European Journal of Soil Biology 49 (2012) 107–111

Contents lists available at SciVerse ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

A molecular perspective on ciliates as soil bioindicators

Enrique Lara [a,*], Dimaris Acosta-Mercado [b]

[a] Laboratory of Soil Biology, University of Neuchâtel, Rue Emile Argand 11, CH-2000 Neuchâtel, Switzerland
[b] Departamento de Biología, Universidad de Puerto Rico, PO Box 9012, Mayagüez 00681-9012, Puerto Rico, USA

ARTICLE INFO

Article history:
Received 15 February 2011
Received in revised form
11 October 2011
Accepted 1 November 2011
Available online 29 November 2011
Handling editor: Christoph Tebbe

Keywords:
Ciliates
Bioindication
Hydrocarbon
Heavy metals
Environmental DNA survey
Pyrosequencing
Ecology

ABSTRACT

Ciliates (or Ciliophora) are probably the most popular group of protists, with some classroom lab favorites such as *Paramecium* spp. These highly conspicuous micro-organisms are easier to identify than many other eukaryotic microbes, at least for trained specialists. As a result, a large amount of data has been produced on their taxonomy, ecology and biogeography. Their relatively short generation times and their ubiquity in virtually all ecosystems on Earth make them excellent bioindicator organisms, particularly in soil systems. However, their practical use is hampered by long and tedious procedures of handling and preparing of specimens for identification, a task which is rendered even more difficult by the existence of cryptic species. A comfortable way to overcome these pitfalls is through the application of environmental molecular diversity screening methods that have been developed in the last two decades to the ciliate communities, and also more modern approaches such as next generation sequencing (454 pyrosequencing). As a complement, several approaches already developed for the prokaryotic communities (functional gene screening, environmental RNA clone libraries, fluorescent in situ hybridization) have not been considered today but could be easily applied to ciliate communities in soils. Still, before these methods can be applied to monitor natural ciliate communities, a considerable effort in species description and culturing (i.e. “classical protistology”) must be achieved in order to interpret optimally the data obtained by molecular-based techniques. Here, we provide an overview of these methods as potential monitor strategies of environmental ciliate communities and its contribution to bioindication in soils, and also their limitations.

1. Introduction

Ciliates colonize and inhabit virtually all environments where eukaryotic life has been found and thus, are one of the most successful groups of protists on Earth. They are extremely diverse with up to 4500 presently described free-living morphospecies, however, surveys of environmental diversity based on the small subunit of the ribosome (SSU rRNA) suggest that this number might still be an underestimation, even at relatively high taxonomical levels [1,2]. Considering the potential tenfold increase in diversity [3], and their predominance within soils, ciliates represent a key functional group and potential biosensors within the soil microbial loop. They have an effect on primary productivity through a top down effect on primary producers, such as diatoms, other microalgae and cyanobacteria [4]. Moreover, they are known to contribute to ecosystem nutrient cycling [5,6] by consuming bacteria and accelerating the turnover of bacterial biomass and soil organic matter [7], and thus releasing sequestered nutrients from the microbial biomass otherwise unavailable for primary producers. This key position within soil food webs, in addition to their life strategies, makes them sensitive to changes that influence their prey, in addition to their own specific tolerance [8]. In fact, one of the bioindicator tools for the management of wastewater treatment plants includes ciliate community structure. Changes in soil ciliate community structure have also been suggested as bioindicator markers of environmental stress. In fact, it provided excellent results for monitoring major soil pollutants (pesticides) and land-use transformations [9–13] . A limiting factor is that active cells are only seldom observed in evolved soils under stable conditions, where only encysted specimens can be found (and therefore impossible to identify).

In contrast to the situation for many other groups of soil protists (with the notable exception of testate amoebae), ciliates often possess a characteristic morphology that allows a general identification by light microscopy Many strains can also be kept in cultures which has fostered ciliate research for over one century. As a consequence, taxonomy and ecology have been far more studied in ciliates than for any other group of soil protists. Although our knowledge on the immense soil ciliate (functional) diversity is still very far from exhaustiveness, the existent database is certainly a robust starting point for bioindication studies.

---

* Corresponding author. Tel.: +41 (0)32 718 22 52.
E-mail address: enrique.lara@unine.ch (E. Lara).

1164-5563/$ – see front matter © 2011 Published by Elsevier Masson SAS.
doi:10.1016/j.ejsobi.2011.11.001

Ciliate tolerance to organic pollutants and heavy metals has been studied and reviewed [9,12,14–17]; the shifts in communities concomitant to soil quality changes have been also extensively studied in the past (see further). Soil ciliate communities provide rapid, unique and non-redundant ecosystem information in comparison with more traditional macroscopic bioindicators such as nematodes [18]. This is due to their unique physiological properties: they consume more food, have higher respiration rate per mass unit, have shorter generation and life times, and reproduce much faster [13].

Our aim in this review is to reframe the potential of the use of ciliate as bioindicators within the context and possibilities offered by new molecular biology technologies. We may gain accuracy and rapid results as shown with the validation of sister species *Stylonychia mytilus* and *Stylonychia lemnae* [19]. The broad responses of ciliates to environmental change in addition to the pioneering genetic studies on soil ciliate biodiversity suggests for the presence of a significant diversity of bioindicator genes. Borrowing the milestones from environmental microbiology methodologies could serve to standardize and successfully develop molecular applications outside the academic world. Nevertheless, we also present the limitations of an exclusive "all-molecular" approach, which should always be used bearing in mind the biological reality of the investigated organisms. Altogether, we wish to promote the use of ciliates for the evaluation of soil quality, in a similar way as they are nowadays commonly used for monitoring freshwater systems (see, for instance, [20]).

2. Non molecular approaches to assess ciliates as bioindicators in soils

Several studies have been conducted in the past that highlight the potential of ciliates for bioindication of soil perturbation. They are known to produce specific responses to fertilizers and pesticides; for example, they responded more dramatically than nematodes, particularly under manure treatments in comparison with fertilized ones [18]. Moreover, after a manipulation experiment with undisturbed soils, Yeates et al. [9] found a lagging effect of 2 yrs for soil protozoan (ciliates included) populations to recover after fumigation with methyl bromide. Similarly, the application of pesticides in Negev, Israel, had an effect on the composition and size of ciliated populations in 1 out of 4 trials [10]. Petz and Foissner [21] have documented the effect of a fungicide (mancozeb) and an insecticide (lindane) on natural soil communities; ciliates were still affected 90 days after the application of a standard or high dose; as expected, mycophagous taxa had disappeared.

Ecotoxicological tests performed *in vitro* suggest that ciliates may be more useful tools for heavy metal assays than other microorganisms [16] despite the fact that they show broad responses to metal concentrations at intra- and interspecific levels. Most of these assessments were performed on activated sludge where Cu seemed to have a high impact [22]; to our knowledge, similar studies have not been performed on soils. For example, Nicolau et al. [17] found that Cu and Zn inhibited growth and grazing activities of *Tetrahymena pyriformis*, similar cytotoxic effects of Cd, Cu and Zn were observed in less charismatic ciliate species such as *Drepanomonas revoluta*, *Uronema nigricans* and *Euplotes* sp. [16]. Moreover, through the usage of fluorophores, Diaz et al. [23] found that different species of class Colpodea showed particular patterns of metal-accumulated granules across the somatic cells when exposed to high heavy metal concentrations. Both, the overall ciliate response at the level of community, and the changes, at the level of morphology, suggest for the presence of potential bioindicator genetic markers. Currently, this molecular bioindicator approach is still hampered by the lack of connectivity between ciliate ecological data and the concomitant molecular signal of transcribed genes under stable and degraded soil ecosystems.

3. Bioindicating groups of soil ciliates

One of the great advantages of ciliates is that some high-level clades (i.e. order-classes) are characterized by common metabolic and life-history features, and can thus indicate certain particularities of the investigated soils. The monophyly of these clades has been demonstrated by molecular phylogenies, mostly based on the SSU rRNA gene [24,25]. There is an extensive literature on these organisms, produced notably by Wilhelm Foissner and co-workers, and summarized in [4]; we give here a short list of high ranked clades that can be unmistakingly detected by molecular methods (based on the SSU rRNA gene) and that provide information on soil condition and particularities (Table 1).

4. Morphological versus molecular identification

Although ciliates are certainly amongst the soil protozoa that are the easiest to identify morphologically, this is certainly not straightforward. Several cell fixation and staining techniques, most often needed in combination, are indispensable for identifying certain ciliates to the species level [26]. These techniques, such as protargol staining or silver nitrate impregnation, are time-consuming and require some expertise. In addition, the existence of cryptic species complexes (i.e. species that are virtually indistinguishable by morphology alone) cannot be ruled out, as suggested for *Carchesium* [27] *Tetrahymena* spp. [28]. Thus, when it comes to species identification, access to high resolution infrastructure, diverse and concomitant staining protocols and original species descriptions are primarily restricted to academic settings rendering a limited usage of ciliates as bioindicators. When it comes to identification, the access to the original species description is not easy. Altogether, these methodological limitations reduce tremendously the treatment of large datasets that would be needed for ecological surveys on soil ciliate diversity. If ciliates have to be used for soil monitoring on a routine base, and not only within the academic context (as it is already the case in freshwater systems, which are more homogeneous and easy to sample; [20]), there has to be a technological leap forward.

The introduction of molecular methods opened the possibility to circumvent these pitfalls, as they are, actually, the same encountered in environmental bacteriology, where identification of single cells is impossible without these tools. As it is usually done for prokaryotes and other eukaryotes, the marker gene chosen for screening environmental ciliate diversity codes for the RNA molecule of the small subunit of the ribosome, called SSU rRNA gene (also called 18S rRNA gene). PCR products obtained from bulk environmental DNA are cloned and sequenced, thus yielding a couple of hundred SSU rRNA sequences of uncultured organisms. For soil ciliate studies, it is advisable to design a taxon-specific protocol in order to get the most sequences within that taxon and

Table 1
Ciliate classes indicating particular characteristics of soils immediately identifiable by molecular methods.

Class	Particularities	Indicates
Colpodea	High growth rate, broad tolerance to harsh conditions	Disturbed soils
Haptoria	Feed exclusively on other eukaryotes	Stable soils
Suctoria	Feed exclusively on other eukaryotes	Stable soils
Armophorida	Exclusively anaerobic	(Temporarily) anoxic soil