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EFFECT OF PESTICIDE PREPARATIONS AND INDOLEACETIC ACID ON YEAST *Saccharomyces cerevisiae* CELLS

ODDZIAŁYWANIE PREPARATÓW PESTYCYDOWYCH ORAZ HETEROAUKSYNY NA KOMÓRKI DROŻDŻY *Saccharomyces cerevisiae*

Abstract: Yeast are often used as a model organism in studies on non-specific effects of xenobiotics. This paper investigates susceptibility of yeast cells of different strains differing in effectiveness of antioxidant system on commercial preparations Betokson Super 025 SL and Fusilade Forte 150 EC which are used as pesticides, and on phytohormone indoleacetic acid (IAA). The experiment was carried out on a wild strain of yeast and mutant *sod1* devoid of cytosolic superoxide dismutase activity, *ctt1cta1* with no catalase activity, and also the mutant C4 with a low level of glutathione. Susceptibility of the cells was estimated upon their ability to grow on solid medium that contained the investigated substances. The results suggest that the enzyme superoxide dismutase plays an important role in protection of yeast cells against the effects of active substances in preparations Fusilade Forte 150 EC, Betokson Super 025 SL and IAA.

Keywords: *Saccharomyces cerevisiae*, antioxidative status, β-naphthoxyacetic acid, fluazifop-*p*-butyl, IAA

Different methodological approaches are used to study of non-specific effects of plant pesticides. One approach focuses on such aspects as assessment of the activity of different physiological groups of microorganisms, which occur in soil environment. Another approach consists in studying the reaction of laboratory strains of microorganisms. This study presents the effect of selected products used in crop protection and phytohormone indoleacetic acid (IAA) on yeast cells *Saccharomyces cerevisiae*. For years they have been used as a model organism in the study of non-specific effect of xenobiotics, such as pesticides, heavy metals and PAHs [1–4]. Contrary to the higher eukaryotes yeast cells tolerate numerous mutations which lead to the change in the activity of their antioxidative system. The *sod1* mutants are devoid of cytoplasmic activity of superoxide dismutase, ie an enzyme which removes superoxide anion-radical

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from cytoplasmic space. The cells of *ctt1cta1* mutant are devoid of cytoplasmic activity of T catalase and peroxysomal A catalase, ie enzymes which remove hydrogen peroxide from these cell compartments. The mutant marked with the symbol C4 is distinguished by a level of glutathione (GSH) lowered by 85 % as compared with the level of this tripeptide in isogenic cells of the wild strain. The reaction of these mutants to different environmental factors has already been extensively described [5–8]. They are also used for the purpose of determining the role of the particular elements of the antioxidative system in the mechanisms of toxicity of studied substances. The aim of the presented study was the assessment of the sensitivity of yeast cells with changed efficiency of antioxidative system to such products as Betokson Super 025 SL and Fusilade Forte 150 EC (containing: β-naphthoxyacetic acid (NOA) and fluazipof-*p*-butyl as active substances, respectively) as well as indoleacetic acid IAA.

Material and methods

The following strains of *Saccharomyces cerevisiae* were used in the experiment:

The SP4 wild strain (wt) of genotype Mat α leu1 arg4, the DSCD1-1C strain devoid of the cytoplasmic activity of superoxide dismutase of genotype Mat α leu1 arg4 *sod1*, the A50 strain of genotype Mat α leu1 arg4 *ctt1 cta1*, completely devoid of catalase activitity and the C4 strain of genotype Mat α leu1 arg4 *als1* which had a low level of glutathione.

The yeast cells of the above-mentioned strains were grown in liquid medium YPD containing peptone (1 %), yeast extract (1 %) and glucose (2 %) in order to obtain a culture of cells at early stationary stage of growth. The culture was diluted so as to obtain a suspension of the cells of each strain of the same density. Drops of 5 mm^3 (μl) of the suspension of cells whose density was 3×10^5, 3×10^4, 3×10^3 and 3×10^2 were used to inoculate solid media containing different concentrations of the preparations under study. The yeast was grown in an incubator for three days in the dark at 28 ^oC. Then the features of their merged and colony growth were assessed. Noticeable differences in the growth of these cells (poor growth, lack of growth) were accepted as a criterion of their sensitivity. The experiments were documented by means of photographs.

Pesticide preparations, ie Betokson Super 025 SL, Fusilade Forte 150 EC and stock solution of IAA in concentration of 10 mM (in DMSO) were diluted with sterile distilled water and added to the liquidated solid medium in such amounts as to obtain the concentrations of active substances (0.05, 0.1, 0.15, 0.2, 0.3, 0.5 $mg \cdot cm^{-3}$).

The experiment was repeated three times in order to verify the repeatability of the obtained results.

Results and discussion

The sensitivity of cells to the active substances of the preparations under study was determined by the assessment of the growth of cells on solid medium in the presence of these substances. Such conditions of culture provide a possibility for assessment of long-term effects of these substances on yeast cells.

Table 1

Sensitivity of yeast cells of studied strains to IAA, NOA and fluazifop-*p*-butyl

Concentrations of active substances [mg · cm^{-3}]	*wt*	*sod1*	*ctt1cta1*	Glutathione-poor mutant
0 (control)	+	+	+	+
IAA				
0.15	+	+	+	+
0.2	+	+/—	+	+
0.25	+	+/—	+	+
0.3	+	+/—	+	+
0.5	+	+/—	+	+
NOA				
0.1	+	+	+	+
0.15	+	+	+	+
0.2	+	+	+	+
0.3	+	+	+	+
0.5	+	+/—	+	+
Fluazifop-*p*-butyl				
0.015	+	+	+	+
0.1	+	+	+	+
0.15	+	+	+	+
0.2	+	+	+	+
0.3	+	+	+	+
0.5	+/—	—	+/—	+/—

+ – means the intensity of growth comparable to that in the control; — – means lack of growth; +/— – means poorer growth as compared with that in the control.

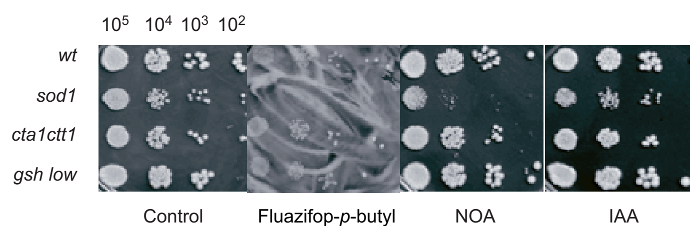

Fig. 1. Growth of yeast cells in the presence of 0.5 mg · cm^{-3} IAA, NOA and fluazifop-*p*-butyl – active substances of Betokson Super 025 SL and Fusilade Forte 150 EC preparations

After a suitable amount of emulsion of Fusilade Forte 150 EC preparation was added to YPD media, it became turbid.

Yeast cells of the wild strain and the C4 and *ctt1cta1* mutants proved to be resistant to the used concentration of indoleacetic acid. Only the cells of the *sod1* mutant manifested sensitivity to higher concentrations of this substance. These strains reacted

in a similar way to the presence of Betokson Super 025 SL preparation. In the case of Fusilade Forte 150 EC preparation clear differences in the growth of the cells of all studied strains were observed after the preparation was applied in the highest concentration ($0.5 \text{ mg} \cdot \text{cm}^{-3}$ as converted into the content of the active substance). The cells of the *sod1* mutant proved to be the most sensitive to the effect of this factor; in these conditions these cells were not able to grow at all. The growth of the cells of the remaining strains under study was very poor: there were small colonies at the place of sowing.

The obtained results suggest that IAA and the synthetic derivative of this hormone – NOA, used as a stimulator of tomato fruit setting, do not have cytostatic properties with respect to the cells of wild strains and such mutants as *ctt1cta1* and C4. According to the data presented in a study by Prusty et al [9], IAA (but not other compounds which have a similar chemical structure) may have different effect on the *Saccharomyces cerevisiae* yeast, depending on the dose used. At low concentrations it enhances both haploid invasion and diploid pseudohyphal growth of yeast cells. Such reaction provides a possibility for infection of the plant by pathogenic yeast strains. When IAA was used at high concentrations inhibition of the growth of yeast of the wild strain was observed both on liquid medium ($EC_{50} = 250\ \mu M$) and on solid medium. In view of the fact that these authors used a different method of assessment of the sensitivity of yeast cells grown on solid medium to IAA, a direct comparison of the obtained results is impossible. According to these authors, the yap-1a strain, devoid of the activity of Yap1p transcription factor which acts as an agent in reaction of yeast cells to pro-oxidative substances, manifested over sensitivity to IAA. According to the present study the *sod1* mutant, devoid of superoxide dismutase – a key enzyme of antioxidative defence, manifested the strongest reaction to IAA.

Fusilade Forte 150 EC containing fluazifop-*p*-butyl used in the highest concentration ($0.5 \text{ mg} \cdot \text{cm}^{-3}$) manifested strong cytostatic effect with respect to the cells of the wild strain and the *ctt1cta1* and C4 mutants; it had a cytotoxic effect on the *sod1* mutant. Consequently, the activity of dismutase is a significant element of the antioxidative system of yeast and it is necessary in order to protect the yeast against the toxic effect induced by this herbicide. As proved in specialist literature, the cells of the *sod1* mutant are also characterised by over sensitivity to many other environmental factors, such as the presence of sodium nitrate(III), iron salts and the atmosphere containing 100 % oxygen [6, 10, 11].

As shown by the result obtained in this study catalase activity and concentration of low-molecular antioxidant GSH play a supporting part in the system of defence of cells against cytostatic effect of Fusilade Forte 150 EC preparation. It probably results from compensatory abilities of yeast consisting in inducing alternative defence and repair mechanisms, such as a response to oxidative stress [12].

Conclusions

1. Fusilade Forte 150 EC herbicide has a cytostatic effect on the yeast cells of the wild strain the *ctt1cta1* and C4 mutants and a cytotoxic effect on the *sod1* mutant.
2. IAA and Betokson Super 025 SL have cytostatic effect only on the *sod1* mutant.

3. Activity of cytoplasmic superoxide dismutase plays a key role in the protection of yeast cells from harmful effects induced by Fusilade Forte 150 EC, Betokson Super 025 SL preparations and phytohormone IAA.

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ODDZIAŁYWANIE PREPARATÓW PESTYCYDOWYCH ORAZ HETEROAUKSYNY NA KOMÓRKI DROŻDŻY *Saccharomyces cerevisiae*

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Abstrakt: Drożdże są często wykorzystywane jako organizm modelowy w badaniach nad niespecyficznym oddziaływaniem ksenobiotyków. W przedstawionej pracy badano wrażliwość komórek drożdży szczepów różniących się sprawnością systemu antyoksydacyjnego na handlowe preparaty: Betokson Super 025 SL i Fusilade Forte 150 EC stosowane jako środki ochrony roślin oraz na fitohormon heteroauksynę IAA. Doświadczenia przeprowadzano na szczepie dzikim drożdży, mutancie *sod1* pozbawionym aktywności cytoplazmatycznej dysmutazy ponadtlenkowej, mutancie *ctt1cta1* pozbawionym całkowicie aktywności katalazowej oraz mutancie C4 o niskim poziomie glutationu. Wrażliwość tych komórek oceniano na podstawie ich zdolności do wzrostu na pożywce stałej zawierającej badane substancje. Uzyskane wyniki sugerują, że enzym dysmutaza ponadtlenkowa odgrywa ważną rolę w ochronie komórek drożdży przed szkodliwym działaniem substancji czynnych preparatów Fusilade Forte 150 EC i Betokson Super 025 SL oraz fitohormonu IAA.

Słowa kluczowe: *Saccharomyces cerevisiae*, system antyoksydacyjny, kwas β-naftoksyoctowy, fluazyfop-*p*-butylowy, auksyna