THE AFFECT OF NUTRIENTS OVER ECOTOXICOLOGICAL RESPONSES OF AQUATIC SPECIES (CANDIDA ALBICANS AND PHAEODACTYLM TRICORNUTUM)

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ABSTRACT

Study shows a primary evaluation on the effect of nutrient loads and toxicant exposure on cell growth of aquatic species comparing results obtained by a well-standardized test on algal specie (Phaeodactylum tricornutum) and yeast, using a recent specie of ecotoxicological interest (Candida albicans). Results obtained suggest a significant effect due to nutrient loads on cells growth in both species affecting toxicity of the tested chemical. Furthermore, yeast responses are faster and comparable to results obtained on algae species.

INTRODUCTION

Ecotoxicological tests on algal species are well standardized both for marine and freshwater samples. In Europe, Microbiotest purchased standard lots for controlled in vitro exposure of different algal species as well as Phaeodactylum tricornutum (marine water) and Selenastrum capricornutum (freshwater). UNI EN ISO 10253 (2006) is the internationally recognized protocol for unicellular algal species and it evaluates the growth-rate inhibition after 72 hours of exposure to toxicants. Recent literature evidences as UNI EN ISO 10253 (2006) is now routinely performed to evaluate water quality thanks to the relatively easiness to perform and the good reproducibility of the standardized protocol (OECD, 2000) [1], [2], [3]. Unicellular algae species are considered suitable target species because of the extensive
knowledge of their biology, ecology and ecotoxicology [4], [5], [6], [7]. In spite of the advantages due to the application of tests on algal species, recently Rumlova and Dolezalova suggest that the yeast Candida albicans could represent a suitable species for ecotoxicological tests due to the chance to perform more rapid and informative growth inhibition tests [7]. Furthermore, yeast is available in its quiescent form and tests with Candida albicans do not need to take care of the reproduction phase of tested organisms reducing both time and space consuming procedures. This paper aims to evaluate on a preliminary basis the applicability of Candida albicans as model specie for ecotoxicological tests comparing results obtained on this specie with a standard test performed on aquatic unicellular algae (P. tricornutum). Furthermore, this study evaluates the effects due to nutrients loads on cell growth inhibition of both species and on toxicological responses after the exposure to chemical dilutions.

MATERIALS AND METHODS

Rationale of Experiments

Two different unicellular aquatic species were tested: yeast (Candida albicans) versus algae (P. tricornutum). Concerning the first specie a literature based exposure protocol was applied, on the other hand, concerning the second one a standardized UNI EN ISO 10253 (2006) procedure was followed. Experiments focus on two steps:

i) Comparison between species related to the effects induced after the exposure to a nutrient gradient. Cell cultures were exposed to scalar dilution (1.0 M - 0.01 M) of sucrose (C12H22O11) in the case of yeast and of orthophosphate (PO₄³⁻) in the case of algae.

ii) Effects induced by the nutrient load on toxicological responses after the exposure of the 1.0 M nutrient concentrations to scalar dilutions of potassium dichromate (K2Cr2O7) [8].

Cell Cultures

Cell cultures were performed as reported by Daniel and David for yeast and as reported by Algal Toxkit for algae species [8]. All cultures were aseptic and bacteria free, experiments
were performed in triplicate (n=3). Concerning yeast, instant dehydrated bakers’ yeast (S. cerevisiae Hansen) was used. Approximately 0.1 g of yeast was suspended in 10 ml of each dilution of the substance solution to test. Cultures were performed aerobically at 30°C and the pH of the medium was monitored within 5.5 - 5.6 units. A water-yeast suspension without any toxicant or nutrient substance was prepared as a control sample. Cell densities were estimated after 5 min, 10 min, 15 min, 30 min and 60 min of exposure. Concerning algae, species were purchased from Ecotox and cell cultures were performed as reported by Renzi et al., according to UNI EN ISO 10253 standardized methods [7]. Cell densities were measured after 0, 24, 48 and 72 hours from the initial exposure. Cell counts were performed by Burker’s chamber standardizing lectures performing 10 independent replicates of 100 µl per each sample. Spectrophotometry technique was not applied to avoid errors due to the effect reported by colored solutions at the wavelength adapt to algae cells counts [7].

Growth inhibition calculations
The cell growth inhibition percentage (I%) was calculated as the difference between the area under the control growth curve (Ac) and the area under the growth curve at each test substance concentration (Ai), as reported by the following equation: I% = (Ac-Ai)/Ac *100. In figures, exposure times are expressed on a logarithmic scale (X axis), while inhibitions are represented as – I% /100. Negative values mean inhibition occurred in tested sample compared to the control, while positive values mean stimulation of cell growth in tested samples compared to control.

RESULTS
The effect induced by nutrients loads on cell culture is reported in (Figure 1) (yeast) and (Figure 2) (algae). Growth inhibition are reported for tested scalar dilution of sucrose (yeast) or orthophosphate concentrations (algae) and represented as ratio between growth inhibition percentages of test and controls. Concerning yeast, doses higher than 0.10 M of sucrose stimulate the population growth compared to control even if, after 60 minutes, growth rates are comparable to controls at 0.50 M. On the contrary, algal specie is stimulated by nutrient loads starting from 0.1 M. Nutrient loads of 1 M for both of
considered species are able to significantly stimulate cell growth till the end of the experiments (respectively 60 min and 72 hours for yeast and algae).

Figure no 1: Effects on cell growth in yeast exposed at different sucrose (C12H22O11) concentrations. Data are reported as average ratio (n=3) between growth inhibition percentages of tested samples vs controls. Positive data means stimulation of growth in tested cells compared to controls while negative data means inhibition. Exposure times are in minutes (logarithmic scale).

Nutrient loads significantly affect both freshwater and marine ecosystems due to discharges of effluents by a wide range of human activities [9], [10]. The eutrophic action of nutrients in aquatic ecosystems on algae species is well documented by the literature [11]. Furthermore, the effect due to the presence of nutrients (sucrose) on yeast metabolic pathway is also well-known. Our data confirm a positive effect on cells growth in both species by the exposure to nutrients. In spite of that, few data are available on additive effects due to nutrients and toxicants exposure both on well standardized model specie (i.e. algae) and on yeast. Results obtained by this preliminary study evidence that nutrients could induce significant changes in ecotoxicological response of tested species. In particular, algae and yeast species are particularly sensitive to nutrient loads due to their specific ecology [12].
Figure no 2: Effects on cell growth in algae exposed at different orthophosphates (PO4-2) concentrations. Data are reported as average ratio (n=3) between growth inhibition percentages of tested samples vs controls. Positive data means stimulation of growth in tested cells compared to controls while negative data means inhibition. Exposure times are in minutes (logarithmic scale).

Figure no 3: Effects on cell growth in yeast exposed at different toxicant concentrations. Data are reported as average ratio (n=3) between growth inhibition percentages of tested samples vs controls. Positive data means stimulation of growth in tested cells compared to controls while negative data means inhibition. Exposure times are in minutes (logarithmic scale). Nut = sucrose 1.0 M. Toxicant dilution tested were: Tox 1 = 15 mg/L; Tox 2 = 7.5 mg/L; Tox 3 = 0.75 mg/L; Tox 4 = 0.075 mg/L, at each toxicant dilution sucrose 1.0 M was added.
Figure no 4: Effects on cell growth in algae exposed at different toxicant concentrations. Data are reported as average ratio (n=3) between growth inhibition percentages of tested samples vs controls. Positive data means stimulation of growth in tested cells compared to controls while negative data means inhibition. Exposure times are in minutes (logarithmic scale). Nut = orthophosphate 1.0 M. Toxicant dilution tested were: Tox 1 = 15 mg/L; Tox 2 = 7.5 mg/L; Tox 3 = 0.75 mg/L; Tox 4 = 0.075 mg/L, at each toxicant dilution orthophosphate 1.0 M was added.

*Candida albicans* has been a valuable asset to human civilization due to its extensive use in the last years, but ecological significance of the *Candida albicans* is also notable as well as it is a ubiquitous species that could be vectored by animals and in particular by wasps [12]. Furthermore, Candida albicans represents useful specie for ecotoxicological tests in freshwater habitats due to cheapness and to easiness to perform and manage its cultures in vitro experiments. Furthermore, its biology and genetics features are well known and widely documented as well as yeast represent a suitable model specie for genetic researches. In spite of that, this specie is few represented in literature for ecotoxicological tests. Obtained ecotoxicological results should be considered only as preliminary on yeast species, due to the needs of further standardization of the method used for in vitro experiments. In spite of that, this specie evidences interesting responses and could represent a useful and interesting test species for ecotoxicological purposes. Furthermore, obtained results evidences that responses of the tested unicellular aquatic species could be significantly affected by nutrient loads. For this reason, the use of tested species to perform acute Ecotoxicological tests on complex environmental aquatic matrices, as well as effluent water or sediment elutriates, should be performed in tight associations to nutrient quantification
in tested water samples to take into some account such “trophic effect” on ecotoxicological results. Preliminary results obtained in this study also suggest that exposure of 60 min and over in yeast tests could be useful to highlight chronical effects of toxicant exposure. Even if further researches are needed to better clarify ecotoxicological responses, obtained results are encouraging and indicate the yeast as interesting model species.

CONCLUSION

_Candida albicans_ represents interesting specie for ecotoxicological purposes. Furthermore, a clear inhibition of cell growth could be detected after a shorter period of time (60 min) compared to algae species (3 days). In spite of that, test method needs to be standardized. Results evidence that acute ecotoxicological tests could be affected by the presence of nutrient in tested water.

REFERENCES


