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Outline of Research Result

Metabolic Symbiosis between Termite Gut Protists and Their Intracellular Bacteria

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Research aims

Termites are one of the few arthropods that exclusively thrive on dead plant matter and efficiently decompose lignocellulose. A dense and diverse microbial community in the gut of termites, comprising of both flagellated protists (single cell eukaryotes) and prokaryotes, is essential for the efficient decomposition, and thus is expected to be an attractive model for efficient utilization of biomass resources¹). Although most members of the community are difficult to cultivate, molecular ecological studies reveal the enormous diversity and novelty of the resident microbial species^{2,3}). A unique and impressive character of the termite-gut community is its highly structured nature; the specific associations of various prokaryotes with the cells of gut protists comprise predominant population in the gut community³⁻⁸). These associations are considered as keys to understand the mechanisms of the efficient decomposition. In this study, the primary metabolism of the host protists and symbiotic interactions with their associated prokaryotes were investigated.

Methods

Culture-independent molecular approaches were mainly applied. Expressed sequence tags (EST) analysis of a cDNA library constructed for mixed-population of gut protists of the termite *Coptotermes formosanus*⁹) and the following comparisons with the public databases were conducted to reconstruct the primary metabolism of gut protists (manuscript in preparation). This termite harbors only the three species of protist belonging to the phylum Parabasalia that carry anaerobic energy-producing organelles, hydrogenosomes¹⁰). Genes encoding hydrogenase homologs were heterologously expressed, and the recombinant enzymes were purified and characterized. The protist origins of the hydrogenase genes were also identified by sequence-specific diagnostic PCR with manually isolated cells of each protist species and whole-cell in situ hybridizations using specific probes. After enrichment of the protist cells, the cellular components were fractionated to evaluate hydrogenase activity¹¹).

Results

In the EST analysis, various genes for cellulases were identified, which were categorized to glycosyl hydrolase families 5, 7, and 45 as shown in the case of gut protists in the termite *Reticulitermes speratus*¹¹). Genes for most glycolytic enzymes and hydrogenosomal functions, such as pyruvate:ferredoxin oxidoreductase and hydrogenase were also identified. It is noted that genes encoding phosphoenolpyruvate (PEP) carboxykinase (PCK) and malate dehydrogenase (MDH) in the cytoplasm as well as hydrogenosomal malic enzyme (ME) were highly expressed when compared to the gene involving in direct transformation of PEP to pyruvate. The results suggest that malate is produced in the cytoplasm and transported into hydrogenosomes to ferment to acetate, CO₂, and H₂ (Fig. 1).

Two genes encoding hydrogenosomal iron-only (Fe-) hydrogenases, which were phylogenetically distinct and carried different N-terminal accessory domains, were identified in the largest cellulolytic protist *Pseudotrichonympha grassii*. The active recombinant enzymes of these two Fe-hydrogenases were successfully obtained and exhibited different specific activity and optimal pH, suggesting the different physiological roles (Table 1). Both Fe-hydrogenases preferentially catalyzed H₂ evolution rather than H₂ uptake. H₂ evolution of at least the short-form enzyme was still active even under high hydrogen partial pressure. The results suggest that termite-gut symbionts are a rich reservoir of novel Fe-hydrogenases whose property is adapted to the gut

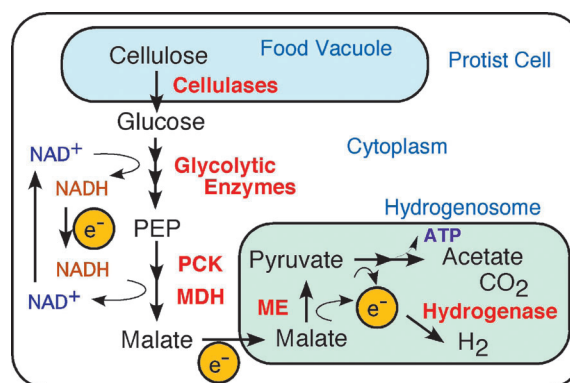


Fig. 1. Schematic showing the reconstructed primary metabolism of parabasalian protists in the gut of termites, with the emphasis on the flow of reducing equivalents (e⁻).

Table 1. Characteristics of two Fe-hydrogenases in *P. grassii*

	Activity		Optimal pH
	H ₂ evolution	H ₂ uptake	
Long-form	121 ± 7.8	ND	6.0
Short-form	2131 ± 54	431 ± 12	8.0

Hydrogenase activity (\pm standard deviation) measured using methylviologen as an electron carrier is expressed as mmol H₂ min⁻¹ mg-protein⁻¹. ND, not detected.

environment.

Fractionation of cellular components of *P. grassii* demonstrated significant H₂ evolution activity in the hydrogenosomal fraction. The vigorous H₂ uptake activity was detected in the fraction enriched in its endosymbiotic bacteria that are affiliated to a novel lineage of the order Bacteroidales⁸⁾, and as a result, the strong H₂ evolution activity of the host protists was compensated. Indeed, living termites emit H₂ at the considerably lower level (0.75 mol H₂ emitted per molar glucose derived from cellulose) than that predicted stoichiometrically (4 mol H₂ per molar glucose). The potential of H₂ production in termite guts had been largely underestimated.

Conclusion

It is generally believed that gut protists phagocytose wood particles and degrade cellulose within their cells. The reconstruction of their primary metabolic pathway supports the cellulolytic property of gut protists. The merit to produce malate in the cytoplasm is that most of the reducing equivalents produced during the cellulose fermentation as a form of NADH were regenerated in the cytoplasm, and transferred as a form of malate to hydrogenosomes where the strong Fe-hydrogenases dispose the reducing power as a form of H₂ (Fig. 1). The endosymbiotic bacteria effectively eliminate the produced H₂. Further studies are necessary to clarify the primary functions of the endosymbionts, particularly the electron acceptor of the endosymbionts that utilize hydrogen. The genome analysis of the endosymbiont is now in progress. Nevertheless, the efficient elimination of reducing equivalents, an important rate-limiting step for anaerobic fermentation, leads to the enhanced cellulose fermentation. The symbiotic associations between termite-gut pro-

tists and prokaryotes as well as the metabolic functions of the protists greatly contribute to the efficiency of cellulose utilization of termites.

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