

SHORT COMMUNICATION

Cellulolytic Activity of an Axenically-cultivated Termite Flagellate, *Trichomitopsis termopsidis*

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Cellulolytic activity of the termite flagellate *Trichomitopsis termopsidis* was demonstrated *in vitro* and *in vivo*. Homogenates of axenically-cultivated flagellates hydrolysed carboxymethylcellulose enzymically indicating that *T. termopsidis* does not depend on living extracellular or endosymbiotic bacteria for cellulose digestion. Cellulolytic activity of the flagellate was also determined by comparing the longevities of defaunated termites (*Zootermopsis*) refaunated by feeding with either *T. termopsidis* or with heat-killed flagellates. The termites refaunated with *T. termopsidis* greatly outlived members of the other group, supporting the view that cellulose digestion by the intestinal flagellate is responsible for the nutrition of termites.

INTRODUCTION

Cleveland (1923) demonstrated that the lower termite *Zootermopsis* is dependent on its intestinal flagellates (protozoa) for survival on a cellulose diet. He proposed that the cellulose-ingesting flagellates produce metabolites which are utilized by the termite. Trager (1932) identified cellulolytic activity in extracts of flagellates from *Zootermopsis* and in extracts of *Trichomitopsis* (syn. *Trichomonas*) *termopsidis* which had been grown in culture with a single extracellular bacterial species. Furthermore, Cleveland (1925) showed that *Trichonympha*, one of the two cellulose-ingesting flagellate genera found in *Zootermopsis* (see Table 1), could support termites indefinitely when present as the only intestinal flagellate, whereas termites harbouring only *Trichomitopsis termopsidis* survived no more than 6 to 7 weeks longer than those animals harbouring commensal flagellates or none at all.

Recent axenic cultivation of *Trichomitopsis termopsidis* (Yamin, 1978) has made it possible to extend this work to flagellates from which extracellular and endosymbiotic bacteria are known to be absent, and to determine whether cultivated flagellates can refaunate termites and contribute to their nutrition.

METHODS

Flagellates. *Trichomitopsis termopsidis* (Cleveland) was grown axenically in an anaerobic medium containing cellulose and autoclaved rumen bacteria; flagellates were harvested from the culture fluid (see Yamin, 1978), 10 to 30 cultures yielding more than 10^9 cells. Flagellates suspended in 1 to 3 ml of culture medium buffer were broken by a single freeze-thaw cycle or by the addition of Triton X-100 (Schwarz/Mann; final concentration 0.2%, v/v) at room temperature.

A homogenate of *Tritrichomonas foetus* (Riedmüller) was kindly provided by Dr D. G. Lindmark, Rockefeller University.

Cellulolytic activity. Homogenates (0 to 100 μ l) were incubated overnight at 37 °C with 1.0 ml of 1.0% (w/v) sodium carboxymethylcellulose (Sigma) in 0.1 M-acetate buffer, pH 5.5, plus one drop of toluene. Reducing sugar was quantified after incubation by a modified Nelson method (Robyt & Whelan, 1968), using glucose as the standard. One unit (U) of cellulolytic activity is defined as the formation of 1 nano-equivalent of reducing sugar h^{-1} at 37 °C. Assays which varied in incubation time, volume of homogenate, pH (4.3 to 6.9) and temperature (18, 27, 37, 55 and 61 °C) were compared with control incubations containing either no homogenate, boiled homogenate, or no substrate. Protein was determined by Lowry's method using bovine albumin fraction V (Miles Laboratories) as the standard.

Defaunation and feeding. Termites (*Zootermopsis* sp., from Dahl Biological Supply, Berkeley, Calif., U.S.A.) were from a single colony of either *Z. angusticollis* (Hagen) or *Z. nevadensis* (Hagen), but could not be identified since alates were absent. Termites were defaunated by exposure to O_2 at 50 lbf in^{-2} (350 kPa) for 3 h in a Mini-Bomb Cell Disruption Chamber (Kontes Glass Co., Vineland, N.J., U.S.A.) and then maintained in Petri dishes on a diet of moist Whatman filter paper at 20 °C in the dark. After 25 d, three small groups of termites were fed either (i) *Trichomitopsis termopsidis* from axenic culture (24 termites), (ii) flagellates from untreated *Zootermopsis* intestinal contents (23 termites), or (iii) heat-killed (100 °C, 5 min) *Trichomitopsis termopsidis* (25 termites), by placing a drop of concentrated flagellate suspension on the termites' mouthparts and holding the termites with forceps until they had opened and closed their mandibles several times. The number of termites surviving in each group was counted periodically, and the flagellate fauna of several termites in each group was examined by gently squeezing out the intestinal contents; this was not injurious. After 1 year, two termites in each surviving group were killed and the total number of *Trichomitopsis termopsidis* per intestine was determined. The living termites had an average mass of 60 mg, and the counts were related to this value.

RESULTS

Cellulolytic activity

Homogenates of axenically-cultivated *Trichomitopsis termopsidis* hydrolysed carboxymethylcellulose; the amount of reducing sugar present in assay mixtures increased linearly with time and with the volume of homogenate used in the assay. Cellulolytic activity showed an optimum at about pH 5.3 and at 37 °C.

The cellulolytic activity of a typical *T. termopsidis* homogenate [3450 U (mg protein) $^{-1}$] was much greater than that of *Tritrichomonas foetus* [56 U (mg protein) $^{-1}$]. Autoclaved rumen bacteria had no cellulolytic activity.

Refaunation

After feeding *T. termopsidis* to defaunated termites, the flagellates multiplied in the intestine to fill the gut with apparently the same total volume of flagellates as in the gut of an untreated termite. The defaunated termites which had been fed heat-killed flagellates remained free of flagellates but became infected with a bacterial flora which resembled that of termites in the refaunated groups. Those defaunated termites fed flagellates from untreated *Zootermopsis* did not become infected with any species of *Trichonympha* (Table 1). The number of *T. termopsidis* per gut after 1 year was inversely proportional to the number of different flagellate species present in the gut: there were 23 times more *T. termopsidis* in termites harbouring this flagellate alone than in untreated termites, and 9 times more *T. termopsidis* in the intestine of termites harbouring it with the three commensal species than in untreated termites (Table 1). Cellulose particles were present in the intestines of all termite groups during their lives indicating that they continued to feed on the filter paper throughout the experiment.

Longevity

As shown in Fig. 1, after 1 year, 46% of the termites refaunated with *T. termopsidis* from axenic culture were still alive, whereas all of the termites fed heat-killed *T. termopsis* had died by 183 d; those termites fed untreated *Zootermopsis* intestinal flagellates survived almost as well (39% alive after 1 year) as those refaunated with axenically-cultivated *T. termopsidis*.

Table 1. *Intestinal faunas in refaunated and untreated termites*

Defaunated termites (*Zootermopsis*) were fed living flagellates as described in Methods. The flagellate faunas of the termites were examined after 1 year and the number of *Trichomitopsis termopsidis* per termite was determined for two termites in each group. Values in parentheses represent the average number of *T. termopsidis* per 60 mg termite (see Methods). Untreated termites which had been maintained in the laboratory during the experiment were similarly examined at this time.

| Termite group | Flagellate fauna |
|---|--|
| Untreated | <i>Trichonympha campanula</i> *, <i>Trichonympha collaris</i> *, <i>Trichonympha sphaerica</i> *, <i>Trichomitopsis termopsidis</i> * (2.1×10^4), <i>Streblomastix strix</i> , <i>Tricercomitus termopsidis</i> , <i>Hexamastix termopsidis</i> |
| Defaunated and then fed intestinal flagellates from untreated <i>Zootermopsis</i> | <i>Trichomitopsis termopsidis</i> * (1.9×10^5), <i>Streblomastix strix</i> , <i>Tricercomitus termopsidis</i> , <i>Hexamastix termopsidis</i> |
| Defaunated and then fed <i>Trichomitopsis termopsidis</i> from axenic culture | <i>Trichomitopsis termopsidis</i> * (4.9×10^5) |

* Cellulose-ingesting species; the others are considered to be commensals (Cleveland, 1925).

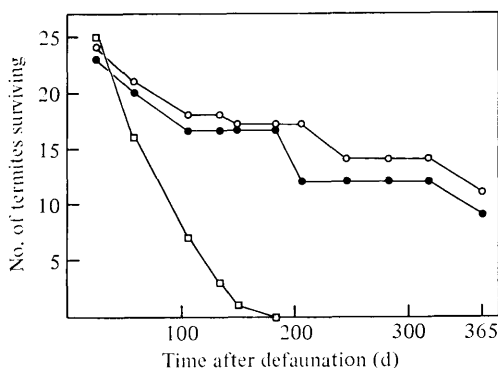


Fig. 1. Survival of defaunated termites after feeding with either *Trichomitopsis termopsidis* from axenic culture (○), intestinal flagellates from untreated *Zootermopsis* (●), or heat-killed *T. termopsidis* (□). Termites were defaunated on day 0 and 25 d later.

DISCUSSION

This study demonstrated that the termite flagellate *Trichomitopsis termopsidis* possesses significant cellulolytic activity and that cultured flagellates can refaunate termites and prolong their lives far beyond that of termites harbouring no flagellates. Whereas Cleveland (1925) found that termites harbouring solely *T. termopsidis* lived only 6 to 7 weeks longer than defaunated termites, the results presented here indicate that *T. termopsidis* can contribute significantly to the nutrition of the termite when present as the sole flagellate. This conclusion is supported by the longevity of the termites fed untreated *Zootermopsis* flagellates which became faunated only with *T. termopsidis* and three commensal species. The failure of *Trichonympha* to become established in this group may have resulted from O_2 toxicity to these fragile flagellates during feeding or from mechanical injury during chewing and swallowing by termites. Under natural conditions, newborn and post-moult termites become refaunated *per os*. This group did, however, provide additional evidence that *T. termopsidis* can multiply in the termite intestine to an extent depending on the number of other flagellate species present. Competition must certainly exist among the cellulose-ingesting flagellates for cellulose, and perhaps among all flagellates for other factors.

Trager (1932) identified cellulolytic activity in extracts of *T. termopsidis* grown in culture with a single extracellular bacterial species. The present study extends this finding to axenic flagellates known not to harbour endosymbiotic bacteria; Pierantoni (1936) postulated that such bacteria may be the source of flagellate cellulase. The cellulolytic activity (per mg protein) of *T. termopsidis* was much greater than that of the bovine parasite *Tritrichomonas foetus*, a related trichomonad flagellate. Although indirect mechanisms could be postulated to explain the longevity of termites refaunated with *T. termopsidis*, it appears likely that the cellulolytic activity of the flagellate, proven *in vitro*, also occurs *in vivo* and provides the termite with usable products of cellulose metabolism.

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