

PH-11**Genetic diversity of protist genes encoding glycoside hydrolase family in the termite gut**Keisuke Gyoji¹, Masahiro Yuki², Takashi Ohtsuki¹,
Moriya Ohkuma², Sadaharu Ui¹, Satoko Noda¹¹Grad. Sch. of Med. and Eng., Univ. of Yamanashi, ²BMEP, BRC-JCM, RIKEN**食性昆虫に共生する原生動物の木質分解酵素遺伝子の多型解析**○行仕 圭佑¹、雪 真弘²、大槻 隆司¹、大熊 盛也²、宇井 定春¹、
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オリソースセンター 微生物材料開発室**Key word** : protist, cellulase gene, termite

Introduction: The relationship between termites and microbial community in their gut is a well-known example of symbiosis, which aids efficient digestion of recalcitrant lignocellulose. Because the symbiotic protists are extremely difficult to cultivate, our knowledge about their physiology and functions are poor. In this study, single cell approach was applied for the symbiotic protist to investigate the process for lignocellulose degradation. **Results & Discussion:** The cells showing typical morphology of the genus *Eucomonympha* and *Trichonympha* were physically isolated from protistan community in the termite gut and used for cDNA synthesis or isothermal whole genome amplification. Three genes that encodes the Glycoside Hydrolase Family (GHF) 7 and 45 cellulase and GHF10 xylanase were amplified by PCR. Each product was cloned and determined the DNA sequences. The result showed that several GHF genes were simultaneously expressed in each cell, and major expressed sequence of each family was common within the protist genera. Genetic variation and the detection rate of the clones possessed nucleotide deletion or stop codon are relatively higher in the sample derived from genomic DNA. These results suggest that the GHF gene was selectively expressed from many homologous genes in the genome, it probably attributed to the efficient degradation of lignocellulose by the gut protists.

ポスター PI**Microbiological methods 方法論****PI-01****Application of electric adhesive device to separate live microbial cells from termite guts**Kumiko Kihara^{1,3,4}, Sumihiro Koyama², Shigeharu Moriya^{3,4},
Yuichi Hongoh^{1,3}¹Grad. Sch. of Biosci. and Biotech, Tokyo Institute of Technology,
²JAMSTEC, ³RIKEN, ⁴Env. Mol. Biol. Div, Yokohama City University**シロアリ腸内から生きた共生細菌だけを電極基板で分けとる方法の開発**○木原 久美子^{1,3,4}、小山 純弘²、守屋 繁春^{3,4}、本郷 裕一^{1,3}¹東工大・生命理工、²海洋開発機構、³理化学研究所、⁴横浜市大・環境分子**Key word** : gut bacteria, isolation, attachment, symbiosis, insect

Environmental samples contain various materials, which potentially inhibit chemical reactions necessary for studies of microbes therein. Therefore, development of methodology to separate live microbial cells from any non-organismal substances is crucially important. Recently, Koyama has invented a device that can collect live cells by adhesion, using a low level electric potential-loaded electrode. Here, with this newly developed device, we attempted to collect live microbial cells from termite guts. Termite guts contain not only live symbiotic microbes, but also a large amount of wood particles, soil particles, and dead microbial cells. We optimized the method of sample preparation, buffer, electrode shape, reaction temperature, concentration of oxygen, and reaction time. As a result, we successfully separated and obtained only microbial cells from the termite guts. In addition to the detailed results, we will present the most recent results and discuss future applications such as single-cell omics analyses.

PI-02**Development of rapid and simple method for total counting of viable denitrifying bacteria in wastewater treatment processes**Takeshi Yamada, Zyunichi Kawashima, Tomoya Hiraoka,
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廃水処理プロセスにおける迅速・簡便な脱窒細菌の生菌数計測方法の開発

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豊橋技科大・環境生命工

Key word : biological denitrification process, denitrifying bacteria, CTC, total viable cell

The biological denitrification processes have been controlled by physicochemical parameters such as temperature, pH, and nitrate removal efficiency. To accurately control the processes, information on numbers of viable microorganisms that play a role for denitrification in the wastewater treatment processes for nitrogen removal is very important. Although some methods such as quantitative RT-PCR and mRNA-based FISH have been known for this purpose, a rapid and simple technique is absolutely nothing for counting viable denitrifying bacteria in the processes. In this study, we consider to apply 5-cyano-2,3-ditoly-2-tetrazolium chloride (CTC) method, which have been used as rapid and simple detection tool for viable aerobic microorganisms, to measurement of viable denitrifying bacteria. For determination of optimum condition of the CTC methods, several nirS and nirK-type denitrifying bacteria and activated sludge under denitrifying conditions are prepared, and CTC concentration, CTC reaction time and the concentration of nitrate respiration inhibitor are explored. The results revealed that the optimum conditions for the CTC method was 0.2 mM CTC, 1 h of reaction time and 1 mM of nitrate respiration inhibitor. Under the optimum condition, the CTC method could detect more than 80% of viable denitrifying bacteria in pure cultures and activated sludge under denitrification condition.