NOVEL OIL-DEGRADING ALGAL-BACTERIAL ASSOCIATIONS FOR THE TREATMENT OF OIL POLLUTION IN THE BALTIC SEA

Elena Safonova and Swetlana König

Institute of Biology of the University Leipzig, Permoserstr. 15, D-04318, Leipzig, Germany

INTRODUCTION

The stability of an ecosystem strongly depends on the biodiversity of its microorganisms population. The network of interactions between microorganisms provides a flexible response to various changes of the coenotic equilibrium. This equilibrium changes drastically if such a network is damaged by oil spills or any other kind of pollution, representing a danger to the existence of a whole ecosystem. Bioremediation is a method employing microorganisms to remove pollutants and to restore the ecology of populations (1,5). Understandably due to this, this approach is considered to be the most gentle and safe one making what it offers attractive. Our focus was to improve the efficiency of the treatment of oil pollution in the Baltic Sea. As a part of ‘Biofend’ project, we aimed to create artificial associations of alkotrophic bacteria and phototrophic partners (algae or cyanobacteria) and to use them as an effective tool for the removal of oil spills.

METHODS

1) Bathometer was used to collect the water samples from the surface and depth of 3 meters (Fig. 3).
2) Colonies growing in the presence of tetradecane’s vapour or on a solid medium with oil added were isolated with the help of a stereomicroscope.
3) Nutrient media used: medium 6 (4), OMB (5), 2216 (5).
4) Algae were incubated at 4°C, 10°C, 15°C, 20°C and light (2000 lux).
5) The cell density of algae was measured by counting the number of cells in a Thoma cell chamber. The populations of bacteria were counted as colony forming units (CFU).
6) Screening was carried out with the stamp method on solid medium with vaporous tetradecane (10°) or addition of phenol (0.05%), rhodamine (Rh) (0.05%), crude oil (0.1%) – 1%, diesel oil (0.1%) – 1% (Fig. 6, Table 1).
7) The effect of salinity from 0.8 to 42 ppm (by adding NaCl) on growth was observed.
8) Oil concentration was determined fluorimetrically on the analyzer Fluorat-Panorama (Lumex Ltd. St. Petersburg) and gravimetrically.
9) The pilot test was conducted in 290-L tanks with the water from the Baltic Sea. Crude oil (164 g) was added to tanks.

RESULTS

Isolation

In the summer and winter 2011-2012 we isolated 157 strains of both algae and cyanobacteria and 199 bacteria from 28 samples. The samples were taken from four different places of the Baltic Sea in the areas of Rostock, St. Petersburg, Kiel and Sassnitz (Fig. 1). Some samples were taken near the oil terminal in ports (Fig. 2). Colonies grown either in the presence of tetradecane’s vapour or on a solid medium with oil added were isolated with the help of a stereomicroscope (Fig. 4).

Screening, Selection, Collection

After the screening we have selected 19 strains of alkotrophic bacteria and 16 strains of green algae and 7 cyanobacteria showing resistance to the pollutants (Fig. 7 and Fig. 8). The screening was performed in the media containing oil, phenol and rhodamine at low temperatures (4°C and 10°C) and different salt concentrations (Table 1).

Degradation of crude oil

15 bacterial strains from the collection were combined in two-component association (with each other) and their degradation of crude oil was observed. As a result, from 116 different combinations we have selected 10 associations with a degradation level of crude oil ranging from 25% to 35%. Six bacterial strains were selected in the experiment (OSB-27, 138, 118, 48, 128, 158) and studied further to examine oil degradation in combinations with bacteria, cyanobacteria (Fig. 10).

Pilot test

In the summer 2013 pilot tests were carried out in tanks (capacity 290 liters) filled with the water from the Baltic Sea (Fig. 9). The oil concentration in the suspension (CFU 5 x 10^4/ml) containing three different bacterial strains (Rhodococcus erythropolis OSB-27, Rhodococcus fascians OSB-138 and Rhodococcus fascians OSB-118) to degrade crude oil was examined. The fluorimetric analysis revealed that the removal efficiency of oil spills by consortium was much higher than that of the control. The concentration of oil decreased up to 44%, comparing to the control (Fig. 14). The number of association’s CFU tested achieved 70.0 x 10^11 (in the first week). In the control tank lacking the associations the number of heterotrophic CFU was higher in the tank with oil (reaches 25.0 x 10^11 in 2 weeks) than in the one without oil (Fig. 14). In the future the research will be extended by introducing the algae and cyanobacteria to the system. Their ability to remove the pollutants will be estimated and compared to our current results.

CONCLUSIONS

1. The collection of algae and bacteria isolated from Baltic Sea with properties suitable for bioremediation of oil spills has been created. The collection includes 19 bacterial, 16 algae and 7 cyanobacteria strains. They have been selected as a result of the screen for the resistance to crude oil and aromatic pollutants and also their ability to grow at low temperatures and tolerance to different pollution types. In the course of our experiments we were able to select several algal-bacterial artificial associations showing the degradation of crude oil at the concentration of 3 g/l in the medium up to 20% (assayed by gravimetry) after 2 weeks of incubation.
2. The experiment with the artificial associations (Rh. erythropolis OSB-27, Rh. fascians OSB-138 and Rh. fascians OSB-118) in the pilot test after 3 weeks revealed oil degradation of up to 44% comparing to the control.
3. In the future the research will be extended by immobilisation of algal-bacterial associations on the binder and testing this system in a field experiment to remove oil spills under natural conditions (direct in sea).

REFERENCES


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