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Application of Protists to Improve Plant Growth in Sustainable Agriculture

13

Alexandre Jousset

Abstract

Plant health and growth are largely dependent on root-associated microbiota. Several bacteria and fungi can provide important services to plants, such as nutrient mineralization or protection against diseases. To date, most of our knowledge is centered on bacterial and fungal taxa. This chapter presents protists as an essential yet often overlooked component of the rhizosphere microbiome, where they play a crucial role in structuring microbial populations. Protists are a keystone group, functioning as predators of bacteria and fungi. They exert a strong pressure on plant-associated microbial communities and shape their functional and phylogenetic composition. They further enhance nutrient turnover and activate bacterial genes needed for pathogen suppression. Protists offer thus new venues to manage plant-associated microbial communities to enhance their functionality and ability to support a high plant growth in agricultural context. This chapter presents the main functional groups of soil protists and explains their distribution and importance for soil fertility. Finally, their applications in biotechnological settings aiming at reducing pesticide and fertilizer input in sustainable agriculture, are discussed.

13.1 Introduction

The growing human population calls for new strategies to improve agricultural yields. Engineering the rhizosphere microbiome to enhance plant yield and health, forms one of the cornerstones of current agricultural research. Plants live in

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association with diverse microbial communities that colonize plant roots and provide essential services to the plant. Plant-beneficial microbial functions encompass, for instance, nutrient mineralization, fine-tuning of the hormonal balance, or pathogen suppression. Together, these functions can contribute to higher plant growth and enhanced nutrition, resistance to abiotic stressors, or reduction of disease symptoms. They may thus offer a great potential to improve agricultural yield while reducing the input of fertilizers or pesticides.

Most current approaches to improve microbiome functionality rely on the introduction of beneficial bacteria or fungi, carrying one or more desired functions. These approaches have led to impressive results, as illustrated in the other chapters of this book. However, they are limited by the survival of the introduced microbiota, which have to compete with the indigenous flora to be able to develop their beneficial activity. In this chapter, I suggest that protists may be used as a keystone group to enhance selected microbes and boost the functionality of the rhizosphere microbiome. I will give an overview of the phylogenetic affiliation of protists, functional groups present in the soil, their potential impact on microbial communities, and applications of protists in the agriculture.

13.2 Phylogeny of Protists

The term “protist” encompasses all eukaryotes with the exception of green plants, fungi, and animals. This paraphyletic concept originates from the scientific tradition of the nineteenth century and has persisted today. Protists were the first organisms discovered by Leeuwenhoek, who named them animalcules. Later, different organisms were investigated by different scientific communities. Algae were investigated by botanists and mobile protists by zoologists, who coined the term protozoa (“primitive animals”). For a long time, all protists were grouped into one separate kingdom (kingdom Protista). This vision radically changed with the development of molecular methods, which brought a complete shift in eukaryote classification. According to the latest phylogenies (Adl et al. 2012; Pawlowski 2013), protists now encompass most eukaryotes, with plants, animal, and fungi appearing as three tiny branches in the eukaryotic tree of life. Eukaryotes contain dozen more of phyla encompassing several lineages with diverse lifestyle and ecological relevance. The whole field of protistology is still influenced by its separate historical background and has developed concepts distinct from the remaining microbiology. These historical legacies still persist in the scientific vocabulary. Protists are for instance often been referred as “microfauna,” small animals, in soil studies, a remnant of ancient scientific traditions. Yet protists are not miniature animals, they encompass phylogenetic groups often unrelated to animals, and are thousands of times more diverse. Similarly, bacterivorous protists have been (and are still being) referred to as grazers, a poetic analogies to cows feeding on a meadow, that is unfortunately in discrepancy with the standard ecological nomenclature. Grazing refers to the partial consumption of the prey, which remains alive and can regrow. Bacterivore prey consumption is best described as predation, which results in prey

death, which had very distinct consequences on population dynamics and evolutionary processes.

13.3 Functional Groups of Soil Protists

Soil is a hotspot of microbial diversity, including protists (Ekelund and Ronn 1994). Besides a few parasitic taxa such as apicomplexan or oomycetes, as well as phototrophic algae, most protists groups are heterotrophs, feeding on bacteria and fungi (Foissner 1987). Protists are often classified on the base of morphological characteristics. Even if these characteristics are not always useful from a taxonomic perspective, with for instance, morphologically almost identical amoebae found in extremely distinct lineages (Pawlowski 2013), they nonetheless offer interesting insights in functionality of protists and their importance for soil fertility (Ekelund and Ronn 1994).

13.3.1 Ciliates

Ciliated protozoa, the only morphotype matching with one defined phylogenetic group (within the phylum alveolates), can consume huge amounts of prey. They are among the biggest protists in soil, ranging from 5 to 500 μm . They are covered with cilia, allowing them to swim rapidly, and possess a large mouth-like opening enabling the rapid consumption of several preys. Ciliates contain, for instance, the model species *Tetrahymena pyriformis* and are one of the most commonly used organisms in food web models (Fussmann et al. 2014; Altermatt et al. 2015). Thanks to their high rate of consumption, it is possible to monitor accurately their interactions with preys under various conditions. Ciliate are excellent bio-indicators of soil conditions, allowing for instance discriminating polluted from pristine soils (Jousset et al. 2009a). Thanks to the large amount of data available, species found in one sample can be readily classified as r or K strategist or biofilm dwelling or planktonic swimmer. Ciliates are an extremely diverse group (Foissner et al. 2004) but depend on the presence of water films for their activity. As a result, ciliate density is low in most soils (Darbyshire et al. 1989), with exception of paddy soils, in which ciliates seem to play an important role as regulator of bacterial communities. Most described ciliates are bacterivorous; however, some species have evolved stylet-like structures allowing them to suck the content of fungal hyphae.

13.3.2 Flagellates

This denomination encompasses several groups of different morphology and phylogenetic affiliation. Flagellates are typically 2–10 μm big and share the property of having one or more flagella, which can serve for locomotion or catching preys. In soils, some of the most common groups of flagellates can be found in the phylum

Rhizaria, with the genera *Cercomonas*, *Heteromita*, in the phylum Euglenozoa, with for instance the kinetoplastida genus *Bodo*, or the excavates, comprising species such as *Jakoba*. Some protists can switch between a flagellate and amoeboid stage during their different life stages. One of the best described example is the amoeba of the genus *Naegleria* (Fritz-Laylin and Fulton 2016).

13.3.3 Naked Amoeba

Naked amoeba shows an irregular shape, with bursting pseudopods. Amoebae are one of the most abundant soil protist functional groups. It also covers several phyla, including Rhizaria, Amoebozoa, and Excavata. Note that the term “Amoebozoa” only refers to one specific eukaryotic phylum, covering only a fraction of all the amoeboid organisms. Isolates of the Amoebozoa, genus *Acanthamoeba*, have been used as model organism in several studies, as they are comparatively easy to isolate and cultivate axenically (Bonkowski 2004; Rosenberg et al. 2009; Jousset and Bonkowski 2010; Neidig et al. 2010).

13.3.4 Testate Amoebae

These amoebae are protected by a shell made of debris, minerals, or secretions from the amoeba. Testate amoebae are particularly abundant in acidic soils such as bogs but can be found in most soils. The empty shells remain identifiable for a long time after the death of the organism, providing useful paleo-records, helping redraw the history of a given site. They are further very sensitive to environmental changes such as fertilization (Krashevskaya et al. 2014). The exact importance of testate amoeba for soil fertility is not elucidated yet, and experiments are complicated because of the difficulty to cultivate many species. No doubt, however, that new insights will come soon.

13.4 Functionality of Protists and Impact on Microbial Communities

Protists have a keystone function in the soil ecosystem. They are primary consumers in the food web, making the link between primary producers and higher soil food web levels such as collembolans and mites. Recent studies have in addition extended this view and protists are now recognized to also feed on animals such as nematodes (Neidig et al. 2010; Geisen et al. 2015; Geisen 2016), showing that the importance of protists may be spread across several trophic levels. This section will focus on the interaction between protists and bacteria, as most of the literature is centered on these taxa.

Protists are, together with nematodes, the main consumers of bacteria in soil (Bonkowski 2004). Predation is so intense that many scientists consider rhizosphere

communities to be top-down regulated: The main constraint on bacterial fitness is not to find nutrients but to escape predation (Moore et al. 2003). Bacterivorous protists appear thus as keystone element that can control microbiome structure and function. Protist populations have long been put in relation with soil fertility and may be used as inoculant in the agriculture to boost soil microbiome functionality. By providing a selective advantage to indigenous microbes that may otherwise be rare and inactive, protists may unlock different functions beneficial for plant growth. Protists can have for instance following effects on the soil microbiome:

13.4.1 Nutrient Turnover

Protists consume bacteria and fungi and typically release the excess of nutrients such as nitrogen, phosphorus, or micronutrients. These nutrients are often limiting in soil and would be without predation kept locked in dormant microbial cells, preventing their use to active ones. One of the most striking effects of protists is their stimulation of nitrogen mineralization and nitrification (Kuikman et al. 1991; Alpehi et al. 1996). Since nitrate is the favored nitrogen source by many plants, protists may stimulate nitrogen uptake in plant, helping in use of resources more efficiently (Alpehi et al. 1996; Kreuzer et al. 2006).

13.4.2 Selection of Specific Bacteria

Due to their activity, protists select for *r* strategists (Swallow et al. 2013). In addition, protists carefully select their prey and discriminate between edible and non-edible bacteria on the base of several characteristics including surface properties or size (Montagnes et al. 2008). Closely related species can have distinct effects on microbial communities. Protist predators play further a key role in promoting toxic microorganisms in the rhizosphere. Secondary metabolites linked to pathogen suppression and may further add protection against predation by protists, which avoid these toxic bacteria and confer them a growth advantage. The productions of broad-spectrum bioactive secondary metabolites such as alkaloids (Klapper et al. 2016), lipopeptides (Andersen and Winding 2004; Jousset et al. 2006; Mazzola et al. 2009) or polyketides (Jousset et al. 2006), and addition of amoebae multiplied the fitness of an introduced biocontrol *Pseudomonas* in rice rhizosphere by a factor of three, by preferentially feeding on nontoxic competitors of the introduced bacteria (Jousset et al. 2008). Similarly, production of gluconic acid, compound-helping bacteria mineralizing phosphorus, can protect bacteria against predation by protozoa from very distinct taxonomic affiliation (Gomez et al. 2010). Thanks to this overlap, protists may thus function as a booster of introduced microbes, ensuring their survival.

13.4.3 Shifts in Microbiome Composition

In addition of the bacterivorous amoeba, *Acanthamoeba castellanii*, to rhizosphere microbial communities results in shift in phylogenetic composition, promoting, for example, actinobacteria or *Herbaspirillum*, two bacterial groups linked to plant growth promotion (Rosenberg et al. 2009).

13.4.4 Enhancement of Plant-Microbe Symbioses

Arbuscular mycorrhizae are an important symbiont of almost all terrestrial plants. They can greatly enhance plant yield and stress tolerance by extending the contact surface with the soil, allowing foraging of regions unreachable for roots alone. Koller and colleagues (2013) showed that mycorrhiza function is largely dependent on protists. Amoebae increased nitrogen turnover around hyphae and stimulated its transfer to the plant. Likely, mycorrhiza fungi themselves were not able to produce the required enzymes required for mineralizing the soil organic material. Instead they secreted plant-derived carbon in their surroundings, fueling associated microbial communities. Without predation, these communities would get blocked by nutrient limitation, a problem solved by adding amoebae to the system. Protists may thus be important to shift plant-mycorrhiza interaction from parasitism (the fungus taking up plant-derived carbon without delivering nutrients) to mutualism in which the fungus provides the plant with the required nutrients such as nitrogen or phosphorus. Protists can further maintain microbiome functioning over evolutionary scales by preventing the emergence of bacterial cheats that consume plant-derived resources but do not contribute to plant health. Since such cheats also become more vulnerable to predation, plant-bacteria cooperation can be maintained by predation by bacterivorous amoebae (Jousset et al. 2009b).

13.4.5 Manipulation of Plant Hormone Balance

Predation by protists favors bacteria producing auxin, a hormone-stimulating root development (Bonkowski and Brandt 2002). Plants co-inoculated with amoebae show for instance a much more ramified root system than control plants (Kreuzer et al. 2006). Such a ramified root system helps plant take up soil nutrients and reducing the use for fertilizer. Although no explanatory mechanism for this selection is available to date, this process appears as a “hormonal” microbial loop (Bonkowski 2004), in which predation by protists such as amoebae cause an increase in nitrate and auxin, which forces the plant to invest more in the root system, feeding more bacteria and ultimately amoebae, completing the cycle.

13.4.6 Stimulation of Beneficial Trait Expression

Some bacterial traits linked to plant growth promotion, including for instance the production of siderophores or toxic secondary metabolites, are strongly affected by the presence of bacterivorous protists. For instance, siderophore production in *Pseudomonas fluorescens* can be stimulated in the presence of amoebae (Levrat et al. 1992). Cyclic lipopeptides (Mazzola et al. 2009) or 2,4-DAPG (Jousset and Bonkowski 2010; Jousset et al. 2010) production increased after confronting bacteria with amoebae or their supernatant. Although the exact nature of the signals involved in this interaction is not known yet, small molecules (<3 kDa) secreted by amoebae are required for the recognition of protists by bacteria (Jousset et al. 2010). Many bacteria can react to chemical cues from predatory amoebae and flagellates (Corno and Jurgens 2006) and such chemical communication may play an important role in structuring rhizosphere communities. Further studies are needed to assess how specific bacteria can recognize and respond to predators. Together, these findings suggest that protozoa may be used to promote the activity of soil microbes. This is illustrated by studies showing that adding protists increased the antagonism of a biocontrol *Pseudomonas* against the plant-pathogenic fungus *Fusarium oxysporum* (Levrat et al. 1991).

13.5 Application of Protists as Microbiome Enhancers

As mentioned above, protists can improve several functions of the soil microbiome relevant to plant health, including making nutrients available to the plant, stimulating plant growth and suppressing diseases. Thanks to the variety of shape and function, they provide a formidable biotechnological pool to improve various soil processes linked to fertility and sustainable crop production (Chen et al. 2007).

Protists cover most of eukaryotes lineages and are thus a huge and untapped source of genetic diversity. The effect of different groups of protists on microbial communities remains to be elucidated, but the sensitivity of protists to secondary metabolites seems to correlate with high level taxonomy (Pedersen et al. 2011). As a result, screening protozoa across the tree of life may help discovering that which group supports which type of antibiotics. In contrast, both closely related *Cercomonas* species and more distantly related protists from different phyla may have very distinct effects on microbial community composition (Rønn et al. 2002; Glucksman et al. 2010). A rapid coevolution process may thus be occurring in soil, potentially on the base of prey recognition receptors and bacterial antigens (Wildschutte et al. 2004). These contrasting results indicate that a huge pool of protists may be used to favor selected functions in the soil microbiome.

Protozoa can be applied in several ways. They can function as an enhancer for introduced plant-beneficial bacteria or fungi: The survival of introduced microbes is often a limiting factor for their impact on plant growth and health. Protists can improve the survival of introduced biocontrol *Pseudomonas* spp. by a 200 % (Jousset et al. 2006), by consuming indigenous species. This effect may be best

obtained with biocontrol agents producing toxic secondary metabolites, yet other traits such as hard cells, biofilm, or filament formation may also provide a competitive advantage (Matz and Kjelleberg 2005; Jousset 2012). For instance, bacteria of the genus *Arthrobacter* seem to be fostered under protists predation, suggesting that several taxa can be enhanced by adding the right protist (Rønn et al. 2002). Different species may be more or less sensitive to specific bacterial metabolites, so that custom pairs of protists and bacteria may best work together. New screenings are needed to uncover the appropriate combinations (Pedersen et al. 2011). Protists may further serve as general enhancer of microbiome function. Protists stimulate nutrient turnover and accelerate the mineralization of organic fertilizer. They are already included in first commercial products, where they speed up nutrient release from organic fertilizer at low temperature, which may be particularly relevant for spring conditions in cold climates.

Finally, protists may be used to directly consume pathogens. Fungivorous amoebae have long been suspected for instance of being able to induce suppressiveness against *Fusarium* (Levrat et al. 1991), although more experimental proofs are needed.

13.6 Protist Preparation

Protists can be grown either on undefined bacteria co-isolated with the species, mono-axenically on one reference bacteria, or axenically in a sterile culture medium (Weekers et al. 1993). Axenic growth is the best option, as it allows high yields, but can be tedious to obtain as protists are typically associated with various bacteria and may not be cultivable in absence of a prey.

Most – if not all – soil protists build cysts, a resistance stage allowing survival in extreme conditions. This property is most useful for biotechnology purposes as it makes dry formulation possible. Once introduced to soil, the cyst hatches and the trophozoites start multiplying. Some cysts can carry bacteria in them. This property has long been known as an issue for potential pathogens (Molmeret et al. 2005) but may as well serve as vector for otherwise vulnerable beneficial soil microbes.

13.7 Precautions

Most protists are free-living organisms. The few obligate parasites species, such as *Trypanosoma* or *Plasmodium*, are not relevant for biotechnological applications aiming at improving soil fertility. However, some bacterivorous species are known to be opportunistic pathogens. As with other opportunistic pathogen, they are ubiquitous in the environment and do not pose objective hazards to healthy individuals. Nonetheless, avoiding them would avoid unnecessary danger for immune-compromised patients and prevent bureaucratic hassles during the registration process.

Some amoebae of the genus *Acanthamoeba* can cause keratitis, a rare but hard to cure eye disease typically associated with poor contact lenses hygiene. Even if only a very few genotypes can cause disease, regulation agencies may not be easy to convince (Siddiqui and Khan 2012). Further, *Naegleria* spp. living in warm waters can cause deadly brain diseases in immune-compromised patient, calling for caution when cultivating them.

13.8 Conclusion

Protists offer new venues to manipulate the soil microbiome and enhance plant health. Several studies on taxonomy and function could be linked together to provide robust biotechnological applications.

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