



High-Throughput Sequencing of the 16S rRNA Gene as a Survey to Analyze the Microbiomes of Free-Living Ciliates *Paramecium*

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Abstract

Ciliates are the largest group of ubiquitous aquatic bacterivorous protists, and many species are easily cultivated. However, only few studies reported prokaryotic communities naturally associated with ciliate cells. Herein, we analyzed the microbiome composition of several strains of *Paramecium* (Ciliophora) originating from different locations and belonging to two morpho-species by high-throughput sequencing (HTS) of the 16S rRNA gene. Possible reasons of HTS results bias were addressed comparing DNA libraries obtained using different primers and different number of ciliate cells. Microbiomes associated with ciliates and their environments were always significantly different by prokaryotic taxonomic composition and bacterial richness. There were also pronounced differences between *Paramecium* strains. Interestingly, potentially pathogenic bacteria were revealed in *Paramecium* microbiomes.

Keywords Ciliates · Microbiomes · Bacterial communities · Single cell sequencing · Metabarcoding · Human pathogens and commensals

Introduction

Water ecosystems are inhabited by numerous and diverse microorganisms, mainly prokaryotes and unicellular eukaryotes. The interactions between pro- and eukaryotes have a great ecological significance, as they provide carbon and energy

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transfer to higher trophic levels and retrieve dissolved carbon into food chains [1]. Planktonic bacteria are efficiently consumed by bacterivorous protists, but many prokaryotes successfully avoid predator’s digestion and survive in the host [2]. Predation was found to be a strong force producing drastic changes in the prokaryotic community composition [3–6].

Ciliates are ubiquitous bacterivorous protists, predominant organisms in the marine zooplankton [7], and a significant component of freshwater communities [8]. Bacterivory is the main but not the only way of interaction between prokaryotes and ciliates. Different cellular compartments of ciliates, especially nuclei and cytoplasm, may be inhabited by intracellular bacterial symbionts, e.g., *Holospira* [9], *Caedibacter* [10], “*Candidatus* Sonnebornia” [11], and “*Candidatus* Megaira” [12]. It is also known that several human pathogenic bacteria can survive and multiply inside the ciliate cell, such as *Legionella* [13], *Mycobacterium* [14], *Listeria* [15], *Salmonella*, *Yersinia*, *Shigella*, and *Campylobacter* [16]. Nevertheless, ciliates as potential reservoir for pathogens are poorly studied. Progress in this field would be promising both for applied epidemiological investigations and for insights into microbial evolution. It has been suggested that protozoan grazing “is a factor driving the evolution of human pathogens in the environment” [17]. Subsequently, Sun et al. [18] proposed to consider determinants of bacterial virulence as