REVIEW ARTICLE

Michael F. Dolan

Speciation of termite gut protists: the role of bacterial symbionts

Abstract At least 12 termite gut protists have been named because of their bacterial symbionts. Dozens more species are diagnosed by epi- and endosymbionts and more still have regular bacterial associations referred to in their species description. Molecular systematic studies have begun to identify these bacteria, but the ecological relations with their protist bionts are still unknown. Recent findings of acetogenic spirochetes in termite guts may explain the peculiar arrangement of spirochetes on some of these protists. Other bacteria function as motility or chemotactic symbionts of these protists. The size and shape of the parabasal body, a Golgi complex, are morphological characters of the Parabasalia (trichomonads, hypermastigids) that may be influenced by regular, heritable epi- and endosymbiotic bacteria.

Keywords Bacterial symbionts · Termite gut protists · Parabasalia · Endosymbiosis · Trichomonads

Nature of termite gut symbiont species designation

Of the 440 species of amitochondriate protists in the groups Trichomonadida, Hypermastigida, and Oxymonadida, the vast majority are found exclusively as gut symbionts of wood-eating termites (Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae, Serritermitidae) and the wood-eating roach, *Cryptocercus*. With the exception of the roach symbionts, apparently all have evolved in the absence of meiotic sex. Their species descriptions are based on cell morphology differences. Morphology-based descriptions often include precise arrangements of bacterial symbionts on the cell surface and in specific regions of the cytoplasm. The use of

gene sequencing and in situ hybridization allows us now to go beyond morphology, to assign more informative identifications to these bacterial symbionts. Here, I review the extent of the bacterial symbioses of the termite gut protists, the diversity of bacteria in the termite gut, and the first attempts at the molecular level to characterize these bacterial-protist symbioses.

Species of trichomonad and other amitochondriate protist symbionts of wood-eating termites and *Cryptocercus* are designated by a collection of morphological characters centered on their motility structures (e.g. the organellar system known as the mastigont) and, in the case of the parabasalia (trichomonads, hypermastigids), the parabasal body (Golgi complex). These characters include the number and arrangement of flagella (undulipodia); the presence and shape of accessory structures, e.g. the costa and cresta, and the arrangement of connected microtubular structures, the axostyle and pelta. The parabasal body, connected to the mastigont in the trichomonads and arranged in multiple copies in the hypermastigids, can have a distinct size and shape and can branch or spiral around the axostyle. While each termite species has a characteristic community of gut protists, protist species are generally not restricted to one species of termite [34].

Bacterial symbionts as eukaryotic species characters

Most descriptions of the termite gut protists occurred more than 50 years ago and were based on traditional protozoological stains, such as hematoxylin and protargol. These provided excellent preparations for morphological species characterization, including the description of unidentified inclusions and epibionts. DNA-specific stains like the Feulgen reaction, although available, were generally not used prior to naming new species. While there was some uncertainty amongst protozoologists over the nature of these inclusions, they were generally thought to be bacterial symbionts. Taxonomists recognized that these bacterial symbionts were integral to the specific nature of the protists.

M.F. Dolan
Department of Geosciences,
University of Massachusetts. Amherst, MA 01003, USA
E-mail: mdolan@geo.umass.edu
Tel.: +1-413-545-3244
Fax: +1-413-545-1200

The presence or absence of bacterial associates was often mentioned in papers describing these new protists. In 12 cases, the bacterial symbionts were acknowledged in the protist name (Table 1). The bacterial symbionts are generally of three types: epibiotic spirochetes, epibiotic rods, and endobiotic rods (such as on the pelta or around the axostyle, the central microtubule cytoskeletal element). Of the 64 species of oxymonads, 8 species diagnoses mentioned the regular presence of bacterial symbionts, and a further 11 species descriptions included symbiotic bacteria (Table 2). Among trichomonads, of the 177 symbionts named to date, the species diagnosis of 56 explicitly refer to bacterial symbionts. The description and figures of a further 18 species mention bacterial associates (Table 3).

Of the four families of trichomonads, the two that are found exclusively as termite symbionts, Devescovinidae and Calonymphidae, have the most bacteria-associated species, whereas the two other families, Monocercomonadidae and Trichomonadidae, which are found as symbionts in a variety of animals, have fewer described

bacterial symbionts. Whole genera of protists were noted for their regular bacterial symbionts, e.g. all species of *Devescovina* have striations due to rod or fusiform epibionts [13]. *Mixotricha*, with its conspicuous cortex, was first described as having a coat of cilia [28]. It was subsequently found to be a trichomonad whose cortex harbors more than 100,000 regularly distributed treponema spirochetes, an equal number of rod bacteria, and several hundred larger spirochetes (*Canaleparolina darwiniensis*) [33]. Spirochetes, which are particularly abundant in wood-eating termite guts, were often reported as occupying discrete locations on the cell surface (FIg. 1). In several cases, two similar protist species were distinguished by the presence or absence of bacterial symbionts (e.g. *Metadevescovina turbula*, *M. nudula*).

Some variation in the description of bacterial symbionts was due to the investigator naming the organism. Protozoologists like Grassé and Kirby usually described the bacteria present, whereas Cleveland, who was more interested in the cell biology of the protists, did not. This can be seen clearly in a detail of the work by Cleveland et al. on *Cryptocercus* [5], in which they distinguish two species of *Spirotrichonympha*: "The description given for the axostyle, flagella, parabasals, and other extranuclear organelles of *S. polygyra* takes care of these organelles in *S. bispira*, and we may proceed immediately with the description of mitosis." Because Cleveland et al. named so many hypermastigids (45 species), original descriptions of symbionts from this order may underrepresent the incidence of regular, heritable bacterial symbionts. Of 206 species of hypermastigids, only 2 were diagnosed and a further 14 were described to have symbiotic bacteria (data not shown).

Major groups of bacteria in the wood-eating termites' gut

Many bacterial species were described from termite guts, first by culturing, which was thought to produce a small

Table 1 Termite gut symbionts whose species names reflect bacterial associations. See [34] for references

Genus	Comment
Devescovina glabra	Bald due to lack of spirochetes; rods on surface
D. insolita	Strange due to mixture of rods and spirochetes
D. striata	Surface fusiform bacteria make striations
D. vestita	Spirochetes and rods cover surface
Dinenympha fimbriata	Fringe of bacteria on surface
Evemonia punctata	Short rods cover surface
Hyperdevescovina balteata	Belt of rods around cell
Metadevescovina nudula	Naked, without spirochetes
M. turbula	Disorderly grouping of spirochetes
Mixotricha paradoxa	Ectosymbiotic spirochetes (two kinds)
Oxymonas pediculosa	"Lousy" with adherent bacteria
Streblomastix strix [6]	Grooves harbor ectosymbionts that function as sensory organs

Table 2 Genera of termite gut oxymonads diagnosed or described in part by bacterial associations. See [34] for references

Genus	No. of species	Diagnosed	Described, but not diagnosed
Monocercomonoididae			
Monocercomonoides	1	0	0
Oxymonadidae			
Barroella	2	1	0
Microrhopalodina	4	1	1
Notila	1	0	0
Opisthomitus	1	0	0
Oxymonas	28	5	3
Saccinobaculus	5	0	0
Sauromonas	2	0	1
Pyrsonymphidae			
Dinenympha	10	1	4
Pyrsonympha	9	0	2
Streblomastix	1	0	0
Total	64	8	11

Table 3 Genera of termite gut trichomonads diagnosed or described in part by bacterial associations. See [34] for references

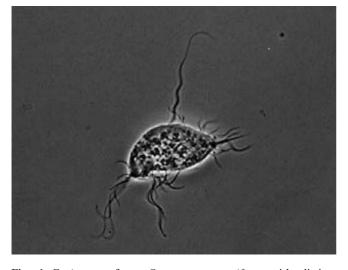| Genus	No. of species	Diagnosed	Described, but not diagnosed
Monocercomonadidae			
Hexamastix	8	1	2
Monocercomonas	2	0	0
Tricercomitus	6	0	1
Devescovinidae			
Achemon	1	0	0
Astronympha	1	1	0
Bullanympha	1	1	0
Caduceia	8	8	0
Devescovina	28	17	0
Evemonia	3	3	0
Foaina	30	0	6
Gigantomonas	1	0	0
Hyperdevescovina	8	8	0
Kirbynia	3	0	1
Macrotrichomonas	10	4	2
Metadevescovina	22	9	2
Mixotricha	1	1	0
Parajoenia	1	0	1
Polymastigoides	1	0	0
Pseudodevescovina	1	0	1
Calonymphidae			
Calonympha	3	1	0
Coronympha	2	0	0
Metacoronympha	1	0	0
Snyderella	4	0	1
Stephanonympha	12	2	1
Trichomonadidae			
Pentatrichomonoides	1	0	0
Pseudotrypanosoma	2	0	0
Trichomitopsis	4	0	0
Trichomonas	8	0	0
Tritrichomonas	4	0	0
Total	177	56	18

Fig. 1 *Foaina* sp. from *Cryptotermes cavifrons* with distinct arrangement of spirochete episymbionts. The anterior most group (lower center) is near the hydrogenosomes. Cell is 35 μm long

percentage of the bacteria present, and recently by molecular systematic studies using, whole gut homogenates (Table 4). The bacteria that can be readily identified to phylum by morphology are spirochetes, methanogens, and mycoplasmas [9]. Other bacteria found include lactic acid bacteria [1], sulfate-reducing bacteria [10], and some that fix nitrogen [23].

Wood-eating termite guts contain a huge abundance and diversity of spirochetes, both free-swimming in the gut and as protist episymbionts. Molecular systematic studies of 16S rRNA genes place these with treponemes [18]. Morphological studies of large spirochetes led to the erection of new genera [4]. The contradictory nature of these two approaches is seen in at least one case [2], in which a large spirochete is put in the treponeme group. Some spirochetes of the termite gut have been shown to be acetogenic [18], which may explain the positioning of spirochetes at the anterior of the cell, near the hydrogenosomes (Fig. 1). The production of H_2 and CO_2 by the protists has attracted acetogens and methanogens as symbionts.

Identifying bacterial affinities through gene sequences

The first uses of gene sequences and in situ hybridization to assign a group identity to bacterial symbionts of termite gut protists were reported by Gunderson and others at American Society for Microbiology meetings

Table 4 Bacteria from intestines of *Cryptocercus* and wood-eating termites

Bacterium	Protist	Termite	Reference
Mastotermitidae			
Acinetobacter calcoaceticus		*Mastotermtes darwiniensis*	[14]
Bacillus cereus		*M. darwiniensis*	[21]
(= *Arthromitus*)			
Burkholderia sp.		*M. darwiniensis*	[9]
Citrobacter freundii		*M. darwiniensis*	[8]
Clostridium sporogenes		*M. darwiniensis*	[14]
Cytophaga/Flavobacterium		*M. darwiniensis*	[3]
cluster			
Desulfovibrio intestinalis		*M. darwiniensis*	[10]
Enterobacter aerogenes		*M. darwiniensis*	[14]
Enterobacter		*M. darwiniensis*	[7]
Enterococccus sp. str. JF1		*M. darwiniensis*	[9]
Flavobacterium		*M. darwiniensis*	[7]
Klebsiella pneumoniae		*M. darwiniensis*	[14]
Methanobrevibacter sp.	*Pentatrichomonoides scroa*	*M. darwiniensis*	[9]
Mycoplasma sp.	*Koruga bonita*	*M. darwiniensis*	[9]
Ochrobactrum anthropi		*M. darwiniensis*	[14]
Pseudomonas aeruginosa		*M. darwiniensis*	[14]
Serratia marcescens		*M. darwiniensis*	[14]
Sphingomonas sp. str. JF2		*M. darwiniensis*	[9]
Streptococcus		*M. darwiniensis*	[7]
Near *Treponema* sp. H1 (11)		*M. darwiniensis*	[2]
Kalotermitidae			
Acetonema longum		*Pterotermes occidentis*	[12]
Bacillus cereus		*Cryptotermes brevis*	[21]
(= *Arthromitus*)			
B. cereus (= *Arthromitus*)		*C. cavifrons*	[21]
B. cereus (= *Arthromitus*)		*Glyptotermes* sp.	[21]
B. cereus (= *Arthromitus*)		*Incisitermes minor*	[21]
B. cereus (= *Arthromitus*)		*Kalotermes approximatus*	[21]
B. cereus (= *Arthromitus*)		*K. flavicollis*	[21]
B. cereus (= *Arthromitus*)		*K. praecox*	[21]
B. cereus (= *Arthromitus*)		*K. schwartzi*	[21]
B. cereus (= *Arthromitus*)		*P. occidentis*	[21]
Near *Bacteroides*		*C. domesticus*	[22]
Desulfovibrio termitidis		*Heterotermes indicola*	[32]
Diplocalyx calotermitidis		*K. flavicollis*	[4]
Enterobacter		*C. primus*	[7]
Hollandina pterotermitidis		*P. occidentis*	[4]
Near *Leuconostoc*		*C. domesticus*	[23]
Near *Methanobrevibacter*		*C. domesticus*	[23]
Pillotina calotermitidis		*K. praecox*	[4]
Streptococcus		*C. primus*	[7]
Near *Treponema*		*C. domesticus*	[23]
Rhinotermitidae			
Alcaligenes sp.		*Reticulitermes hesperus*	[29]
Arthrobacter sp.		*R. hesperus*	[29]
Arthrobacter-like		*R. santonensis*	[14]
Aureobacterium liquefaciens		*R. santonensis*	[14]
Bacillus cereus		*R. hesperus*	[29]
B. cereus		*R. santonensis*	[14]
B. cereus (= *Arthromitus*)		*Coptotermes formosanus*	[21]
B. cereus (= *Arthromitus*)		*R. flavipes*	[21]
B. cereus (= *Arthromitus*)		*R. hesperus*	[21]
B. cereus (= *Arthromitus*)		*R. tibialis*	[21]
B. firmus		*R. santonensis*	[14]
B. licheniformis		*R. santonensis*	[14]
Bacillus		*C. acinaciformis*	[7]
Bacillus		*Schedorhinotermes intermediatus*	[7]
Bacteroides		*R. flavipes*	[27]
Bacteroides termitidis		*R. flavipes*	[24]
Citrobacter amalonaticus		*R. santonensis*	[14]
C. freundii		*C. lacteus*	[8]
Citrobacter		*R. flavipes*	[27]
Clevelandina reticulitermitidis		*R. tibialis*	[4]
Desulfovibrio sp.		*R. santonensis*	[15]
Enterobacter agglomerans		*C. formosanus*	[24]

Table 4 (Contd.)

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Bacterium	Protist	Termite	Reference
E. cloacae		*R. flavipes*	[27]
E. cloacae		*R. santonensis*	[14]
Enterobacter sp.		*R. santonensis*	[14]
Enterobacter		*C. acinaciformis*	[7]
Enterobacter		*C. lacteus*	[7]
Enterobacter		*Heterotermes ferox*	[7]
Enterobacter		*S. intermediatus*	[7]
Near *Enterococcus faecalis*		*R. flavipes*	[1]
Enterococcus strain RFL6		*R. flavipes*	[30]
Fusobacterium		*R. flavipes*	[27]
Lactobacillus		*R. flavipes*	[27]
Near *Lactococcus lactis*		*R. flavipes*	[1]
Listeria innocua		*R. santonensis*	[14]
Methanobrevibacter curvatus		*R. flavipes*	[16]
M. cuticulatus		*R. flavipes*	[16]
M. filiformis		*R. flavipes*	[17]
Methanobrevibacter sp.	*Dinenympha parva*	*R. speratus*	[31]
Ochrobactrum anthropi		*R. santonensis*	[14]
Pseudomonas aeruginosa		*R. santonensis*	[14]
Serratia ficaria		*R. santonensis*	[14]
S. marcescens		*R. hesperus*	[29]
Streptococcus cremoris		*R. flavipes*	[27]
S. lactis		*R. flavipes*	[27]
Streptococcus		*C. lacteus*	[7]
Streptococcus		*H. ferox*	[7]
Streptococcus		*S. intermediatus*	[7]
Treponema cluster II	*Dinenympha porteri*	*R. speratus*	[11]
Termopsidae			
Bacillus cereus (= *Arthromitus*)		*Zootermopsis angusticolis*	[21]
Methanobrevibacter sp.	*Dinenympha* sp.	*Hodotermopsis sjoestedti*	[31]
Methanobrevibacter sp.	*Hexamastix termopsidis*	*Z. angusticollis*	[19]
Methanobrevibacter sp.	*Microjoenia*	*H. sjoestedti*	[31]
Methanobrevibacter sp.	*Tricercomitus termopsidis*	*Z. angusticollis*	[19]
Methanobrevibacter sp.	*Trichomitopsis termopsidis*	*Z. angusticollis*	[19]
Gamma-proteobacterium	*Trichonympha* sp.	*Zootermopsis* sp.	[7]
Treponema ZAS 1 and ZAS 2		*Z. angusticollis*	[18]
Treponema cluster II	*Dinenympha* sp.	*H. sjoestedti*	[11]
Treponema cluster II	*Pyrsonympha* sp.	*H. sjoestedti*	[11]

between 1996 and 2000. They concluded that the rods covering the *Barbulanympha* spp of *C. punctulatus* were related to the *Bacteroides*/*Porphyromonas* complex. In a subsequent report, this group concluded that one of the epibionts (they did not indicate which) of *Caduceia versatilis* from *Cryptotermes cavifrons* was also related to this bacterial complex. Most spirochete epibionts of *Dinenympha porteri* from *Reticulitermes speratus* and *Pyrsonympha* sp. and *Dinenympha* sp. from *Hodotermopsis sjoestedti* were from the *Treponema bryantii* subgroup of treponemes but, in some cases, the ectosymbionts on a single protist were shown to be of at least three phylogenetically distinct spirochetes [11, 20, 25].

As more episymbionts are identified by gene sequence analysis, their role in the speciation of two related protists can be better investigated. While probably some of the epibionts have a weak association with the protists, the provision of "docking sites" and other specialized cell-surface features at the site of bacterial attachment [26] suggests that the associations are more integrated. Endobionts are probably even more tightly integrated in protist metabolism. If they receive gene products or in-

duce the protist to produce specific membrane-associated proteins, the bacterial symbionts may influence the location, number, size, and shape of the parabasal bodies, one of the key sets of morphological characters used in delineating species.

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