北海道海岸の原油分解菌群の構造

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Community structures of crude-oil degrading bacteria in seacoasts of Hokkaido

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The oil fields at Sakhalin Island, Russia, reached a commercial level of operation in 2008. The Japanese island of Hokkaido is located to the south of Sakhalin, and is at risk of oil spills from the Sakhalin oil fields. Physical, chemical, and biological treatments are indispensable in cleaning up any contaminated regions, such as seas and seashores. Compared to the physical and chemical treatments, the biological technology, bioremediation (BR), is considered relatively cost-effective and environmentally friendly. However, there are problems to be solved also in the BR method including biostimulation (BS) and bioaugmentation (BA) technologies. To overcome the problems of the existing BR methods (BS and BA), we proposed a new BR technology: autochthonous bioaugmentation (ABA) (Fig. 1; refs 1) and 2)), to clean up oil-contaminated sites, in particular, the seacoasts of Hokkaido.
In the ABA, contaminant-degrading microorganisms indigenous to the contaminated site or predicted contamination site are isolated and used. It is considered that information on the types of contaminant-degrading bacteria and sea sands or on physical and chemical factors of environments is requisite to use the ABA more effectively.
Sea sand samples were collected from several seacoast places of Hokkaido including those of Shari Town (Shiretoko areas), facing the Sea of Okhotsk in March and/or in September. Sea sand was mixed with 2 % crude oil and minimum salts medium (MSM), and then incubated at 20 ˚C or 4 ˚C for two and four weeks. After incubation, DNA was extracted from sea sand cultures and it was subjected to PCR targeting 16S rRNA genes. Amplificates were analyzed by denaturant gradient gel electrophoresis (DGGE). After PCR-DGGE, band profiles were compared, and relatively dense bands were excised and then sequenced.
The PCR-DGGE profiles of DNA prepared from the sea sand samples collected at several places of Shari Town were similar one another. However, the DGGE band profiles showed more diversity in the samples collected in September than in March. When BS treatment using crude oil and the sea sand collected at Onnebetsu, Shari Town, was carried out, several new bands appeared. These results suggest that crude oil-degrading bacteria inhabit seacoasts as very minor species, unless oil contamination happens and that such crude-oil degrading bacteria could be used for practical performance of ABA in the fields. Not only the Shiretoko areas but also other seacoasts of northern Hokkaido are at a risk of oil spills caused by the Sakhalin oil field. We will present data on the microbial community structure of these places and discuss their biological and practical significances, considering the feasibility of practical usage of ABA.

2008 年ロシア・サハリン島の油田開発は商業段階に入った。北海道沿岸域はサハリン油田開発を原因とする原油流出事故の危険に曝されている。原油などによって汚染された場所の浄化には、物理的、化学的そして生物学的な手法などが用いられる。その中でも、生物学的な手法、特に微生物環境修復技術(BR)は、比較的低コストで、環境への負荷も少ない。しかし、現在主に用いられているBR技術であるBiostimulation(BS)やBioaugmentation(BA)には様々な欠点がある。そこで、我々は、新しいBR技術として原地性バイオオーグメンテーション(ABA)を提唱し、北海道沿岸域の原油汚染に備えようと考えている(Fig. 1)。
ABAとは、汚染された場所、または汚染される可能性が高い場所に元々生息している汚染物質分解菌を用いて汚染物質の除去を行う方法である。そのため、ABAをより有効活用するためには、その場に生息する汚染物質分解菌の

情報や、海岸の物理的、化学的な性質についての情報を得ておく必要がある。そこで、網走市や斜里町を含む、北海道各所の海岸から3月または９月に海砂を採集し、それに原油と最少塩培地(MSM)を添加し、2週間、または4週間培養した。培養後、海砂（培養物）からDNAを抽出し、PCRによって16S rRNA 遺伝子断片を増幅した後、変性剤濃度勾配ゲル法(DGGE)によって微生物群集構造を比較した。また、DGGEのゲルからバンドを切り出し、シークエンス解析を行った。
斜里町の海砂の微生物群集構造は、採集した場所に関係なく、それぞれ似通っていた。また、微生物群集の多様性は、９月に採集したサンプルの方が高かった。斜里町(遠音別)の砂と原油を用いて BS を行ったところ、BS を行う前には見られなかったいくつかの新しい DNA バンドが見られた。これらの結果から、北海道沿岸の海砂中に生息する原油分解菌は、平素は数が少ないが、原油流出事故が起きた際には急激に増殖し、優占してくることがわかった。このように優占してくる種は実地における ABA 試験に有効に利用できると考えられる。知床地方だけではなく、北海道北部の海岸はサハリン油田を原因とする原油流出事故の危険に曝されている。そこで本研究では、実用的な ABA 手法の確立を目指し、北海道北部の海岸の微生物群集構造について調査した。

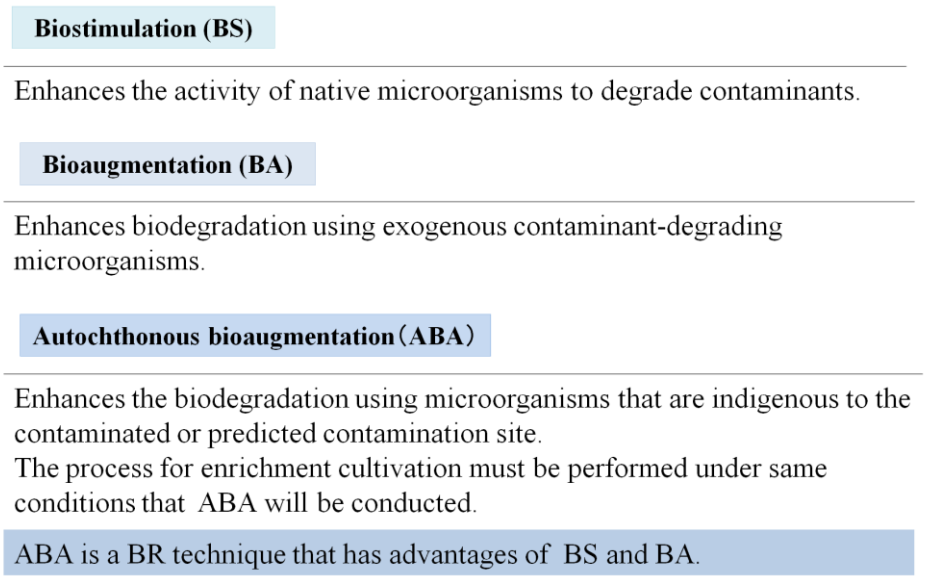

Fig. 1 The features of typical BR techniques

References

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