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Response of the bacterial symbiont *Holospora caryophila* to different growth conditions of its host

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Abstract

Previous studies on bacterial symbionts of ciliates have shown that some symbionts can be maintained relatively well under standard laboratory conditions whereas others are frequently lost, especially when the host is cultivated at a high division rate. In this study, the variation in infection level by the endosymbiont *Holospora caryophila* within its host population *Paramecium octaurelia* was investigated in response to three alimentary treatments and a subsequent starvation phase. The response of the ciliates was determined as a nearly exponential growth rate with different slopes in each treatment, proportional to the amount of food received. The initial infection level was higher than 90%. After 24 days of exponential host's growth, the prevalence remained stable at approximately 90% in all treatments, even after a subsequent starvation phase of 20 days. However, at intermediate time-points in both the feeding and the starvation phase, fluctuations in the presence of the intracellular bacteria were observed. These results show that *H. caryophila* is able to maintain its infection under the tested range of host growth conditions, also due to the possibility of an effective re-infection in case of partial loss.

Keywords: Ciliates; Endosymbiosis; *Paramecium*; Parasites; Macronuclear

Introduction

Intracellular bacteria of ciliates are generally termed endosymbionts, independently of the kind of relationship with their hosts. Nowadays they are considered frequent rather than exceptions, while in the past their abundance was probably overlooked and thus underestimated (Fokin 2004, 2012). Indeed, although this topic still remains largely underexplored, several detailed reports were published recently (e.g. Boscaro et al. 2013a,b; Gong et al. 2014; Sun et al. 2009; Vannini et al. 2013, 2014). These investigations revealed that many symbionts of ciliates are phylogenetically closely related to putative pathogens of humans and other vertebrates, e.g. belonging to the families "*Candidatus* Midichloriaceae" (Montagna et al. 2013), *Rickettsiaceae* (Schrallhammer et al. 2013; Vannini et al. 2014) or the genus *Francisella* (Schrallhammer et al. 2011).

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A topic which has been rarely addressed is the stability and prevalence of the association between ciliates and their bacterial endosymbionts. For instance, *Polynucleobacter necessarius* has always a 100% prevalence in populations of the host *Euplotes aediculatus*, and is indeed an essential symbiont (Heckmann 1975), whereas *Caedibacter varicaedens* can be lost if the host *Paramecium biaurelia* reproduces at a high division rate (Preer 1948). In the absence of proved functional dependencies, such results cannot be directly extrapolated to natural populations, but can provide useful information for their understanding. In this regard the endosymbiotic genus *Holospora*, which displays very distinguishing features and interactions with its host, is especially intriguing.

Holosporas occur in two forms, the reproductive and the infectious form (Fujishima 2009; Fujishima and Fujita 1985). The reproductive form displays the typical shape of a Gram-negative rod and reproduces by binary fission in the host nuclei. Under certain conditions (frequently connected to starvation), the reproductive form develops into an elongated and distinctively organised infectious form, which is unable to divide but can be released in the external medium (Fujishima 2009; Gibson et al. 1986; Görtz et al. 1989). When a free infectious form is ingested by a compatible host cell, it is activated by the local acidification in the food vacuole. Hence, it acquires the ability to escape the lysosomal digestion and to reach the target nucleus exploiting the host cytoskeleton (Sabaneyeva et al. 2009). After entering the nucleus it undergoes multiple fissions, which result in several reproductive forms (Fujishima 2009).

According to Boscaro et al. (2013a), *Holospora* and the HLB (*Holospora*-like bacteria) are considered the only representatives of the family *Holosporaceae* (*Rickettsiales*, *Alphaproteobacteria*). Its monophyly is well supported by 16S rRNA gene sequences (Amann et al. 1991; Boscaro et al. 2013a,b; Hori et al. 2008; Rautian and Vakkerov-Kouzova 2013; Vakkerov-Kouzova and Rautian 2011) available up to now. Traditionally, holosporas are subdivided into two groups based on the dispersal mechanism of the infectious forms, i.e. the connecting piece-forming species, which are the object of most studies (for review, Fokin and Görtz 2009; Fujishima 2009) and the non-forming ones (Fokin et al. 1996).

Holospora caryophila (Preer 1969; Preer and Preer 1982), a macronuclear symbiont described in a few species of the *Paramecium aurelia* complex as well as in *Paramecium caudatum* (Fokin and Görtz 2009), belongs to non-connecting piece forming group. Besides the ability to be released by vacuoles in the environment, it also presents some peculiar adaptations, which allow the maintenance of the macronuclear infection during host sexual processes even without intermediate dispersal (Fokin 1998; Preer 1969).

This study is aimed to investigate the maintenance ability and the prevalence dynamics of *H. caryophila* infecting *Paramecium octaurelia*, in response to three quantitavely different alimentary treatments of the host, followed by a starvation period. This approach demonstrated that *H. caryophila* is able to maintain or re-establish a high infection prevalence in all tested conditions.

Material and Methods

Strain origin and maintenance

Paramecium octaurelia strain GFg infected with *Holospora caryophila* was provided by Alexey Potekhin (St Petersburg State University, Russia). It was maintained at 21 °C ± 1 K by feeding with a bacterised medium inoculated with *Raoultella planticola* (strain DSM-3069) according to Boscaro et al. (2013a).

Growth experiment conditions

A growth experiment was designed to test the effects of different alimentary treatments on the infection level of the symbiont. It was carried out at 21 °C ± 1 K.

During a preliminary adaptation phase, an aliquot of the stock culture GFg was fed every second day by the addition of 1/4 volume of fresh (within 4 days after inoculation) bacterised medium for a period of 10 days (days I–X, Fig. 1a). After the adaptation phase (on the eleventh day), the 24 day long feeding phase of the experiment started, in which three alimentary treatments were applied (Fig. 1a). The treatments differed in the relative amount of bacterised medium provided at alternate days: “full” (1 volume), “half” (0.5 volume), and “quarter” (0.25 volume). Such an approach allowed to analyse over a period of time the three combinations of cell and food concentration produced by the differential feeding, expanding on this specific point previous analysis on other *Paramecium* and *Holospora* experimental systems (e.g. Kaltz and Koella 2003; Restif and Kaltz 2006). Each treatment was applied to three replicate cultures, therefore the adaptation culture was split into nine sub-cultures (three for each alimentary treatment). Due to experimental constraints, the initial sub-culture volume differed among treatments (9 ml for “full”, 30 ml for “half” and 110 ml for “quarter”), under the assumption that the initial difference would have been irrelevant since the treatments were volume-independent.

After the completion of the feeding phase, all replicate cultures were starved for 20 days (starvation phase) independently on the previous alimentary treatment (Fig. 1a), until almost no *Paramecium* cells were detectable.

Cell density measurements

The *Paramecium* cell density was controlled at regular time intervals during the course of the experiment. During the feeding phase, samples for enumeration were taken always prior to feeding (i.e. on every other day) and at equivalent time intervals during the starvation phase (Fig. 1b).