EFFECTS OF SPILLED OIL ON MICROBIAL COMMUNITIES IN TIDAL FLAT

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Spilled oil has significant impacts on constituent organisms of tidal flat ecosystems. Microorganisms play major roles in energy transformations and fundamental biogeochemical processes. Many bacteria have been isolated and characterized which have an ability to degrade spilled oil. However, little is known about the effects of the oil on the population balance of microbes in a tidal flat ecosystem. The purpose of this study is to clarify the effects of spilled oil on microbial communities in tidal flats by using a simulator for tidal flat ecosystem.

The simulator for tidal flat ecosystem is composed of a tidal flat, wave generator and tide control device, and the wave, the tide cycle and the temperature were controlled by computer system. Sediments inclusive of macrobenthos were collected at a natural tidal flat in Hiroshima Bay, Japan. After several weeks for stabilizing benthic organisms, fuel oil C was added on the surface of the flat at 3 liter per m^2 (O flat). Sediments in the simulator were sampled and populations of macrobenthos, amounts of chlorophyll-a, and total microbial numbers were analyzed once a week. Total DNA in the sediments was purified and 16S rRNA gene of bacteria (from 341 through 536, numbering of 16S rRNA gene for *Escherichia coli*) were amplified by polymerase chain reaction. The DNA mixture was then separated by using Denaturing Gradient Gel Electrophoresis and the migration of each DNA fragment was compared. Bacterial colonies were isolated by using agar medium of Bacto Marine Broth (Difco Co.) and the susceptibility against the presence of hydrocarbons was examined.

The oil spill caused significant decrease in population density of macrobenthos such as annelida, common organisms in natural tidal flats. Results of direct counts of microorganisms seemed not to be influenced by oil spill and 10^9 cells /g dry weight were kept before and after the addition of the oil. However population analyses by using 16S rRNA gene clearly showed the drastic change in microbial communities after the addition of fuel oil C. Microorganisms in O flat were categorized into three groups: Group 1, oil-insusceptible microbes which populations were enhanced by spilled oil; group 2, oil-susceptible microbes which populations were decreased by spilled oil; group 3, oil-insusceptible microbes which populations were not influenced by spilled oil. Microbes categorized in the group 1 are characteristic of bioremediation because these populations seem not preceded without oil and carrying oil-degrading ability. Microbes in the group 2 are unable to multiply in the tidal flat polluted with oil, and these organisms seem characteristic of clean environments or an indictor monitoring the completion of bioremediation.