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CONTENT

TARGETING THE ETHER-À-GO-GO ION CHANNELS IN CANCER THERAPY: CURRENT KNOWLEDGE AND FUTURE PERSPECTIVES

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Abstract: Members of the Ether-à-go-go (Eag) group of potassium channels, including the human Ether-à-go-go (hEag) and the human Ether-à-go-go-related gene (hERG) ion channels, have been shown to play important roles in cancer pathogenesis and to regulate many aspects of tumour development. It was shown that they are frequently overexpressed or ectopically expressed in different human cancers, which, combined with their cell surface expression, led to different attempts at finding their therapeutic potential as promising cancer therapy targets. This review summarizes the current knowledge drawn from the results of independent studies performed to identify sought-after alternatives of targeting these ion channels in cancer therapy.

INTRODUCTION

Ion channels are transmembrane proteins that allow the passive passage of specific ions through the plasma membrane. Their cell surface expression is correlated with a high therapeutic potential, since many drugs targeting them may not need to enter into cells to exert their beneficial effects. Such therapeutic effects usually consist either in blocking the ion conduction or oppositely, to activate the channel's function and sustain an overly increased flow through them. Although ion channels form a large pharmacological target group, no ion channel is currently targeted in antitumour therapy, despite recent intense efforts directed towards deciphering their role, expression and therapeutic potential, which lead to an impressive accumulation of experimental data (Becchetti et al, 2013).

There are many identified ion channels which play roles in the progression of cancer, but evidence accumulates in favor of potassium (K^+) channels, which are frequently overexpressed in tumour cells and have been demonstrated to play roles in basically all cancer cell-specific physiological processes (Huang and Jan, 2014). A particular group in the K^+ channels superfamily is named Ether-à-go-go, or Eag. In humans, this group is formed by the Ether-à-go-go (hEag), Ether-à-go-go-related gene (hERG) and Ether-à-go-go-like (hELK) ion channels. They all share several structural features, but have different physiological parameters. Each one of the channels may be coded by different genes (for example, hEag1 is coded by *KCNH1* while hEag2 is coded by *KCNH5*) and may present different isoforms after differential splicing. All channels have various, but different, functions in the human body. The hEag channel is expressed in fusing myoblasts, the retina and the central nervous system. The hERG channels are expressed in a wide variety of tissues, being critically responsible for conducting the rapid depolarizing current which follows the cardiac action potential, which is why several non-specific hERG1 blockers have been withdrawn from the market, as the channel became an important pharmaceutical antitarget (Raschi et al, 2008).

The Eag channels have important roles in cancer cells of various origins (Tang et al, 2016). They have been shown to possess oncogenic properties when their expression is enlarged and are also frequently expressed or overexpressed in tumour cells (Camacho, 2006; Hemmerlein et al, 2006). A large amount of evidence emerged, all of which show how hEag and hERG channels interact and regulate specific cancer cells functions. In fact, hEag has a limited tissue distribution, but combined with its ectopic expression in cancer makes it a highly suitable biomarker and potential antitumour target, unlike hERG, which is expressed more ubiquitously and its blockade has serious cardiac side effects. The channels play important roles in cancer cells and regulate several aspects of cancer pathogenesis and progression (Arcangeli and Becchetti, 2015). Here, we present several lines of evidence that emerged during recent years regarding the potential of Eag channels as promising anticancer targets and the various strategies that were demonstrated to have limited experimental benefits. Then, we present several perspectives, which could well be considered to be further employed in order to draw new information about these channels and to ultimately target them in cancer therapy.

TARGETING EAG CHANNELS WITH SMALL MOLECULES

Targeting ion channels by small molecules is currently used in treating a variety of conditions, due to the accessible location of ion channels (Bagal et al, 2013). An impressive number of compounds have been found to inhibit the hERG ion channel, due to its unique large cavity which can accommodate many types of blockers. Interestingly, most known blockers of hERG and hEag, like imipramine or astemizole, have been shown to inhibit cell proliferation in some cancer cell lines by targeting hERG (García-Ferreiro et al, 2004). However, most hERG blockers are also blockers of hEag, therefore it was assumed that the anticancer effects could be due to blocking both K^+ currents at once. Nevertheless, hERG blockers usually have a lower affinity for the hEAG channels, which is usually attributed to the lack of inactivation of the latter. Inactivation of hERG is thought to favor a much better placement of important residues in the lower cavity, to contact the blocking compounds (Perry et al, 2010), even though there are some identified exceptions to this general rule, (see below). In fact, to date, it was difficult to make any correct interpretation of the role of hEag1, since there is no specific potent hEag1 blocker. Such a blocker would have important consequences, as it should have important antitumour properties, given the fact that specific hEag1 antibodies have been shown to significantly decrease proliferation of cancer cells (explained in another part of the manuscript). An interesting challenge could be finding through drug design processes or high-throughput screening certain compounds which could bind differently to the ion channels' inner cavity, thus having an increased blocking effect on hEag1 and not on the related hERG channels. Such a hypothesis was recently launched as it is believed that clofilium, another non-specific blocker, could be chemically modified to specifically target hEag1, given its particular blocking determinants (Șterbuleac and Maniu, 2016). Particular structural features of the two channels (on the intracellular activation gate), combined with a new binding mode of clofilium to the hERG channel, would mean that increasing the polarity of the molecule near the phenyl group should generate the needed larger affinity for the hEAG1 channel (Șterbuleac and Maniu, 2018). Since hERG blockers exert different types of side effects, not all of which are very dangerous, it was also hypothesized that they could be repurposed for cancer therapy under careful monitoring (Huang and Jan, 2014).

Another promising potential was brought up, regarding the involvement of hERG channels in leukemias. The hERG-encoding gene, *KCNH2*, expresses at least two different hERG isoforms, A and B, corresponding to two different ion channels, hERG1A and hERG1B (Gasparoli et al, 2015). It was noticed that hERG1A is the dominant form expressed in myocytes, but hERG1B is frequently overexpressed in leukemias. A promising drug candidate has been identified, which is able to block the hERG1B ion channel without interfering with hERG1A function, named CD-160130. It was also shown that this drug has important *in vitro* and *in vivo* antileukemic effects, thus opening the door to subsequent research in this promising alternative anticancer combination between target and targeting compound.

TARGETING EAG CHANNELS WITH ANTIBODIES

Employing antibody therapy in cancer therapeutics is being given full consideration, due to recent advances in this research field (Wold et al, 2016), but significant progress is still awaiting in order to use this approach to block ion channels function and treat human diseases (Sun and Li, 2013). In order to identify novel therapeutic ion channels antibodies, several issues have to be addressed (Wilkinson et al, 2015). In fact, the first monoclonal antibody that specifically

inhibited only one K^+ channel from this group, hEag1, showed that specific targeting of this channel has important antitumour activity (Gómez-Varela et al, 2007). This antibody had antigrowth effect *in vivo* and on several cell lines expressing hEag1. As such, it showed that sole blockade of hEag1 is sufficient for good anticancer effects. However, as far as we know, no other similar studies were designed.

TARGETING GENE EXPRESSION BY siRNA

Small-interfering RNA (siRNA) therapy is a promising new field of research and relies on using specific RNA molecules which should combine with cellular RNA transcripts and silence the respective gene (Gavrilov and Saltzman, 2012). Nevertheless, it faces serious challenges, probably the most important one relying on the challenges to design carriers to deliver siRNA to target specific cells. This method consists in applying small RNA molecules complementary to the mRNA, therefore silencing specific gene expressions. In line with other studies previously presented, silencing the Eag channels' gene expression also revealed antitumour properties, but these studies were performed only using cancer cell cultures. For example, hEag1 siRNA significantly reduced proliferation and colony formation (Wu et al, 2015; Weber et al, 2006). Little to no effect was observed on apoptosis, suggesting that the effect is cell cycle-specific, but significant beneficial effects were observed on cell adhesion and migration of osteosarcoma cell lines. A similar study was performed on hERG (Zeng et al, 2016) and showed comparable results, although in this case siRNA induced apoptosis. These studies are important since they brought additional information regarding the complex cellular signaling processes in which Eag channels are involved and include STAT3, VEGF or NF-κB, proteins known to be involved in cancer progression. Interestingly, different cellular signaling pathways were activated by interfering with each one of the channels.

DUAL-TARGETING OF EAG CHANNELS

As presented so far, it can be noticed that the studies performed on the anticancer therapeutic potential of these channels either need further extension or show potential limitations. However, several lines of evidence exist, showing that using multiple types of channel targeting has combined effects. It was demonstrated that a hEag1 specific antibody coupled to the soluble tumour necrosis factor-related apoptosis-inducing ligand (sTRAIL), which is a known promising anticancer candidate, led to an improved cancer cell-targeting on different cancer cell lines than application of antibody alone (Hartung et al, 2011). Using calcitriol (which blocks hEag1 while having other beneficial effects) and astemizole combined showed improved *in vivo* effects than application of either one alone, which also attests that more efficient results can be seen by using a combined targeting strategy (García-Quiroz et al, 2014).

PERSPECTIVES

As mentioned, there are several lines of research that merit full consideration towards finding novel anticancer therapeutics or to repurpose other drugs. Clofilium is an interesting candidate to be chemically altered and to identify the first hEag specific blockers. Recent structures of hERG1 and hEag1 were identified and could be used to aid the drug design processes. Then, it would be interesting to see if such blockers could be optimized for lesser toxicity and would have anticancer effects. Indeed, an alternative approach is a comprehensive screening of a large compound library, in order to identify specific potent hEag1 blockers or chemical features of

such a blocker. Such a study is much sought-after and would bring important information and knowledge in this field.

Other pieces of information are still missing. An integration of the two channels in the complex signaling pathways of normal and cancer cells would be quite challenging, but it should rely on a comprehensive biochemical and cellular experimental perspective. It should also be based on deciphering the precise biogenesis and trafficking of the two channels, which could then be integrated altogether in the complex so-far-known architecture of the cellular machinery. Such machinery is highly unregulated during cancer, which also leads to a genomic instability, which then influences, through mutations, the structure and function of ion channels. It has to be analyzed whether disfunction of ion channels occurs as a consequence of genome instability and if this can be reversed.

Antibody therapeutics is another promising line that could bring new evidence about the role of Eag channels in cancer cells. Yet, targeting ion channels in cancer with antibodies faces serious challenges and the difficulties regarding it might also indicate the scarcity of studies in this specific field. Nevertheless, given the highly relevance of the Eag channels in cancer pathogenesis and the recent evidence, studies should rely on deciphering whether multiple ion channels targeting, used in conjunction with other types of anticancer targeting, would bring improvements. Interesting results were obtained by combining multiple types of targeting, even if such studies were limited to cancer cell cultures. This merits further studies to be also used in a clinical basis, thus avoiding hERG-related side effects while personalizing the treatment independently for each patient. Targeting Eag could well serve as a means of improving the overall classical chemotherapeutic treatment, by integrating Eag blockers in the treatment scheme while taking account of patient's genetic profile or compound metabolism. This is why the involvement of Eag channels should also be highlighted using pharmacogenetics approaches, which will definitely yield important cellular interactions specific to these channels and that may be further employed in other studies.

CONCLUSIONS

The Eag channels have important roles in cells and their function is linked to various physiological processes. Despite their relatedness, the hERG and hEag play significantly different roles in various cell types. It is acknowledged that Eag channels play significant roles in cancer progression and their targeting in anticancer therapy is one of the most promising research premises in this research area. Several studies showed that interfering with the channels' function leads to anticancer effects. All of the presented strategies merit further development in order to relate Eag channels to specific roles played in cancer and to identify clinically-relevant active molecules to target Eag channels in cancer therapy.

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ASPECTS OF THE BENIGN EXTRASYSTOLIC ARRHYTHMIA IN THE CHILD AND TEENAGER WITH TETANY

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Keywords: benign extrasystolic arrhythmia, child and teenager, tetany

Abstract: The tetany in children represents a state of pathological hyperexcitability of the central and peripheral nervous system. The aim of this study is to present aspects of the benign extrasystolic arrhythmia in the children and teenagers with tetany, starting from the assumption that electrolyte imbalances of calcium and magnesium ions might be the cause of these dysrhythmias. The patients with hypocalcemic and hypomagnesemic tetany had the greatest share, followed by the patients with normocalcemic and normomagnesemic tetany, with no statistically significant difference between the atrial, respectively ventricular extrasystoles in any of the forms of tetany. The percentage of the occurrence of extrasystoles in the patients with latent tetany was higher than in the patients with manifest tetany, with a significant statistical difference between the types of extrasystoles, in both types of tetany.

INTRODUCTION

The tetany in children represents a state of pathological hyperexcitability of the central and peripheral nervous system (Kurdziel K et al., 2016). It is well known that Na+, K+, OH ions increase the neuronal excitability while the Ca++, Mg++, H^- ions decrease it. From the point of view of the etiopathogenic mechanism, there can be hypocalcemic tetany: due to hypoparathyroidism or to the lack of D vitamin, hypomagnesemic and psychogenic tetany: the normocalcemic tetany in the big child and the teenager. Clinically, there are two main types of tetany: manifest and latent tetany. Irrespective of the primary cause of tetany, the symptomatology is heterogeneous, represented by motor manifestations of the peripheral nervous system: the carpopedal spasm, the contraction of the facial muscles, laryngospasm, cardiac manifestations: precordial pricking pain and palpitations, sensitive manifestations: paresthesias or psychical manifestations: asthenia, anxiety and even depression. The patient with latent tetany does not present spontaneous clinical manifestations, thus, a specific motor answer can be reached through the excitation of nerves by the action of tapping over (Matasaru, 2007) (Ito et al., 2007). The Chvostek sign is a clinical clue indicative of the latent tetany in children and teenagers which should become the practice for each general practitioner (Hasan et al., 2014). The aim of this study is to present aspects of the benign extrasystolic arrhythmia in the children and teenagers with manifest or latent tetany, starting from the assumption that electrolyte imbalances of calcium and magnesium ions might be the cause of these dysrhythmias appearing on a healthy cord.

MATERIALS AND METHODS

The batch included 41 children aged 12-18 years, diagnosed with tetany at the medical check-up and in the case of whom extrasystoles were identified on the standard electrocardiogram. The examined batch included a number of 41 children aged 12-18 years, diagnosed with tetany at the general examination and in whom extrasystoles were detected on the standard electrocardiogram. All the children presented the Chvostek sign at the objective examination, 12 children having symptoms, with clinical light, heterogeneous manifestations. The forms of tetany in the examined children were the following ones: 25 cases of hypocalcemic tetany associated with light hypomagnesemia (61%), 15 cases of normocalcemic tetany (36.60%) and 1 case of hypocalcemia (2.40%). According to the ectopic focus, 27 children presented atrial extrasystoles - AE (65.85%) and 14 children presented ventricular extrasystoles - VE (34.15%).
The assessment of each patient was based on the past medical history, the clinical examination and paraclinical investigations (the measurement of the total and ionized calcium, magnesium levels, electrocardiogram investigation). The children suffering from a cardiac condition have not been taken into account for the analysis. The patients were followed up for a period of 18 months.
The statistical analysis was carried out by applying the Chi-square test, a non-parametric test used for statistical hypotheses, in the case of two or more samples taken randomly from a population and for which the frequencies are differently distributed between them.

RESULTS AND DISCUSSIONS

Atrial extrasystoles: there were 27 patients identified, representing 65.85% of the total number. The distribution of cases by sex highlights a higher frequency in the female patients, 17 cases (62.96%) compared to the male patients, 10 cases (37.04%).

Only in the case of 2 patients could we detect extrasystoles on the medical examination. The non-standardized stress tests were carried out for all the children, the absence of symptoms being noticed in all the cases. Based on the associated symptoms, the atrial extrasystoles were diagnosed by electrocardiography in 23 asymptomatic (85.2%) and 4 symptomatic (14.8%) patients.

Regarding the plasmatic level of calcium and magnesium, 1 patient (3.7%) was hypocalcemic, 16 patients (59.3%) had hypocalcemia associated with hypomagnesemia and 10 (37%) had normal levels of calcium and magnesium.

The asymptomatic patients presented: hypocalcemia - 1, hypocalcemia and light hypomagnesemia - 16, normocalcemia and normomagnesemia - 6; all 4 symptomatic patients were normocalcemic and normomagnesemic.

After examining the morphological aspect, all the atrial extrasystoles registered were monomorphous and isolated (sporadic), repetitive atrial extrasystoles not being registered (couples or atrial run). In none of the cases, the presence of arrhythmia had any hemodynamic consequence. As the atrial extrasystoles were monomorphous, isolated and appeared on a normal-sized heart, no antiarrhythmic teatment was administered, taking into account their benign character. Since they had no particular clinical significance, the prognostic is excellent. All the patients were treated with magnesium over a period of 3 months and the patients with hypocalcemia were administered calcium, 10 days a months, for 3 consecutive months, with a view to correcting the magnesium and calcium deficiencies.

Ventricular extrasystoles: there were 14 patients identified, respectively 34.15% of the total number. The distribution of cases by sex highlights a relatively equal distribution, 8 girls (57.14%) and 6 boys (42.86%).

At the medical examination, we suspected the presence of extrasystolic arrhythmia in 3 patients, the standard electrocardiogram subsequently confirming it. In one of the children, the extrasystolic arrhythmia was associated with respiratory arrhythmia. This situation was caused by the significant variations of the sinus rhythm, over 30 beats per minute, as a consequence of the variations of the vagal tone determined by respiration: the cardiac rhythm increases at the end of inspiration, the vagal tone being decreased and the cardiac rhythm decreases at the end of expiration, the vagal tone being increased. The non-standard stress test led to the disappearance of arrhythmia in all the cases, this one representing an important prognostic factor (Beaufort-Krol GC et al., 2008). Ventricular extraystoles were detected on the electrocardiogram of 6 asymptomatic (42.9%) and 8 symptomatic children (57.1%).

After testing the plasmatic level of calcium and magnesium, 9 children had hypocalcemia associated with light hypomagnesemia (64.3%) and 5 had normal levels of calcium and magnesium (35.7%).

Out of the asymptomatic patients, 3 had hypocalcemia and hypomagnesemia, 3 had normal levels of calcium and magnesium; out of the symptomatic children, 6 were hypocalcemic and hypomagnesemic and 2 had normal levels of calcium and magnesium.

In none of the cases, the presence of arrhythmia had any hemodynamic consequence. In the cases we encountered, there were not any ventricular extrasystoles that should lead to the R/T phenomenon, situation in which the ventricular extrasystole overlaps the descending part of the T wave of the previous QRS complex. In all the cases, the ventricular extrasystoles were isolated (sporadic), rare, monomorphous and non-systematic. No antiarrhythmic treatment was administered since they were well tolerated by children.
In the patients with hypomagnesemia and hypocalcemia, the treatment with magnesium was assigned for a period of 3-6 months, followed by the treatment with calcium, the magnesium and calcium levels being tested by biochemical tests while in the patients with normal values of calcium and magnesium, only treatment with magnesium was administered. The clinical and electrocardiographical evolution was favourable in all the cases, the patients being re-evaluated every 3 and 6 months since the extrasystoles were diagnosed.
Although the 24-hour Holter ambulatory monitoring has a significant role in the quantitative and qualitative analysis of extrasystolic arrhythmia (Ciudin R et al., 2003), its performance was conditional on the child's and the family's compliance, on costs, being performed only in 3 cases. Extrasystolic dysrhythmia was identified in 15 (36.6%) (10 with AE, 5 with VE) of the patients having normocalcemic tetany and in 25 (61%) (16 with AE, 9 with VE) of the patients with hypocalcemic and hypomagnesemic tetany, only one patient (2.4%) with hypocalcemia presenting atrial extrasystoles (Figure 1). The percentage in the patients with hypocalcemic and hypomagnesemic tetany was 1.6 higher than in the patients with normocalcemic and normomagnesemic tetany.

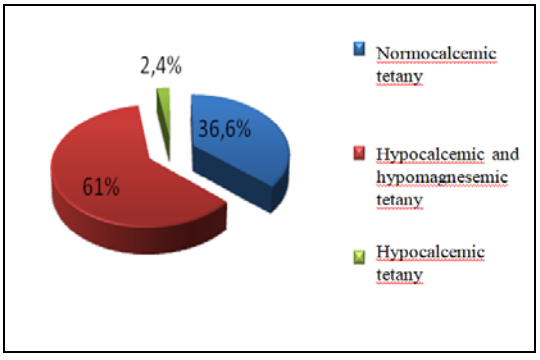

Figure 1. The distribution of pacients based on calcium and magnesium level

For the patients having normal values of Ca and Mg, the identified percentage (37%) of atrial extrasystoles was slightly higher than the percentage of ventricular extrtasystoles (35.7%). In the patients with low values of Ca and Mg, the percentage of atrial extrasystoles identified was 3.7% while the venticular extrasystoles have not been detected (0%) (Figure 2). After aplying the Chi-square test, the statistical significance was not obtained.

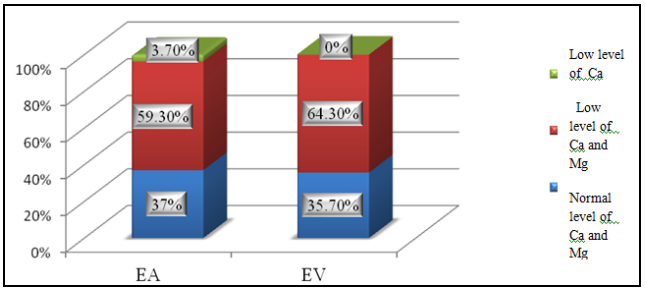

Figure 2. EA/EV at pacients with normal values of Ca and Mg, low Ca si Mg and low Ca

The test calculation: The Chi-square value for alpha=5% (probability - 95%) and 3-1 the degree of freedom is 5.99. The value obtained for Chi square (0) is lower than the carrying value, therefore the percentages are not statistically different.
As concerns the type of tetany, arrhythmia was detected in 12 symptomatic patients with manifest tetany (29.3%) and in 29 asymptomatic patients with latent tetany (70.7%) (Figure 3). The percentage in the patients with latent tetany was 2.4 higher than in the patients with manifest tetany.

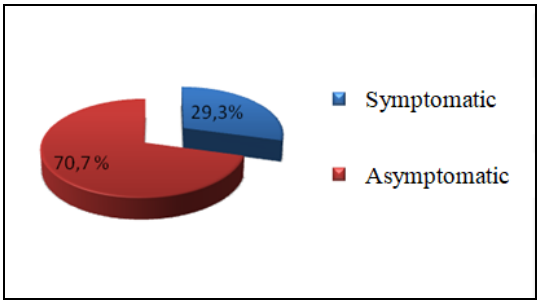

Figure 3. The distribution of symptomatic (manifest tetany)/asymptomatic (latent tetany) patients

In the symptomatic patients with manifest tetany, the percentage of ventricular extrasystoles (57.1%) detected was approximately 4 times higher than that of atrial extrasystoles (14.8%). In the asymptomatic patients with latent tetany, the percentage of detected atrial extrasystoles (85.2%) was almost double compared to that of ventricular extrasystoles (42.9%) (Table 1, Figure 4). After applying the Chi-square test, we obtained an important statistical significance between the ratios.

Table1. EA/EV – symptomatic/asymptomatic patients

Rhythmic disorders * SimptomatologieCrosstabulation					
			Symptomatology		
			AS	S	Total
Rhythmic disorders	EA	Cases	23	4	27
		% ↔	85.2%	14.8%	100.0 %
		% ↕			
	EV	Cases	6	8	14
		% ↔	42.9%	57.1%	100.0 %
		% ↕			
Total		Cases	29	12	41
		% ↔	70.7%	29.3%	100.0 %

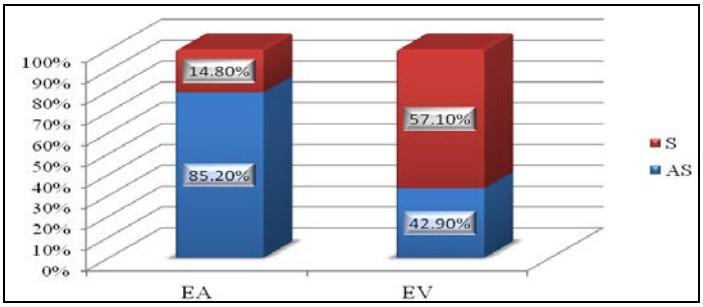

Figure 4 - EA/EV - symptomatic/asymptomatic patients

Theoretical frequencies

Theoretical frequencies	AS	S	Total
EA	19,09	7,91	27
EV	9,90	4,10	14
Total	28,99	12,01	41

The Ch-square value is calculated basen on the following formula: $\chi_c^2 = \sum_i \sum_j \frac{(O_{ij} - T_{ij})^2}{T_{ij}}$,

where O_{ij} represents the frequencies noticed and T_{ij} the theoretical frequencies. For the "heart rhythm disorder" variable that has two variants, AE and VE, we will use the index: i with the values: 1 și 2. In the case of the "Symptomatology" variable with two variants: AS and S, we will use the j index having the values 1 and 2. The theoretical Chi-square value for alpha=5% (95% probability) and 1 ((2-1)(2-1)), the degree of freedom is 3.84. Consequently, the value obtained for Chi square (7.97) is greater than the carrying value, thus the ratios differ, as confirmed by the statistically high percentage.

The analysis of the data of this study shows that the patients with hypocalcemic and hypomagnesemic tetany had the greatest share, followed by the patients with normocalcemic and normomagnesemic tetany, only one case with hypocalcemia having isolated atrial extrasystole. It is worth mentioning the fact that the hypocalcemia and hypomagnesemia of the patients examined were not serious.

It is well known that hypocalcemia is a common manifestation of hypomagnesemia. Up to a third of the patients with hypomagnesemia can have hypocalcemia. There is a positive correlation between the plasmatic levels of magnesium and those of calcium, even a slightly low level of magnesium can lead to a significant decrease of the calcium level. Only the treatment with magnesium can restore to normal the serum concentration of calcium (Gärtner, 2003; Swaminathan, 2003).

Magnesium is the second most common intracellular cation, having the role of important metabolic co-factor for over 300 enzymatic reactions in the whole human body. Among its different roles, magnesium modulates the entry and the release of calcium from the sarcoplasmic reticulum and regulates the ATP pomps in the myocite and the neuron, thus controlling the cardiac and neurone excitability. Consequently, the deficiency in this essential mineral can lead to cardiovascular disorders (Gröber et al., 2015; Mawri et al., 2017).

The use of magnesium as an antiarrhythmic agent in ventricular and supraventricular arrhythmias is a more and more discussed and controversial subject in recent years. Experimental studies have proved the importance of magnesium in maintaining the electrical stability of myocardial cells. That is why its use in the treatment of arrhythmias seems to be reliable (Zehender, 1996). Clinical trials show that the incidences of extrasystoles as well as patients' symptoms are reduced during oral magnesium therapy (Stühlinger et al., 2000).

The favourable evolution of our cases after the treatment with magnesium highlights the fact that the lack of this ion would explain the heart rhythm disorders that occur without any cause in teenagers, on a normally-sized heart. The magnesium deficiency, quite often detected in patients within this age range, due to a poor food intake, is usually latent, but can have an influence on the electrical activity of the heart, as evidenced by the electrocardiography. Determining the magnesium value is not useful many times since the magnesium deficiency is intracellular and plasma is only a transit sector. This would explain the appearance of extraystoles also in the patients with normocalcemic and normomagnesemic tetany. Therefore, in any patient who has an extrasystolic arrhythmia, magnesium therapy appears to be beneficial (Pignide et al.,1985).

Generally, tetany is caused by imbalances of the calcium and magnesium ions, although it is debated, in recent years, that it is caused by several electrolytic imbalances: hypokalemia, alkalosis or the electrolytic imbalances after hyperventilation (Gryglas et al., 2015). Magnesium ion coordinates a multitude of events in the heart cell, so its role is very complex. As mentioned above, there is evidence in the literature that the magnesium deficiency may be the cause of ventricular and supraventricular arrhythmias. At the same time, there is a discussion of the coexistence of a significant depletion of potassium, rejecting the idea that isolated hypomagnesemia may be the cause of arrhythmia. However, there is enough evidence to indicate that hypomagnesemia will significantly exacerbate the proarrhythmic effect of hypocalcaemia (Millane et al., 1992).

On the other hand, in the normocalcemic and normomagnesemic tetany – the psychogenic form, the psychical stress can be the main triggering factor although it coexists with the magnesium deficiency. It is well known that the psychogenic form is more frequently encountered in

teenagers and young women (Toruńska, 2003), females being prevalent in the analysed group (65.85%).
In our analysis, we did not obtain a correlation between the type of extrasystoles and the value of calcium and magnesium levels, the percentage of patients with atrial extrasystoles being comparable to that of patients with ventricular extrasystoles, both in the children with hypocalcemic and hypomagnesemic tetany and in the children with normocalcemic and normomagnesemic tetany.
Concerning the occurrence of extrasystoles based on the clinical manifestations of the patient, the latent tetany prevailed, which shows that identifying the Chvosteck sign must be taken into consideration. The Chvostek sign is very significant in children and teenagers, losing its value in adults, being most of the time negative. A study conducted in 2003 concluded that up to 25% of the healthy adults and up to 29% of those with hypocalcemia may have the positive Chvostek sign (Méneret et al., 2003). The detection of the Chvostek sign in all our cases represented a starting point in the measurement of calcium and magnesium levels in the children with latent tetany.
From the point of view of the type of extrasystoles, the atrial extrasystoles were prevalent in the asymptomatic patients with latent tetany while the ventricular extrasystoles were prevalent in the symptomatic patients with manifest tetany.

CONCLUSIONS

The percentage of patients with hypocalcemic and hypomagnesemic tetany exhibited extrasystoles at a rate of 1.6 times higher than that the patients with normocalcemic and normomagnesemic tetany, with no statistically significant difference between the atrial, respectively ventricular extrasystoles in any of the forms of tetany.
The percentage of the occurrence of extrasystoles in the patients with latent tetany was 2.4 higher than in the patients with manifest tetany. There is a significant statistical difference between the types of extrasystoles, both in the case of latent tetany and in the manifest tetany.
Since all the extrasystoles were isolated, monomorphous and non-systematic, we administered treatment with magnesium, followed or not by treatment with calcium, according to the type of tetany.
In a patient with tetany, the treatment with magnesium should not depend on its plasmatic value, most of the times it is an ionic deficiency at the intracellular level.

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BIOSYNTHESIS OF β-GLUCANS AND MORPHOLOGICAL FEATURES *SACCHAROMYCES CEREVISIAE* CNMN-Y-20 YEASTS UNDER THE ACTION OF ZINC OXIDE NANOPARTICLES

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Abstract: The paper provides new information on β-glucans biosynthesis capacity and the morphological features modification of cells and colonies of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain known as producer of β-glucans. It was found that the development cycle and bioproductive capacity of the yeast was affected by cultivation in the presence of ZnO in a concentrations and the contact duration manner. Within 6-24 hours, the reproduction of the nanomodified cell was decreased compared to the control, but after 120 hours of submerged cultivation an insignificant increase in biomass content relative to 1L culture medium was recorded predominantly related to nanoparticle concentrations 0.5-1.0 mg/L. ZnO nanoparticles (<100 nm). Concentrations of 0.5-15 mg/L initiated a 13-15% increase in average dimensions of *Saccharomyces cerevisiae* CNMN-Y-20 cells and 7-12% increase in the ability to form of β-glucans, especially at concentrations of 1 and 5 mg/L. The correlation coefficient between the cells area and the β-glucans amount is strong (R^2 = 0.8021). The results provide the possibility of enhancing of the range of analyzes and the formation of reference bases necessary for the strategy to enlarge the biotechnological performance of yeasts.

INTRODUCTION

Glucan is a complex polysaccharide present in the yeast cell wall, composed of β-(1→6) and β-(1→3) linked D-glucopyranose units (Lesage et al., 2006). The β-glucans extracted from the yeast cell walls possess high biological activity, in particular, immunomodulatory, anticancer, antimicrobial (Hunter et al., 2002; Volman et al., 2008; Yoon et al., 2008; Pillai et al., 2005; Thammakiti et al., 2007) and are widely used at production of medical preparations, as active part of cosmetic remedies and in other fields. β-glucans obtained from yeasts were approved by the European Food Safety Authority as new food ingredients (EFSA, 2011). The genus *Saccharomyces* presents a great deal of scientific and practical interest among other β-glucans producing yeasts (Cabib et al., 2012; Novak et al., 2012).

The analysis of fields of β-glucans utilization of microbial origin demonstrates the importance of searching for new ways of producing this polysaccharide component. Currently, more research has been done on the use of metal oxides nanoparticles in the biotechnology of microorganisms (Rai, Duran, 2011). Nanotechnologies have the potential to influence different areas, including the food, pharmaceutical and cosmetic industries, and microbial biotechnology (Mrinmoy De Patha et al., 2008). According to some authors, the application of nanoparticles in the biotechnology of microorganisms cultivation ensures increased absorption of the necessary nutrients and may change the metabolic processes (Ban, et al., 2014).

A new approach for increasing the β-glucans production potential would be the application of metal oxides nanoparticles in the biotechnology processes of yeast cultivation. The search for nanoparticles suitable for yeasts frowth and development is of major importance. Among metal oxides nanoparticles, preferential are zinc oxide nanoparticles. The perspectives of applying ZnO nanoparticles in different fields are mentioned in several researchers publications (Espita et al., 2012; El-Diasty Eman et al., 2013). The microbial metabolic complexity complicates the analysis and identification of the nanoparticle-cell interaction. According to some investigations, the mechanism of ZnO nanoparticles influence on microbial cell is complex and induces changes in both cell membrane and cytoplasm (Ya-Nan Chang et al., 2012).

In order to develop the technological possibilities for production of new bioproducts, including β-glucans with polyvalent properties, it is important to investigate in detail the impact caused by nanoparticles on the development and production of metabolites by yeasts with broad potential in biotechnological applications.

Thus, the present paper discusses the results of the elucidation of β-glucans biosynthesis potential and changes in cellular and colonial morpho-cultural characteristics of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain under the action of zinc oxide nanoparticles.

MATERIALS AND METHODS

Strains, culture medium, cultivation conditions. The yeast strain *Saccharomyces cerevisiae* CNMN-Y-20 was used as a model organism for β-glucans producer (Chiseliţa et al., 2010). The strain is preserved in the collection of Yeasts Technology Laboratory and in the Collection of Nonpathogenic Microorganisms of within Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova.

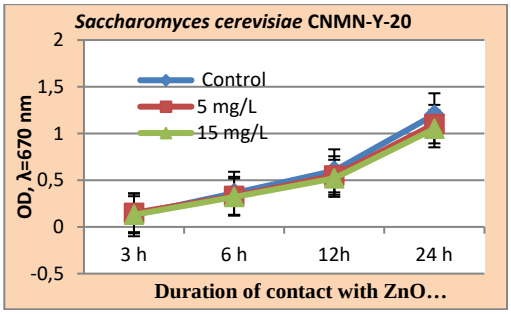
The YPD fermentation medium (Aguilar-Uscanga et al., 2003) specific for selected yeast strains was used for inoculation and submerged cultivation of yeasts. The submerged cultivation was carried out in depth capacity 1 liter Erlenmeyer flask, shaker (200 rpm) at temperature of 25ºC, aeration rate 80.0-83.0 mg/L, for 120 hours. Yeast cells at a concentration of $2x10^6$ cells/ml were inoculated on the liquid medium at 5% ratio.

Zinc oxide nanoparticles (ZnO Np) nanopowder, <100 nm particle dimension were from SIGMA-ALDRICH. The suspension was prepared according to the method specified (Oterro-Gonzalez et al., 2013). The final concentrations of nanoparticles used at the yeast cultivation were 0.5; 1.0; 5.0; 10 and 15 mg/L. The variant without application of nanoparticles served as control sample.

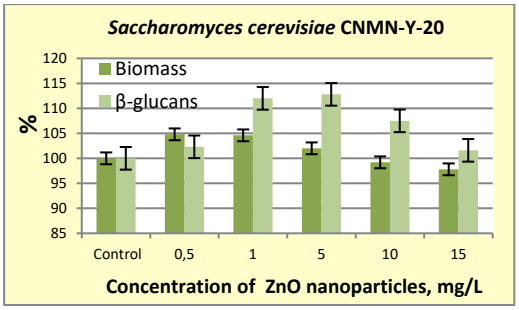
Methods. The total number of cell obtained at liquid medium was determined spectrofotometrically according to the known methods (Mitcell et al., 2004; Dobias, 2013). Yeasts biomass was determined gravimetrically (Hong-Zhi et al., 2009). The β-glucans content in the yeast biomass was determined gravimetrically as described (Thammakiti et al., 2004). Morphological characters of yeast strain were established according to indices described in (Barnet, et al., 2000; Kreeger-Van Rij, 1984; Anghel et al., 1991). The shape and dimension of the cells was examined at YPD nutritive medium, recommended for yeasts. After inoculation, samples were incubated at 25-28°C. Preparation of yeast cells by tehnique of fixation was effectuated after 6, 24 and 120 hours of cultivation. Fixed yeast smears were stained using the gentian violet solution staining technique. The cell shape, budding mode, cell dimensions were determined using XSZ-500 microscope, 100x/1.25 OIL, 160/0.17 and MEM1300 video camera and Future WinJoe special program. Colony morphology was established according to the principles and techniques of general microbiology (Zarnea et al., 1992). Macromorphological investigations were made by inoculation of yeast cultures on solid beer wort using the inoculation loop. Incubation was carried out at 28°C for 5 days. The morphological assessment of the colonies was achieved by noting the shape of the colonies, the size, the profile, the gloss, the transparency, the color, the edge of the colony, the consistency. The degree of correlation between morphological characters and β-glucans content was established using Microsoft Excel. Statistical processing of results was carried out using statistical software kit 7. Statistical processing of obtained results was effectuated electronically with the calculation of the standard errors for the relative and average values, the differences between the experimental and control data were established using Student's t-test and P value.

RESULTS AND DISCUSSIONS

The results of the analysis of cell multiplication dynamics, biomass and β-glucans production by *Saccharomyces cerevisiae* CNMN-Y-20, under the influence ZnO nanoparticles (<100 nm), revealed new data on the effects of nanoparticles produced according to concentration and duration of contact with yeasts. As a result of this investigation, we showed that cells of the control sample and those cultivated in the presence of nanoparticles had the identical *lag* phase (Figure 1). Over the period of 6-24 hours, the process of cell multiplication is declining in relation to the control. In the case of utilization of 5 and 10 mg/L nanoparticles, a decrease in optical density was observed, the difference from the control after 24 hours being 8.3-12.5%.



a)



b)

Figure 1. The dynamics of cell multiplication (a), biomass content and β-glucans (b) at *Saccharomyces cerevisiae* CNMN-Y-20 under the ZnO nanoparticles action (<100 nm).

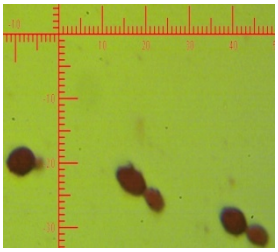
After the growth text, the biomass production after 120 hours of submerged cultivation on the YPD medium was evaluated. The biomass was collected by centrifugation and subjected to biochemical analysis. The presented figure 1b reveals data on the accumulated biomass content. In these experiments, there was an insignificant increase in biomass content, predominantly related to nanoparticle concentrations of 0.5-1.0 mg/L.

To a far greater extent, ZnO nanoparticles (<100 nm) have influenced the β-glucan biosynthesis process. An increase in the β-glucans content in samples cultivated in the presence of nanoparticles in concentrations of 1, 5 and 10 mg/L was recorded (Figure 1b). In these variants the β-glucans amount in the yeast biomass was increased by 7.5-12.8%.

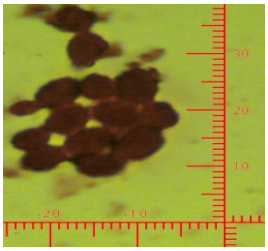
Generalizing research results, it can be mentioned that cell cycle and bioproductive parameters of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain in cultivation in the presence of ZnO nanoparticles (<100 nm) were modified depending on used concentrations and duration of contact. The increase of the rates of β-glucan accumulation in yeasts biomass was observed at the cultivation on the nutrient medium supplemented with ZnO nanoparticles in limits of concentrations of 1-5 mg/L.

Research that involved the analysis of morpho-cultural characters of cells and colonies caused by ZnO nanoparticles (<100 nm) has revealed different changes depending on applied concentrations. Thus, yeast cultures in the liquid medium both control and experimental samples produce cloudiness after 24 hours of incubation at 25°C forming a white foam, often a creamy ring is formed at the surface of the culture medium. After 72 hours of submerged cultivation, a compact sediment is formed, the yeast cells retain their flocculation ability.

After examination of *Saccharomyces cerevisiae* CNMN-Y-20 cells being in contact with ZnO nanoparticles (<100 nm) under a light microscope, it can be mentioned the lack of nanoparticle effects on the budding process of yeast. In both experimental and control variants, cells have demonstrated unipolar budding (Figure 2).



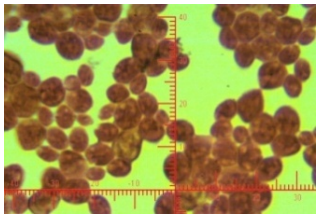
Control



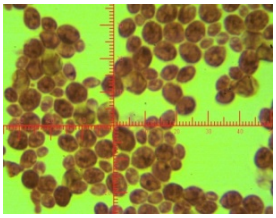
15 mg/L ZnO Np (<100 nm)

Figure 2. Budding *Saccharomyces cerevisiae* CNMN-Y-20 cells 6 hours of cultivation (100x/1,25 OIL, 160/0,17)

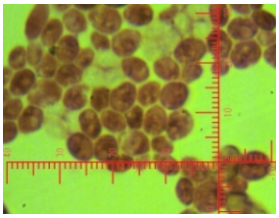
It should be noted that the average size of yeasts cells have changed depending on the applied concentrations of ZnO nanoparticles (<100 nm). After 120 hours of contact with ZnO nanoparticles (<100 nm), the cells cultivated on the YPD medium are generally elliptical, some being more elongated, some more rounded. The arrangement of the cells were in isolation, in pairs and in chains. Sometimes, after 120 hours of cultivation, partial or total cellular overflow occurs as a result of damage to cell membranes. The results obtained by the microscopy methods were confirmed by the images, which are presented below in Figure 3.



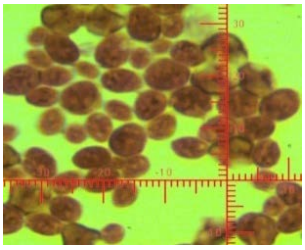
Control



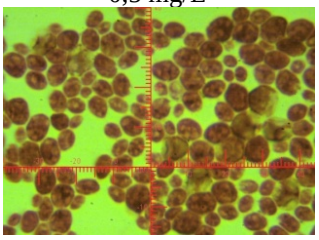
0,5 mg/L



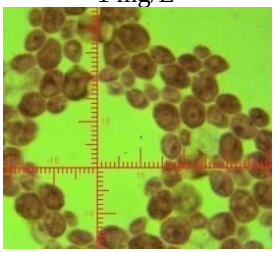
1 mg/L



5 mg/L



10 mg/L



15 mg/L

Figure 3. Morphological features of *Saccharomyces cerevisiae* CNMN-Y-20 cells, cultivated in the presence of ZnO nanoparticles (<100 nm), (contact time 120 hours), 100x/1.25 OIL 160/0.17

In a study effectuated to elucidate the specificity of the zinc oxide nanoparticles influence on the cellular parameters of yeast growth, the measurements of the cell length and width were performed, and the area of the cell was subsequently calculated. The mean values of the length, width and area of the examined yeast strain cells are presented in Table 1. From the table it is apparent that the average cell dimension range between 5.77-6.5 μm length and 4.92-

5.4 μm width. These cell characteristics, with some little deviations, are also seen in control samples. From the obtained data, the benefic influence of the concentrations of 1 mg/L and 5 mg/L is observed, the average area of the cells is slightly increased compared to the control and it is 26.28-26.63 μm^2, which is more with 13.8 and 15.4%.

Table 1 Average dimensions of yeast cells of Saccharomyces *cerevisiae* CNMN-Y-20 yeast strain, at the cultivation for 120 hours in presence of ZnO nanoparticles (<100 nm)

Nr	**Sample, ZnO NP** <100 nm	**Number of examinated cells**	**Average lenght (D), μm**	**Average width (d), μm**	**Cell area (A=π/4xDd)**	
					μm^2	**% of control**
1	Control	55	5.94±1.43	4.95±1.45	23.08	100
2	0.5 mg/L	50	6.24±1.67	5.02±1.68	24.58	106.4
3	1mg/L	44	6.50±1.35	5.22±1.53	26.63	115.4
4	5 mg/L	40	6.20±1.54	5.40±1.33	26.28	113.8
5	10 mg/L	63	6.05±1.53	5.11±1.68	24.26	105.1
6	15 mg/L	40	5.77±1.19	4.92±1.37	22.28	96.5

In order to determine the morphological features modification of *Saccharomyces cerevisiae* CNMN-Y-20 colonies, the both control and experimental variants of yeast culture were seeded on solid YPD medium. The results of the colony examinations are presented in Table 2. Research has demonstrated that ZnO nanoparticles (<100 nm) do not significantly modify the morphological features of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain. Thus, yeasts form on solid medium white or pale pink colonies, surface glossy, smooth, umbonate, the diameter varies between 3-8 mm, the characters observed for control and experimental variants. At the same time, in experimental samples with nanoparticles applied in concentrations 0.5 mg/L and 15 mg/L, colonies with special characters compared to the control were found. The differences were expressed by the undulate margins of the colonies and crateriform profile.

Table 2 Morphological features of *Saccharomyces cerevisiae* CNMN-Y-20 colonies, cultivated in the presence of ZnO nanoparticles (<100 nm), (contact time 120 hours)

ZnO NP <100 nm**)**	**Form of colonies**	**Profile of the yeast colonies**	**Transparency**	**Consistency**	**Colour**	**Dimension, mm**
Control	Circular, entire margins	Umbonate, glossy surface	opaque	slightly creamy	white or pale pink	3-5
0.5 mg/L	Circular, entire margins or undulate	Umbonate, or umbilical, glossy surface	opaque	slightly creamy	white or pale pink	3-8
1 mg/L	Circular, entire	Umbonate, glossy	opaque	slightly creamy	white or pale	3-5

	margins	surface			pink	
5 mg/L	Circular, entire margins	Umbonate, glossy surface	opaque	glutinous	white or pale pink	3-5
10 mg/L	Circular, entire margins	Umbonate, glossy surface	opaque	slightly creamy	white or pale pink	4-7
15 mg/L	Circular, entire margins or undulate	Umbonate, or umbilical, glossy surface	opaque	slightly creamy	white or pale pink	4-6

Morphological features of *Saccharomyces cerevisiae* CNMN-Y-20 colonies, cultivated in the presence of ZnO nanoparticles (<100 nm) were confirmed by the images presented below in Figure 4.

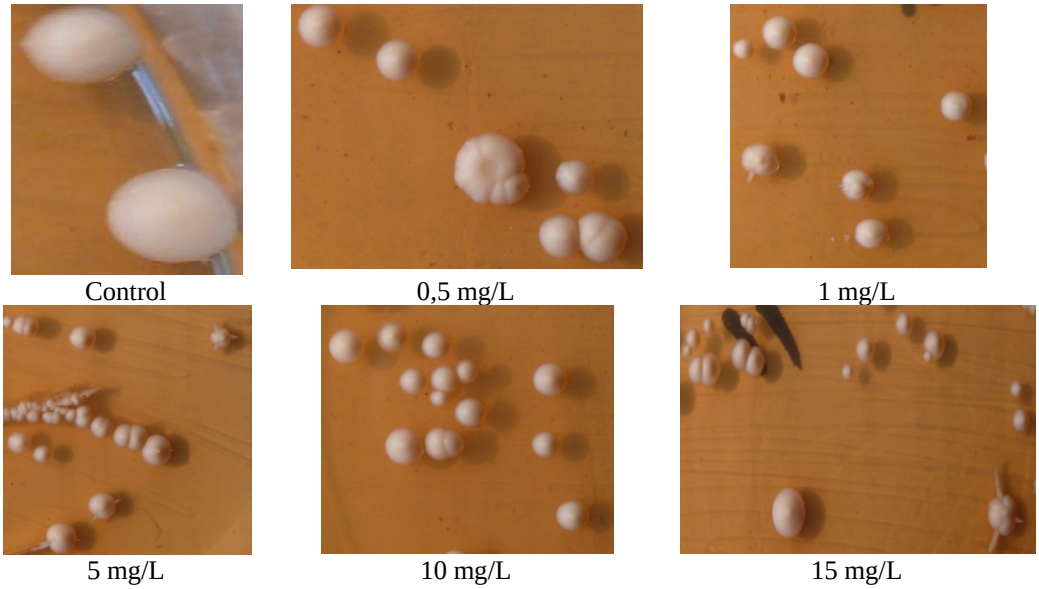


Figure 4. Morphology of *Saccharomyces cerevisiae* CNMN-Y-20 colonies at the cultivation in the presence of ZnO nanoparticles (<100 nm), contact duration 120 hours

In order to enhance the research and to establish the degree of correlation between morpho-cultural features and the bioactive principles production under the action of ZnO nanoparticles (<100 nm), the correlation between the average cell area and β-glucans content in yeast biomass has been determined. It was established that β-glucans content is concomitant ascending with average area of cell with the maximal effect at the nanoparticles concentrations of 1mg/L and 5 mg/L. In the samples where the cells were in contact with higher nanoparticles concentrations (10-15 mg/L), the dynamics of both tested parameters was decreased (Figure 5).

The correlation coefficient between the β-glucans amount and the average cell range is R^2 = 0.8021, which is considered to be a strong correlation.

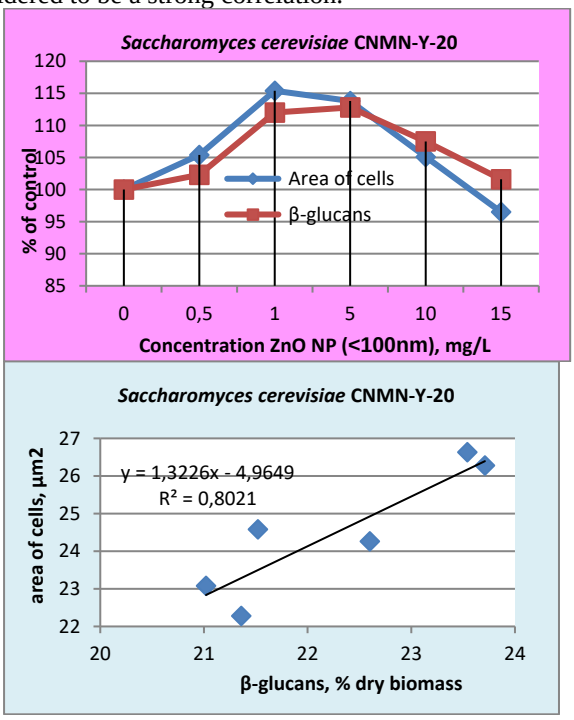


Figure 5. The correlation between the β-glucans content and cells area of *Saccharomyces cerevisiae* CNMN-Y-20 at ZnO nanoparticles (<100 nm) action, contact time 120 hours

Generalizing the latest information, it can be mentioned the importance of studying β-glucans due to their application in various fields such as medicine, food industry, pharmaceutical industry, etc. β-glucans can also be used as bioproducts with polifunctional properties, in particularly, immunomodulatory, anticancerigen and antimicrobial (Rondanelliet et al., 2009; Chan GC, 2009; Espita et al., 2012). The functionality of β-glucans and their production, to a large extent, depends on nutritive media composition and cultivation conditions. Specific literature reflects data on the use of metal oxides nanoparticles in biotechnology of cultivation of microorganisms (Vaseem et al., 2010; Dobias, 2013). In the present study, we analyzed the effects of ZnO nanoparticles (<100 nm) on *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain in terms of determination of β-glucans formation capacity and morphological features modification. The effectuated experiences have been made elucidate effects in a different way.

CONCLUSIONS

We showed that the development cycle and the bioproductive parameters of the yeast cultivated in the presence of ZnO nanoparticles (<100 nm) changed depending on the used concentrations and the duration of contact. Within 6-24 hours, the reproduction of nanomodified

cell was decreased compared to the control, but after 120 hours of submerged cultivation there is an insignificant increase in biomass content relative to 1L culture medium, predominantly related to nanoparticle concentrations 0.5-1.0 mg/L.

ZnO nanoparticles (<100 nm), at concentrations of 0.5-15 mg/L initiated a 13-15% increase in average dimensions of *Saccharomyces cerevisiae* CNMN-Y-20 cells and 7-12% of the ability to form of β-glucans, especially at concentrations of 1 and 5 mg/L. The correlation coefficient between the area of cells and the β-glucans amount is strong.

Cellular morphology data (correlated to cell dimensions) correlated with those of the bioactive principles of biotechnology interest, β-glucans, may offer the possibility of enhancing of the range of analyses and the formation of reference bases necessary for the strategy to enlarge the biotechnological performance of yeasts in the case of metal oxides nanoparticles application.

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About Associate Professor Mihai LAZĂR, PhD

Conf. univ. dr. Mihai LAZĂR

”**Cinstită este înaintea Domnului Moartea cuviosului**” se spune în **Sfânta Psaltire**. Așa trebuie să fie cinstită plecarea dintre noi a profesorului care și-a făcut în mod cinstit, cu dăruire și cu har de la Dumnezeu profesiunea; așa trebuie să fie cinstiți toți cei care, cu cinste și cu sacrificii și-au făcut sfânta datorie pentru care au fost chemați.

Conducem astăzi pe ultimul drum pe eminentul profesor universitar Mihai Lazăr un cadru didactic cu har, cu o structură intelectuală de tip enciclopedic, proiectată parcă în mod special pentru un fiziolog, profesiune care solicită un spirit de observație cu totul deosebit, abilități de performanță în activitățile de laborator și o capacitate ieșită din comun de a face corelațiile necesare între structurile și procesele fiziologice atât de complexe de la animale și de la om.

În anul universitar 1961-1962, fiind în ultimul an la Facultatea de Biologie aveam în planul de învățământ și un curs de **Activitate nervoasă superioară**. Eram conștienți de importanța acestui curs și ne mândream, considerând că ne va fi folositor în înțelegerea ființei umane și a vitalului.

Amănunțisem lumea plantelor, a animalelor și a evoluției lor cu profesori de înaltă ținută academică, făcusem un curs de **Anatomia comparată a animalelor și a omului** cu un specialist de excepție, academician Olga Necrasov, care ne-a facilitat și cunoașterea științifică a lui **Homo sapiens sapiens** în cadrul cursului de Antropologie.

Îmi amintesc cu câtă plăcere, dar și cu câtă emoție îl așteptam pe domnul asistent Mihai Lazăr la primul seminar de Activitate nervoasă superioară.

Făcându-și apariția în sala de seminar, cu un calm deosebit, bine dispus, îmbrăcat elegant și zâmbitor, ne-a cucerit de la început.

Cu un ton prietenesc ne-a comunicat ceea ce știam deja, că vom face împreună seminarul de Activitate nervoasă superioară și ne-a invitat la colaborare.

Această invitație a cântărit greu în sufletele noastre și a avut darul de a ne descătușa de grijile nemăsurate. Consideram cursul și seminarul de Activitate nervoasă superioară ca pe o împlinire pe plan profesional, ca o încununare a muncii noastre de șlefuire profesională.

Associate Professor Ph.D. Mihai LAZĂR

"**Honestly is before the Lord the Death of the Benevolent**," says the Holy Psalter. That is the way to be honored the departure among us of the professor who has done his profession, with abnegation and grace, from God; in this way. It must be honoured all those who, with honor and sacrifices, have done their holy duty for which they have been called.

We are leading today on the last road the eminent university professor Mihai Lazăr, a didactic specialist with grace, with an intellectual encyclopedic structure, designed especially for a physiologist, a profession demanding a very special observation spirit, performance skills in the activities laboratory and an outstanding ability to make the necessary correlations between structurers and physiological processes so complex from animals and from man.

In the university year 1961-1962, being in the last year at the Faculty of Biology, I had in the education plan also a course of **Upper Nervous Activity**. I was aware of the importance of this course and we were proud, considering that it would be useful in understanding the human being and of the vital.

We had detailed the world of plants, animals, and their evolution with high-school professors, and I had made a course of C**ompared Anatomy of Animals and of Man** with an outstanding specialist, academician Olga Necrasov, who also facilitated us the scientific knowledge of ***Homo sapiens sapiens*** within the course of Anthropology.

I remember with what pleasure, but also with how much excitement I was expecting Mr. assistant Mihai Lazăr to the first seminar of Upper Nervous Activity.

Making his appearance in the seminar room, with a special calm, in a good temper, dressed elegantly and smiling, he conquered us from the beginning.

With a friendly tone, he communicated us what we already knew, that we would do the upper nervous activity seminar together and invited us to collaboration.

This invitation has weighed heavily in our souls and it had the gift of unleashing us from the

Nu ne-am înșelat; domnul asistent ne-a convins, în dezbaterile noastre că omul devine om numai prin educație; că la naștere copilul nu este încă un om, dar că va deveni om datorită capacității scoarței cerebrale de a primi informații, de a le stoca și prelucra și de a le depozita apoi în conștient, inconștient și în subconștient colectiv.

Ne-a convins, în dezbaterile noastre, că omul reprezintă statuia care se sculptează singură și că fiecare ființă este rezultatul muncii sale. Ca un adevărat pedagog ne îndeamnă să conștientizăm aforismul lui Nicolae Iorga, conform căruia ”*Școala cea mai bună este școala care te învață să înveți*”.

Dar, iată că există un dar, cine mai dă astăzi importanță unei astfel de profunde gândiri?

Domnul profesor Mihai Lazăr a văzut lumina zilei la 6 septembrie 1925, în satul Palade, comuna Bârlădeni, din județul Hotin, în familia unor oameni gospodari, iubitori de viață și de copii. Dar mama i-a părăsit încă din 1931, pe când avea doar șase ani, așa că tatăl a preluat grijile familiei și a făcut tot ceea ce poate face un om cinstit și muncitor pentru a-și educa copiii și pentru a-i lansa în viață.

Școala primară a urmat-o în satul Palade, iar prima clasă secundară la C.F.R. Lipnic, Soroca.

Venind cu familia în România a fost înscris la **Liceul de Aplicații** din Iași. Studiile liceale le-a urmat la Craiova, la **Liceul ”Frații Buzești”,** urmându-și tatăl în funcție de serviciul său.

Dovedind o inteligență nativă și dragoste față de natură, după obținerea cu succes a bacalaureatului a primit accepțiunea tatălui său de a merge la facultate.

Atras de meleagurile moldave a optat pentru Facultatea de Științele Naturii de la Universitatea ”Al.I.Cuza” din Iași.

Fiind un student deosebit de manierat, liniștit și cu o mare putere de muncă a reușit să obțină nu numai rezultate foarte bune în pregătirea profesională, ci și aprecierea colegilor și a profesorilor săi.

Așa se explică faptul că la 15 sept. 1950, pe când era student în anul al IV-lea, a fost numit ca preparator la Laboratorul de Biologie Generală și Genetică, la conferențiarul Petru Șuster, un adevărat fenomen didactic; avea o memorie care depășea imaginația; putea să citeze la orele de curs pagini întregi din opera unor mari biologi și avea un har didactic ieșit din comun.

Eruditul entomolog Petru Șuster l-a atras pe tânărul preparator să studieze un grup de diptere

unmeasured worries. We were considering the course and the seminar of upper nervous Activity as a professional fulfillment as a crowning of our work of professional grinding.

We have not deceived ourselves; the Mr. assistant convinced us, in our debates, that man becomes man only through education; that at birth the child is not yet a human being, but that he will become a human being due to the ability of the cerebral cortex to receive information, to store them and process them, and then to store them in the conscious, unconscious and subconscious collective.

He convinced us in our debates that man represents the statue that carves itself and that every being is the result of his work. As a true educator, he urged us to become aware of Nicholas Iorga's aphorism, according to which **"The best school is the school that teaches you how to learn."**

But, there is a but , who still gives today importance to such profound thinking?

Mr. Professor Mihai Lazăr saw the light of the day in the Republic of Moldavia, on September 6, 1925, in the Palade village, Bârlădeni Commune, Hotin County, in the family of some peasants, lovers of life and children. But his mother left them in 1931, when he was only six years old, so the father took cares of the family and did everything an honest and labored man could do to educate his children and launch them in life .

He followed the primary school in the Palade village, and the first secondary class at Romanian railways, Lipnic, Soroca.

Coming with the family in Romania, he was enrolled at the **High School of Applications** in Iasi. The High school studies followed in Craiova, at the **"Buzești Brothers" High School**, following his father according to his service.

Proving a native intelligence and love for nature, after successfully obtaining a baccalaureate, he received his father's acception to go to secondary school.

Attracted by the Moldavian parts, he opted for the Faculty of Natural Sciences from the "Alexandru Ioan Cuza" University of Iași.

Being a particularly mannered student, quiet and with a big power of work, he managed to achieve not only good results in professional training, but also the appreciation of his colleagues and professors.

In this way, it is explained the fact that on September 15,1950, when he was a student in the fourth year, he was appointed as a university

din familia Conopidae deosebit de interesantă care, chiar merită să fie cercetată. Fiind specialist în studiul Dipterelor: Tachinidae și Syrphidae, era și firesc ca magistrul să-și apropie noul discipol în studiul unor diptere. De altfel, la Centenarul Universității ”Al.I.Cuza”, în 1960, domnul asistent Mihai Lazăr a prezentat prima sa lucrare științifică: ”**Contribuții la cunoașterea faunei de Conopidae (Diptera) din R.S.R**”.

După regretabila dispariție a magistrului său domnul asistent Mihai Lazăr a lucrat sub conducerea profesorului universitar Dumitru Cărăușu.

Simțind afinități deosebite pentru disciplina de **Fiziologie animală** s-a apropiat de eminentul profesor și apoi academician Petru Jitariu, care îl aprecia foarte mult. Domnul asistent Mihai Lazăr s-a transferat, prin concurs, la **Catedra de Fiziologie animală**, unde avea să-și dedice restul existenței sale.

Chiar în 1960, publică împreună cu Petru Jitariu și Gh. Botez prima lucrare științifică de fiziologie animală. Publică apoi, împreună cu profesorii săi Petru și Matilda Jitariu, cu Napoleon Topală, Gheorghe Dimitriu și Ștefan Agrigoroaie o serie de lucrări, care probează cât de frumos s-a încadrat în colectivul de fiziologie condus de academicianul Petru Jitaru.

În cercetările de fiziologie animală a abordat o tematică largă:

- Fiziologia păstrăvului și a crabului *Carcinus moenas*;
- Determinarea acțiunii fiziologice a unor substanțe noi preparate în România;
- Fiziologia splinei de iepure;
- Determinarea unor indici fiziologici la oile brumării etc.

În 1972 își susține cu succes teza de doctorat: ”**Date privind acțiunea câmpului electromagnetic asupra organismelor animale**” elaborată sub conducerea academicianului Petru Jitariu.

Astfel de cercetări au fost inițiate și apoi fundamentate de Petru Jitariu în România, punând astfel bazele unei puternice Școli de Fiziologie animală, recunoscută pe toate meridianele lumii.

În 1987 domnul conferențiar dr. Mihai Lazăr a fost răsplătit cu **Premiul Academiei Române ”Emil Racoviță”** pentru setul de lucrări: ”**Acțiunea câmpului magnetic asupra organismelor animale**”

Consacrarea în acest domeniu important de cercetări avea să fie încununată cu succes de

preparator at the Laboratory of General Biology and Genetics, at the Reader Petru Şuster, a true didactic phenomenon; he had a memory beyond imagination; he was able to quote at the course hours full-time pages from the work of some great biologists and he had a distinguished didactic talent.

The erudite entomologist, Petru Şuster, drew the young preparator to study a very interesting group of Diptera from the Conopidae family, particularly interesting which is really worth exploring. Being a specialist in the study of Diptera: Tachinidae and Syrphidae, it was natural for the Master to take for himself his new disciple in the study of some Diptera. At the Centenary of "Alexandru Ioan Cuza" University, in 1960, Mr. assistant Mihai Lazăr presented his first scientific paper: **"Contributions to the Knowledge of the fauna of Conopidae (Diptera) in the Socialist Republic of Romania".**

After the regrettable disappearance of his master, Mr. Assistant Mihai Lazăr worked under the leadership of Professor Dumitru Cărăușu.

Feeling special affinities for the discipline of **Animal Physiology**, he approached the eminent professor and then the academician Petru Jitariu, who appreciated him very much. Mr Assistant Mihai Lazăr was transferred to the **Department of Animal Physiology**, where he would dedicate the rest of his life.

Even in 1960, he published together with Petru Jitariu and Gh. Botez the first scientific paper of animal physiology. He then publishes, together with his professors Petru and Matilda Jitariu, with Napoleon Topală, Gheorghe Dimitriu and Ștefan Agrigoroaie a series of papers that prove how beautiful he fit into the physiology collective led by the academician Petru Jitaru

- In the animal physiology researches, he tackled a broad theme:
- The Physiology of the trout and of the crab ***Carcinus moenas***;
- The Determination of the physiological action of new substances prepared in Romania;
- The rabbit spleen physiology;
- The Determination of some physiological indexes in the light grey sheep etc.

In 1972 he successfully presented his doctoral thesis: "**Data on the action of the electromagnetic field on animal organisms**" elaborated under the leadership of the academician Petru Jitariu.

Such researches were initiated and then

lucrarea realizată în colaborare cu un important colectiv de specialiști, să publice în Editura Academiei Române: ”**Acțiunea câmpului magnetic și electromagnetic asupra organismelor animale**”.

Nu întâmplător vorbeam de structura intelectuală de tip enciclopedic a domnului conf.dr. Mihai Lazăr.

În ceea ce privește activitatea didactică universitară domnia sa a onorat mai multe discipline la Facultatea de Biologie și la Institutul Pedagogic de 3 ani de la Iași: - **Fiziologia animalelor și a omului, Activitatea nervoasă superioară, Anatomia și fiziologia omului și igiena școlară și Citohistofiziologia animală.**

Aceste discipline au fost asigurate cu manuale universitare pentru a veni în spiritul studenților și a specialiștilor din domeniu.

Prelegerile sale erau bogate în informații științifice, cu noutățile aduse la zi, bine structurate și prezentate cu un desăvârșit har didactic. Când am afirmat că domnia sa era predestinat pentru a fi expert în Fiziologie animală m-am bazat pe aprecierile mele ca student care a participat la cursurile și seminariile sale, pe baza cursurilor de Anatomia și fiziologia omului (sistemul nervos, funcția de nutriție și funcția de reproducere) care reprezintă un model de ceea ce înseamnă manuale universitare.

Încă nu am spus totul; nu am vorbit de OMUL care a fost domnul conferențiar dr. Mihai Lazăr.

Un om cu suflet nobil, dedicat muncii, profesiunii și familiei; un adevărat gentleman: manierat, modest, apropiat de oameni și iubitor de viață. Nu l-am auzit niciodată vorbind de rău pe cineva. A fost destul de brutal lovit de politica de cadre practicată de regimul trecut. Nu a avut bucuria, așa cum ar fi meritat, să ajungă în ierarhia didactică universitară ca profesor titular, însă acest aspect ne pune în lumină o altă caracteristică particulară a domniei sale: a fost cinstit, drept și nu a acceptat nici un fel de compromisuri. Personal, în calitate de decan al Facultății de Biologie am militat mult pentru a fi răsplătit cu titlul de **Profesor Emeritus**, așa cum merita pentru serviciile aduse învățământului academic românesc.

În repetate rânduri l-am auzit vorbind cu sfințenie despre familie: despre soția sa, Maria Vârtolaș, colegă de facultate, cu care s-a căsătorit în 1951, pe când erau studenți și care a funcționat cu rezultate deosebite ca **Șefă de Secție la Grădina**

proved scientifically by Petru Jitariu in Romania, thus laying the foundations for a strong Animal Physiology School, recognized on all the world's meridians.

In 1987, Mr. Reader Mihai Lazăr was rewarded with the "**Emil Racoviţă" Romanian Academy Award** for the set of works: "**The Action of the Magnetic Field on Animal Organisms".**

The devotion in this important field of researches was to be successfully crowned by the work realized in collaboration with an important collective of specialists, to publish in the Romanian Academy Publishing House: "**The action of the magnetic and electromagnetic field on animal organisms".**

It was not by chance that we talked about the intellectual structure of the encyclopedic type of Mr. Reader PHD Mihai Lazăr.

As concerns the university didactic activity he honored several disciplines at the Faculty of Biology and at the Pedagogical Institute for 3 **years in Iasi: - The Physiology of animals and of the man, Upper nervous activity, The Anatomy and physiology of the man and school hygiene and Animal cyohistophysiology.**

These disciplines were provided with university textbooks to come to the spirit of students and of specialists in the field.

His lectures were rich in scientific information, up-to-date novelties, well-structured and presented with a perfect didactic grace. When I stated that he was predestined to be an expert in Animal Physiology, I relied on my appreciation as a student who attended his courses and seminars on the basis of the Anatomy and Human Physiology courses (the nervous system, the nutrition function and the function of reproduction) that represents a model of what the university textbooks mean.

I have not said yet all; I did not talk about the MAN who was Mr. Reader PH D. Mihai Lazăr.

A man with a noble soul, dedicated to work, profession and family; a true gentleman: modest, close to the people and lover of life. I never heard him talking badly about anyone. He was brutally struck by the policy of cadres practiced by the past regime. He did not have the joy, as he would have been worth, to get into the academic teaching hierarchy as a titular professor, but this aspect brings to light another particular feature of his personality: he was honest, just and did not accept any compromises. Personally, as a Dean of the Faculty of Biology, I militated much as he to be

Botanică a Universității ”Al.I.Cuza”, fiind o botanistă de înaltă ținută academică.

Cât despre fiica sa nu avea decât cuvinte de laudă și de admirație pentru slefuirea sa profesională și pentru comportamentul de mamă și de fiică iubitoare. Când vorbea despre nepotul său vorbea cu toată ființa sa; este adevărat că toți bunicii vorbesc elogios despre nepoții lor, dar puțini sunt cei care s-au bucurat de un asemenea nepot, cu totul ieșit din comun.

Acum la despărțire nu pot decât să spun că: cinstită trebuie să fie de noi, cei care l-am cunoscut și apreciat, întreaga existență a celui ce a fost un **MARE OM, PROFESOR** și **FAMILIST** – Mihai Lazăr.

Să-l purtăm în inimile noastre, în întreaga noastră existență și să-l rugăm pe Bunul Dumnezeu să-l pomenească în Împărăția Sa!

Dumnezeu să-l ierte!

29 ianuarie 2018

MUSTAȚĂ GHEORGHE, Profesor Universitar Asociat Emeritus Dr., Membru Titular al Academiei Oamneilor de Știință din Romania, Facultatea de Biologie, Universitatea „Alexandru Ioan Cuza” Iași, ROMÂNIA

rewarded with the title of **Emeritus Professor**, as he deserved for the services provided to the Romanian academic education.

I have repeatedly heard him speak with holiness about the family: about his wife, Maria Vartolaş, colleague of faculty, whom he married in 1951 as a student, and who worked with great results **as Head of Section at the Botanical Garden** "Alexandru Ioan Cuza" University, being a highly respected botanist.

As for his daughter, he had only words of praise and admiration for her professional schooling and for behavior of mother and loving daughter. When he talked about his nephew, he spoke with all his being; it is true that all grandparents speak eloquently about their grandchildren, but few are those who have enjoyed such a grandson, totally out of the ordinary.

Now at the sad separation, I can only say that: honest must be by us, those who have met and appreciated him, the whole existence of the one who was a **GREAT MAN, PROFESSOR and FAMILIST - Mihai Lazăr.**

Let us carry him in our hearts, in the whole of our existence, and pray the Good God to remind him in his kingdom!

God forgives him!

January 29, 2018.

MUSTAȚĂ GHEORGHE, Associate Professor PH D, Emeritus, Titular Member of the Academy of Men of Sciences of Romania, Faculty of Biology, „Alexandru Ioan Cuza” University of Iași, ROMANIA

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