ЕРЕВАНСКИЙ ГОСУДАРСТВЕННЫЙ УНИВЕРСИТЕТ
YEREVAN STATE UNIVERSITY

СТУДЕНЧЕСКОЕ НАУЧНОЕ ОБЩЕСТВО
STUDENT SCIENTIFIC SOCIETY

ISSN 1829-4367

СБОРНИК НАУЧНЫХ СТАТЕЙ СНО ЕГУ

МАТЕРИАЛЫ ЕЖЕГОДНОЙ НАУЧНОЙ СЕССИИ 2016 ГОДА

COLLECTION OF SCIENTIFIC ARTICLES OF YSU SSS

PROCEEDINGS OF THE ANNUAL SCIENTIFIC SESSION OF 2016

1.5 (22)

Естественные и физико-математические науки

Natural and Physical-Mathematical Sciences

ЕРЕВАН - YEREVAN
ИЗДАТЕЛЬСТВО ЕГУ - YSU PRESS
2017

ԵՐԵՎԱՆԻ ՊԵՏԱԿԱՆ ՀԱՄԱԼՍԱՐԱՆ
ՈՒՍԱՆՈՂԱԿԱՆ ԳԻՏԱԿԱՆ ԸՆԿԵՐՈՒԹՅՈՒՆ

ISSN 1829-4367

ԵՊՀ ՈՒԳԸ ԳԻՏԱԿԱՆ ՀՈԴՎԱԾՆԵՐԻ ԺՈՂՈՎԱԾՈՒ

2016 Թ. ՏԱՐԵԿԱՆ ԳԻՏԱԿԱՆ ՆՍՏԱՇՐՋԱՆԻ ՆՅՈՒԹԵՐ

1.5 (22)

Բնական և ֆիզիկամաթեմատիկական գիտություններ

ԵՐԵՎԱՆ
ԵՊՀ ՀՐԱՏԱՐԱԿՉՈՒԹՅՈՒՆ
2017

Հրատարակվում է ԵՊՀ գիտական խորհրդի որոշմամբ
Издается по решению Ученого совета ЕГУ
Published by the resolution of the Academic Council of YSU

Հրատարակիչ՝ ԵՊՀ հրատարակչություն
Հասցե՝ ՀՀ, ք. Երևան, Ալ. Մանուկյան 1, (+374 10) 55 55 70, publishing@ysu.am

Հրատարակության նախապատրաստող ստորաբաժանում՝ ԵՊՀ ուսանողական գիտական ընկերություն
Հասցե՝ ՀՀ, ք. Երևան, Ալ. Մանուկյան 1, (+374 60) 71 01 94,
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COMPARATIVE INVESTIGATION OF WATER-SOLUBLE PROTEINS OF X-RADIATED YEASTS *CANDIDA GUILLIERMONDII* NP-4 BY ELECTROPHORESIS

Proteins are the most important biopolymers of each organism. Taking into account that the requirement of proteins is increasing day after day, in nowadays the investigation of metabolism, in particular the yeast proteins metabolism, has an important role. From this point of view the scientific interest has the study of proteins fractions of alimentary yeasts. Some literary data have shown that yeast proteins are considered as full-fledged proteins, they have a large amount of essential amino acids [1].

Nowadays, connecting with the development of technical opportunities, the increase of risks of living organisms to expose to ionizing radiation takes place. In different extreme conditions, including thermal shock, X-radiation etc., organisms are adapted to survive [2]. Certain biochemical changes occur in organisms, particularly there are very different changes in proteins and DNA. In this point of view the great scientific interest has the synthesis of protective proteins, called Heat shock proteins (HSPs). Stress or heat shock proteins (HSPs) are the most conserved proteins present in both prokaryotes and eukaryotes [3].

Proteins are charged molecules, and it is caused by the radicals of amino acids which are on a protein molecule. So proteins migrate under the influence of electric field. By this point of view the very sensitive method of protein analysis is developed, the electrophoresis. The method of electrophoresis gives the possibility to separate the mixtures of proteins by the size (molecular weight), cooperative charge, and structure [4, 5]. This method can provide information about the molecular weights and charges of proteins, the subunit structures of proteins, and the purity of a particular protein preparation. The most common use of gel electrophoresis is the qualitative analysis of complex mixtures of proteins. The technique provides the highest resolution of all methods available for separating proteins. Polypeptides differing in molecular weight by as little as a few hundreds of Daltons and proteins differing by less than 0.1 pH unit in their isoelectric points are routinely resolved in gels [6]

This work is applied to the comparative study of water-soluble proteins of X-irradiated and repaired yeasts. This scientific research is very actual. It is a part of

comprehensive research applied to study the metabolic changes in yeasts under influence of ionizing radiation and post-radiation repair.

The results of study will help to explain the high radio resistance of yeasts *Candida* and to work out the molecular mechanisms of humans and animals protection from radiation.

The object and methods of investigation. The object of investigations were yeasts *Candida guilliermondii* NP-4, which were received from Moscow Institute of protein synthesis. Yeasts were kept in 2-4 ^{0}C degree in refrigerator, on the plates with 2 % wort agar. Before experiment yeast cells were reseeded and incubated in thermostate, for 48 hours, at 30 ^{0}C.

Obtaning the yeast biomass. *C. guilliermondii* yeasts were grown in liquid synthetic medium, which contained 0.2 mM $NH_4H_2PO_4$, 0.5 mM $(NH_4)_2HPO_4$, 0.6 mM K_2SO_4, 0.8 mM $MgSO_4$, 100 mM D-glucose (pH 5.5). As growth factor was used biotin (vitamin H) by concentration of $0{,}8 \cdot 10^{-6}$ g/l. The incubation of yeasts was realized on the shaker (200-250 rpm), during 24 hours, at 30 ^{0}C, under 4000 Lux of light. Then the yeast biomass was separated from cultural medium by centrifugation (3500 g, 10 min) and was twice washed with distilled water. The yeast biomass quantity was determined as a weight of dunk biomass by weighing on a scale or by spectrophotometer (Genesis 10S UV-VIS, λ=590 nm).

X-irradiation of yeast cells. *C. guilliermondii* NP-4 yeasts were exposed to X-radiation on X- machine Dron 3 (Russia) by dose 30kR (Cu X-tube, U=25 KkV, I=15 mA, t=20 min, λ=$1.54 \cdot 10^{-8}$ nm). After that a part of irradiated yeast biomass was incubated in the same liquid medium as native cells for 24 hours. The biomasses of radiated and repaired yeasts were separated from cultural medium as was described above for non-radiated yeasts.

Separation of water soluble proteins. The yeast cell walls frozen in -10 ^{0}C have broken by French press. The homogenates were mixed with distilled water on magnetic shaker, during 20 minutes, by temperature 0 ^{0}C. Then the obtained extracts were centrifuged by 15000 g, during 15 min. The supernatants were collected as water soluble proteins fractions.

Definition of protein amount. Amount of proteins in non-radiated, radiated and repaired yeast extracts were defined by Lowry method [7].

The investigation of water soluble proteins by vertical agar electrophoresis. The investigation of water soluble proteins of yeasts *C. guilliermondii* was realized by vertical polyacrylamide gel-electrophoresis. The electrophoresis was performed in the 14 % of stacking gel and 7.7 % of resolving gel. The electrophoresis implemented in the tris-HCl buffer: in stacking gel the pH value was 6.7 and in resolving gel – 8.9. The electrophoresis was realized in tris-glicin buffer, under conditions of U=100-120 V. As electrophoretic marker the bromfenol blue was used. The electrophoregramms were visualized using

Coomassie Brilliant Blue R-250. It is an anionic dye, which non-specifically binds to proteins. Proteins in the gel were fixed by acetic acid and simultaneously stained. The excess dye incorporated into the gel was removed by destining with the same solution without the dye. The proteins were detected as blue bands on a clear background.

The obtained data and their discussion:

The two most important physical properties of proteins are their electrophoretic mobility's and their isoelectric points. The electrophoretic mobility of a protein depends on its charge, size, and shape, whereas its isoelectric point depends only on its net overall charge. The rate of migration of a protein per unit of field strength is called its "electrophoretic mobility". Separations between proteins result from differences in their electrophoretic mobility's. It has been shown that in free solution the electrophoretic mobility of a protein is a function of the ratio of its charge to its frictional coefficient (shape). Electrophoretic mobility's are influenced by some factors, such as pH of medium. Proteins are amphoteric molecules, and they can carry different charges depending on the pH of their local environment. For every protein there is a specific pH value at which its net charge is zero. This pH is called the "isoelectric point", or *pI*. This pH obviously affects the mobility's of proteins and the direction of migration [6].

During electrophoresis, proteins move through the pores of a gel. From a macroscopic point of view, migrating proteins segregate into discrete regions, or zones, corresponding to their individual gel-mediated mobility's.

In our work the 7.7 % polyacrylamide gel was used. The water soluble protein samples from non-radiated, X-radiated and repaired yeasts were used for investigations. Each electrophoretic well was loaded with 20 μl (1 on the electrophoregramm below) or 30 μl (2 on the electrophoregramm) of samples. The protein amounts of these samples are shown in table 1.

Table 1.

Amount of proteins in *C. guilliermondii* yeast extracts and in electrophoretic wells

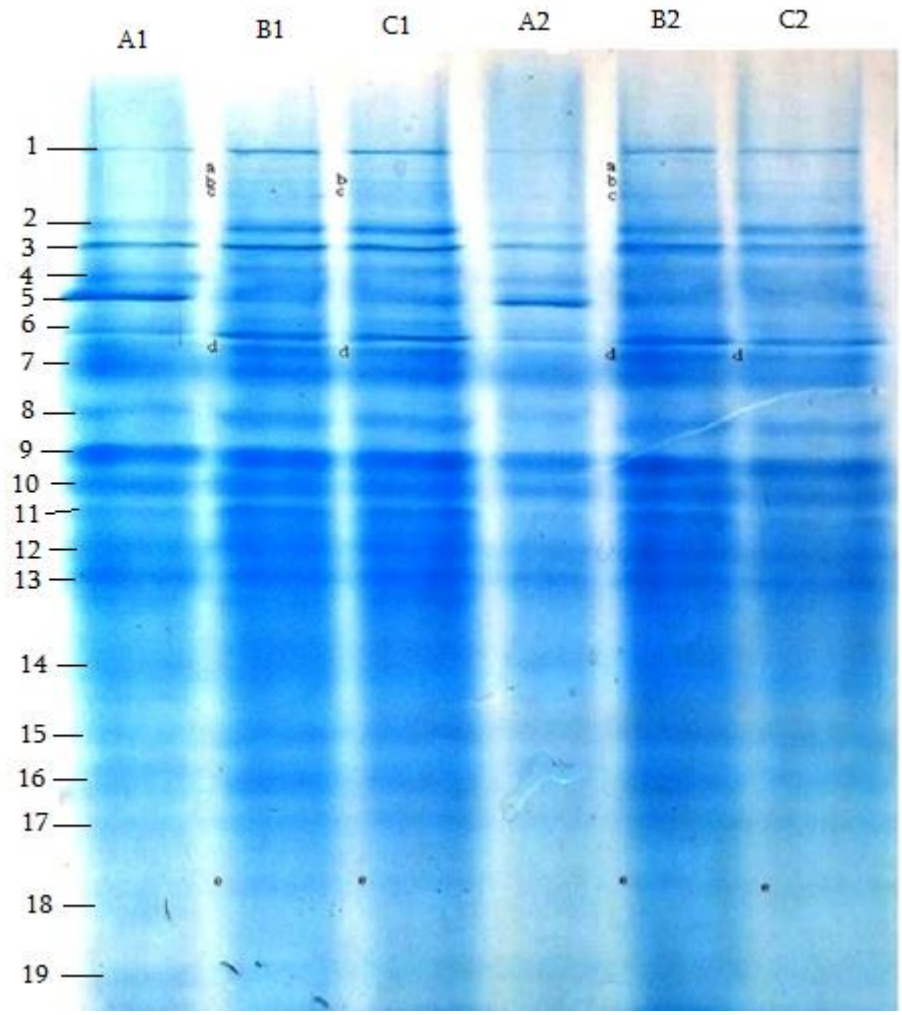
	Protein amount (mg)	Amount of proteins in wells (μg)	
		1 on the electrophoregramm	2 on the electrophoregramm
Non-radiated yeasts (A on the electrophoregramm)	365.4 ± 24.0	24.3 ± 2.0	36.5 ± 2.8
X-radiated yeasts (B on the electrophoregramm)	348.2± 27.0	25.0 ± 2.1	37.5 ±2.9
Repaired yeasts (C on the electrophoregramm)	385.6± 3.2	23.4 ± 2.3	35.0 ± 3.1

As the obtained electrophoregramm has shown (pic.1) in non-radiated yeasts water soluble protein fraction (A1, A2) there are 19 bands of proteins with different mobility (1-19 on the electrophoregramm).

For radiated yeasts proteins (B1, B2) on the electrophoregramm there are 24 bands, so in comparison with non-radiated yeasts in case of X-radiated cells there are 5 additional protein bands (a,b,c,d,e).

In repaired yeasts water soluble proteins electrophoregramm we can see 23 bands, and in comparison with non-radiated yeasts there are only 4 additional bands (b,c,d,e). So, in comparison with X-radiated yeasts, in repaired cells the “a” band of additional proteins is absent.

Summarizing obtained by us data’s of water soluble protein separation from non-radiated, X-radiated and repaired yeasts, we can conclude, that in X-radiated and repaired yeasts there is obvious the new protein synthesis (bands b, c, d, e), which perhaps carry out certain protective functions. These proteins can be members of Heat Shock proteins family, which were synthesized in yeast cells in reply to radiation stress. What about the band “a”, which is present only in X-radiated yeasts, we can suppose, that this protein has a protective function during the radiation of cells and lose it after exposition of cells to X-radiation. For final conclusion about functions and some properties of these new synthesized proteins subfractions and their final identification the further experiments are required.



Pic.1. The electrophoregramm of *C. guilliermondii* NP-4 yeasts water soluble proteins: A1, A2 - non-radiated yeasts, B1, B2 – X-radiated yeasts, C1, C2 – repaired yeasts (The explanations are in the text).

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Գայանե Պետրոսյան, Սյուզան Մարության, Լիլիթ Արշակյան, Սեդա Մարության

ՌԵՆՏԳԵՆՅԱՆ ՃԱՌԱԳԱՅԹՄԱՆ ԵՆԹԱՐԿՎԱԾ *CANDIDA GUILLIERMONDII* ՀՊ-4 ԽՄՈՐԱՍՆԿԵՐԻ ՋՐԱԼՈՒԾ ՍՊԻՏԱԿՈՒՑՆԵՐԻ ՀԱՄԵՄԱՏԱԿԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒՄԸ ԷԼԵԿՏՐԱՖՈՐԵԶԻ ԵՂԱՆԱԿՈՎ

Բանալի բառեր՝ խմորասնկեր, Candida guilliermondii, ջրալուծ սպիտակուցներ, ռենտգենյան ճառագայթում, վերականգնում, էլեկտրաֆորեզ

Ամփոփում

Ներկայացված աշխատանքի նպատակն է՝ իրականացնել ռենտգենյան ճառագայթման և հետճառագայթային վերականգնման ենթարկված *C.guilliermondii* ՀՊ-4 խմորասնկային բջիջների լուծամզվածքներում ջրալուծ սպիտակուցների ուսումնասիրություն ուղղահայաց ժել-էլեկտրաֆորեզի մեթոդով: Ցույց է տրվել, որ ճառագայթված և ռեպարացված խմորասնկերի սպիտակուցների էլեկտրաֆորեգրամներում առկա են նոր սինթեզված շերտեր, որոնք բացակայում են չճառագայթված խմորասնկերում: Այս սպիտակուցները կարող են լինել պաշտպանական սպիտակուցներ և, հավանաբար, պատկանում են ջերմաշոկային սպիտակուցների ընտանիքին:

Гаяне Петросян, Сюзан Марутян, Лилит Аршакян, Седа Марутян

СРАВНИТЕЛЬНЫЙ АНАЛИЗ ВОДОРАСТВОРИМЫХ БЕЛКОВ, ПОДВЕРГНУТЫХ РЕНТГЕНОВСКОМУ ОБЛУЧЕНИЮ ДРОЖЖЕЙ *CANDIDA GUILLIERMONDII* НП-4, МЕТОДОМ ЭЛЕКТРОФОРЕЗА

Ключевые слова: дрожжи, Candida guilliermondii, водорастворимые белки, рентгеновское облучение, восста, электрофорез

Аннотация

Целью настоящей статьи является исследование водорастворимых белков в экстрактах дрожжей *C.guilliermondii* НП-4 методом вертикального полиакриламидного гель-электрофореза после воздействия рентгеновского облучения и восстановления клеток. Нами было доказано, что в электрофореграммах дрожжевых белков, подвергнутых рентгеновскому облучению, после пострадиационного восстановления обнаруживаются новые белковые полосы, которые отсутствуют в необлученных дрожжях. Эти белки могут быть защитными белками и возможно принадлежат семейству белков теплошока.

Gayane Petrosyan, Syuzan Marutyan, Lilit Arshakyan, Seda Marutyan

A COMPARATIVE INVESTIGATION OF WATER-SOLUBLE PROTEINS OF X-RADIATED YEASTS *CANDIDA GUILLIERMONDII* NP-4 BY ELECTROPHORESIS

Key words: yeasts, candida guilliermondii, water soluble proteins, X-radiation, repair, electrophoresis

Summary

The aim of this work was to investigate water soluble proteins by vertical polyacrylamide gel-electrophoresis in extracts of *C. guilliermondii* NP-4 yeasts after the influence of X-rays and repair. It has been shown that in electrophoregramms of X-radiated and repaired yeast proteins there are newly synthesized protein bands which are absent in non-radiated yeasts. These proteins can be protective proteins and can belong to the Heat Shock proteins family.