PHOTODYNAMIC INACTIVATION OF HARMFUL AND PATHOGENIC MICROORGANISMS

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Summary. Photodynamic treatment is based on the interaction of two absolutely non-toxic agents – photosensitizer, accumulated in the microorganism and visible light. This interaction is named photosensitization and in the presence of oxygen induces radical-based citotoxic reactions. The described phenomenon is widely used to erradicate tumors in oncology, to cure arthritis and atherosclerosis. This work has been carried out to define the possibility to use photosensitization for inactivation of pathogenic and harmful microorganisms. First data obtained show that patogenic yeasts *Saccharomyces cerevissiae* and fungi *Ulocladium oudemansii* might be effectively inactivated by this new treatment.

Keywords: photodynamic treatment, inactivation of microorganisms, food processing and safety.

FOTODINAMINĖ KENKSMINGŲ IR PATOGENIŠKŲ ORGANIZMŲ INAKTYVACIJA

Santrauka. Fotodinaminio poveikio esmę sudaro dviejų absoliučiai netoksiškų agentų – fotosensibilizatoriaus, kuris kaupiasi, pvz., mikroorganizme, ir matomos šviesos – sąveika. Ji vadinama fotosensibilizacija ir deguonies aplinkoje indukuoja radikalines citotoksines reakcijas. Šiuo reiškiniu pagrįsti metodai plačiai taikomi onkologijoje, gydant aterosklerozę arba artritus. Darbo tikslas – įvertinti fotosensibilizacijos panaudojimo galimybes inaktyvuojant patogeninius mikroorganizmus. Pirmųjų tyrimų rezultatai rodo, kad fotodinaminis poveikis, naudojant du skirtingus fotosensibilizatorius – hematoporfiriną ir protoporfiriną, gali sustabdyti tiek *Saccharomyces cerevisiae*, tiek *Ulocladium oudemansii* grybų augimą.

Raktažodžiai: fotodinaminis gydymas, mikroorganizmų inaktyvacija.

Introduction. In 1900 Raab firstly observed the death of *Paramecium caudatum* after light exposure in the presence of acridine arange. At that time it was impossible to understand the mechanism and reasons of death. Now it is worldwide accepted that this phenomenon might be named "photosensitization". In general, this treatment involves the organic dye (for instance acridine orange, methylene blue, hematoporphyrin what usually accumulates in target microorganism) and subsequent irradiation with visible light. This combination of two absolutely nontoxic elements – dye and light – in oxygenated environment induces damage and total destruction of microorganisms. This phenomenon in 1924 was applied to cure skin cancer. Afterwards in 1978 Daugherty in USA started successful application of this novel technique for treatment of different cancers. Moreover, numerous investigators demonstrated possible practical usefulness of photosensitization in the broad field of different sciences: virusology, microbiology, imunology, dermatology. It is necessary to note that microorganisms and cancer cells have some common features. One of them – high proliferation and active metabolism. Since microorganisms are able to accumulate different photosensitizers, it is believed that photodynamic inactivation of them might be really effective.

Let's run initially through the photodynamic inactivation of different patogens which usually induce different diseases in humans and animals. For instance, oral pathogens, such as *Porphyromonos gingivalis, Actinomyces viscosus* might be inactivated by photodynamic treatment (Materski et al., 2003). Human papilloma virus, which is associated with cervical, anal, vaginal intraepithelial neoplasia might be eradicated by this treatment (Stanley, 2003). In addition, representatives of wound microflora – *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Proteus mirabilis* might be killed by photodynamic action as well (Tolstykk et al., 2001). Several cutaneous microbial spiecies – *Streptococcus pyogenes, Corynebacterium minutissimum, Propionibacterium acnes, Candica albicans* are sensitive to this treatment (Zeina et al., 2001). There are data, persented by North (North et al., 1992) that blood products can be effectively decontaminated from viruses in this way.

Moreover, plethora of microorganisms, which are able to destroy different biological objects (including plants, food products and etc.) or have impact on quality of industrial food processing are identified in ourdays. The point is that majority of them are resistant to heating, uv-irradiation or antibiotic treatment. Undoubtly, that it limits effective prevention of industrial food from, for instance, fungal contamination. As a fact, fungi that cause damage of millions dollars fruit-juice branch are *Byssochlamys nivea, Neosartorya fischeri, Eupenicllium brefeldianum*. Moreover, a lot of yeast spiecies are heat-resistant and causes troubles in beer processing (*Saccharomyces carlsber gensis*). In other words, it is easy to understand, that food processing and food safety problems are not still solved and need novel and advanced approaches.

The aim of this article is to shed more light on the possible uses of this phenomenon to inactivate plethora of harmful microorganisms.

According to the first experimental data an effective and non-reversible killing effect has been observed irradiating yeasts with visible light in the presence of

photosensitizer. Data presented in Fig. 1 clearly indicate, that yeasts *Saccharomyces cerevisiae* might be inactivated by photosensitization, using two different photosensitizers: hematoporphyrin (HP) and protopophyrin (PP). By the way, in our experimental setup inactivation of yeasts was evaluated as inhibition of growth, counting the number of cells in control sample and in treated samples. Therefore, photodynamic activity of tested photosensitizers diverged: HP was about twice as much in yeasts compared to PP. Thereupon, the following study was undertaken to determine whether other representative of harmful microorganisms *Ulocladium oudemansii* might be inactivated by photodynamic treatment. Data obtained are statistically significant and indicate, that increasing concentration of photosensitizer is diminishing notably the growth of these microorganisms.Thereby it means, that a lot of different patogenic microorganisms might be inactivated in such a way.

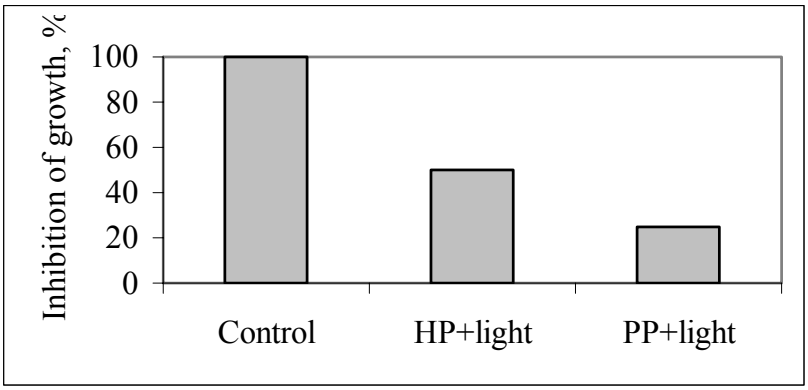

Fig. 1. **Inhibition of growth of *Saccharomyces cerevisiae* after photodynamic treatment using different photosensitizers: HP – hematoporphyrin, PP – protoporphyrin (10^{-5} M, 18 hours incubation)**

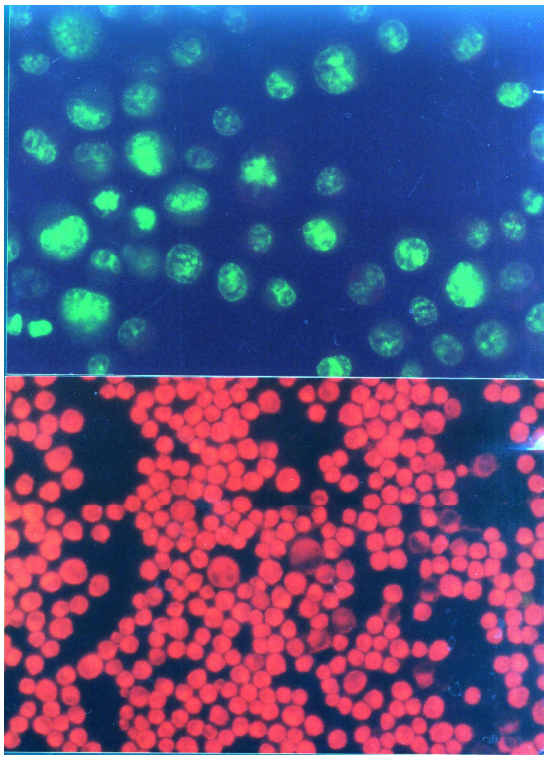

Fig. 2. **Fluorescence of Reh cells after incubation with hematoporphyrin: a - control cells with green autofluorescence, b - red fluorescence of cells, incubated with hematoporphyrin**

The other interesting approach might be to use photodynamic action as novel and effective tool to detect microorganizms. Data, presented in Fig.2 clearly show, that red fluorescence of Reh cells incubated with hematoporphyrin might be significant marker to detect living and fastly proliferating objects.

Conclusions. The first experimental evidences and results obtained in our department allow us to draw a conclusion, that photodynamic treatment might be effectively used against different pathogenic and industrially harmful microorganisms, which destroy biological objects, damage food processing and safety.

Moreover novel approach to detect microorganisms is suggested.

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