Effect of DMSO on metabolic activity of *Saccharomyces cerevisiae* cells

Agata Święciło

Department of Environmental Microbiology, University of Life Sciences in Lublin, Leszczyńskiego 7, 20-069 Lublin, Poland, agata.swiecilo@up.Lublin.pl

Introduction

Dimethyl sulfoxide (Me_2SO; commonly referred to as DMSO) is a natural compound present in low nanomolar concentrations in a variety of environments, mainly in water, but also in the atmosphere or in sediments. It is also produced anthropogenically in large amounts and used on a large scale in various branches of industry. As a polar aprotic solvent which dissolves both polar and apolar compounds, with an exceptional ability to penetrate biological membranes and cellular barriers, DMSO is often exploited as a carrier in research in the fields of biochemistry and cell biology. DMSO itself has various therapeutic and pharmaceutical properties, such as anti-inflammatory, radioprotective, and local and systemic analgesic, and anticancer properties. In scientific research and in cryosurgery, DMSO is used as a cryoprotectant for storing various types of cells, tissues, and cell- and tissue-based products for long periods of time at low temperatures.

The aim of the study was to determine the effects of short-term (one-hour) exposure to DMSO at high concentrations characteristic of the anthropogenic environment (2-20% v/v/) on *S. cerevisiae* yeast. The effects of short-term exposure to DMSO on the biochemical and physiological parameters of yeast cells were studied. In particular, such parameters as metabolic activity, functionality of cytoplasmic membranes and their ability to proliferate were analyzed, as well as whether this xenobiotic is able to modulate the genetic and metabolic activity of the cell (i.e. to activate the environmental stress response (ESR) programme and induce oxidative stress).

Materials and Methods

The following yeast strains were used:

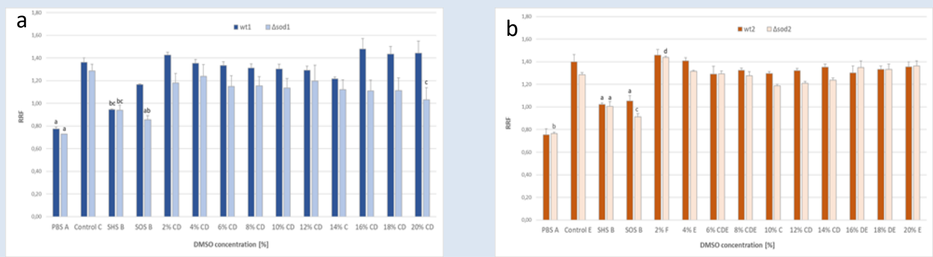
Name & Number	Strain symbol	Characteristic
SP4	wt1	a wild-type strain
DSCD1-1C	Δ*sod1*	a mutant lacking cytoplasmic superoxide dismutase (Cu,ZnSOD) activity
EG103	wt2	a wild-type strain
EG110	Δ*sod2*	a mutant lacking mitochondrial superoxide dismutase (MnSOD) activity
By4741	wt3	a wild-type strain
Δmsn2	Δ*msn2*	a mutant lacking transcription factors (Msn2p) which activate ESR programme
Δmsn4	Δ*msn4*	a mutant lacking transcription factors (Msn4p) which activate ESR programme
Δmsn2msn4	Δ*msn2msn4*	a mutant lacking both (Msn2p, Msn4p) transcription factors

Yeast cells were incubated in liquid YPD medium at 22°C for one hour with various concentrations of DMSO (0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% v/v), after which the yeast were centrifuged at 2,000 x g for 5 min at 25 C. The supernatant was discarded, and the sediment containing yeast cells was re-suspended in PBS (0.1 M, pH = 7.4) buffer in such quantity as to obtain approximately 1–5 x 10^7 cell/mL, which were examined for metabolic activity (using the resazurin reduction assay), survival rate (as a marker of their proliferative capacity), and catalase T activity (as an indicator of general stress response). In addition, the electrical conductivity (EC) of the yeast environment test (as a marker of cell membrane damage) and production of superoxide radicals were performed.

The results were statistically analysed using Statistica ver. 13.3 software (StatSoft, Kraków, Poland). A multivariate analysis of variance (ANOVA) was carried out at the α = 0.05 level of significance to determine significant differences between the biochemical and physiological parameters between untreated and DMSO-treated samples.

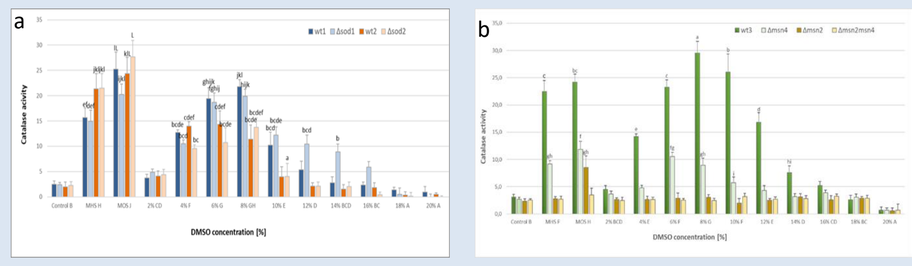
Results

Fig 1. Metabolic activity of yeast cells in the presence of DMSO (a) for cells of wt1 and Δ*sod1* strains; (b) for cells of wt2 and Δ*sod2* strains.



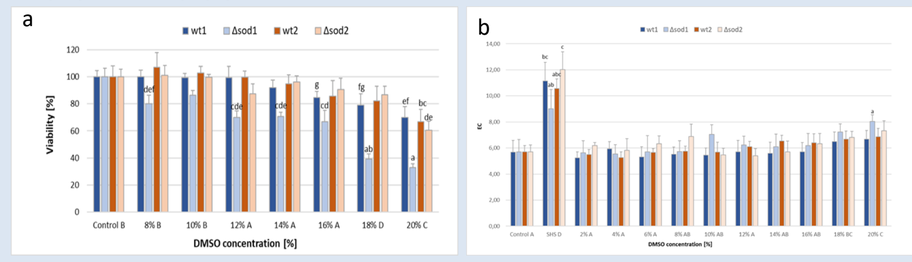
Explanations: wt1 – wild-type strain SP4, Δ*sod1* – its isogenic mutant lacking cytoplasmic dismutase activity, wt2 – wild-type strain EG 103, Δ*sod2* – mutant isogenic to wt2 lacking mitochondrial superoxide dismutase activity, PBS – phosphate-buffered saline, Control – cells not exposed to DMSO and any other stress factor, SHS – cells subjected to strong heat stress (48°C for 30 min), SOS – cells subjected to strong oxidative stress (0.75% H_2O_2 for one hour), RRF – resazurin reduction factor

Fig 2. Catalase activity of yeast cells in the presence of DMSO (a) for cells of wt1, wt2, Δ*sod1*, Δ*sod2* strains; (b) for cells of wt3, Δ*msn4*, Δ*msn2* and Δ*msn2msn4* strains.



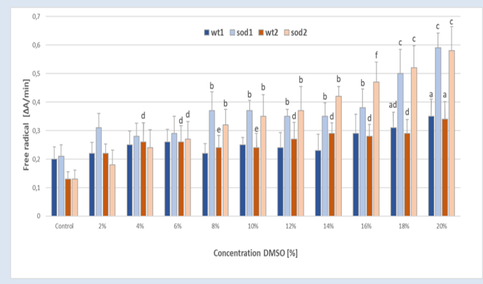
Explanations: Control, wt1, wt2, Δ*sod1*, Δ*sod2*, as in Figure 1, wt3 - wild-type strain BY4741; Δ*msn2*, Δ*msn4*, Δ*msn2msn4* - its isogenic mutants lacking Msn2p, Msn4p or both transcription factors activating the general stress response, MHS – cells subjected to mild heat stress (37°C for 30 min), MOS – cells subjected to mild osmotic stress (0,3 M NaCL for one hour)

Fig 3. Effect of DMSO on the viability (a) of yeast cells and membrane stability (EC test) (b)



Explanations: Control, wt1, wt2, Δ*sod1*, Δ*sod2*, as in Figure 1.

Fig 4. Superoxide radical anions level in yeast cells exposed to DMSO.



Explanations: Control, wt1, wt2, Δ*sod1*, Δ*sod2*, as in Figure 1.

Conclusions

1. Short-term exposure to DMSO even at a wide range of concentrations (2–20%) had little effect on the metabolic activity of the yeast cells and the stability of their cell membranes, but induced free radicals and clearly altered their proliferative activity
2. Cells of the Δ*sod1* mutant showed greater sensitivity to DMSO in these conditions
3. DMSO at concentrations from 4 to 10–14% (depending on the strain and genetic background) activated the ESR programme, which determines resistance to various types of environmental factors
4. The obtained results indicate that DMSO, despite being considered a neutral and safe compound for cellular systems, may induce harmful effects in yeast cells with a reduced level of antioxidant defense and is capable of activating universal defense mechanisms (ESR), which may be an indicator of its toxic potential.