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The golden root, *Rhodiola rosea*, prolongs lifespan but decreases oxidative stress resistance in yeast *Saccharomyces cerevisiae*

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ABSTRACT

The effect of aqueous extract from *R. rosea* root on lifespan and the activity of antioxidant enzymes in budding yeast *Saccharomyces cerevisiae* have been studied. The supplementation of the growth medium with *R. rosea* extract decreased survival of exponentially growing *S. cerevisiae* cells under H_2O_2-induced oxidative stress, but increased viability and reproduction success of yeast cells in stationary phase. The extract did not significantly affect catalase activity and decreased SOD activity in chronologically aged yeast population. These results suggest that *R. rosea* acts as a stressor for *S. cerevisiae* cells, what sensitizes yeast cells to oxidative stress at exponential phase, but induces adaptation in stationary phase cells demonstrating the positive effect on yeast survival without activation of major antioxidant enzymes.

Introduction

Rhodiola rosea (also known as golden root or roseroot) is a perennial plant of the *Crassulaceae* family that grows in the Arctic and in the mountainous regions of Europe, Asia, and North America. The rhizome and roots of this plant have been long used in traditional medicine in Eastern Europe and Asia for enhancing the human physical and mental performance. Studies with cell cultures, animals, and humans have demonstrated many health-promoting effects of *R. rosea*, including antidepressant, anticancer, antioxidant, cardioprotective activities, and central nervous system performance (Brown et al. 2002). The *R. rosea* root was found to contain compounds known as adaptogens that allow an organism to counteract adverse physical, chemical, and biological stressors increasing non-specific resistance (Kelly 2001). Since an increase in stress resistance has frequently been reported to coincide with extended lifespan in variety of organisms (Longo 1999; Lithgow and Walker 2002), the involvement of plant adaptogens in the prevention of age-associated diseases and the deceleration of senescence have been studied intensively. In recent studies, it was shown that *R. rosea* extracts were able to extend the lifespan of fruit fly *Drosophila melanogaster* (Jafari et al. 2007; Lushchak et al. unpublished data) and nematode *Caenorabdidis elegans* (Wiegant et al. 2008) in a dose-dependent manner.

The mechanisms, in which *R. rosea* increases stress resistance are still unknown, although several studies have shown an involvement of pathways leading to synthesis of heat shock proteins as well as a development of oxidative stress resistance (Boon-Niermeijer et al., 2000; Wiegant et al., 2008; Panossian et al. 2009). In this work, we have investigated whether aqueous extracts from *R. rosea* root could extend the lifespan of the baker's yeast *Saccharomyces cerevisiae*, which, due to its easy genetic manipulation, sequenced genome, and short generation time, is widely used as a model system to study many aspects of eukaryotic cell biology (Longo 1999; Costa and Moradas-Ferreira 2001). Two types of aging have been described in *S. cerevisiae* cells. Individual yeast cells have limited number of divisions. An age-dependent decrease in number of cell divisions is defined as replicative aging. The second type of aging, termed chronological aging, is attributed for yeast populations. Chronological lifespan is defined as the ability of stationary phase cultures to maintain viability over time in a non-dividing state. It has been considered that stationary phase yeast cells are good model system for aging of somatic cells of higher eukaryotic organisms, because they are postmitotic cells and rely on mitochondrial respiration to maintain viability. A variety of experimental data suggests a key role of antioxidant defense in the extension of chronological lifespan in non-dividing yeast cells. Thus, it was shown, that deletion of *SOD1* (encoding cytoplasmic superoxide dismutase) or both *SOD1* and *SOD2* (encoding mitochondrial superoxide dismutase) dramatically reduced chronological lifespan in *S. cerevisiae* (Longo et al. 1996). On the contrary, overexpression of *SOD1*, *SOD2* and *CTT1* (cytosolic catalase T) at various combinations increased *S. cerevisiae* lifespan (Fabrizio et al. 2003). Taking these data into account, we examined the effect of *R. rosea* root aqueous extracts on stationary phase survival and antioxidant enzyme activities in *S. cerevisiae* cells. For the estimation of potential

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stress-protective effects of *R. rosea*, the resistance to hydrogen peroxide in exponential phase yeast cells, which were grown without and in the presence of roseroot extracts, was compared.

Materials and methods

Strains, growth conditions, and stationary phase survival

The *S. cerevisiae* strain YPH250 (*MAT***a** *trp1-Δ1 his3-Δ200 lys2-801 leu2-Δ1 ade2-101 ura3-52*) used in this study was kindly provided by Dr. Youshiharu Inoue (Kyoto University, Japan). Overnight cultures were grown in YPD medium (1% w/v yeast extract, 2% w/v peptone, 2% w/v glucose), inoculated into flasks with the same medium supplemented with aqueous extracts from *R. rosea* root to final concentration about 0.3×10^6 cells ml^{-1}, and grown at 28 °C with shaking at 175 rpm.

To determine the number of viable yeast cells, starting at day 3, aliquots were removed from each flask, serially diluted and plated. The viability was defined as the ability of an individual yeast cell to reproduce and form a colony within 72 h (colony forming unit, or CFU) on YPD agar plates (Fabrizio and Longo 2003). The amount of dead cells in yeast culture was measured by methylene blue staining (Smart et al. 1999).

Preparation of plant aqueous extracts, and determination of salidroside

Commercial preparations of dried roots of *R. rosea*, collected in Ukrainian Carpathians, were used in the work. The herbal raw materials were comminuted to obtain particle fraction of 1–2 mm in size. The aqueous extraction was performed on the boiled water-bath in the ratio of 1:20 (herbal dried crushed rhizome material:distilled water) for 30 min. All liquid extracts were filtered, sterilized by boiling for 20 min, and kept at 4 °C for 2 days. The content of saliroside in aqueous extracts and in dried rhizome material was estimated spectrophotometrically at 486 nm according to the method approved by the Soviet Pharmacopoeia (NPhUSSR 1990).

H_2O_2 treatment

Exponential-phase growing cells were harvested and resuspended in an equal volume of 50 mM potassium phosphate buffer (pH 7.0). Aliquots of the experimental cultures were exposed to different concentrations of hydrogen peroxide during 1 h at 28 °C. Cell survival after hydrogen peroxide exposure was monitored by measuring of colony-forming units' number as described.

Preparation of cell-free extracts and assay of enzyme activities

Cell extracts were prepared by vortexing yeast cells with glass beads (0.5 mm), as described previously (Lushchak et al. 2005). The activity of superoxide dismutase (SOD) was assayed at 406 nm as the inhibition of quercetin oxidation by superoxide anion (Lushchak et al. 2005). Dismutation of hydrogen peroxide by catalase was measured at 240 nm using an extinction coefficient for hydrogen peroxide of 39.4 M^{-1} cm^{-1} (Lushchak et al. 2005).

Polyacrylamide gel electrophoresis

The native polyacrylamide gel electrophoresis (PAGE) was carried out by the method of Davis (1964). Superoxide dismutase isoenzymes were detected on the gels according to Beauchamp and Fridovich (1971). Catalase activity was visualized by incubating the gels in 0.003% H_2O_2 for 15 min at room temperature, followed by treatment with solution, containing 2% (w/v) $FeCl_3$ and 2% (w/v) $K_3Fe[CN]_6$ (Woodbury and Spencer 1971). Relative band intensities were estimated, measuring density with TotalLab Quant Software.

Protein concentration and statistical analysis

Protein concentration was determined by the Coomassie brilliant blue G-250 dye-binding method (Bradford 1976) with bovine serum albumin as a standard. Experimental data are expressed as mean ± SEM. For statistical analysis of data, Student's *t*-test, Dunnett's and Student–Newman–Keuls tests were used.

Results and discussion

Toxicity and aging

The content of salidroside, a marker compound of *R. rosea*, in dried rhizomes of *R. rosea* was 1.77 ± 0.05%. According to the Soviet Pharmacopoeia, the raw material of *R. rosea* in dried form must contain at least 0.8% of salidroside (NPhUSSR 1990). Thus, our experimental stock plant material corresponded to minimal standard requirements to *R. rosea* raw material. The concentration of salidroside in prepared aqueous extract from *R. rosea* root was 0.080 ± 0.009%, namely 1 μl of roseroot extract contained 0.8 μg of salidroside.

It is well known that many phytochemicals being beneficial at low concentrations may become toxic at higher levels (Wiegant et al. 2008). To test the toxicity of *R. rosea* extracts for *S. cerevisiae* cells, we examined yeast growth in the presence of different concentrations of *R. rosea*. The incubation media were supplemented with 2–50 μl of roseroot extract per ml medium that has given the final concentrations of salidroside 1.6–40.0 μg/ml medium. The supplementation of the medium with *R. rosea* extracts at concentrations 2–20 μl/ml medium did not affect the growth rate of *S. cerevisiae* YPH250 cells (Fig. 1), but in the presence of 50 μl/ml *R. rosea* extract yeast cells showed reduced growth at 10–48 h. In further experiments aqueous roseroot extracts were added to the culture medium at concentration of 20 μl/ml which corresponded to final concentration of salidroside of 16 μg/ml medium and demonstrated no toxicity on yeast.

For the study of anti-aging effects of *R. rosea* extracts, yeast cells were cultivated with them for 19 days. Fig. 2A demonstrates the effect of *R. rosea* extracts on the yeast viability measured by monitoring cell ability to form colonies on complete medium (CFUs). The number of viable cells which could reproduce and form colonies decreased in cultures with and without *R. rosea* extracts over time.

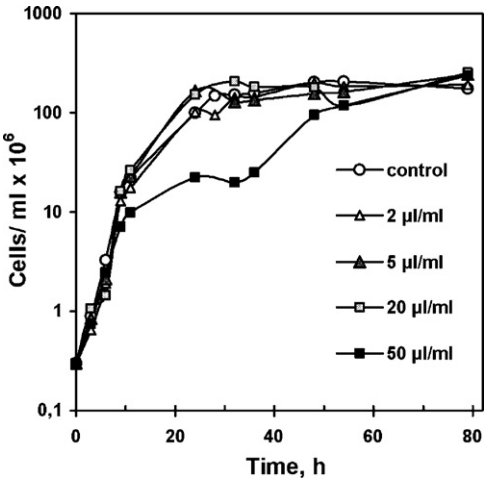

Fig. 1. Growth curves of *S. cerevisiae* incubated with different concentrations of *R. rosea* aqueous extracts.

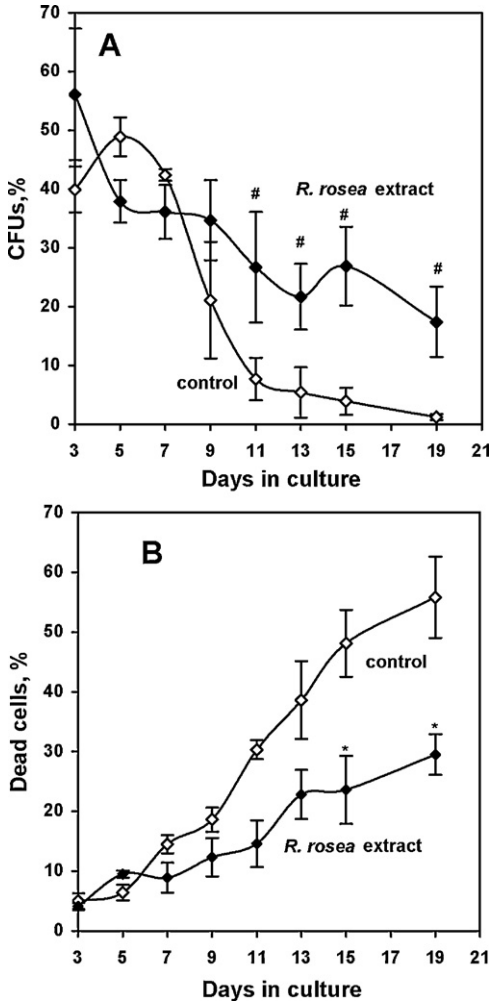

Fig. 2. Viability of *S. cerevisiae* cells in the presence of *R. rosea* aqueous extracts. Yeast viability was determined by counting the number of colony-forming units (CFUs) (A) and by staining of dead cells with methylene blue (B). CFU was calculated as the ratio between the numbers of cells forming colonies by plating to the total number of cells plated. *Significantly different from respective control values with $P < 0.05$ by Student–Newman–Keuls test. #Significantly different from respective control values with $P < 0.01$ using Student's *t*-test ($n = 3–6$).

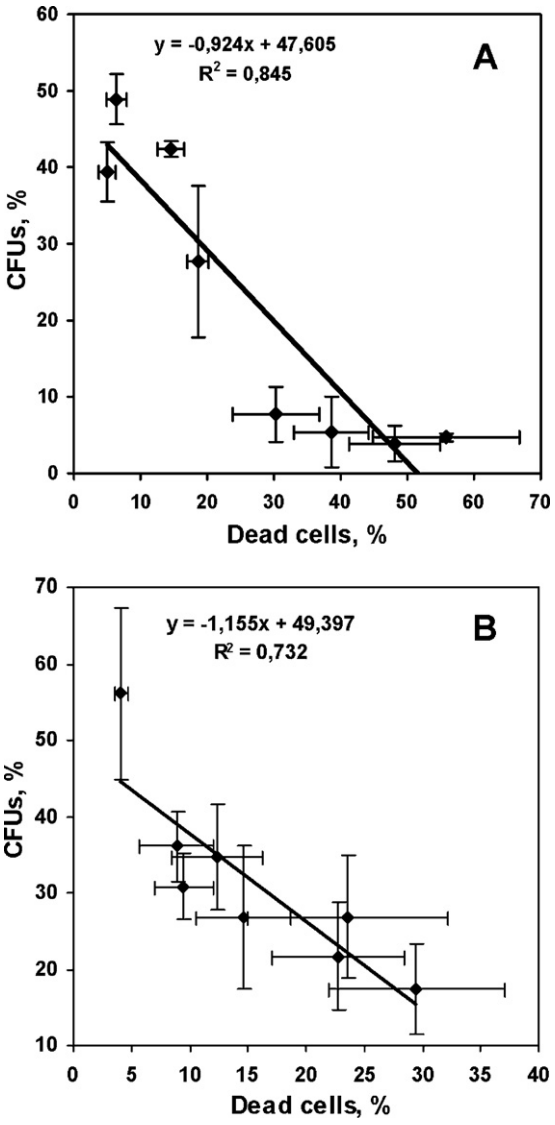

Fig. 3. Correlation analysis of data obtained with *S. cerevisiae* cells by long-term survival: between the numbers of CFUs and of dead cells in cultures grown without (A) and in the presence of *R. rosea* aqueous extracts (B). Results are shown as mean ± SEM ($n = 3–6$).

The number of reproductive active cells was higher in control cultures at day 5, but at final period of cultivation (at days 15–19) the percentage of cells which kept reproductive capability was higher by 15–20% in cultures with roseroot preparations, than that in control. The viability was also measured by counting dead cells after methylene blue staining. The age-dependent increase in the amount of dead cells in cultures was observed (Fig. 2B). The percentage of dead cells was higher in cultures grown with *R. rosea* extracts at day 5, but at days 15–19 the roseroot-supplemented cultures had 25% smaller proportion of dead cells, than control ones. It indicates that *R. rosea* extracts decrease yeast survival at first days of cultivation and have a favorable effect on yeast cell long-term viability. A strong negative correlation was found between yeast viability determined with plating assay and dead/live cells staining, i.e. between CFUs and cell death (Fig. 3). This relationship was observed in cultures grown without and with *R. rosea* extracts. It is consistent with previous studies reported that the loss of the ability to reproduction was related with yeast cell death (Fabrizio and Longo 2003). The time-dependent loss of viability was compared with the concentration of the protein released into the medium by damaged and dead cells. In agreement with measurements of cell viability, the increase in the protein concentration in the media from cultures of different ages was observed (Fig. 4). Moreover, a strong positive correlation between the protein concentration and the number of dead cells was found (Fig. 5), suggesting that protein concentration in culture medium may be used as a marker of yeast cell viability. A major increase in the protein concentration was detected in the medium taken off from control cultures at day 19 that corresponded to a significantly lover number of alive cells in these cultures (Fig. 4). However, the release of protein in cultures with *R. rosea* was ~1.7-fold lower, than in control, at final day of cultivation. It confirms the above observations which demonstrated higher yeast viability in cultures supplemented with roseroot extracts. Taken together, these results suppose that *R. rosea* extracts prevented cell death at a long-term cultivation of *S. cerevisiae* and increased the reproductive potential of aged yeast population.

Superoxide dismutase and catalase: activity and isoforms

Oxidative phosphorylation is a main source of energy to maintain the viability in yeast cells at stationary phase. Reactive oxygen

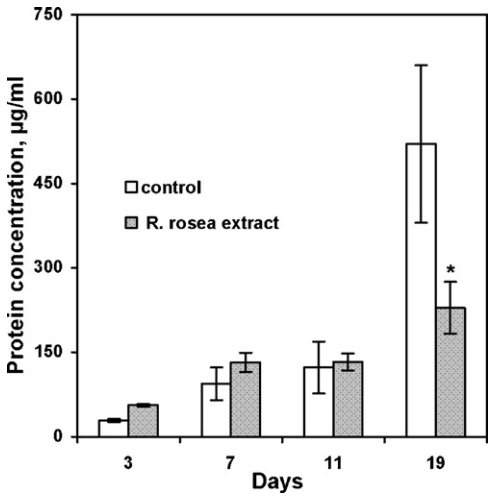

Fig. 4. Protein concentration in the medium from the yeast cultures of *S. cerevisiae* grown in control and *R. rosea* aqueous extract supplements from days 3 to 19. *Significantly different from respective control values with $P<0.05$ by Student–Newman–Keuls test ($n=3$–6).

species (ROS) produced during respiratory metabolism have been proposed to cause cumulative oxidative damage to cellular components and with depletion of antioxidant defense seem to be key factors leading to yeast cell aging and death (Jakubowski et al. 2000; Costa and Moradas-Ferreira 2001). To elucidate whether lifespan extending effect of *R. rosea* on yeast was connected with the influence on antioxidant defense, we examined the activities of SOD and catalase, which are known, to play an important role in yeast survival at prolonged cultivation (Longo et al. 1996; Fabrizio et al. 2003). Table 1 shows SOD activity of during the yeast cultivation with *R. rosea* extracts. SOD activity did not differ in cells grown without and with roseroot extracts at day 3 and was lower in cells cultivated with *R. rosea* extracts as compared to control at further cultivation. Higher SOD activity at *R. rosea* supplementation was observed at day 7, compared to ones at day 3, and this increase was higher in control cultures (2.4-fold versus 1.7-fold). The enzyme activity in control cells remained at the same level to day 11 and decreased by 1.8-fold at final day of cultivation. At the same time, in cells cultivated with *R. rosea* extracts SOD activity significantly decreased at day 11, compared to day 7, and further did not change to the end of cultivation. Using native PAGE following the quantification of SOD activity we showed that the relative level of SOD isoforms (Fig. 6A) was similar in cells from both cultures at day 7 (Mn-SOD – 25 and 22%, Cu,Zn-SOD – 75 and 78% in control cells and cells incubated with roseroot preparations, respectively). Thus the higher SOD activity in control cells, compared to cells incubated with *R. rosea* extracts, at this period was not caused by changed distribution of SOD activities between two isoforms.

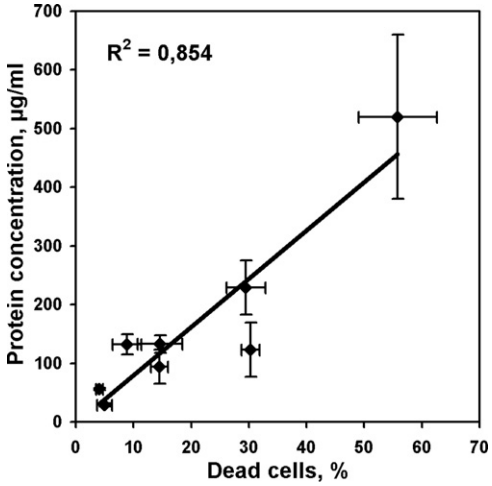

Fig. 5. Correlation between protein concentration in the medium and the number of dead cells in cultures *S. cerevisiae* grown without and in the presence of *R. rosea* aqueous extracts at days 3–19. Results are shown as mean ± SEM ($n=3$–6).

Table 1
Activities of superoxide dismutase and catalase in *S. cerevisiae* YPH250 grown in the presence of *R. rosea* aqueous extracts.

Days of cultivation	SOD (units/mg protein)		Catalase (U/mg protein)	
	Control	*R. rosea*	Control	*R. rosea*
3	87 ± 12	61 ± 10	102 ± 6	51 ± 3[#]
7	213 ± 16[a]	106 ± 9[a,#]	120 ± 16	119 ± 17[a]
11	237 ± 18[a]	73 ± 12[b,#]	95 ± 13	132 ± 13[a]
19	132 ± 21[a,c]	58 ± 8[b,#]	100 ± 20	86 ± 27

[a] Significantly different from respective values at day 3 with $P<0.05$ by Student–Newman–Keuls test.
[b] Significantly different from respective values at day 7 with $P<0.05$ by Student–Newman–Keuls test.
[c] Significantly different from respective values at day 11 with $P<0.05$ by Student–Newman–Keuls test..
[#] Significantly different from respective control values with $P<0.01$ using Student's *t*-test. Results are shown as mean ± SEM ($n=4$–6).

Superoxide dismutase is a major antioxidant enzyme in yeast stationary phase, and its both isoenzymes, are required for long-term yeast survival, protecting the cells against damaging effects of superoxide anion and, in turn, other ROS (Longo et al. 1996; Fabrizio et al. 2003). The production of ROS has been shown to increase during prolonged incubation of stationary cultures of *S. cerevisiae* (Jakubowski et al. 2000). Therefore, the observed increase in SOD activity at first 7 days of yeast stationary phase may be considered as adaptive response to enhanced oxidative danger. The decrease in SOD activity at late stages of cultivation can indicate that there is the increased exposure of macromolecules to ROS. In this case,

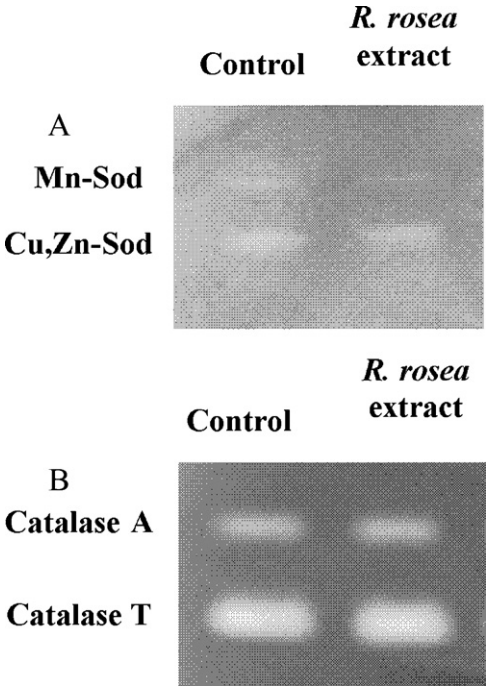

Fig. 6. Native PAGE electrophoresis of SOD (A) and catalase (B) activity in *S. cerevisiae* cells grown during 7 days without and in the presence of *R. rosea* aqueous extracts. An amount of total protein applied to each well was 50 µg and 10 µg for SOD and catalase, respectively.

SOD itself could become a target for ROS attack. It is known, that SOD is inactivated by exposure to hydrogen peroxide, especially high sensitivity to oxidation was demonstrated for its cytosolic isoenzyme – Cu,Zn-SOD (Costa et al. 2002). However, an alternative explanation that the decreased activity of SOD is due to decreased protein turnover or protein synthesis in stationary cultures cannot be excluded. Decrease in SOD activity during long-term incubation may promote oxidative damage, resulting in a loss of viability. It is confirmed by studies reported age-dependent inactivation of mitochondrial aconitase which is the primary target of superoxide and may contribute to aging and death in yeast (Fabrizio et al. 2003).

In the case of catalase, the activity of this enzyme in control cells was unchanged during all periods of cultivation (Table 1). In the cells, grown with *R. rosea* extract the catalase activity was lower by 50% at day 3 but did not differ from controls during next days of cultivation. The PAGE also demonstrated no differences in relative level of catalase isoenzymes in cells, cultivated with and without roseroot preparations at 7 day (Fig. 6B) (catalase A – 29 and 28%, catalase T – 71 and 72% in control cells and cells grown with *R. rosea* extracts, respectively).

The obtained results suggest that an elevation of activity of the major antioxidant enzymes did not require for life-extending action of *R. rosea* in *S. cerevisiae* cells, and moreover the decrease in SOD activity was observed. Recently similar results demonstrating protective role *R. rosea* on human cells against oxidative stress without activation of antioxidant defences were reported (Schriner et al. 2009). It cannot be excluded, that *R. rosea* may act in other manner, such as providing DNA stability or via interaction with mediators of signaling pathways that provide stress resistance by mechanisms including not only antioxidant enzymatic defences. That fact, that in cultures with *R. rosea* extracts the number of dead cells increased and the CFUs' number decreased at first days of cultivation compared to control, suggest that initially *R. rosea* may act as mild stressor, causing observed effects. An adaptation to *R. rosea* results in increase in CFUs and yeast survival (on days 8–19). In animal studies, some mechanisms of stress-protective activity of *R. rosea* has been identified, such as regulation of molecular chaperon synthesis (Panossian et al. 2009) and interaction with insulin/IGF-1-like/daf-2 pathway by activating DAF-16, a FOXO-family transcription factor, that influences the synthesis of proteins involved in stress resistance and thereby declines the rate of aging (Wiegant et al., 2008). In *C. elegans*, among the genes regulated by-he daf-2 pathway are mitochondrial Mn-SOD and several heat shock proteins (Cherkasova et al. 2000). In *S. cerevisiae* Ras2/Cyr1/cAMP/PKA and the Sch9 pathways are similar to that identified in worms and regulate stress resistance and longevity by modulating activity of transcription factors Msn2/Msn4, which induces the expression of similar proteins (heat shock proteins, catalase, Mn-SOD and other maintenance proteins) (Fabrizio and Longo 2003). Msn2/Msn4p are considered as components of general stress response and they will be activated under different stress conditions (Costa and Moradas-Ferreira 2001). Therefore it may be supposed, that in our experiments *R. rosea* extract may act, at least partly, due to interaction with Msn2/Msn4 transcription factors inducing synthesis of protective proteins. We did not show the difference in activity of catalase and detected the decrease in SOD activity in yeast treated with aqueous *R. rosea* extract. It can indicate that *R. rosea* influences other, than antioxidant, cell defense mechanisms, such as a level of the heat shock proteins, analogically to worms, or may prevent the depletion of glutathione, a low-molecular mass antioxidant, which play an important role in yeast survival at stationary phase (Stephen and Jamieson 1996), as it was shown in cell cultures (Calcabrini et al. 2010) and mice (Kim et al. 2006). In this case the increase in activity of antioxidant enzymes in *R. rosea* extract treated yeast is not necessary, and the modulation activity of the other protective components may be sufficient to provide stress resistance and yeast longevity during the definite time. However the weakness of defense mechanisms and accumulation of damaged molecules at late stages of cultivation, as was mentioned above, led to decrease in yeast survival as in control as in roseroot treated cultures.

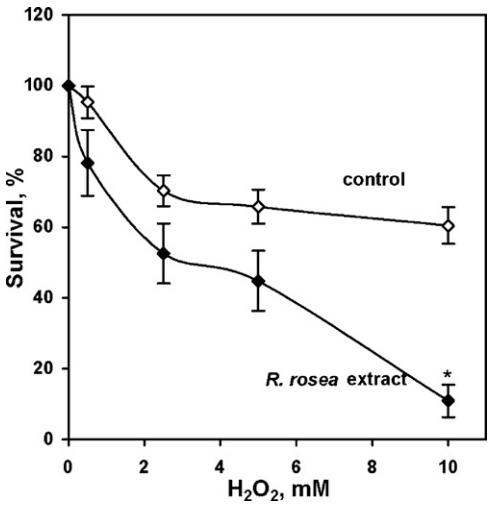

Fig. 7. Survival of *S. cerevisiae* cells grown with *R. rosea* aqueous extracts to middle exponential phase under treatment with different H_2O_2 concentrations for 1 h ($n = 4–6$). *Significantly different from respective control values with $P < 0.01$ using Student's *t*-test.

Susceptibility to hydrogen peroxide

One of the major properties of plant adaptogens is that they have been shown to increase resistance to different stresses in model organisms (Boon-Niermeijer et al. 2000; Wiegant et al. 2008). Therefore, we addressed the question whether extracts from *R. rosea* root were able to enhance resistance against oxidative stress in *S. cerevisiae* cells. Oxidative stress was induced in exponential-phase yeast cells by hydrogen peroxide. Yeast cells grown in the presence of *R. rosea* aqueous extracts showed greater susceptibility to H_2O_2 compared to control (Fig. 7). After treatment with 10.0 mM H_2O_2 the viability of the cells grown without and in the presence of *R. rosea* extracts amounted to 11% and 55% of the untreated cell viability (without H_2O_2), respectively.

Such antioxidant enzymes as catalase and SOD play a critical role in protection of *S. cerevisiae* against damaging effects of H_2O_2. It was previously reported that biosynthesis and activities of catalase and SOD in *S. cerevisiae* were elevated upon cell exposure to low concentrations of hydrogen peroxide (Godon et al. 1998; Bayliak et al. 2006). Therefore, in the present study we investigated also the effect of *R. rosea* extracts on the cell response to H_2O_2-induced stress. Data presented in Table 2 demonstrate that the activity of catalase in YPH250 cells grown with *R. rosea* aqueous extracts did not differ, while the activity of SOD was by 60% lower, than in control cells. Exposure to 0.5 and 2.5 mM H_2O_2 enhanced SOD activity by 1.4-fold in cells grown with roseroot preparations and in controls, but there was no marked difference in the activity between cells untreated and incubated at 10.0 mM H_2O_2. At the same time, 0.5 mM hydrogen peroxide increased catalase activity by 1.3-fold in control cells, but did not change it in cells cultivated with *R. rosea* extracts. Higher concentrations of hydrogen peroxide resulted in the decrease in catalase activity. A degree of enzyme activity decline was higher in cells cultivated in the presence of *R. rosea* extracts. Thus, hydrogen peroxide induced weaker adaptive response in cells grown with *R. rosea* extracts, compared to control that was in parallel with lower survival of these cells under H_2O_2-exposure. When we plotted the activity of SOD against

Table 2
Activities of superoxide dismutase and catalase in *S. cerevisiae* YPH250 cells grown with *R. rosea* aqueous extracts to middle exponential phase under treatment with various H_2O_2 concentrations for 1 h.

H_2O_2 (mM)	SOD (units/mg protein)		Catalase (U/mg protein)	
	Control	*R. rosea*	Control	*R. rosea*
0	182 ± 15	111 ± 10*	7.48 ± 0.47	6.80 ± 0.82
0.5	255 ± 24[a]	163 ± 22*,[a]	10.2 ± 0.58[a]	7.58 ± 0.45*
2.5	255 ± 22[a]	173 ± 10*,[a]	9.28 ± 0.95	3.68 ± 1.26*,[a]
5.0	225 ± 30	128 ± 24*	7.23 ± 0.68	4.03 ± 0.95*,[a]
10.0	170 ± 14	94 ± 11*	6.05 ± 0.34[a]	3.43 ± 0.86*,[a]

* Significantly different from respective control values with $P < 0.05$ using Student's *t*-test.

[a] Significantly different from respective values for untreated cells (without H_2O_2) with $P < 0.05$ by using Dunnet's test for multiple comparisons. Results are shown as mean ± SEM ($n = 4–6$).

catalase activity, a positive correlation between catalase and SOD activity was found in control cells treated with different H_2O_2 concentrations (Fig. 8), but not in cells grown with *R. rosea* extracts (not shown). A similar relationship between the two enzymes was found in our previous studies (Lushchak et al. 2005; Bayliak et al. 2006). It suggests the coordinated action of these enzymes under oxidative challenge, but in some cases, as in the case of cells cultivated with roseroot extracts, this relationship is, probably, more complicated and can be realized not only at the level of enzyme activities. The concentration-dependent changes in the activities of antioxidant enzymes under H_2O_2-exposure may reflect a balance between processes of their activation and inactivation that depend on concentration of ROS in the cell. The lower activity of antioxidant enzymes together with higher sensitivity of yeast cells grown in roseroot cultures to H_2O_2 may suggest that *R. rosea* act as a relatively strong stressor in actively reproducing cells and under an additional exposure by H_2O_2 cell defense mechanisms are not capable to cope with both challenges that leads to cell death. Similar results were shown in cell cultures studies reported that *R. rosea* extract at high doses facilitated the toxic action of oxidants (Schriner et al. 2009). Nevertheless, it cannot be ruled out, that *R. rosea* extracts can act through suppression of gene expression or synthesis of protective enzymes.

In summary, our data show that extracts from *R. rosea* root exhibit geroprotective action in *S. cerevisiae*, being able to prevent viability and reproduction decline of yeast cells in stationary phase. Extracts of *R. rosea* at concentrations extending yeast lifespan did not demonstrate protective effects against oxidative stress induced by H_2O_2 in *S. cerevisiae* at exponential phase and significantly increased sensitivity of exponentially growing cells to this oxidant. Low concentrations of hydrogen peroxide induced a weaker adaptive response and were already toxic for cells, growing in the presence of *R. rosea* extracts, in contrast to control cells. These observations suggest that in exponential-phase yeast cells *R. rosea* acts as stressor which did not provide pre-adaptation to next oxidative challenges. The extracts did not effect on catalase activity and decreased SOD activity in chronologically aged yeast cells. These effects, probably, are not connected with positive influence of *R. rosea* on *S. cerevisiae* lifespan. The opposite effects of *R. rosea* extracts on stress resistance and lifespan of the yeast may suggest that beneficial doses for "young" exponentially growing cells and "old" non-dividing yeast cells in stationary phase are different. Taking into account that exponential phase yeast culture is somewhat relevant to proliferating cells of metazoas, whereas stationary phase – differentiated cells (Longo 1999), and received here date may expect that *R. rosea* preparations may be especially efficient for protection of the cells of nervous system.

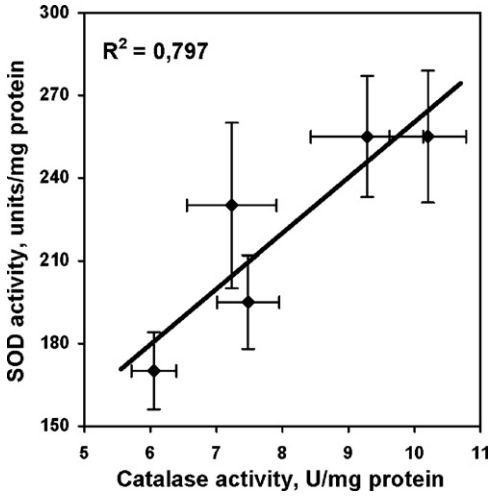

Fig. 8. Correlation between SOD and catalase activities in *S. cerevisiae* exponential cells in control cultures treated with different H_2O_2 concentrations for 1 h ($n = 4–6$).

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