

論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Molecular ecological studies of uncultured rare bacterial groups in the termite gut
著者(和文)	UTAMI YUNIAR DEVI
Author(English)	Yuniar Devi Utami
出典(和文)	学位:博士(理学), 学位授与機関:東京工業大学, 報告番号:甲第10937号, 授与年月日:2018年9月20日, 学位の種別:課程博士, 審査員:本郷 裕一,田中 寛,増田 真二,二階堂 雅人,山田 拓司
Citation(English)	Degree:Doctor (Science), Conferring organization: Tokyo Institute of Technology, Report number:甲第10937号, Conferred date:2018/9/20, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	要約
Type(English)	Outline

Thesis Outline

Molecular ecological studies of uncultured rare bacterial groups in the termite gut

Graduate School of Biosciences and Biotechnology

Department of Biological Sciences

Yuniar Devi Utami

Supervisor: Yuichi Hongoh, D. Sc.

The termite gut is populated with microbes, comprising protists, bacteria, and archaea. Most of these are unique to termites and extremely resistant to cultivation. Among them, I focused on the non-photosynthetic cyanobacterial class “*Melainabacteria*” and the candidate phylum Termite Group 2 (TG2 or ZB3), both of which are less abundant but consistently found bacterial clades in the termite gut. These clades are uncultured, and only limited information has been available to date. I performed fluorescence *in situ* hybridization (FISH) analyses, single-cell genomics, and metagenomics to understand their ecological and physiological characteristics as well as their symbiotic functions in the termite gut ecosystem.

The 16S rRNA gene of phylotype Rs-H34 (AB089123) was previously found from the gut of termite *Reticulitermes speratus*, and now it has been recognized as a member of “*Melainabacteria*”. The 16S rRNA gene amplicon sequencing analysis was performed and melainabacterial sequences were detected from 48 out of the 68 termite and cockroach species with low abundances (0.02–1.90%). A single-cell genome of a melainabacterium, designated as phylotype Tpq-Mel-01, was obtained using a fluorescence-activated cell sorter (FACS) and whole genome amplification (WGA), from the gut of the termite *Termes propinquus*. The Tpq-Mel-01 genome shared basic features with other melainabacterial genomes previously reconstructed from human and koala fecal metagenomes. It has a small genome (~1.6 Mb), lacked genes for photosynthesis and the carbon fixation pathway, and was predicted to be fermentative, possibly using sugars and chitobiose for its energy and carbon sources. The bacterium is putatively motile and does not appear to directly play critical roles in the mutualism between the gut microbiota and the termite host, such as cellulose hydrolysis and nitrogen fixation; it may be a commensal gut bacterium. On the basis of the genome sequence and FISH data, I propose a novel species, “*Candidatus Gastranaerophilus termiticola*”, for this Tpq-Mel-01 bacterium.

The uncultured candidate phylum TG2/ZB3 was previously proposed based on 16S rRNA sequences from the gut of the termite *Reticulitermes speratus* and a sulfide-rich spring, and recently incorporated to the candidate phylum “*Margulisbacteria*”. The 16S rRNA gene amplicon sequencing analysis showed that TG2/ZB3 sequences were found from the guts of 40 out of 72 termite/cockroach species with low abundances (0.01–1.58%). All these sequences exclusively formed a monophyletic cluster and shared $\geq 94\%$ 16S rRNA sequence identity; I propose a novel genus for these uncultured bacteria, “*Candidatus Termitenax*”, which represents a new class-level clade in the candidate phylum “*Margulisbacteria*”. FISH analyses revealed that they are short or curved rods and that they specifically attached to ectosymbiotic spirochetes of oxymonad protists in the gut of lower termites. Draft genome sequences of four “*Ca. Termitenax*” phlotypes were obtained by single-cell genomics using the FACS from the termite *Hodotermopsis sjostedti* or by binning metagenomic contigs obtained from bacterial communities associated with single oxymonad protist cells from the termites *R. speratus* and *Neotermes koshunensis*. Draft genome sequences of four “*Ca. Termitenax*” phlotypes and a partner spirochete species were reconstructed. The functional genome analyses suggested that “*Ca. Termitenax*” has a potential for cellulose hydrolysis and fermentation of the resulting glucose to H₂, CO₂, ethanol, and acetate. The draft genome of the spirochete partner encoded genes responsible for reductive acetogenesis from H₂ and CO₂; I suggest that “*Ca. Termitenax*” is a commensal symbiont of the spirochetes, exploiting them as H₂ sinks.

The results from this study provide novel insight into the complex, multi-layered termite gut ecosystem and also greatly expand our knowledge of these underrepresented, yet-uncultivable bacterial groups.