

Screening of Genes from Subseafloor Sediments and Co-Rich Ferromanganese crust Using Substrate-induced Gene Expression (SIGEX) Method.

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The substrate-induced gene expression (SIGEX) method is a gene-screening approach that uses a promoter trap-type vector based on the assumption that general gene expression is induced by substrates/metabolites of catabolic enzymes and that the regulatory elements are often positioned in proximity to the catabolic genes¹. The DNA fragments from environmental samples are inserted upstream of the gene of green fluorescence protein, and induction-positive clones can be selectively isolated by fluorescence-activated cell sorting in a high-throughput manner. One of the advantages of this method is that we can presume the potential gene-function without relying on its sequence information, which can lead to explore new, unknown functions of DNA sequences buried in environmental metagenomes. In this study, we applied SIGEX method to metagenomes of ultra-*oligotrophic* South Pacific Gyre (SPG) subseafloor sediment samples and covalt-rich ferromanganese crust from Dakuyo-Daigo seamount. In SPG sample, we tested substrates of methane, sodium acetate, or manganese chloride as inducing compounds, and isolated in total 87 induction-positive clones. We then performed DNA sequencing on 72 clones (CH₄: 13, CH₃COONa: 52, MnCl₂: 7). On the other sample, we tested substrates of sodium formate, manganese chloride, manganese sulfate, cobalt chloride, nickel chloride, sodium tangstate, and others metallic ions. We isolated in total 58 induction-positive clones and the inserted sequences of 42 clones were determined (HCOONa: 5, MnCl₂: 10, MnSO₄: 8, Na₂WO₄: 14, CoCl₂: 2, FeCl₂(II), NiCl₂, Na₂MoO₄:1). Through database search, we often encountered to the situation that the sequence in induction-positive clones show low similarity to the sequences in the database. One of the notable characteristics for the sequences was that there were highly frequent observation of inverted repeat sequences that we similarly identified in previous studies^{2,3}, but the fragment that we isolated from Takuyo-Daigo seamount didn't have these sequence. We will discuss the results of databases search and functional prediction analyses using available tools for the 114 sequences. The linkage between gene-induction response and gene fragments found in this study indicates that there is still a large room of the unknown genetic function to be explored in the environmental metagenomes.

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2)Futagami T *et al.*, (2013) *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **368**, 20120249.

3)Wakamatsu T *et al.*, (2018) *J. Appl. Microbiol.*, **125**, 1396-1407.

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