible wood-decaying mushroom *Pleurotus ostreatus,* which is wide-spread in the forests and commercial mushroom *Lentinula edodes*, under influence of such an abiotic factor as the extremely high frequency waves in the interval of 45 GHz – 53 GHz during 20 and 40 min on the 7th day of mycelial culture's growth. After the treatment of cultures we continued their growth and on the 3th day we examined the influence of these waves on fermentative activity of mycelial extracts. The some conditions of such treatment led to significant rising of β-glucosidase activities in the extracts of mycelial cultures.

Basidiomycetes – betta-glucosidase – Lentinula edodes – mm-waves – Pleurotus ostreatus

Բազիդիալ մակրոմիցետները օժտված են ոչ միայն բարձր սննդային հատկություններով, այլ նաև ծառայում են որպես արժեքավոր հումք այնպիսի կենսաակտիվ միացությունների համար, ինչպիսիք են գինեստին β-գլյուկանները, գլիոկսալ-օկսիդազը և այլն: Աշխատանքում օգտագործվել են գերբարձր հաճախությունների էլեկտրամագնիսական ճառագայթների տարբեր հաճախություններ՝ նպատակ ունենալով ստանալ սնկերի կուլտուրաներ (մշակույթներ), օժտված ավելի բարձր ֆերմենտային ակտիվությամբ, փոփոխելով միցելիումի աճի ընթացքում պեպտոնային միջավայրում պայմանները: Ուսումնասիրված է ոչ ջերմային գերբարձր հաճախությամբ էլեկտրամագնիսական ճառագայթման ազդեցությունը 45 ԳՀց –53 ԳՀց միջակայքում երկու տեսակի փայտ քայքայող սնկերի β-գլյուկոզիդազ ֆերմենտի ակտիվության վրա:

Աշխատանքում հետազոտված են ուտելի փայտ քայքայող սնկեր՝ *Pleurotus ostreatus,* լայնորեն տարածված լայնատերև անտառներում, և առևտրային սունկ *Lentinula edodes*, այնպիսի աբիոտիկ գործոնի ներքո, ինչպիսիք են գերբարձր հաճախության էլեկտրամագնիսական ճառագայթները 45 ԳՀց – 53 ԳՀց միջակայքում՝ 20 րոպե և 40 րոպե տևողությամբ միցելիումի աճի 7-րդ օրը: Միցելիումի, մշակումից հետո 3-րդ օրը հետազոտվել է ԳԲՀ ԷՄՃ ազդեցությունը միցելիալ լուծամզուկների ֆերմենտային ակտիվությունը: Ցույց է տրվել, որ մշակման որոշակի պայմանները խթանում են β–գլյուկոզիդազ ֆերմենտի շոշափելի աճ միցելիալ լուծամզուկներում:

Բազիդիոմիցետներ – բետտա-գլյուկոզիդազ – ՄՄ-ալիքներ – Lentinula edodes – Pleurotus ostreatus

Базидиальные макромицеты обладают не только большой питательной ценностью, но служат также источником таких биологически активных соединений как гинестин, β-глюканы, глиоксаль-оксидазы и др. В работе были использованы различные частоты крайне высоких частот электромагнитного излучения с целью получения культуры грибов с повышенной активностью ферментов, модулируя условия выращивания в течении роста мицелия на пептонной среде. Исследовано воздействие нетепловых крайне высокочас-тотных электромагнитных волн в интервале 45-53 ГГц на активность β-глюкозидаз двух видов дереворазрушающих грибов.

В данной работе исследованы съедобные дереворазрушающие грибы: *Pleurotus ostreatus*, широко распространенный в лиственных лесах, и коммерческий гриб *Lentinula edodes* под воздействием такого абиотического фактора как электромагнитные волны крайне высоких частот в интервале 45 ГГц - 53 ГГц в течение 20 и 40 мин на 7 день роста мицелиальной культуры. После обработки культуры мы продолжали культивирование и на 3 день после обработки исследовали воздействие этих волн на ферментативную активность мицелиальных экстрактов. Некоторые экспозиции такой обработки приводили к значительному возрастанию активности β-глюкозидаз в экстрактах мицелиальных культур.

Базидиомицеты – бета-глюкозидаза – ММ-волны – Lentinula edodes – Pleurotus ostreatus

Cellulose is the main polymeric component of the plant cell wall, the most abundant polysaccharide on Earth, and an important renewable resource. Basidiomycetous fungi belong to its most potent degraders because many species grow on dead wood or litter, in environment rich in cellulose. Fungal cellulolytic systems differ from the complex cellulolytic systems of bacteria. For the degradation of cellulose, basidiomycetes utilize a set of hydrolytic enzymes typically composed of endoglucanase, cellobiohydrolase and β-glucosidase [3]. β -glucoside glucohydrolase commonly called β-glucosidase catalyzes the hydrolysis of alkyl and aryl- β-glucosides such as diglucoside and oligosaccharides. These enzymes are widely used in various biotechnological processes, including the production of fuel ethanol from cellulosic agricultural residues [4] and used for the synthesis of β-glycosides. In the production of flavors in food, β-glucosidase is also a key enzyme in the enzymatic release of aromatics glucosidic precursors present in fruits and enzymatic products. On the contrary, many aromatic organic compounds such as monoterpenols, C-13 norisoprenoids, shikimate-derived compounds accumulate in fruits as flavorless precursors linked to mono- or diglycosides and requires enzymatic or acidic hydrolysis for the liberation of their flavors [11]. Finally, β-glucosidase activity may also improve the organoleptic properties of citrus fruit juices, in which the content of the bitterness caused by glucosidic compounds, for example of naringin for hydrolysis of which requires the sequential processing by β-rhamnosidase and β - glucosidase [10].

It has been established that certain monoterpenoly grapes (linalool, geraniol, nerol, citronellol, β -terpineol and linalool oxide) connected to diglicosids such as 6-O-β-L-rhamnopyranosyl, 6-O-β-L-arabinofuranosil and 6-O-β-D-apiofuranozil-β-D-glucosides, which define the quality of the wine flavoring [5]. For the enzymatic hydrolysis of these compounds are required sequence of reactions cleave 1…6 bond, and then, aromatic compounds are released from the monoglucosides by influence of β-glucosidase [9]. In contrast to acid hydrolysis, enzymatic hydrolysis is very efficient and does not lead to a modification of aromatic character. However, grape and yeast glucosidase show up least activity on glucosides- monoterpenes in the wine industry, and most aromatic fractions predecessors left untreated. Addition of exogenous β -glucosidase during or after the fermentation is more effective to improve hydrolysis glyucose-binding aromatic components to improve the flavor of the wine. Addition of exogenous β-glucosidase during or after fermentation

proved to be more effective for improving hydrolysis of the glucose-binding of aromatic components in order to improve the flavor the wine. Ideal β-glucosidase should operate and be sustained at low pH values (around pH 2.5 to 3.8) and should to keep activity at high glucose concentrations (10-20%) and in the presence of 10-15% ethanol. However, most of the bacterial β-glucosidase are very sensitive to inhibition by glucose and glucono-β-lactone, and to compounds produced by the grapes-striking mushrooms [9].

The aim of this study was to identify and obtain the optimal exposure of processing of edible mushrooms` cultures by mm-waves for increasing the activity of the enzyme β-glucosidase in mycelial extracts of mushrooms. Such cultures with increased activity of the enzyme will be a good source for obtaining an activity and stability enzyme for further use in the food and wine processing.

Materials and methods. *Treatment of mushrooms` mycelial cultures.* Culture of a mushrooms treated with low intensively coherent extremely high frequency of electromagnetic irradiation (EHF EMI) duration of 20 and 40min on the 7^{th} day of growth mycelial fungal cultures of *Pleurotus ostreatus* and *Lentinula edodes*. The source of irradiation served as a high-frequency signal generator G4-141 EMI (Russia) in the range 45 GHz - 53 GHz. Irradiation was carried out during 20 and 40 min with irradiation power 0.64 mW/cm^2. Culture continued to grow in the thermostat after a single exposure. On the 3rd (10 days mycelium) days after irradiation of cultures obtained intracellular extracts of the fungus for further their studies. As a control used unexposed culture extract of the mushrooms.

Obtaining mycelia extracts. For intracellular extract mycelium gently scraped from the agar surface in a Petri dish, weighed, and triturated in a pre-cooled mortar (to prevent inactivation of peroxidases) with glass beads by adding 0.15 M Tris-HCl buffer, pH = 8.0 [7]. The buffer was added at the rate of 0.2 ml buffer per 100 mg of mycelium. The extract was separated by centrifugation on the cold for 20 min at a speed of 18,000 rev / min in a centrifuge Mechanika Precyzyjna (Poland). For further analysis of the protein amount and enzymatic activity of the mycelial extracts was used supernatant.

Determination of activity of β-glucosidase. The enzyme activity of mycelial extracts of cultures was determined in a reaction mixture, containing 1 ml of mycelia extract, 2 ml 5 mM nitrocellulose prepared in 100 mM acetate buffer pH = 5.0. The reaction was initiated by incubating the homogenate for 15 min at 50^0C and stopped by cooling on ice or by addition of 1 M Na_2CO_3 [9]. The reaction was followed and estimate absorption at A = 400 nm on spectrophotometer.

Statistical analysis of the data. The graphs show the average arithmetic value and a standard deviation of four biological replicates of each. In processing the data by the method of the t-test of Student's determined confidence intervals for the average impact of EHF EMR at 5% significance level.

Results and Discussion. The need to find β-glucosidase enzymes with preferred properties has led many researchers to find new suitable sources. Recently it has been shown that extracellular glucose-stable and pH-resistant β-glucosidase activity can be produced by strains of Aspergillus [9]. However, the enzyme having interest is consist only a minor fraction of the entire β-glucosidase activity, and a large fraction of highly sensitive to inhibition by glucose**.** As shown Aspergillus orizae has proved effective producer of the minor form of glucose-tolerant β-glucosidase enzyme when grown on quercetin, phenolic flavonoid found in plant cell walls [9]. Researches and searching for other sources of plant and mushroom β-glucosidase, tolerant to glucose inhibition, continues. Perhaps modifying the growth conditions also can lead to producing resistant forms of the enzyme.

The need for more suitable enzymes had led us and other workers to search for novel β-glucosidases with desired properties. In view of the foregoing by the facts it was initiated search for suitable fungal cultures that rich in β-glucosidase enzyme by modulating the growth conditions to achieve increasing of enzyme activity. It allows using such crops as a raw material for producing an enzyme required in many areas of the food industry. Our selection was focused on wood-destroying fungi, because they contain two major enzymatic system-cellulolytic and ligninolytic. Basidiomycetes are the most potent destructors of

cellulose, since many of them grow on dead wood – in an environment rich in dietary fiber. Classic range of mushroom cellulolytic enzymes consists of endo- and exolytic enzymes acting on cellulose. Obtaining cellobiose further processed by β-glucosidases till produce glucose, is illustrated in the scheme (fig. 1).

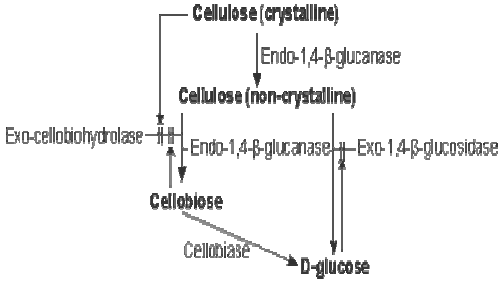

Fig. 1. Outline of the relationship between the enzyme activities in the hydrolysis of cellulose. || represents inhibitory effects. Endo-1,4-b-glucanase is the rate-controlling activity and may consist of a mixture of enzymes acting on cellulose of different degrees of crystallinity. It acts synergistically with both exo-1,4-b-glucosidase and exocellobiohydrolase. Exo-1,4-b-glucosidase is a product-inhibited enzyme. Exocellobiohydrolase is product inhibited and additionally appears to be inactivated on binding to the surface of crystalline cellulose.

The enzyme β-glucosidase was detected by us in the wood decaying fungi *Pleurotus ostreatus*, which is also serve as an important taxonomic index in determining the systematic affiliation of wood Basidiomycetous fungi [5, 8].

We used different frequencies EHF EMI to modulate the growth conditions of the oyster mushroom and shiitake cultures in order to obtain optimal high enzymatic activities of the mycelial extracts in response to stressful impact of external abiotic factors. In our experiments we were obtained oyster mushroom culture with a high activity of the β-glucosidase enzyme by changes in growth conditions. Thus, in the diagram presented on fig. 2, shows that at frequencies of 49 GHz treatment during 20 min the β-glucosidase activity is maximized – increasing by 2 times relative to the control culture. To the increase of the enzyme activity also results treatment at frequency of 51.8 GHz, with an exposure of 20 min (1.2-fold) and a frequency of 46 GHz, 40 min (1.5-fold) relative to the control. The smallest value of β-glucosidase activity reaches at 53 GHz for 20 min and 45 GHz for 40 min, among all of the studied exposures.

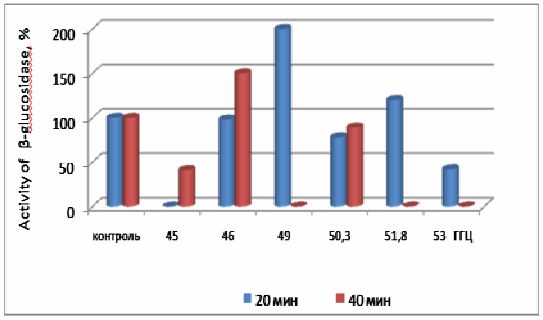

Fig. 2. Dependence of -glucosidase activity in mycelial extracts of *P.ostreatus* from frequency and duration of mm-waves treatment.

In addition, all biological systems producing hydroxyl radicals based on cellobiose dehydrogenase, quinine redox cycling or glycopeptides–based Fenton reaction are involved in the degradation of several plant cell wall components, including cellulose. The complete cellulolytic complex used by a single fungal species is typically composed of more than one of the above mechanisms that contribute to the utilization of cellulose as a source of carbon or regulation of cellulose degradation differs among wood-rotting, litter-decomposing, mycorrhizal or plant pathogenic fungi and yeast due to the different roles of cellulose degradation in the physiology and ecology of the individual groups [11].

We examine influence of mm-waves on β-glucosidase activity and on commercial Chinese medicinal mushroom *Lentinula edodes,* or better known as shiitake. Data on the changes in β-glucosidase activity in extracts of shiitake culture are presented in fig. 3. Studies have demonstrated positive effects on the culture of the fungus at mm-wave frequencies of 53 GHz for 20 min by increasing the activity of this enzyme is almost 2 times. Increasing exposure time to 40 min resulted in more sustained beneficial effect: at frequencies of 45 GHz, 49 GHz and 53 GHz was observed an increase in enzyme activity is more than two times. Processing mm-wave frequencies at 50.3 GHz and 51.8 GHz for 40 min also led to an increase in enzyme activity of about 1.5 and 1.2 times, respectively.

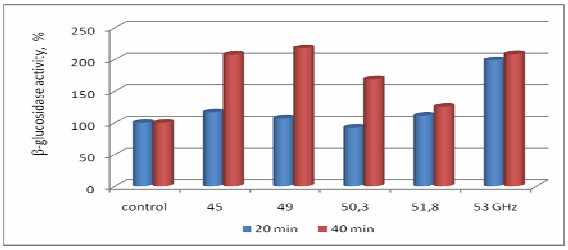

Fig. 3. Dependence of -glucosidase activity in mycelial extract *Lentinula edodes* from frequency and duration of mm-waves treatment.

It has been previously shown effects of mm-wave in the same range of frequencies on the peroxidase activity of mycelia extracts from three types of wood-decaying mushrooms, as the peroxidase enzyme is part of the ligninolytic system of fungi. The most sensitive effects have been obtained en treated by mm-waves on culture of wood-destroying fungi *P.ostreatus* [2]. Processing cultures of the fungus is not only increased metabolic and fermentative activities, but also enhanced the anti-inflammatory activity of studed extracts [1].

Researches and searching for other sources of plant and mushroom β-glucosidase, tolerant to glucose inhibition are continuing by many researchers. Perhaps such modifications of conditions during growth of wood-destroying mushrooms` culture can also be a good source for resistant forms of the enzyme. However, these studies need to continue to identify the optimum conditions of treatment by mm-waves to produce highly tolerant to inhibition by glucose β-glucosidase.

Thus, the obtained changes in the metabolic activity of the cultures we studied wood-destroying fungi under the influence of external abiotic factors that are expressed as a change in biomass and the increase of enzymatic activity of two studied fermentative complexes – ligninolytic and a cellulolytic.

The data of our study can be used for resolving the problem of the bioconversion - for utilization of hard degradable polymers of the wood cell walls, and as an enzyme-rich raw materials for use in the food industry and pharmaceutics [1, 2].

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Received on 18.01.2016