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New Test-system Based on the Evaluation of Yeast Cells Resistance to Dehydration-rehydration Stress

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Abstract: A new test-system was developed for rapid evaluation of the cytoprotective potential of substances which can be included in cosmetics and health care compositions. It was evaluated by changes of the viability of yeast cells after dehydration/rehydration (D/R) treatment. To check the efficiency of proposed test system we studied the effects of water extracts of the natural peloid, which are used in balneotherapy procedures. It was revealed that the resistance to D/R of cells incubated in peloid water extracts (PWE) was significantly increased (by 30-38%) already after their short incubation in the solutions with low concentrations of the PWE. Meanwhile the membrane permeability was significantly decreased after incubation with PWE. The viability of yeast cells which were more sensitive to D/R stress was increased by PWE to larger extent than the viability of the cells that were initially more resistant to this stress. The positive effect PWE upon yeast cell viability after D/R treatment can be linked with the stabilisation of the cells' plasma membrane. The results obtained in this study indicate that this new test-system can be recommended as fast, simple and cost-efficient assay for the evaluation of various substances effects upon eukaryotic cells including also the ingredients for cosmetic products.

Keywords: Cell viability, dehydration/rehydration, peloid, permeability, plasma membrane, stress resistance.

INTRODUCTION

The yeast, Saccharomyces cerevisiae is an excellent cell model for multitude of physiological processes in higher eukaryotes due to the similarities in the structure of the cells, main pathways of cellular metabolism, DNA replication, recombination and repair, RNA transcription and translation, intracellular trafficking etc. [1, 2]. For the development of the test systems yeasts are excellent candidate organisms due to their rapid and cost-efficient reproduction and due to the lack of the ethical problems associated to the animal models. The ability of yeast to grow under a wide variety of conditions allows the selection, screening, identification of mutant yeast phenotypes and creation of new strains [1, 3-6]. During the last decades yeasts have been frequently used as an efficient model in the studies of various processes in higher eukaryotic organisms including cancer research and to identify molecular mechanisms of human diseases [1, 2, 6], test possible toxicity of new drugs [7, 8], screen for the antioxidant activity [9], study oxidative modifications of proteins [10], in ageing and apoptosis research [11-13]; neurodegeneration research [6], for the understanding the mechanisms of drugs activities [14], etc.

The goal of this research was to develop a new yeast based test system for rapid evaluation of effects of various compounds upon eukaryotic cells and to check its efficiency at the studies of peloid effects.

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MATERIALS AND METHODS

Yeast Strain and Culture Conditions

The yeast *Saccharomyces cerevisiae* 14, obtained from the Microbial Strain Collection of Latvia was grown in flasks with YPM medium containing 10 g/L yeast extract, 20 g/L peptone and 20 g/L maltose with initial pH 5.00 on an orbital shaker at 30 °C for 48 h. Yeast biomass was harvested by centrifugation, washed twice with distilled water and then used for incubation with PWE and for the exposure to the dehydration stress.

Preparation of Peloid Samples

Peloid obtained from Rider-Ta Ltd, Latvia was heat sterilized at 121°C for 20 min before the use. Humidity of peloid was determined by drying to constant weight at 105 °C. To prepare the experimental extracts for further studies 11 g of fresh peloid were mixed with 40 mL of distilled water. This suspension was stirred during 24 h using the rotary shaker followed by centrifugation for 20 min at 3200 g. The upper phase was diluted at 1:50; 1:25, 1:5 with distilled water and used in the further incubation procedure.

Incubation of the Yeasts with PWE

3 g of fresh yeast biomass was incubated in 40 mL of PWE using rotary shaker for 25 min or 3 h. Thereafter the yeast biomass was harvested by centrifugation during 10 min at 2600 g at room temperature for its further exposure to dehydration. The control samples were incubated in water.

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Dehydration Procedure

After centrifugation yeast biomass was pressed through a sieve (mesh diameter – 1 mm) and subjected to the convective drying in a partially lid covered plastic Petri dish placed in an oven at 30 °C for 20 h. Dehydration procedure leads to the anhydrobiosis: decrease of cells' relative humidity till 8-10% and temporary reversible delay of the metabolism. The changes of the surface area exposed to the air convection at dehydration significantly influence the viability of the yeast cells after rehydration. At harsh dehydration process, if the Petri dish is fully open, rapid convection takes place and the cell viability after rehydration is low, ca. 30-40%; at mild dehydration process, if the Petri dish is half-open, slower convection takes place and the cell viability after rehydration is higher, ca. 50-60%.

The Viability of Yeast after Dehydration

The viability of dehydrated yeast samples was determined after 10 min rehydration in distilled water at room temperature using fluorescent microscopy and the fluorochrome primulin [15].

Cell Membrane Permeability

The changes of the permeability of the cell plasma membrane during the D/R stress were quantified by the losses of cells dry substance as measured by the difference of dry weight of the yeast samples before and after rehydration.

Statistical Analysis

Experiments were done in triplicate. Results were expressed as the mean ± SD. A Student's t-test was used for comparisons between two groups. $P<0.05$ was defined as the threshold of statistical significance.

RESULTS AND DISCUSSION

As the basis of this study we have taken into account our previous results on the yeast cell response upon the D/R stress. It was shown that dehydration of yeast which is transferring cells into the state of anhydrobiosis (temporary reversible delay of metabolism) can lead to serious changes in all intracellular organelles [16, 17], switch on a number of intracellular protective reactions [16, 18] and result in the appearance of "new" physiological and biotechnological characteristics of the cells [19, 20]. The conclusion has been made that cell resistance against D/R stress is an integral characteristic of the physiological state of eukaryotic cells. Different approaches of controlling and improving the stress resistance of the cells have been proposed [21-25]. Considering all information accumulated during the studies of anhydrobiosis in yeast we proposed the idea that application of dehydration-rehydration stress to a yeast model can be rather helpful for the understanding of possible changes in the physiological state of the cells in response to their treatments by any substance which can be interesting for researchers. To check this hypothesis we used yeast cells treatment with peloid water extracts (PWE). Such study was interesting because of the possibilities to use peloid's components for new cosmetic products. In our experiments yeast cells from the stationary growth phase of the culture were incubated in PWE for 25 min and for 3 h. After the incubation yeast samples were subjected to D/R stress and their viability was evaluated. The results which have been obtained are presented at the Fig. (**1**), showing by 16-23% increased stress resistance of the PWE treated cells in

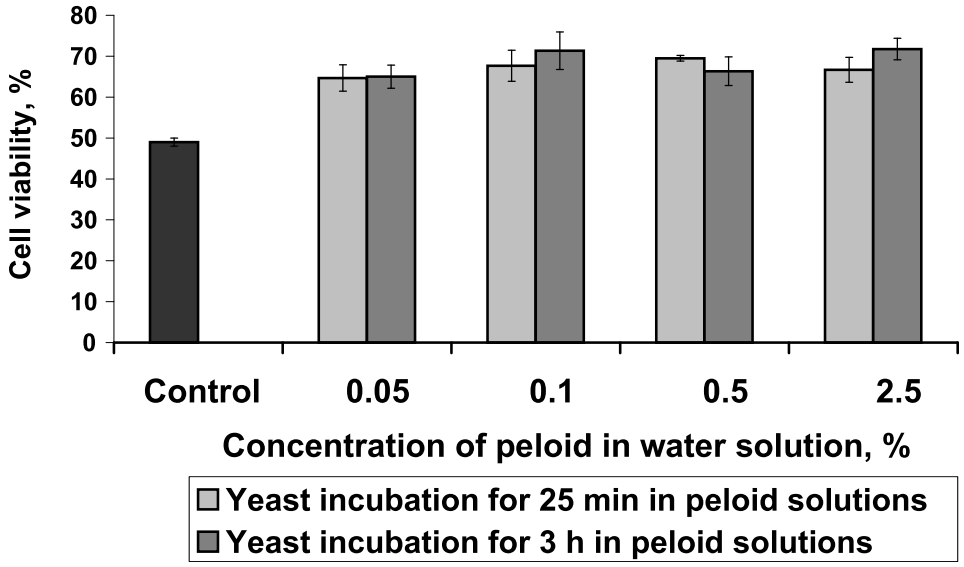

Fig. (1). Influence of incubation of yeast cells in the solutions with different concentrations of peloid extracts upon their viability after dehydration. SD are indicated by error bars. The differences in comparison to the control at significance level $p<0.05$ are marked by (*), at $p<0.01$ are marked by (**)

comparison to the control samples. The incubation of the yeast in PWE during 25 min gave practically the same effect as the incubation during 3 h. In the next series of experiments we checked also the effects of yeast cells incubation in PWE if the cells then were subjected to more soft or severe drying procedures. The cells that had undergone more harsh drying conditions and had decreased viability, ca. 38% after rehydration, exhibited more strong protective effect, additional 30-40% points of viable cells, after PWE treatment than the cells that had undergone soft drying, and having ca. 60% viability after rehydration, increased viability by ca. 10-15% points after PWE treatment (Table **1**).

In general, it became clear that the pre-treatment of the yeast cells with PWE in all cases increases their resistance to D/R stress until a maximum resistance level. In our experiments this maximum of viability was 68-76% of the total cell number. To understand the mechanisms of the positive influence of the PWE upon yeast viability after D/R stress we examined the changes of plasma membrane permeability. It is known that the plasma membrane state is a crucial element for the maintenance of the cell homeostasis. Usually various pathological processes as well as just "weakening" of different higher organisms correlates with changes of functional characteristics of cellular and intracellular membranes [26-30]. Our previous studies have shown that stabilisation of cell plasma membrane is one of the most important properties for the maintenance of cell viability at D/R stress [23, 31]. We have addressed the correlation of the membrane permeability with the increased viability of PWE treated cells to clarify, if the loss of substances through the destabilized cell membranes may be one of the reasons of decreased viability of the cells after D/R stress, what may be counteracted by the PWE treatment. Our experiments showed that the PWE treatment before the dehydration decreases the permeability of plasma membrane of the yeast cells after D/R stress and prevents the efflux of the cells' internal substances (Table **2**).

Already the incubation with PWE for 25 min at low, 0.05% - 0.10% concentrations of the extract lead to the significant increase of cells' resistance and to the stabilisation of their plasma membrane. Within the used range of the peloid dilutions the effect was not concentration dependent, the increase of peloid concentrations from 0.05% to 1.0 and 2.5% was not accompanied with additional increase of the observed positive effects.

We conclude that the increase of the yeast viability after D/R stress and concomitant decrease of the efflux of substances from the cell through the plasma membrane may be used as a sensitive and fast assay to assess the anti-stress effect of any tested substance and in our case of natural peloid and their components. The peloid fractions obtained from Latvian Kemeri mire contain biologically active compounds, which can increase the D/R stress resistance of eukaryotic cells. Of course, our data is only preliminary and provides just a phenomenology of the anti-stress activity; to elucidate the exact molecular mechanism of the observed effect additional studies are needed. At the same time, our data on the increase of plasma membrane stability after PWE treatment clearly indicate further pathways for the exploration.

Table 1. Influence of incubation of yeast cells (which differ in their resistance to D/R) in the solutions with various concentrations of peloid water extracts upon their viability after dehydration.

Viability of cells after dehydration, %			
	Control	Incubation in peloid water extracts with various concentrations, %	
		0.5	2.5
Harsh drying conditions	38 ± 4	68 ± 6.2 **	76 ± 5.1 **
Soft drying conditions	60 ± 4	72 ± 6.2 *	70 ± 3.4 *

The differences in comparison to the control at significance level $p<0.05$ are marked by (*), at $p<0.01$ are marked by (**)

Table 2. Influence of yeast cells incubation in peloid water extrracts upon the permeability of their plasma membrane evaluated by the total losses of dry substance after yeast D/R.

Yeast samples	Total losses of substances in dry weight, %	
Control	24 ± 0.5	
Concentration of peloid in water extracts, %	Incubation of samples in peloid water extracts for 25 min	Incubation of samples in peloid water extracts for 3 h
0.05	22 ± 0.09 *	22 ± 0.8 *
0.1	21 ± 0.4 *	21 ± 0.1 *
0.5	20 ± 0.1**	20 ± 0.9 *
2.5	20 ± 0.6 *	20 ± 0.6 *

The differences in comparison to the control at significance level at $p<0.01$ are marked by (*), at $p<0.001$ are marked by (**)

In our previous studies we have shown that the viability of the cells after D/R stress can be increased by the pre-incubation before the drying in the solutions, which contain either Mg^{2+} and Ca^{2+} ions [24], or sucrose, lactose, xylitol and/or glycerol at concentrations increasing the osmotic pressure [21, 23, 25]. Practically in all the cases the improvement of the cells' stress resistance was paralleled with the increase of plasma membrane stability. The role of plasma membrane in the stress resistance is corroborated by the fact that the plasma membrane permeability and temperature of phase transitions of membrane lipids of *Debaryomyces hansenii*, a yeast species, which is extremely resistant to various environment stresses, strongly differs from the corresponding parameters of conventional *S. cereviseae* membrane [22, 31]. It is plausible that the increase of plasma membrane stability can provide substantial contribution to the improvement of the cells' stress resistance in the present study.

Peloids are natural organic-colloidal formations of various origins (silt, peat etc.), which have great plasticity, high heat capacity and slow heat transfer [32]. This complex heterogeneous system consists of two solid and one liquid phase. Solid phase consists of crystalline skeleton which determines the mechanical structure of peloids and of colloidal skeleton which defines thermal properties and consistency of peloids. Liquid phase is the peloid solution which serves as a carrier of the therapeutic effect [33]. The therapeutic effect of peloids is due to their organic and mineral composition presented by various biologically active substances such as mineral salts, organic matter and gases. The mineral part of peloids consists of water insoluble minerals, water hardly soluble salt compounds, different ions and gases. Additionally, compounds of iron, sulphur, phosphorus, nitrogen, microelements such as iodine, bromine, lead, and molybdenum can be found in peloids. Composition of gases is presented by hydrogen sulphide, carbon dioxide, nitrogen, methane and oxygen. Many biologically active compounds can be found such as B-group vitamins: riboflavin and folic acid; vitamins C and D, hormone compounds, and the trace elements: bromine, iodine, boron, manganese, copper, iron and others [32]. The organic macromolecules presented in the peloid are represented by humic substances, including humic acids, fulvic acids, ulmic acids and humins [32]. Humic acids are high molecular polymeric aromatic substances having complicated structure of polyaromatic and heterocyclic chemicals with the multiple carboxylic acid side chains, showing outstanding physicochemical properties [34]. Humic acids are known to elicit hepatoprotective, antihypoxial, antitoxic, antioxidant effects due to the affinity for biological membranes, involvement in the ion transport and because of enzyme activity modulation [35, 36]. It was shown also that the antioxidant activity of humic acids is dependent on the presence of carboxy- and hydroxyl groups in their macromolecules [36].

Special role belongs to peloid`s microflora which define the biological processes occurring in the peloids. It was shown the formulation of thermal muds must include a maturation stage – the procedure of mixing of the solid and liguid phases [32, 37].

It was proposed that the regulations and suitable quality criteria should be established for peloids used for therapeutic purposes. Different data should be taken into consideration: mineralogy, grain size, geochemical composition, microbiological content, pH, plasticity etc. [37].

The given examples of peloid active components show their possible protective effects can be created by the peloid applications in eukaryotic cells and organisms. These effects should be considered when addressing the mechanism of the anti-stress effects observed with PWE.

The observation that the PWE treatment creates more pronounced stabilizing effect with the cells, which have lower viability due to the suboptimal drying conditions, is of special importance for their eventual use in cosmetic recipes. Skin ageing is associated with the loss of water from the skin cells and concomitant effects of dehydration. Our data on the recovery of the survival rate of the more vulnerable dehydrated cells by PWE treatment can be considered in context of revitalization of senescent dehydrated skin cells. PWE samples are prospective candidates to be included and further studied as eventual components for the skin anti-ageing compositions, using, i.e., the yeast D/R stress and membrane integrity models.

Summarizing the results of this study it is necessary to mention that it gave important information for 2 different issues. First, it is interesting data on the prospects of further detailed investigations of components of PWE for new cosmetic skin anti-ageing creams. At the same time these results showed the efficiency of application of a new model based on the evaluation of yeast cells subjected to dehydration-rehydration stress. Such approach gives us new fast, simple and cost-efficient assays for the evaluation of possible effects of various chemical substances upon the physiological state of eukaryotic cells.

ABBREVIATIONS

D/R = dehydration/rehydration;

PWE = peloid water extracts.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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REFERENCES

[1] Barrientos A. Yeast models of human mitochondrial diseases. IUBMB Life 2003; 55: 83-95.

[2] Pray L. L.H. Hartwell`s yeast: a model organisms for studying somatic mutations and cancer. Nat Educ 2008; 1: 183.

[3] Botstein D. Why yeast? Hospital Practice 1991; 26: 157-61.

[4] Botstein D, Chervitz SA, Cherry JM. Yeast as a model organism. Science 1997; 29: 1259-60.

[5] Mattiazzi M, Petrovič U, Križaj I. Yeast as a model eukaryote in toxinology: a functional genomics approach to studying the molecular basis of action of pharmacologically active molecules. Toxicon 2012; 60: 558-71.
[6] Pereira C, Bessa C, Soares J, Leao M, Saraiva L. Contribution of yeast models to neurodegeneration research. J Biomed Biotechnol 2012; 2012: 1-12.
[7] OECD Guidelines for the testing of chemicals Test No. 480: genetic toxicology: Saccharomyces cerevisiae, gene mutation assay 1986; 4: 1-5.
[8] OECD Guidelines for the testing of chemicals Test No. 481: genetic toxicology: Saccharomyces cerevisiae, mitotic recombination assay 1986; 4: 1-6.
[9] Slatnar A, Jakopic J, Stampar F, Veberic R, Jamnik P. The effect of bioactive compounds on *in vitro* and *in vivo* antioxidant activity of different berry juices. PloS ONE 2012; 7(10): e47880.
[10] Lushchak VI. Budding yeast Saccharomyces cerevisiae as a model to study oxidative modification of proteins in eukaryotes. Acta Biochim Pol 2006; 53(4): 679-84.
[11] Breitenbach M, Madeo F, Laun P, *et al*. Yeast as a model for ageing and apoptosis research. Top Curr Genetics 2003; 3: 61-97.
[12] Rego A, Trindade D, Chaves SR, *et al*. The yeast model system as a tool towards the understanding of apoptosis regulation by sphingolipids. FEMS Yeast Res 2014; 14: 160-78.
[13] Carmona-Gutierrez D, Eisenberg T, Büttner S, Meisinger C, Kroemer G, Madeo F. Apoptosis in yeast: triggers, pathways, subroutines. Cell Death Differ 2010; 17: 763-73.
[14] Bjornsti M-A. Cancer therapeutics in yeast. Cancer Cell 2002; 2: 267-73.
[15] Rapoport AI, Meysel MN. Survival rates of yeast organisms after dehydration as determined by fluorescence microscopy. Microbiology (Moscow) 1983; 54: 53-5.
[16] Beker MJ, Rapoport AI. Conservation of yeasts by dehydration. Adv Biochem Eng/Biotechnol 1987; 35: 127-71.
[17] Rapoport A. Anhydrobiosis of yeasts: Main intracellular reactions. Comp Biochem Physiol 2008; A151: S31.
[18] Guzhova I, Krallish I, Khroustalyova G, Margulis B, Rapoport A. Dehydration of yeast: changes in the intracellular content of Hsp 70 family proteins. Proc Biochem 2008; 43: 1138-41.
[19] Muter O, Millers D, Grigorjeva L, Ventina E, Rapoport A. Cr(VI) sorption by intact and dehydrated Candida utilis cells: differences in mechanisms. Proc Biochem 2001; 37: 505-11.
[20] Rapoport A, Borovikova D, Kokina A, *et al.* Immobilisation of yeast cells on the surface of hydroxyapatite ceramics. Proc Biochem 2011; 46: 665-70.
[21] Rapoport AI, Beker ME. Effect of sucrose and lactose on resistance of the yeast Saccharomyces cerevisiae to dehydration. Microbiology (Moscow) 1983; 52: 556-9.
[22] Khroustalyova GM, Adler L, Rapoport AI. Exponential growth phase cells of the osmotolerant yeast Debaryomyces hansenii are extremely resistant to dehydration stress. Proc Biochem 2001; 36: 1163-6.
[23] Rapoport AI, Khroustalyova GM, Crowe LM, Crowe JH. Anhydrobiosis in yeast: stabilization by exogenous lactose. Microbiology (Moscow) 2009; 78: 624-9.
[24] Trofimova Yu, Walker G, Rapoport A. Anhydrobiosis in yeast: influence of calcium and magnesium ions on yeast resistance to dehydration-rehydration. FEMS Microbiol Lett 2010; 308: 55-61.
[25] Rozenfelde L, Rapoport A. Anhydrobiosis in yeasts: is it possible for yeast grown in anaerobic conditions? 1st Congress of Baltic Microbiologists. Riga: Book of Abstracts 2012; p 20.
[26] Bansal D, Miyake K, Vogel SS, *et al*. Defective membrane repair in dysferlin-deficient muscular dystrophy, Nature 2003; 23: 168-72.
[27] Andrews NW. Membrane repair and immunological danger (Review). EMBO Reports 2005; 6: 826-830.
[28] Callahan JW, Bagshaw RD, Mahuran DJ. The integral membrane of lysosomes: its proteins and their roles in disease (Review). J Proteomics 2009; 72: 23-33.
[29] Howard AC, McNeil AK, McNeil PL. Promotion of plasma membrane repair by vitamin E. Nat Commun 2011; 2: 1-8.
[30] Zhang D, Iyer LM, He F, Aravind L. Discovery of novel DENN proteins: Implications for the evolution of eukaryotic intracellular membrane structures and human disease. Front Genetics 2012; 3: 283.
[31] Rapoport AI, Khroustalyova GM, Camanis GJ, Beker MJ. Yeast anhydrobiosis: permeability of the plasma membrane. Microbiology (Moscow) 1995; 64: 229-32.
[32] Cholopov AP, Shashel VA, Perov JuM, Hastenko VP. The pelotherapy. Eco Nedra, Moscow (In Russian) 2005.
[33] Potpara Z, Duborija-Kovacevic N. Effects of the peloid cream from the Montenegrin Adriatic coast on skin humidity, transepidermal water loss and erythema index, examined with skin bioengineering *in vivo* methods. Farmacia 2012; 60: 524-34.
[34] Vašková J, Veliká B, Pilátová M, Kron I, Vaško L. Effects of humic acids *in vitro*. *In Vitro* Cell Dev Biol Anim 2011; 47: 376-82.
[35] Smirnova OV, Efimova IV, Khil'ko SL. Antioxidant and pro-oxidant activity of ascorbic and humic acids in radical-chain oxidation processes. Rus J Appl Chem 2012; 85: 252-5.
[36] Efimova IV, Khil`ko SL, Smirnova OV. Antioxidant activity of humic acids in radical chain oxidation processes. Rus J Appl Chem 2012; 85: 1351-4.
[37] Quintela A, Terroso D, Ferreira Da Silva E, Rocha F. Certification and quality criteria of peloids used for therapeutic purposes. Clay Minerals 2012, 47: 441-51.

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