

Towards an understanding of the distribution, dynamics and ecological significance of bacterial symbioses in protists*

Hans-Dieter GÖRTZ

Abstract: Bacterial symbionts in protists have been observed for more than a century. Whereas cytological details and certain biological features of the symbioses were investigated, little is known about the geographical distribution, the dynamics and ecological significance of such associations, and namely about the question of endemism versus cosmopolitanism of intracellular bacteria in ciliates as in other protists. Many of the bacterial symbionts that are belonging to various higher bacterial taxa are host specific and restricted to certain compartments of the host cell, e.g. to micro- or macronucleus. It is obvious that many of the symbionts are well adapted to intracellular life, but in only few cases the bacteria are of selective advantage for the host. Different symbionts may interact and compete within a cell and some observations indicate competition between intracellular symbiont species in host populations. Though data about geographical distribution are scarce, it appears that certain symbionts occur wherever the host species occurs, while on the other hand certain symbiont species seem to exclude one another. For definite conclusions about these questions more investigations are needed, the more so as protists may harbor potential new pathogens of humans.

Key words: Bacterial symbioses, ciliates, geographical distribution, intracellular bacteria, macronucleus, micronucleus, *Paramecium*, protists.

Introduction

Epidemiologies and biogeography of human infectious diseases are fairly well understood and may help to predict and avoid epidemics. Parasites, infectious microorganisms and symbionts may be of higher significance than predators for population dynamics of protists as they are for animals. Symbioses sensu lato may therefore be important factors in ecosystems. Our understanding of the distribution, dynamics and ecological significance of bacterial symbioses in protists is, however, insufficient. Background of the considerations in this article is the debate on cosmopolitanism versus endemism of ciliates and other protozoa (FINLAY et al. 1996; FOISSNER 1999); are protozoa generally cosmopolitans or are many of them endemists? The geographical distribution of intracellular bacteria may be questioned similarly: are intracellular bacterial species in ciliates (and in other protists) spread all over the world or are they rather found in certain geographical areas, restricted to host populations or even parts of

populations? In this short review some aspects of this topic that has been neglected up to the present shall be considered with emphasis on ecology and epidemiology of bacterial symbioses in protists, chiefly in *Paramecium* where intracellular symbioses have been investigated in most detail.

Host specificities of bacterial symbionts

Already in the middle of the nineteenth century, intracellular bacteria have been observed in ciliates. In 1890, HAFKINE described three species of bacteria found in the nuclei of *Paramecium caudatum*. These were *Holospora obtusa*, *H. elegans* and *H. undulata*. However, even in the middle of the 20th century the bacterial nature of many intracellular particles was still questioned. PREER et al. (1974) ended this debate about the bacterial nature not only for endosymbionts in *Paramecium*. In other ciliates, too, intracellular „particles“ were recognized as bacteria and given binomial names. Intracellular bacteria typically do not grow outside their host cells. This made their description impossible with classical methods of microbiology. Biological features were used and in addition molecular and biochemical ones. Among biological features, host specificity was espe-

* This article is dedicated to Wilhelm FOISSNER in honour of his 60th birthday.

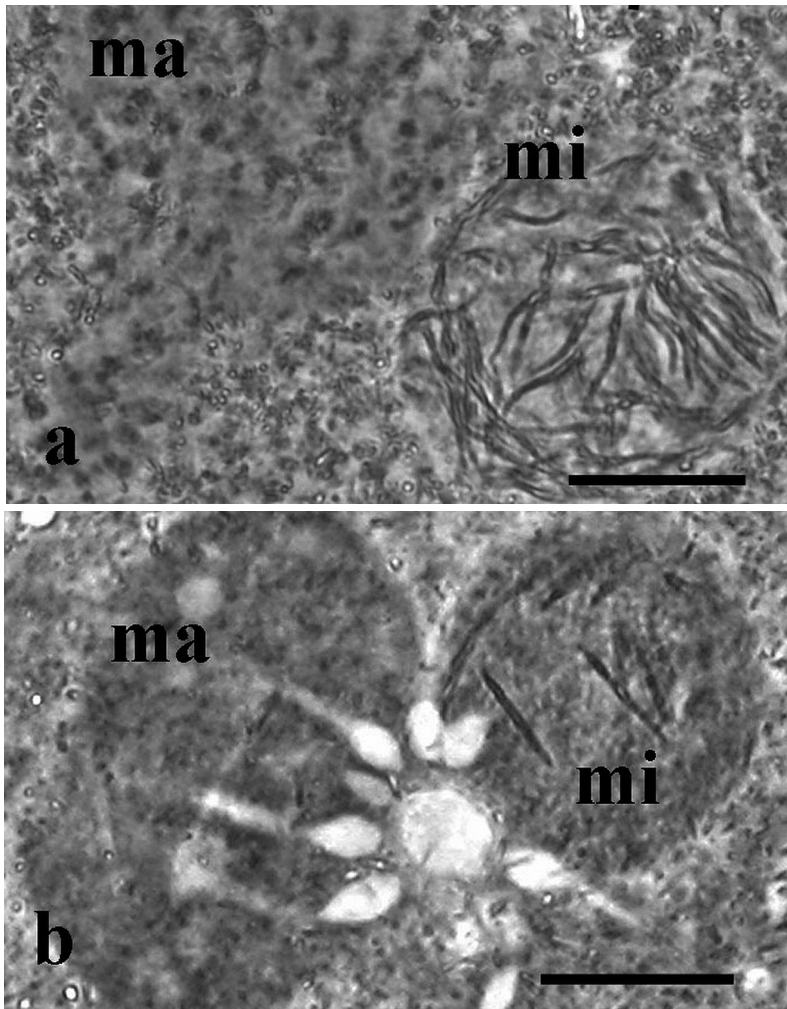


Fig. 1a, b: Micronuclei of *Paramecium caudatum* infected with *Holospora*, **a:** *H. undulata*, **b:** *H. elegans*. mi – micronucleus, ma – macronucleus. Living cells, phase contrast. Zeiss Axioskop, AxioCam. Bars – 10 µm. Courtesy of Dr. Yoshimitsu NAKAMURA.

cially regarded significant. Bacteria were observed to be species-specific, in some cases even host strain-specific. At least in some cases the strain specificity could be correlated with a certain genetic situation in the host (SONNEBORN 1943; FUJISHIMA & FUJITA 1985).

Selective advantages of infected host ciliates

The killer trait in *Paramecium* has been accepted as an example of competitive advantage brought about by intracellular bacteria (SONNEBORN 1938; LANDIS 1981, 1987). Toxins produced by the endosymbionts may kill competitors, whereas hosting cells killer bacteria are resistant. Killer symbioses are complex, as the toxins are encoded on bacteriophage or plasmid genomes and the advantage for the host may depend upon the ecological conditions (LANDIS 1988; KUSCH & GÖRTZ 2006). Other examples of endosymbionts that are of advantage for their protozoan hosts are e.g. the *Polynucleobacter*

species that are essential for certain fresh water *Euplotes* (HECKMANN 1975, 1983) and methanogens in anaerobic protists (FENCHEL et al. 1977; VAN BRUGGEN et al. 1983). Endosymbiotic or epibiotic methanogens generate methane using H_2 that is produced in hydrogenosomes of anaerobic protists by the conversion of pyruvate into acetyl-CoA (LINDMARK & MÜLLER 1973; YARLETT et al. 1981; HACKSTEIN & STUMM 1993). Earlier, SOLDI & GODOY (1973a) found evidences that *Lyticum flagellatum* (PREER et al. 1974; originally called lambda particles) may provide its host, *Paramecium octaurelia* with folic acid.

An interesting interaction of symbiont and host was found by HORI & FUJISHIMA (2003), who showed that endonuclear *Holospora* bacteria may enhance heat-shock gene expression in host cells (FUJISHIMA et al. 2005). *Holospora* infected paramecia of the strains tested tolerate higher temperatures. A further example of selective advantage brought about by symbiotic bacteria was described by (ROSATI et al. (1999): in the ciliate *Euplotidium* epibiotic bacteria play a significant role in defending its host against predators. Less well proven is the suggestion that endocytobionts keep host cells from being infected by other, potentially harmful microorganisms (GÖRTZ 1982, 1983). While it may be too early for substantial general conclusions on the significance of intracellular bacteria in protists, the various examples indicate very specific interactions that may be even strain specific for both, symbiont and host.

Evolution and diversity of intracellular bacteria

The small genome size of intracellular bacteria in ciliates have been one argument among others for, why these bacteria were regarded as ancient symbionts SOLDI & GODOY 1973b; PREER 1977; HECKMANN 1983; SCHMIDT 1982). Still today, we have little information about metabolic interactions between symbionts and hosts. *Caedibacter* and *Holospora* were found to be energy parasites. Like certain human pathogens, e.g. rickettsias and chlamydias, they express an ATP / ADP antiporter (LINKA et al. 2003). The authors suggest that horizontal gene transfer is responsible for the distribution of nucleoside-triphosphate-transporter (NTT) paralogs in various intracellular bacteria.

The phylogenetic position of *Holospora obtusa*, endonuclear bacterium in *Paramecium caudatum*, based upon SS rDNA sequence was determined by AMANN et al. (1991). In the following years the positions of more and more symbionts were elucidated, sometimes making necessary a revision of earlier systematics (BEIER et al. 2002). It became apparent that intracellular bacteria in

protists are belonging to various higher bacterial taxa, among them Proteobacteria, Chlamydiales and Bacteroidetes. Free-living protists seem to offer attractive niches for Eubacteria and protists from anaerobic habitats are even colonized by Archaea (e.g. STUMM et al. 1982; FINLAY & FENCHEL 1991; GÖRTZ 2002; HORN & WAGNER 2004).

Yet, bacterial infections and establishment of symbioses may need pre-adaptations of bacteria and host cells. SCHNELLER et al. (1959), BALSLEY (1967) and FUJISHIMA & FUJITA (1985) showed that a certain genetic condition in paramecia is needed for the maintenance of killer symbionts. On the other hands, LOHSE et al. (2006) found that paramecia may achieve resistance against infectious *Holospira* bacteria by de novo evolution. Many observations indicate that protists are still training ground for the evolution of symbiosis.

Interactions between different intracellular bacteria

Most endosymbionts were shown to colonize the host cell as single infections: but one symbiont species at a time was found in the cells. In some cases endosymbionts were even suggested to prevent infections by harmful microorganisms (GÖRTZ 1983). Though this seemed not to hold true for most symbioses, from laboratory observations we know that certain intracellular bacteria may outcompete others in the host cell. The number of a given endosymbiont in *Euplotes daidaleos* was reduced, when a second endosymbiont species occurred in the host cell (HECKMANN et al. 1983). *Caedibacter caryophilus*, e.g., was found to outcompete *Holospira obtusa* in the macronucleus of *Paramecium caudatum* (GÖRTZ 1987).

Nevertheless, it is not rare that a population is infected by more than one bacterium, sometimes individual cells may be bearing two or more different endocytobionts. In case of more than one symbiont species in a ciliate, it appears that each prefers a certain site, not overlapping with the sites of the others. In the cells of a strain of *Spirostomum teres*, FOKIN et al. (2005) observed three different bacteria. One symbiont was living in the macronucleus, another one all over the central cytoplasm and a third, very tiny bacterium could only be found in the cortex just beneath the alveoli. We have little knowledge about the dynamics of such multiple infections neither in its host cells nor in host populations. Competitive interactions between intracellular bacteria may be of ecological as well as evolutionary interest.

Prevalence of intracellular bacteria in ciliate populations

Most natural populations of paramecia and other ciliates are infected by intracellular microorganisms. Infection rates differ. For cytoplasmic and macronuclear endocytobionts high infection rates of up to 100 % are found. This holds true namely for killer symbionts, because symbiont free cells are killed, but also non-killer symbionts may display similarly high infections rates. The macronuclear *H. obtusa* is a non-killer symbiont. Often, however, natural populations are completely infected. The reasons seem obvious. The infectious form of *H. obtusa* displays a perfect mode to be released from the host nucleus and then may infect other cells of the population. On the other hand, the reproductive forms of the bacterium segregate into the daughter nuclei during host cell division making sure the infection to be maintained. Micronuclear-specific bacteria display lower infection rates. GÖRTZ & FUJISHIMA (1983) found evidences that micronuclei are harmed by infection with *H. elegans*. *Paramecium caudatum* bearing *Holospira elegans* – even paramecia having been cured of this bacterium – may be genetically dead. After conjugation they may not be able to produce intact macronuclei. High infection rates of micronuclei would result in the genetic death of the population. This would imply that *H. elegans* strains being as infectious as *H. obtusa* would kill the host population and thereby itself.

Geographical distribution

Bacterial endosymbionts in ciliates have been described chiefly by researchers from Europe, USA, Japan. These are the continents, where intracellular bacteria have been reported from almost exclusively. Only very little is known about the occurrence of ciliates with intracellular bacteria in Africa, Southern America, Australia and most parts of Asia. The overall observations on geographic distributions are, however, scarce and mostly not systematic. PREER et al. (1974) reviewed the bacterial endosymbionts in *Paramecium* known at that time and their host specificities. Most of these symbionts were reported from species of the *P. aurelia* group of species, the geographical distribution of which is known (NYBERG 1988; PRZYBO & FOKIN 1997). Nothing is known, however, about the geographic distribution of the symbionts, with few exceptions.

From 1980 till 1988 the late Josef DIECKMANN repeatedly investigated water samples from ponds and lakes in various parts of Germany, sometimes together with the author, for the occurrence of *Holospira* species, namely *H. obtusa*, *H. elegans* and *H. undulata*. The places where in Nordrhein-Westfalen (North-Rhine-

Westfalia), Niedersachsen (Lower Saxonia) and Nordhessen (northern Hesse), all northern Germany. All of these endonuclear bacteria were host-specific for *Paramecium caudatum*. *Holospira obtusa* was found all over the area. Most interesting was the changing distribution of the two micronuclear specific species, *H. elegans* and *H. undulata* (Fig. 1). The infectious form of *H. elegans* is straight, that of *H. undulata* is undulated, wavy.

Whereas *H. elegans*, too, was found in waters at many places in western Germany, *H. undulata* was rare until 1983. In fact, even in 1983, *H. undulata* was only found in small lakes in the east of the area under investigation, close along a line from Hamburg to about Göttingen and Fulda. West of this line, *H. undulata* had not been found by us before until 1983. In the following years, *H. undulata* seemed to move west and *H. elegans* became rare. Pittifully, the dynamics has not been monitored by us. In 1988, we tried hard to find *H. elegans* in Westfalia, where it had been wide spread and abundant up to 1983. We did not find it, but in the waters we had found *H. elegans* even five years ago, *H. undulata* was now present. At that time, *H. undulata* was also found in the area around Stuttgart by S.I. FOKIN (personal communication). At that time already, we knew that both species were clearly distinct. While both are host-specific to *Paramecium caudatum* and both are micronuclear-specific, they display a clearly different morphology and are different species also due to sequence data. It must be mentioned that occurrence of *Holospira elegans* and *H. undulata* in Russia had been reported repeatedly.

Conclusions

Intracellular bacteria in ciliates and other protists have been investigated for more than one century. However, data about the dynamics, prevalences and epidemiologies of infections as well as ecological significance and biogeography are scarce. More systematic investigations are needed. The need of detailed investigations, however, is not only due to scientific interest: it is well known that protists may harbour bacteria that are closely related to human pathogens (GÖRTZ & MICHEL 2003; HORN & WAGNER 2004). The sudden emergence of new pathogens such as *Legionella pneumophila* may well be correlated with environmental changes influencing the dynamics of bacterial infections in free-living protists.

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Address of author:

Prof. Dr. Hans-Dieter GÖRTZ
 Biologisches Institut
 Universität Stuttgart
 Abt. Zoologie
 Pfaffenwaldring 57
 70569 Stuttgart
 Germany
 E-mail: goertz@bio.uni-stuttgart.de

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Jahr/Year: 2008

Band/Volume: [0023](#)

Autor(en)/Author(s): Görtz Hans Dieter

Artikel/Article: [Towards an understanding of the distribution, dynamics and ecological significance of bacterial symbioses in protists 307-311](#)