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論 文 要 旨

THESIS SUMMARY

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学生氏名： Student's Name	UTAMI YUNIAR DEVI	指導教員（主）： Academic Supervisor(main)	本郷裕一
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

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 the diverse microbes detected in the termite gut, 16S rRNA genes from two bacterial lineages, namely non-photosynthetic cyanobacterial class "*Melainabacteria*" and an uncultured bacterial clade, Termite Group 2 (TG2), are consistently found from the gut of diverse termite and cockroach species. In this study, 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 within the termite gut ecosystem.

The phylum *Cyanobacteria* has recently expanded its assemblages from only photosynthetic bacterial class *Oxyphotobacteria*, to that also accommodating the candidate classes "*Melainabacteria*" and "*Sericytochromatia*", both of which lack the photosynthetic machineries. 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 presence of this phylotype suggests that the termite guts are potential habitats for this rare bacterial group.

The 16S rRNA gene amplicon sequencing analysis using the Illumina MiSeq platform was performed for the gut microbiota of 60 termite and eight cockroach species, and melainabacterial sequences were detected from 48 out of the 68 insect species with low abundances (0.02-1.90%). Most of these sequences (amplicon sequence variants: ASVs) were affiliated with the strictly anaerobic order "*Gastranaerophilales*" and two ASVs were with the microaerophilic order "*Obscuribacterales*". 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. Fluorescence in situ hybridization (FISH) analyses using a specific probe for Tpq-Mel-01 showed that the bacterium is a short rod with dimensions of 0.8-1.2 μm by 0.4-0.6 μm. 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 other target of this study, TG2, was previously discovered based on 16S rRNA sequences, namely Rs-A23 (AB089067) and Rs-J96 (AB089068) from the gut of *R. speratus*. These sequences did not cluster with any known bacterial phyla at that time. This TG2 clade is currently recognized also as ZB3 and as a member of the candidate phylum "*Margulisbacteria*". I aimed to reveal the phylogenetic diversity, localization, and ecological functions of the TG2 bacteria in the termite gut ecosystem and also to clarify the taxonomic status of the termite-derived TG2/ZB3 bacteria within "*Margulisbacteria*".

Amplicon sequencing of 16S rRNA genes was performed similarly as above, using 62 termite and 10 cockroach species. TG2 sequences were found from 34 out of 72 insect host species with low abundance (0.01-1.58%). Additional cloning analysis using a newly designed primer set specific to 16S rRNA of TG2/ZB3 further explored its sequences from additional 6 insect host species. All these sequences exclusively formed a monophyletic cluster within the phylum "*Margulisbacteria*" and shared ≥ 94% 16S rRNA sequence identity; I propose a novel genus, "*Candidatus* Termitenax", which represents a class-level clade in "*Margulisbacteria*". FISH analyses using a probe specific to the 16S rRNA of "*Ca.* Termitenacis" revealed their specific attachment to ectosymbiotic spirochetes of oxymonad protists in the gut of three lower termite species. They are short rods or curved rods with dimensions of 0.6-1.4 μm by 0.2-0.4 μm.

Draft genome sequences of four "*Ca.* Termitenax" phylotypes were obtained by single-cell genomics using FACS from the termite *Hodotermopsis sjoestedti* or by binning metagenomic contigs obtained from bacterial communities associated with single oxymonad protist cells from the termite *R. speratus* and *Neotermes koshunensis*. Two

high-quality draft genomes (completeness ~90%) and two partial genomes (completeness <10%), as well as a draft genome (completeness ~60%) of a partner spirochete species, were reconstructed and analyzed. The functional genome analyses suggested that "*Ca.* Termitenax" has potential to hydrolyze cellulose and ferment glucose to H_2, ethanol and acetate. Since the partner spirochete genome encoded genes responsible for the reductive acetogenesis from H_2 and CO_2, I suggest that "*Ca.* Termitenax" are commensal symbionts of the spirochetes, having exploited them as H_2 sinks.

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