UNIVERSITÄT LEIPZIG

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

Elena Safonova and Swetlana König

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

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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 (1-3). Understandably due to its nature, this approach is considered to be the most gentle and safe one what makes it very attractive. Our focus was to improve the efficiency of the treatment of oil pollution in the Baltic Sea.

As a part of "BioBind" project, we aimed to create artificial associations of alkanotrophic bacteria and phototrophic partners (algae or cyanobacteria) and to use them as an effective tool for the removal of oil spills.

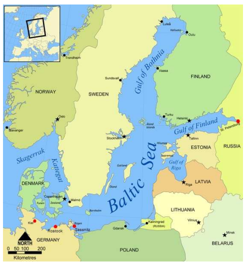
METHODS

1) Bathometer was used to collect the water samples from the surface and depth of 3 meters (Fig. 3).
2) Colonies grown either in the presence of tetradecane's vapoure or on a solide medium with oil added have been isolated with the help of a stereomicroscope.
3) Nutrient media used: medium 6 (4), OMB (5), 2216 (5).
4) Algae were incubated at 4℃, 10℃, 15℃, 20℃ 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 (TD) or addition of phenol (Phl) - 0,05%, phenanthrene (Phn) - 0,05%, crude oil (RO) - 1%, diesel oil (LO) - 1 %, black oil (HO) - 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 (IOW - Leibniz Institute for Baltic Sea Research, Warnemünde).
9) The pilot test was conducted in 290-L tanks with the water from the Baltic Sea. Crude oil (184 g) was added to tanks.

RESULTS

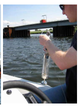
Isolation

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 from water near the oil terminal in ports (Fig. 2). Colonies grown either in the presence of tetradecane's vapoure or on a solide medium with oil added have been isolated with the help of a stereomicroscope (Fig. 4).

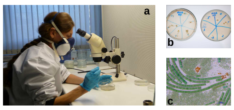


▲Fig. 1. Sampling places in the Baltic Sea are marked as red

▲Fig. 2. Oilport of Rostock



▲ Fig. 3. Sampling with Bathometer



▲ Fig. 4. Isolation of colonies
a - Dr. E.Safonova isolating colonies under the stereomicroscope
b - petri dishes with bacterial colonies
c – enrichment culture with algae and cyanobacteria

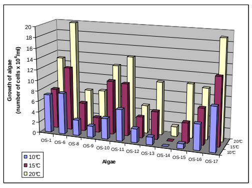
Screening, Selection, Collection

Selection criteria for bacteria: Growth on media containing phenol, phenanthrene, crude oil, black and diesel oil, good growth in a wide salt concentration range (Fig. 6) .

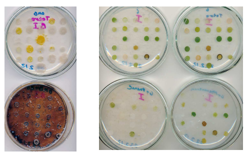
Selection criteria for algae and cyanobacteria: Resistance to oil and aromatic contaminants (Fig. 6), as well as good growth at low temperatures (Fig. 5) and various salt concentrations.

After the screening we have selected 19 strains of alkanotrophyc 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 phenanthrene at low temperatures (4℃ and 10℃) and different salt conc entrations (Table 1).

All the species of the bacteria we have selected belong to the genus *Rhodococcus* (Fig. 9).

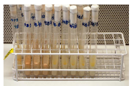


▲ Fig. 5. Algal growth at 10℃, 15℃ and 20℃ after 16 days

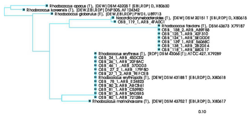


▲ Fig. 6. Screening for the resistance of bacteria and algae to the pollutans

▲ Fig. 7. Algal cultures



▲ Fig. 8. Bacterial strains



▲ Fig. 9. The genetic distance dendrogram of the 19 bacterial strains (Dr. M. Labrenz)

▼ Table 1. Characterization of the strains in the collection

Strains	Place	Gr	10°C	Salinity	TD	Phl	Phn	LO	RO	HO
OS-74	SP	A	-	2,7-40,2	++++	-	++	-	+++	-
OS-15	R	A	+	2,7-40,2	++++	-	++++	+	+++	+++
OS-11	R	A	+++	2,7-30,2	++++	++++	+++	-	++	NT
OS-112	R	C	-	2,7-40,2	++++	-	++++	+++	++++	++++
OSB-24	R	B	NT	2,8-42,8	++++	++++	++++	+++	+++	-
OSB-118	K	B	NT	2,8-42,8	++++	+	+	++	+++	+
OSB-138	K	B	NT	2,8-42,8	++++	-	-	++	++++	+
OSB-158	SP	B	NT	2,8-42,8	++++	+	++	++	+++	+

R-Rostock, SP-St.Petersburg, A-Algae, C-cyanobacteria, B-bacteria, K-Kiel, TD-tetradecane, Phl - phenol, Phn-phenanthrene, RO - crude oil, LO - diesel oil, HO- black oil, (+) – growth, (-) - no growth

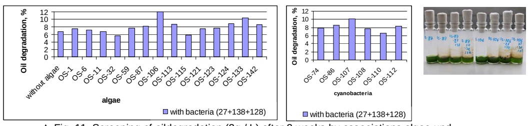
Degradation of crude oil

15 bacterial strains from the collection were combined in two-component association (with each other) and their degradation of crude oil (at the concentration of 2 g/L) after 1 month was estimated. As a result, from 190 combinations we have selected 10 associations with a degradation level of crude oil ranging from 25% to 35%.

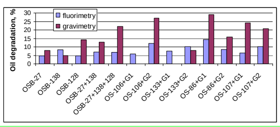
Six bacterial strains were selected in the experiment (OSB-27, 138, 118, 48, 128, 158) and studied further to examine oil degradation in combinations with bacteria, cyanobacteria and algae (Fig. 10).

The highest degradation ability of oil was observed in the combinations consisting of two (G1 = OSB-27+OSB-138) and three bacterial strains (G2 = OSB-27+OSB-138+OSB-128). The next screening was performed including the associations of bacteria, algae and cyanobacteria from the collection (Fig. 11). After 2 weeks of incubation the most efficient crude oil degradation was in the following associations (Fig. 12): OS 86 + G1 (14,5% fluorimetry and 29,0% gravimetry), OSB 27 + 138 +128 (6,7% fluorimetry and 22,0% gravimetry), OS-106 + G2 (12,0% fluorimetry and 27,0% gravimetry), OS-107 + G2 (10,2% fluorimetry and 21,0% gravimetry).

▲ Fig. 10. Degradation of crude oil (5 g/l) after 1 week by bacterial strains (top) and association



▲ Fig. 11. Screening of oildegradation (2g / L) after 2 weeks by associations algae und cyanobacterien with three bacteria strains (27 +138 +128) by fluorimetry

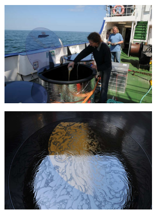


◄ Fig. 12. Degradation of oil (2g/L) by algal-bacterial associations was determined by fluorimetric and gravimetric analysis after 2 weeks.
G1 and G2: mixed bacterial strains
G1 = OSB-27+OSB-138
G2 = OSB-27+OSB-138+OSB-128

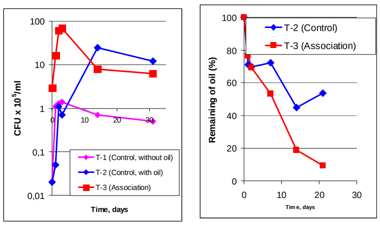
Pilot test

In the summer 2013 pilot tests were carried out in tanks (capacity 290 liters) filled with the water from the Baltic Sea (Fig.13). The ability of the suspension (CFU 9,0 x 10^8/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 10^5/ml 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 10^5/ml 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.



▲ Fig. 13. The experiment with the bacterial association in the tank containing crude oil to treat. In the photo are Dr. E.Safonova and S.Tech



▲ Fig. 14. Growth of heterotrophic bacteria (left) and decrease in oil (right)

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 algal and 7 cyanobacterial 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 salt concentrations.
2) In the course of our experiments we were able to select several algal-bacterial artificial associations showing the degradation ability of oil (present at the concentration of 2g/l in the medium) up to 29% (assesed by gravimetry) after 2 weeks of incubation.
3) The experiment with the artificial bacterial 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.
4) 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 the sea).

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