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Do Nutrients Affect Ecotoxicological Responses of Aquatic Species? Preliminary Results on *Saccharomyces cerevisiae* and *Phaeodactylum tricornutum*

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Research Article

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ABSTRACT

This study performs a preliminary evaluation on the effect of nutrient loads and toxicant exposure on cell growth of two unicellular aquatic species comparing results obtained by a well-standardized test on algal specie (Phaeodactylum tricornutum) and yeast, using a recent specie of ecotoxicological interest (Saccharomyces cerevisiae). 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 standardised 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 [4-7]. In spite of the advantages due to the application of tests on algal species, recently Rumlova and Dolezalova suggest that the yeast *Saccharomyces* cerevisiae 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 S. *cerevisiae* 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 S. *cerevisiae* 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.

MATERIAL AND METHODS

Rationale of Experiments

Two different unicellular aquatic species were tested: yeast (S. *cerevisiae*) *versus* algae (*P. tricornutum*). Concerning the

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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 ($C_{12}H_{22}O_{11}$) in the case of yeast and of orthophosphate (PO_4^{-2}) 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 ($K_2Cr_2O_7$) [8].

Cell Cultures and Lectures

Cell cultures were performed as reported by Rumlova and Dolezalova for yeast and as reported by AlgalToxkit® 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 coloured 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).

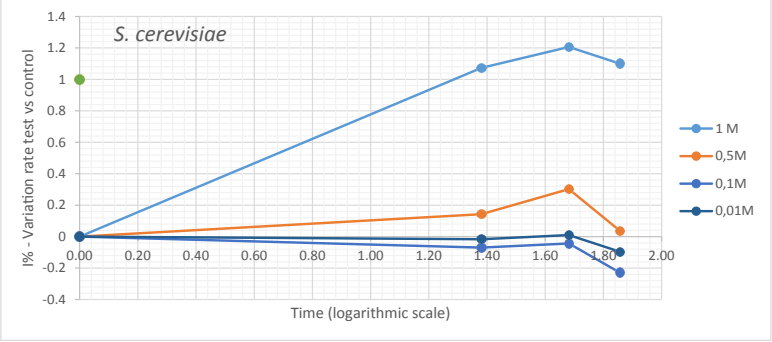

Figure 1. Effects on cell growth in yeast exposed at different sucrose ($C_{12}H_{22}O_{11}$) concentrations. Data are reported as average ratio (n=3) between growth inhibition percentages of tested samples vs controls. Positive data means stimulation of growth in tested cells compared to controls while negative data means inhibition. Exposure times are in minutes (logarithmic scale).

In **(Figure 3 and Figure 4)** are reported, respectively, effects induced on cells growth of yeast and algae by the exposure to different toxicant dilutions ($K_2Cr_2O_7$) when nutrient load is fixed at 1.0 M for all dilutions of the toxicant. Both species show inhibition at higher doses of toxicant in presence of 1.0 M nutrients. Comparing Toxicant n. 4 curves with Nutrient ones, yeast evidences significant responses even at the lowest tested dose, while algae does not show toxicity at the lowest dilution tested.

DISCUSSION

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 [8]. 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

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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.

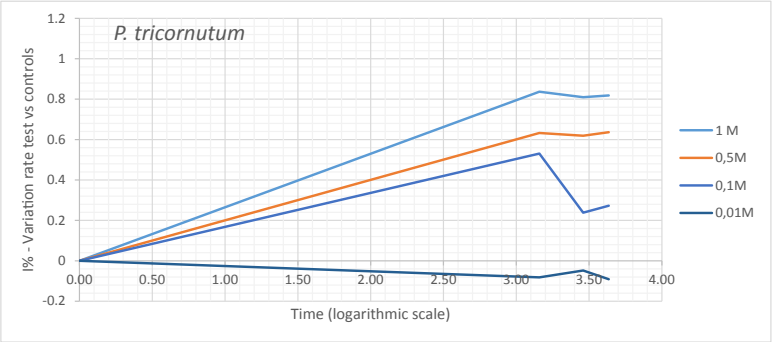

Figure 2. Effects on cell growth in algae exposed at different orthophosphates (PO_4^{-2}) concentrations. Data are reported as average ratio (n=3) between growth inhibition percentages of tested samples vs controls. Positive data means stimulation of growth in tested cells compared to controls while negative data means inhibition. Exposure times are in minutes (logarithmic scale).

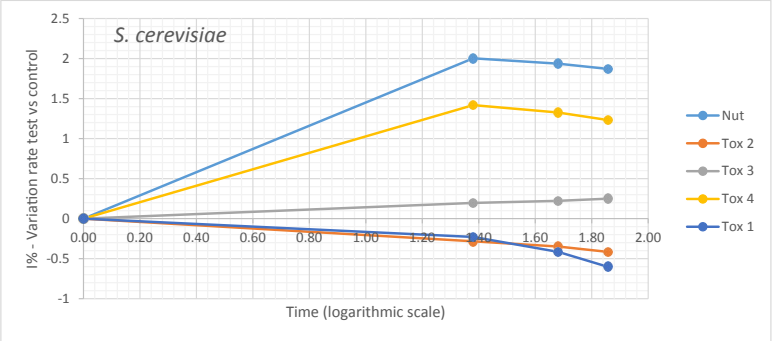

Figure 3. Effects on cell growth in yeast exposed at different toxicant concentrations. Data are reported as average ratio (n=3) between growth inhibition percentages of tested samples vs controls. Positive data means stimulation of growth in tested cells compared to controls while negative data means 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.

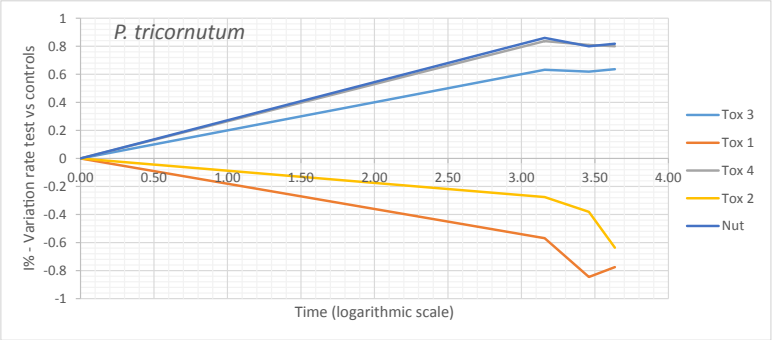

Figure 4. Effects on cell growth in algae exposed at different toxicant concentrations. Data are reported as average ratio (n=3) between growth inhibition percentages of tested samples vs controls. Positive data means stimulation of growth in tested cells compared to controls while negative data means 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.

S. cerevisiae has been a valuable asset to human civilization due to its extensive use in the last 9,000 y, but ecological significance of the *S. cerevisiae* is also notable as well as it is a ubiquitous species that could be vectored by animals and in particular by wasps [12]. Furthermore, *S. cerevisiae* represents a 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 genetic 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.

CONCLUSIONS

S. cerevisiae represents an 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 samples that induce significant interferences on cell growth rates.

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