

**B033**

### The Product of Biosurfactants According to Microbial Medium.

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Chemical surfactant is used in a lot of industrial fields. However, this chemical surfactant has a problem called the second pollution environmentally. Biodegradable surfactant became presentation as an alternative, but hardly spends it by a high unit cost.

This study is the culture medium optimization that can mass-produce surfactant producing microorganism (*C. bombicola* sp., *S. chungbukensis* sp., *S. yanokuyae* sp) in a low unit cost. A progress process of study, compare with biosurfactant of the optimum culture medium state after culturing a microbe under various condition after that in a molasses culture medium. Measured growth curve in cultivation in a comparative way and observed it and sampling did this culture medium, and sampling did suspension after centrifugal disconnection and measured CMC and HLB Value. It is experiment to have recognized possibility of produced biosurfactant in the molasses culture medium that a unit cost is low by this measurement. Biosurfactant was generated in molasses by this experiment results and followed trial, and it was different, and microbe growth and a product generation speed were known.

**B034**

### Development of Nested PCR Procedure for the Detection of *Clostridium botulinum* Toxin Types A, B, E, and F in Soil Samples

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The presence of *C. botulinum* spores in soil is one of a potential source of human botulism. Our goal was to develop a specific PCR procedure for detecting *C. botulinum* types A, B, E, and F, which are associated with human botulism, all together in soil samples. A newly developed nested PCR consisted of two steps of amplification. The first step was performed with a set of primers (BT-F, BT-R) that include common region of type A, B, E and F neurotoxin genes. The amplicon of first step was used as template for the second amplification with internal sets of primers specific for four toxin types (BT-AR, BT-BR, BT-ER and BT-FR). The specificity of this assay was verified by evaluating with genomic DNA from 11 genera of 14 strains, concluding that the PCR assays could detect *C. botulinum* specifically. Ability to detect *C. botulinum* from marine sediment was confirmed by spiking spores of four types into soil sample. These results suggest that newly developed nested PCR procedure can be applicable to determine a prevalence of *C. botulinum* types A, B, E, and F in soil environments.

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### Effect on of Mer Gene by Cell Density in *Pseudomonase* spp

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The heavy metals most frequently found in polluted environments by many bacteria was survival. The effects of these metals on bacterial growth and protein synthesis were existence and bacterial diversity on polluted environments were existence. Mercury of these metals is toxic metal on living cells. Diversity of Mercury resistance genes on some mines and one of *Pseudomonase* spp. On relationship of cell density and mer genes were studied. Diversity of mer genes on mines was similar to area of polluted environments. Mer gene expression effect on cell density of *Pseudomonase* spp.

**B036**

### Influence of Elevated CO<sub>2</sub> on the Community Structure of Denitrifiers and Methangens in Wetland Soil Investigated with Terminal Restriction Fragment Length Polymorphism (T-RFLP)

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To investigate the effect of elevated CO<sub>2</sub> on the community structure of denitrifiers and methanogens in wetland soils, the genetic heterogeneity of the nitrite reductase gene (*nirS*) and methyl-coenzyme M reductase gene (*mcr*) was analyzed by using terminal restriction fragment length polymorphism (T-RFLP) analysis. Seven species of wetland plants have been continuously exposed to ambient and elevated to CO<sub>2</sub> at twice-ambient concentration (740ppm) for 110 days in growth chambers. *nirS* gene and *mcr* gene fragments could be amplified from all analyzed soil samples. PCR products were purified and digested with restriction enzymes such as HhaI, HaeIII, and MspI. The result showed that different genetic structures were observed between each samples treated with various conditions. The effect of CO<sub>2</sub> treatment was increase, decrease or nothing in the diversity of bacterial species. Overall, the data in this study showed that the response of denitrifying and methane-producing community in the wetland soil to elevated CO<sub>2</sub> might be impacted by another environmental factor such as nutrient and vegetation type.

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