

Chapter 13

SYMBIOTIC ASSOCIATIONS WITH PROTISTS

¹Tetsushi Inoue, ²Osamu Kitade, ³Tsuyoshi Yoshimura and ⁴Ikuo Yamaoka

¹Center for Ecological Research, Kyoto University, Otsu 520-2113 Japan: ²Faculty of Science, Ibaraki University, Mito 310-8512, Japan: ³Wood Research Institute, Kyoto University, Kyoto 611-0011, Japan: ⁴Faculty of Science, Yamaguchi University, Yamaguchi 753-8512, Japan.

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Abstract: Progress in understanding the symbiosis between protists and termites has not matched that between prokaryotes and termites. Methods are now available for the isolation of pure cultures of trichomonads and hypermastigids, although only a few have been cultivated. Sufficient molecular data are now available to construct tentative phylogenetic trees. Molecular data indicate that these organisms are amongst the most primitive eukaryotes lacking mitochondria; the trichomonads also use a prokaryote-like 70S ribosome. In metabolic terms, more is known about the cellulolytic protists but only in outline.

1. INTRODUCTION

The association of flagellated protists with termites is, for most biologists, a textbook example of symbiosis but it has proved difficult to define the role of protists in that symbiosis. The general view that they are cellulolytic, mutualistic organisms digesting cellulose for the termite is simplistic. If this were true, why are they absent in the higher termites which constitute the majority of species? Perhaps the answer to this question will come from molecular phylogenetic studies which may help us to understand not only the phylogenetic relationships between termite protists (and bacteria) but also provide clues to the origin of the symbiotic association. Much of the work published since *Biology of Termites* [34] on termite protists has been concerned with descriptions of new taxa [49, 50, 60, 61] or detailed studies such as those on rotary motors

of flagellates [66, 81] or ultrastructural studies [10, 46, 63]. While such studies have added to our knowledge of described species, they tell us little about the symbiotic association and are really of more interest to protozoologists than termite scientists and thus will not be examined in detail in this chapter.

There are many examples of termite protists having, in addition, symbiotic associations with bacteria. For example, one of Cleveland's last contributions to termite biology was the demonstration that the locomotion of *Mixotricha paradoxa*, a trichomonad flagellate from *Mastotermes darwiniensis*, is in fact propulsion by the helical undulations of adherent spirochaetes [16]. A comparable system is the bacterial-devescavinid motility system in *Cryptotermes cavifrons* [65]. Many termite protists also harbour methanogenic bacteria [45, 47, 56, 69]. It is slightly ironic that it has been easier to define the role of the bacteria in these associations than the advantage to the protist. Accordingly, it is more

appropriate to describe such associations in Chapter 10.

For termite scientists, the major advances of interest have been the isolation of pure cultures of protists and the successful application of the techniques of comparative molecular biology to protist phylogeny. The isolation of pure cultures of two protists by Yamin [77, 80] has made it possible to explore with confidence the metabolic potential of protists and thus obtain a more precise idea of their symbiotic roles.

The second advance has been the application of the techniques of comparative molecular biology to protist phylogeny. This has enabled specific phylogenetic information to be obtained without having to isolate the protists in pure culture, previously the main difficulty in studying termite protists. Although to date the number of such studies is small, they have immediately established and defined (in molecular terms) the uniqueness of many of the protist (and bacterial) symbionts.

Complementary to these approaches, classical light and electron microscopy studies have yielded useful information on the distribution of protists in the hindgut [44, 74, 88] and the ingestion of wood [87], while experimental feeding of termites on varying dietary regimens has clarified the nutritional requirements of some of the protists [2, 36, 43, 70, 71, 83].

2. ISOLATION

The paucity of studies on pure isolates of termite protists comparable to those now available on termite bacteria (see Chapter 10) is a major reason for our uncertainty on the symbiotic role(s) of the protists. The isolation of a microorganism in pure culture is usually the first step when attempting to define its metabolic capability, but this has proved technically demanding for termite protists.

Adding to the difficulty of isolating termite protists has been that of removing the bacterial endosymbionts that many of them contain. Hence the importance of Yamin's pioneering

work in the isolation of *Trichomitopsis termopsidis* [77] and *Trichonympha sphaerica* [80] from *Zootermopsis* sp. Preliminary experiments were carried out by Yamin on their nutritional potential. Critically, he established that they can metabolise cellulose [79, 80, 82] and that the principal products were acetate, CO₂ and H₂. Using ¹⁴C-cellulose, 80-90% of the labelled end products were ¹⁴C-acetate and ¹⁴CO₂; the remaining end product(s) containing 10-20% of the label were unidentified, indicating that the production of end products may not be quite as simple as the notional summary equation



Yamin's work was extended to more detailed nutritional and biochemical studies on *Trichomitopsis termopsidis* [55, 56]. This flagellate normally contains methanogenic bacteria which can be readily removed without apparently affecting the flagellate. For optimal growth, *Trichomitopsis termopsidis* requires particulate forms of cellulose (but not wood) and heat-killed bacteria, preferably of termite origin [56]. Crude extracts of *Trichomitopsis termopsidis* could hydrolyse crystalline cellulose, carboxymethylcellulose and cellobiose, evidence of endo-β-1,4-glucanase and β-glucosidase activities. Other polymers which could be hydrolysed were starch, xylan and protein [55]. This remains the only biochemical profile of a protist living in a culture without living prokaryote associates.

Termite protists are also known predators of bacteria (Figure 1). For example, a trichomonad, *Trichomitus trypanoides*, has been isolated from the hindgut of *Reticulitermes santonensis*, which feeds on living bacteria [3]. The cellulolytic *Trichomitopsis termopsidis* requires bacterial cell material of termite origin for optimal growth [56], suggesting that the protists may have some difficulty in taking up amino acids (present in the hindgut, see Chapter 10) or that these nutrients are not readily available in that environment.