Phytomedicine 18 (2011) 1262–1268

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

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

Maria M. Bayliak, Volodymyr I. Lushchak*

Department of Biochemistry and Biotechnology, Vassyl Stefanyk Precarpathian National University, 57 Shevchenko Str., Ivano-Frankivsk 76025, Ukraine

ARTICLE INFO

Keywords:
Saccharomyces cerevisiae
Rhodiola rosea
Lifespan
Hydrogen peroxide
Antioxidant enzymes

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

* Corresponding author. Tel.: +380 342 714683; fax: +380 342 714683.
E-mail address: lushchak@pu.if.ua (V.I. Lushchak).

0944-7113/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2011.06.010

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.

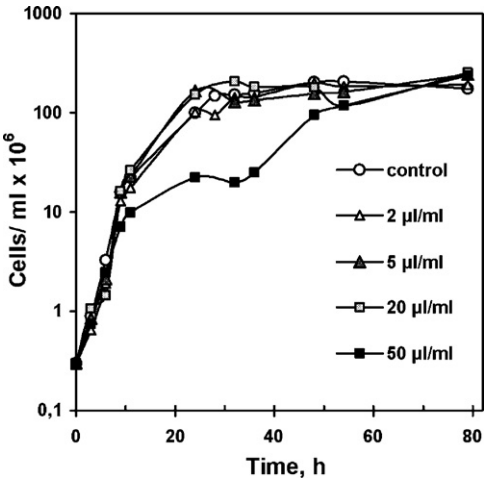

Fig. 1. Growth curves of *S. cerevisiae* incubated with different concentrations of *R. rosea* aqueous extracts.