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MENADION EFFECT IN *PHAFFIA* CELLS: INDUCER OR MUTAGEN?

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The yeast strains of *Phaffia rhodozyma (Xanthophyllomycesdendrorhous)* are able to synthesize polar (astaxanthin or torularhodine) and non-polar (beta-carotene or torulene) carotenoids. One of the ways of reactive oxygen species (ROS) detoxification is realized through mechanisms of the non-fermentative systems defense of cells. That's why selective compounds are used for carotenoids induction [Johnson J. A., 2003]. Menadione (2-methyl-1,4-naphthoquinone) causes ROS generation and high accumulation of cellular ROS levels, which affects cell viability and cell morphology [Kim I. S., 2011].

The aim of this work is to explain the action of menadione in selective medium as possible inducer of carotenogenesis in *P. rhodozyma* strains or not, and to discuss its role in mutagenized yeast cells.

The yeast *P. rhodozyma* are cultivated into medium which consists (g/L) of: KH2PO4 — 1.0; $MgSO_4x7H_2O$ — 0.5; $(NH_4)_2SO_4$ — 0.9; $CaCl_2x2H_2O$ — 0.1; yeast extract — 2.0; biotin — $2x10^{-6}$. As Carbon source is added sucroseorglucose (20 g/L).Yeast biomassa recultivated in Erlenmayer flasks with work volume 10 % (200 rpm, 20 °C).

Cell viability of the yeast *P. rhodozyma* in media with 0.2 mM menadione is 0.16 %, but in lower concentrations about 0.1 mM menadione — 71.76 %, on 0.05 mM menadione — 92.75 % and 0.025 % menadione– 83.21 %. Yeast cells mitochondria are damaged by menadione. When the menadione concentration was 0.025 mM, the addition of both SOD and catalase decreased menadione-induced cytotoxicityin *S. cerevisiae* [http://nhsjs.com/2010/a-study-of-menadione-induced-cytotoxicity-reduction-in-yeast-cells-by-selected-enzymes/]. When the menadione concentrations were 0.1 mM and 0.5 mM, however, only catalase significantly decreased cytotoxicity of yeast. Actually, menadione can induce cytotoxicity in yeast cells. The menadione-induced oxidative stress can damage DNA and protein of mitochondria causing the death of cells [Nutter L. M., 1992].

Mutagen lethality of N-methyl-N›-nitro-N-nitrosoguanidine (0.67 g/LNG, 15 min) in 1-old day culture yeast 21091 *P. rhodozyma* was about 3.4 % and by ultraviolet radiation (5 min UV) forstrain 8UV *P. rhodozyma*in 2-old days cells was approximately 2.9 %.There are no colonies (yeast treatment suspension was 10^6 CFU) in selective 0.12 mM menadione mediafor both variants. Menadione, however, if consumed in too large quantity, induces oxidative stress by releasing reactive oxygen species, including hydrogenperoxide and superoxide anions. Accumulated reactive oxygen species damage mitochondrial DNA and protein eventually destroying the mitochondria and killing the cell [Nutter L. M., 1992].

From a toxicological perspective, two major mechanisms for the cytotoxic action of quinones, such as menadionein a variety of biological systems are proposed [Castro F. A. V., 2008]. The first, quinones undergo one electron reduction by enzymes such as microsomal NADPH-cytochrome P-450 reductase or mitochondrial NADH ubiquinone oxidoreductase, yielding the corresponding semiquinone radicals. Under aerobic condition, semiquinone radical participates in redox cycling to generate ROS like superoxide anion and hydrogen peroxide. The second, quinones are potent electrophiles, capable to reacting with the thiol groups of protein and glutathione (GSH). In fact, generation of GS-conjugates catalyzed by glutathione transferase isoforms with depletion of GSH has been associated with menadione-induced cytotoxicity and oxidative stress. Cellular redox homeostasis, ROS metabolism, and cellular xenobiotic detoxification (GSH transferases, GSH biosynthethic pathway) are activated by xenobiotics such as menadione. Glutathione transferases are also importantin processes such as protection against oxidative stress, regulation of gene expression or signal transduction.

Mutagenesis (NG or UV) of*Phaffia* yeast into selective menadione medium has provoked synergism of cytotoxicity as result excessive generation ROS in cells.