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The Exometabolite Assemblage of Aquatic Macrophytes May Enhance the Growth and Oil-Destructive Activity of *Pseudomonas melochlora*

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Abstract: Oil and oil-products are wide-spread pollutants of natural waters that need to be eliminated to maintain ecological balance and biodiversity. Oil-degrading microorganisms are extensively used for bioremediation purpose. In nature, however, there are complex interactions between organisms that may influence the process of oil biodegradation. This article is intended to reveal the role of the exometabolite assemblage isolated from various aquatic macrophytes in the activity of oil-oxidizing microorganism *Pseudomonas melochlora.* It was detected that addition of the exometabolite assemblage to growth medium modified the number of *Pseudomonas melochlora* cells as well as their oil-degrading activity. The observed effects depended on the type of aquatic macrophyte and on the time when the exometabolites were isolated from the plants.

Key words: Aquatic macrophyte · Oil · Biodegradation · Exometabolites · *Pseudomonas melochlora*

INTRODUCTION

Increasing industrialization world wide resulted in serious pollution of the environment and natural waters are at the main terminal points for capturing these pollutants. Oil and oil-products are wide-spread pollutants of natural waters that need to be eliminated to maintain ecological balance and biodiversity [1-3]. Remediation of the polluted natural waters may be achieved by application of various methods including a biological one. The latter seems more attractive due to the absence of negative consequences of the physico-chemical methods. Many works were devoted to the study of the oil-degrading activity of microorganisms of various ecological niches [4, 5]. There are, however, complex interactions between the living organisms in the environment that may influence the process of oil biodegradation. Taking into account the prominent sanative role of aquatic macrophytes [6] and content of their exometabolites [7], it is reasonable to suggest that growth and oil-destructive activity of microorganisms may be influenced by these factors. The aim of this article was to check this assumption with *Pseudomonas melochlora* as a model object.

MATERIALS AND METHODS

Pseudomonas melochlora (Winslow, Broadhurst, Buchanon, Krumwiede, 1917, 555) was obtained from Kazan Institute of Epidemiology and Microbiology (Kazan, Russia). It is an aerobic bacterium with the bacillus-like morphology and it has blue-green fluorescent pigments. The bacterium is able to oxidize oil and its products. *P. melochlora* was grown in Muntz growth medium with oil at 28°C for 2 to 6 days till stationary growth phase. The growh medium contained (g per L of water): NH_4NO_3-0.8, $NH_4H_2PO_4$-1.0, KH_2PO_4-0.2, $MgSO_4 \cdot 7H_2O$-0.1, NaCl-0.1, desalinized oil (Romashkinskoe oil field (Tatarstan, Russia)-600 mg/L, pH-7.0-7.2. For cultivation of bacteria, 2 mL of bacterial subculture was inoculated into 400 mL flasks (after that the concentration of microorganisms was about 0.2-0.3x10^6 cells/mL) with the exometabolite assemblage isolated from aquatic macrophytes.

Growth rate of *P. melochlora* was evaluated on Petri dishes with agar and the indicated supplements and without them (control). The amount of the used oil in growth medium was checked at the end of the experiment. The extraction of unoxidized oil was performed with the use of carbon tetrachloride (in proportion 1:20). To

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eliminate the dead bacteria, oil extract was centrifuged at 8000 *g*; then optical density of the solution was analyzed using infrared spectrophotometer at λ = 3350 nm. The respiration of *P. melochlora* in a mixture of oil-oxidizing culture was analyzed by Warburg method [8]. The analysis was performed during 3 h at 28°C with half-hour periodicity. The initial optical density of *P. melochlora* at λ = 400 nm was about 0.6 (approximately $590x10^3$ cells/mL), oil concentration-2.59 g/L (one drop) and 5.18 g/L (two drop).

Exometabolites of the following aquatic macrophytes were taken for this research: rush (*Phragmites australis*), various species of reed mace (*Typha angustifolia* and *Typha latifolia* L.), bulrush (*Scirpus lacustris* L.) and elodea (*Elodea canadensis* R.). the exometabolite assemblage was obtained as described before [7].

RESULTS

We found in this study that the single-step or divided addition of exometabolite assemblage (or fractions), isolated from the aquatic macrophytes under study in different stages of vegetation, resulted in stimulation of the growth of *P. melochlora* and the process of oil biodegradation.

The maximal effect was detected after 3 days of addition of the exometabolite assemblage isolated from *P. australis* in the first decade of August (Fig. 1A). For example, a number of *P. melochlora* cells increased up to $625x10^6$/mL vs $330x10^6$/mL (the control value) (Fig. 1A); also we observed 21% increase in oil biodegradation (in comparison with the control) (Fig. 2, I). Exogenous fractions of amino acids and carbohydrates produced in

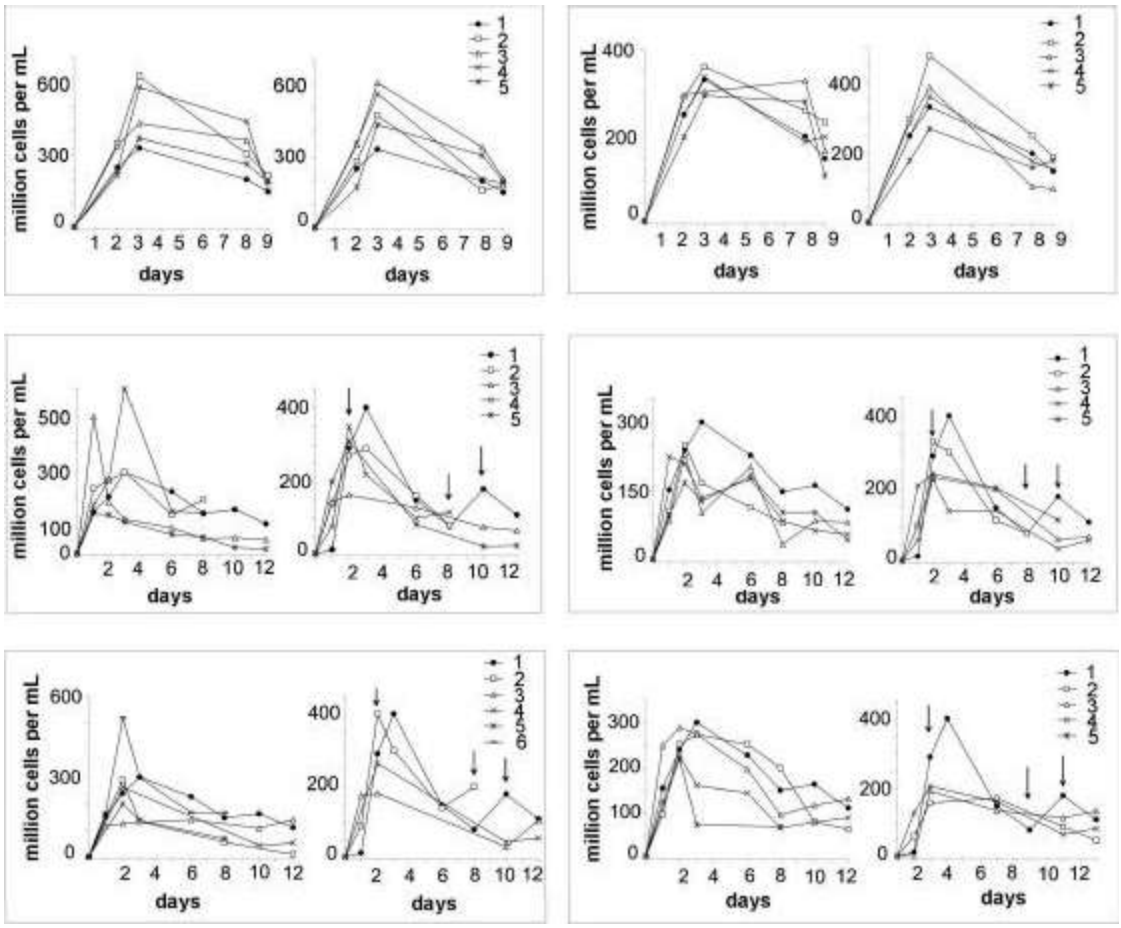

Fig. 1: The influence of exometabolites isolated from various aquatic macrophytes on the growth of *P. melochlora* cells. *Note:* A-F = 1-control; 2-the exometabolite assemblage; 3-fraction of amino acids; 4-fraction of carbohydrates; 5-fraction of organic acids. C-F = I-the single-step addition of exometabolites; II-the divided addition (at the beginning of the experiment and other periods are indicated by arrows). A-B = August, C-F = September. E = I, 6-2-fold concentration of the exometabolite assemblage.

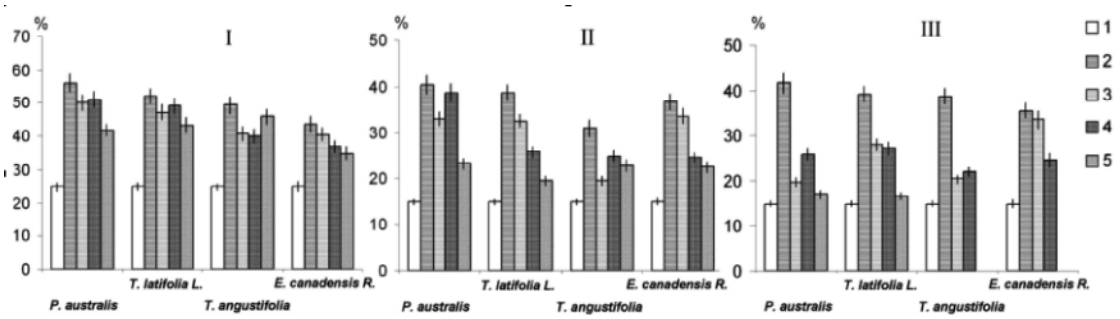

Fig. 2: The influence of exometabolites from aquatic macrophytes on oil degradation (%) by *P. melochlora* cells. Note: I-August, the single-step addition; II-September, the single-step addition; III-September, the divided addition; 1-control; 2-the exometabolite assemblage; 3-fraction of amino acids; 4-fraction of carbohydrates; 5-fraction of organic acids

this period by *P. australis* induced more significant stimulation of oil biodegradation in comparison with the analogous exometabolites isolated from other aquatic macrophytes (Fig. 2, I).

During the second decade of September, the process of bacterial destruction of oil in the presence of exometabolites of *P. australis*, *T. angustifolia* and *T. latifolia* L. was lowered in comparison with August (Fig. 2, II, III). There were no significant differences in the growth of *P. melochlora* in comparison with control (Fig. 1, C-F). In the same time, we detected an increase in oil-degrading activity of *P. melochlora* after addition of the exometabolite assemblage and the amino acid fraction (22% and 19%, respectively) isolated from *E. canadensis* R. (Fig. 2). This may be connected with the autumnal maximal excretive activity of the macrophyte.

The divided addition of the macrophyte exometabolites (isolated in the second decade of September) to growth medium resulted in maintenance of *P. melochlora* number similar to those with the divided addition despite the fact that the concentration of each of them was significantly less. The process of bacterial destruction of oil in variants with the divided addition of the exometabolite assemblage isolated from the macrophytes under study was more active as opposed to the single-step addition of fractions of amino acids, carbohydrates and organic acids (Fig. 2).

The 2-fold increase of the concentration of the exometabolite assemblage of *T. angustifolia* in the growth medium resulted in enhancement of *P. melochlora* number by the second day and in the increase of oil-degrading activity (by 37% and 39% after the single-step and divided addition, respectively) (Fig. 1). This fact suggest the potential ability to enhance the activity of oil-degrading bacterial culture during the attenuation of the symbiotic relationships between aquatic macrophytes and the bacteria.

DISCUSSION

The representatives of the *Pseudomonas* genus (*P. meridiana, P. antarctica, P. effusa, P. crucuviae, P. tralucida* and many others) were reported able to degrade crude oil [9, 10] and the possible mechanisms were discussed [11]. We presented here new data on another *Pseudomonas* species-*P. melochlora* and its oil-degrading activity. It is clear from the displayed material that natural compounds, dissolved in water and produced by emergent aquatic macrophytes as well as by other living organisms, may actively influence this process. Despite the fact that oil components (like naphthenic acids) may have phytotoxic effect for aquatic macrophytes, the same plants may help to degrade oil [12].

As was stated above, the maximal growth stimulation was observed when exometabolites were isolated in August. It is not surprising since rich qualitative and quantitative content of macrophyte exometabolites was detected during the period of active vegetation [7]. Due to impoverishment of the content of exometabolites produced by aquatic macrophytes by the end of the vegetation period, the effect of stimulation of *P. melochlora* growth and oil biodegradation was reduced. Although there is a controversy on a correlation between a number of oil-degrading bacterial cells and the efficiency of oil degradation [13], we found a strong dependence between these two parameters (Fig. 1 and 2).

CONCLUSION

Thus, *P. melochlora* showed an evident biodegrading potential. We demonstrated that its oil-degrading activity may be influenced by addition of exometabolites isolated from the aquatic macrophytes. The effect depends on the time when the exometabolites were extracted as well as the type of macrophyte and a way of their addition (the single step or divided).

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