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Cell Biology

The Effect of Detergents on Active Metabolite Production by Microorganisms

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(Presented by Academy Member M. Gordeziani)

ABSTRACT. The paper presents evaluation of the effect of ionic and nonionic detergents on the well-established biodestructor $11^{5(1)}$*y Nocardiopsis dawsonvillei* laboratory strains. Functional characteristics of detergent treated $11^{5(1)}$*y Nocardiopsis dawsonvillei* laboratory strains have been investigated. Ionic and non-ionic detergent treated samples were compared. Based on the obtained results, we have attained reliable decrease of microbial destructive activity in SDS treated cells culture. Under current experimental conditions different ways of influence of ionic and nonionic detergents on the tested microorganisms are discussed. *© 2008 Bull. Georg. Natl. Acad. Sci.*

Key words: *microorganisms, detergents, peroxidation, acid phosphatase.*

The ability of microorganisms to deteriorate various materials and goods is a characteristic of considerable microbiological interest in terms of both degradation of recalcitrant xenobiotic compounds and the microbes that have this enzymatic activity.

It is well established that microbial aggressive metabolites, i.e. organic acids, extra cellular enzymes, nitric oxide, reactive species of oxygen promote destructive activity. There is some evidence indicating the essential role of peroxide as well as reactive oxygen species generated during microbial metabolic reactions [1]. Different physical and chemical factors cause considerable changes of cell permeability, leading to derangement of metabolic and functional activity of microorganisms. Moreover, causing considerable structural changes of membranes, detergents serve as tools for investigation of the functional characteristics of several microbial cells [2].

The present paper describes the effect of ionic - SDS and non-ionic - Tween-80 detergents on viable cells amount, concentration of secreted proteins, acid phosphatase activity and intensity of lipid peroxidation of tested microorganism $11^{5(1)}$*y Nocardiophsis dawsonvillei* cells. In order to control the effect of detergents on $11^{5(1)}$*y Nocardiopsis dawsonvillei* cells were grown on mineral liquid medium supplemented with ionic - SDS and non-ionic - Tween-80 detergents - final concentration 10^{-7} M, 10^{-5} M, and 10^{-2} M respectively. Based on our previous experiments, 9-day old $11^{5(1)}$ *y Nocardiopsis dawsonvillei* cell cultures were employed.

The cells from culture medium were separated by filtration and secreted protein concentration [3], acid phosphatase activity [4], peroxidation intensity [5] were determined in filtrate. All the experiments were repeated at least twice and the samples were assayed in triplicate. The data points represent the mean values within ± 4 to 5% of the individual values. The data were treated by one-way ANOVA analysis.

Experiments of submerged culture of the fungus were carried out on SDS/Tween-80 containing medium alternatively and an amount of viable cells, secreted protein concentration, peroxidation intensity and acid phosphatase activity in culture medium were assessed. The results are summarized in Fig.1. A and B show

differences of the investigated parameters in culture medium containing SDS/Tween-80 rising concentrations - 10^{-7}M, 10^{-5}M, 10^{-2}M. C and D - at single cell level respectively. Quantitative changes of *$11^{5(1)}$y Nocardiopsis dawsonvillei* viable cells grown on medium containing appropriate concentrations of detergents have shown that non-ionic detergent (Tween-80) stimulated the growth of the cells compared to the control, while appropriate concentrations of SDS significantly decreased the cell amount. Such discrepancy between the influence of ionic and non-ionic detergents may be due to differing composition of detergents and/or specific interactions with membrane lipid bilayer [6]. In the same conditions secreted protein concentration, acid phosphatase activity and peroxidation intensity were examined. In culture medium containing Tween-80 initial concentration all the investigated parameters are retained at control level (B) and only highest tested concentration of Tween-80 (10^{-2}M) increased the secretion of acid phosphatase. The data revealed that low concentrations of SDS decreased the amount of secreted protein and MDA without altering acid phosphatase activity. High SDS concentration enhanced protein secretion, suggesting that only a high concentration of detergents destroys cell membrane. The functional relationship between the relative degrees of investigated parameters (protein/cell, mda/cell, acid phosphatase/cell) was elucidated and the values at single cell level were assessed in *$11^{5(1)}$y Nocardiopsis dawsonvillei* cells grown on medium supplemented with both detergents in appropriate concentrations – C, D. We have observed a gradual increase of secreted protein amount, secreted acid phosphatase activity and

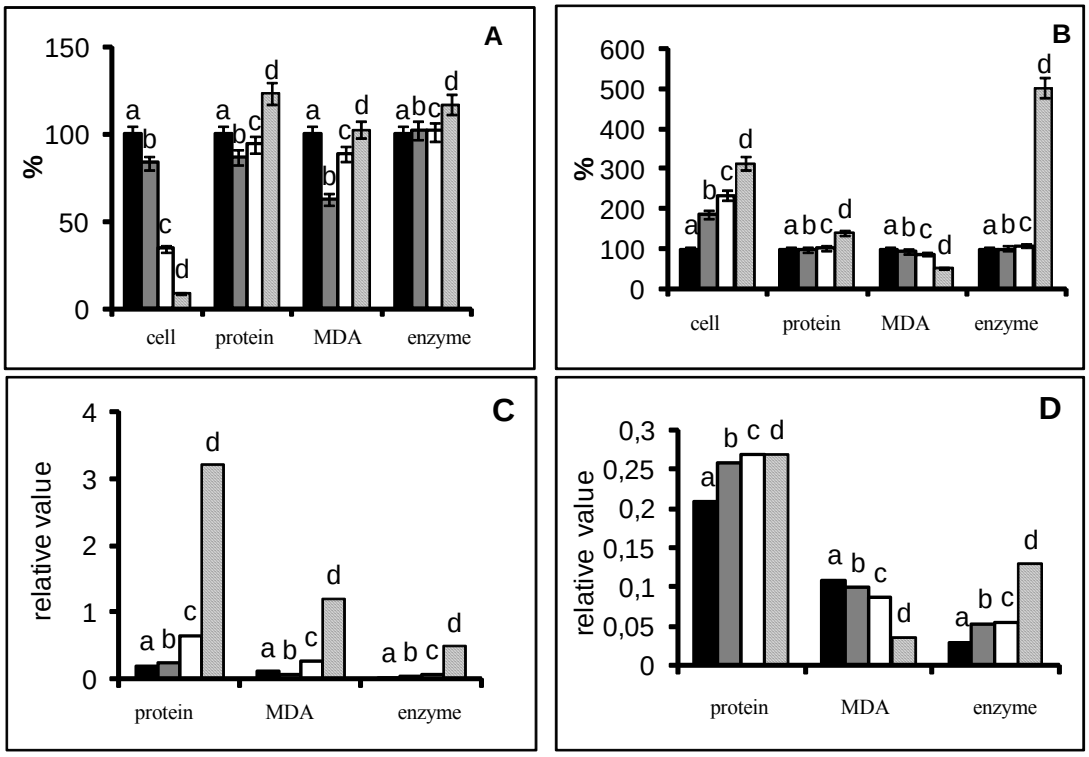

Fig. 1. **A**-Viable cells amount, protein concentration, peroxidation intensity and acid phosphatase activity in culture medium of *$11^{5(1)}$ y Nocardiopsis dawsonvillei* cells grown on mineral medium containing SDS different concentrations. *Cultures were inoculated (1% [vol/vol]) into 100 mL of mineral liquid medium supplemented with detergents10^{-7}M, 10^{-5}M, 10^{-2}M concentrations,incubated for 9 days at 28°C. The results are mean values of triplicate samples.Key: a-control, b-10^{-7}M, c-10^{-5}M, d-10^{-2}M.*

B-Viable cells amount, protein concentration, peroxidation intensity and acid phosphatase activity in culture medium of *$11^{5(1)}$ y Nocardiopsis dawsonvillei* cells grown on mineral medium containing different concentrations of Tween-80. *Cultures were inoculated (1% [vol/vol]) into 100 mL of mineral liquid medium supplemented with detergents10^{-7}M, 10^{-5}M,10^{-2}M concentrations, incubated for 9 days at 28°C. The results are mean values of triplicate samples.Keys: a- control, b- 10^{-7}M, c-10^{-5}M, d-10^{-2}M.*

C-Comparison of exogenous protein concentration, peroxidation intensity and acid phosphatase activity at single cell level in *$11^{5(1)}$ y Nocardiopsis dawsonvillei* cell culture medium supplemented with different concentrations of SDS. *Cultures were inoculated (1% [vol/vol]) into 100 mL of mineral liquid medium, supplemented with detergents' 10^{-7}M, 10^{-5}M, 10^{-2}M concentrations, incubated for 9 days at 28°C. The results are mean values of triplicate samples. Keys: a-control, b- 10^{-7}M, c-10^{-5}M, d-10^{-2}M.*

D-Comparison of exogenous protein concentration, peroxidation intensity and acid phosphatase activity at single cell level in *$11^{5(1)}$ y Nocardiopsis dawsonvillei* cell culture medium supplemented with different concentrations of Tween-80. *Cultures were inoculated (1% [vol/vol]) into 100 mL of mineral liquid medium, supplemented with detergents'10^{-7}M,10^{-5}M,10^{-2}M concentrations, incubated for 9 days at 28°C. The results are mean values of triplicate samples. Keys: a-control, b-10^{-7}M, c-10^{-5}M, d-10^{-2}M.*

peroxidation intensity in *11[5(1)]y Nocardiopsis dawsonvillei* cells grown on SDS containing medium (C). Results are in compliance with our previous observation that the enhancement of secretion of proteins is the result of detergent's damaging effect on *11[5(1)]y Nocardiopsis dawsonvillei* cells. The same hint may be rational in interpreting the examined elevation of peroxidation intensity, suggesting that SDS high concentration significantly modifies cell membranes, making lipoperoxidation substrates more accessible for reactive oxygen species. Complete positive correlation (r=0.99 ±0.01) between peroxidation intensity/acid phosphatase activity has been revealed for cells grown on SDS containing medium. We consider that SDS dependent changes of enzyme activity of *11[5(1)]y Nocardiopsis dawsonvillei* cells may perhaps be mediated by peroxidation reactions.

Comparing the microbial capacity of cultures grown on alternative detergent, it is obvious that in Tween-80 containing medium secreted protein, the content and acid phosphatase activity are increased along with biomass augmentation. Complete positive correlation (r=0.99±0.01) between cell amount/acid phosphatase has been established and no correlation between enzyme activity/peroxidation intensity was detected. Altogether the data results indicate the existence of acid phosphatase secretion of different origin [7].

The suitability of the applied approach tested in our investigations seem to be adequate for evaluation of microbial destructive activity under the action of various damaging factors.

უჯრედული ბიოლოგია

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ნაშრომში განხილულია SDS და Tween-80-ის გავლენა ცნობილი ბიოდესტრუქტორის **11[5(1)] *y Nocardiopsis dawsonvillei*-ს** დამაზიანებელი აქტივობის განმსაზღვრელ ზოგიერთ ფუნქციურ მახასიათებელზე. გამოვლენილია უჯრედებზე იონური და არაიონური დეტერგენტების ზემოქმედების განსხვავებული ხასიათი. შეფასებულია SDS-ის როლი **11[5(1)] *y Nocardiopsis dawsonvillei*-ს** დამაზიანებელი აქტივობის შემცირებაში.

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