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Minireview

# Ecological and evolutive implications of bacterial defences against predators

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## Summary

**Bacterial communities are often heavily consumed by microfaunal predators, such as protozoa and nematodes. Predation is an important cause of mortality and determines the structure and activity of microbial communities in both terrestrial and aquatic ecosystems, and bacteria evolved various defence mechanisms helping them to resist predation. In this review, I summarize known antipredator defence strategies and their regulation, and explore their importance for bacterial fitness in various environmental conditions, and their implications for bacterial evolution and diversification under predation pressure. I discuss how defence mechanisms affect competition and cooperation within bacterial communities. Finally I present some implications of bacterial defence mechanisms for ecosystem services provided by microbial communities, such as nutrient cycling, virulence and the biological control of plant diseases.**

## Introduction

Bacterial communities form the backbone of virtually all known ecosystems, and play a fundamental role in nutrient cycling, primary production and consumption, or pollutant degradation. Bacteria form also the base of many food webs, and are subject to strong predation pressure by eukaryotes in aquatic and soil environments (Gasol *et al*., 2002; Rønn *et al*., 2002). Predation is a major cause of bacterial mortality (Pernthaler, 2005), and a driver of the genetic and functional structure of bacterial communities (Griffiths *et al*., 1999; Rønn *et al*., 2002; de Mesel *et al*., 2004; Bell *et al*., 2010). Predation also modulates the metabolic characteristics and the activity of bacterial communities, thereby contributing to nutrient cycling (Microbial loop, Clarholm, 1985; Bonkowski, 2004). Predation pressure is largely due to microfaunal predators (Ekelund and Ronn, 1994; Pernthaler, 2005), a functional group including protozoa and nematodes. A large fraction of all protozoan species are predators of bacteria, and free-living bacterivorous protozoa are present in all ecosystems ranging from sea to soil including deserts (Bouwman and Zwart, 1994; Ekelund and Ronn, 1994; Rodriguez-Zaragoza *et al*., 2005). Bacterivorous protozoa embrace many functional feeding groups, such as free swimming ciliates (one of the best known examples being the model organism *Tetrahymena thermophila*), heterotrophic flagellates, and amoebae. Each of these functional types has its own hunting characteristics and ecological niche (Coûteaux and Darbyshire, 1998). For example, naked amoebae dominate soil systems where they can access very small pores (Ekelund and Ronn, 1994), while in aquatic systems heterotrophic nanoflagellates are the main consumers of bacteria (Pernthaler, 2005). Nematodes, such as the well investigated *Caenorhabditis elegans*, are common in compost, soil as well as aquatic systems (Jensen, 1987; Neher, 2001), and form the second main group of bacterivorous organisms. Resisting predation improves survival in top-down controlled communities, and numerous bacteria from all phyla developed an array of defence mechanisms reducing predation pressure (Matz and Kjelleberg, 2005). During the last years, the investigation of bacterial defence strategies has been gaining in momentum. Technical advances, such as the availability of genome sequences and the solid investigation of pro- and eukaryotic model organisms, the better understanding of regulatory networks by bacteria and high-throughput analytics, allowed understanding a number of molecular mechanisms involved in bacterial defence. Additionally, the similarities between predator resistance and pathogenesis (Adiba *et al*., 2010) have fostered research on this subject by both environmental and medical microbiologists. The detection mechanisms involved in innate immunity and prey detection by free-living protozoa are very similar, and the data

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from immunological studies can help to better understand ecological processes (Stuart and Ezekowitz, 2008). Our understanding of how predator–prey interactions affect population dynamics and ecosystem stability has greatly improved during the last decades and provides a powerful interpretation framework to understand the causes and consequences of bacterial defence (Jeschke *et al*., 2002; Yoshida *et al*., 2003; Brose, 2008). Merging molecular tools with ecological models is a promising path of ecological research allowing to reliably predict the impact of different traits on the functioning and development of bacterial communities. This review aims at providing an interdisciplinary overview on these recent advances. First, I will present the known defence mechanisms involved in reducing predation pressure. Then, I will place these mechanisms in a more general ecological framework to discuss how distinct mechanisms affect predator–prey interactions. Finally, I will discuss direct and indirect consequences of bacterial defence on other ecological and evolutive processes. I will focus on interactions between bacteria and eukaryotic predators. Non-eukaryotic consumers, such as bacterial predators (e.g. *Bdellovibrio*) and viruses, also play a fundamental role in microbial ecology and evolution (see for example Weinbauer, 2004; Sockett, 2009), but for the sake of brevity and conciseness these will not be considered. Throughout this review, I will use the terms of 'predators' and 'predation' to describe the consumption of bacteria. Microbial ecologists often use grazing for what ecologists from other research fields would describe as predation, and both terms can be in the present context understood as synonyms.

## Predation and the evolution of bacterial defences

Predation is a complex process involving a number of components such as prey finding, recognition, consumption and digestion (Jeschke *et al*., 2002). First I will give a short overview on predator–prey interactions and then consider the numerous morphological and chemical adaptations of bacteria against predators according to their putative effect on predators.

The predator functional response describes the relationship between prey density and consumption rate, and can take various shapes according to the type of interaction (Fig. 1). Two types of functional response are commonly found at all trophic levels, the type II (similar to Michaelis-Menten kinetics) and type III functional response (sigmoid). Prey defence affecting certain components of predation may have distinct effects on the shape of the functional response curve depending on which component of the predator–prey interaction is affected (Fig. 1). Bacteria evolved a wealth of defence strategies, affecting virtually all components of predator–prey interaction (Table 1). In aquatic and terrestrial ecosystems, the most described strategies are morphological adaptations, such as filament formation or clumping (Hahn *et al*., 1999; 2004) and the production of toxic secondary metabolites (Jousset *et al*., 2006; Deines *et al*., 2009; Mazzola *et al*., 2009), but these are only part of the broad spectrum of antipredator adaptations (Matz and Kjelleberg, 2005). Bacterial defence mechanisms can increase the search time spent by the predator until finding prey or the handling time required for capturing, consuming and digesting prey. For commodity, I will classify the different components of the predator–prey interaction and the prey adaptations based on the model

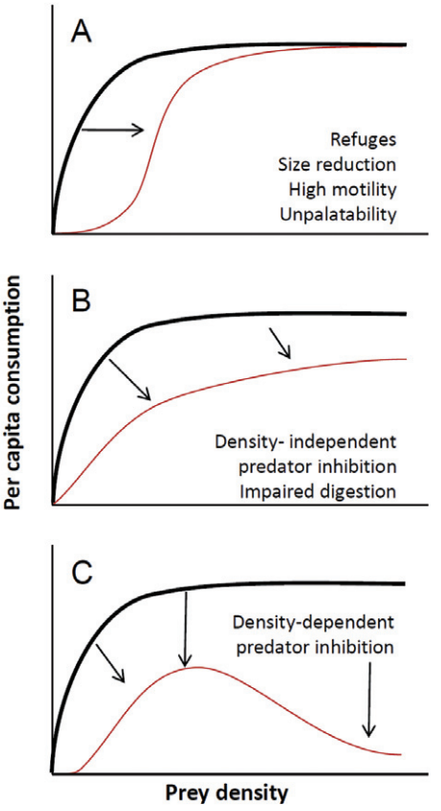


**Fig. 1.** Potential effects of bacterial defence on a model type II functional response curve of a microfaunal predator. Per capita consumption depends on the prey density, and different antipredator adaptations of the prey will have distinct effects on the shape of the functional response. The black curve shows a type II functional curve on a hypothetical undefended prey, the red curve shows the consumption of defended prey.
A. Prey adaptations like motility, hiding or size reduction increase the time the predator requires to find a prey, and lead to a reduced consumption at low prey density.
B. Prey adaptations like indigestibility or constitutive toxin production reduce predation regardless of prey density.
C. Quorum sensing-regulated traits (e.g. secondary metabolite production by *Pseudomonas* spp.) are increasingly expressed as prey density increase until potentially reducing predation pressure to zero (type IV functional response).

**Table 1.** List of described antipredator adaptations by bacteria.

| Defence mechanism | Prey | Predator | Affected predation step | Reference |
|---|---|---|---|---|
| *Morphological* | | | | |
| Filament formation | *Flectobacillus* sp. | *Ochromonas* sp. | Ingestion | Corno and Jurgens (2006) |
| | *Serratia liquefasciens* | *Acanthamoeba polyphaga* | | Queck *et al.* (2006) |
| Biofilm | *S. liquefasciens* | *A. polyphaga* | Ingestion | Queck *et al.* (2006) |
| | *Pseudomonas aeruginosa* | *Bodo saltans* | Search, ingestion | Weitere *et al.* (2005) |
| Microcolonies | *P. aeruginosa* | *Rhynchomonas nasuta* | Ingestion | Matz *et al.* (2004a) |
| *Surface properties* | | | | |
| LPS modification | *Salmonella enterica* | *Negleria gruberi* | Recognition | Wildschutte *et al.* (2004) |
| S-layer | *Actinobacteria* | *Poterioochromonas* sp. | Digestion | Tarao *et al.* (2009) |
| Flagellum modification | *Helicobacter pylori* | Macrophages | Recognition | Galkin *et al.* (2008) |
| EPS capsule | *Pseudomonas* sp. | *Ochromonas* sp. | Ingestion/digestion | Hahn *et al.* (2004) |
| Spore formation | *Bacillus* sp. | *Tetrahymena thermophila* | Digestion | Klobutcher *et al.* (2006) |
| *Secondary metabolites* | | | | |
| Cyclic lipopeptides | *Pseudomonas fluorescens* | *Negleria americana* | Kills predator (disrupts membranes) | Mazzola *et al.* (2009) |
| | *P. fluorescens* | *Hartmanella vermiformis* | | Andersen and Winding (2004) |
| | | *Acanthamoeba* sp. | | |
| HCN/DAPG/PRN | *P. fluorescens* | *Acanthamoeba castellanii* | Kills predator (inhibits respiration) | Jousset *et al.* (2010) |
| HCN | *P. aeruginosa* | *Caenorhabditis elegans* | Kills predator (paralytic death) | Gallagher and Manoil (2001) |
| Gluconic acid | *Enterobacter intermedium* | *Colpoda steini* | Search/attack | Gomez *et al.* (2010) |
| | | *Vahlkampfia* sp. | Causes encystment | |
| | | *Neobodo designis* | | |
| Violacein | *Chromobacterium violaceum* | *Ochromonas* sp. | Kills predator | Matz *et al.* (2004b) |
| | | *B. saltans* | | |
| Shiga toxin | *Escherichia coli* | *Tetrahymena pyriformis* | | Lainhart *et al.* (2009) |
| Exoproteases | *P. fluorescens* | *C. steini* | Cause encystment | Jousset *et al.* (2006) |
| | | *Vahlkampfia* sp. | Kills predator | |
| | | *Neobodo designis* | | |
| | *Vibrio cholerae* | *C. elegans* | Kills predator | Vaitkevicius *et al.* (2006) |
| | | *T. pyriformis* | | |
| | | *Cafeteria roenbergensis* | | |
| Serrawettin W2 | *Serratia marcescens* | *C. elegans* | Search | Pradel *et al.* (2007) |
| *Secretion systems/effectors* | | | | |
| Type III SS | *P. aeruginosa* | *A. castellanii* | Effectors kill predator | Matz *et al.* (2008b) |
| Type VI SS | *V. cholerae* | *Dictyostelium discoideum* | Digestion (intracellular survival) | Jani and Cotter (2010) |
| | *Burkholderia cenocepacia* | | | |

For each adaptation, the main step of the predator–prey interaction affected is described. The preys and predators indicated are those presented in the corresponding reference. Some reported effects are putative, and it is advised to consult the corresponding reference for detailed information.

described by Jeschke and colleagues (2002), with a few simplifications (Fig. 2). The different components might be hard to disentangle, and different classifications exist (Montagnes *et al.*, 2008). Moreover, a single defence strategy may affect different components of the predation process. The provided classification should therefore be rather considered as a guide that allows connecting biochemical processes such as prey recognition to models on population dynamics.

### *Search phase*

Predators may spend a large fraction of their time searching for prey. The search efficiency (the 'attack rate' of the predator) largely depends on the time needed to find his prey (Jeschke *et al.*, 2002). Predators may move randomly through their environment until encountering potential prey. The search time can be however greatly reduced by chemotaxis: microfaunal predators, such as nanoflagellates (Mohapatra and Fukami, 2007), amoebae (Konijn, 1969; Fenchel and Blackburn, 1999) and nematodes (Beale *et al.*, 2006), use chemical cues from their prey to locate and reach it more effectively. For example, quorum sensing signals (a category of signal compounds allowing bacteria to sense population density and diffusion gradients) such as the *N*-acylhomoserine lactones (AHLs) produced by Gram-negative bacteria, attract the nematode *C. elegans* (Beale *et al.*, 2006) as well as human neutrophils (Zimmermann *et al.*, 2006), suggesting widespread use of these signals as cue for prey detection. A first line of defence for bacteria therefore might consist in impairing

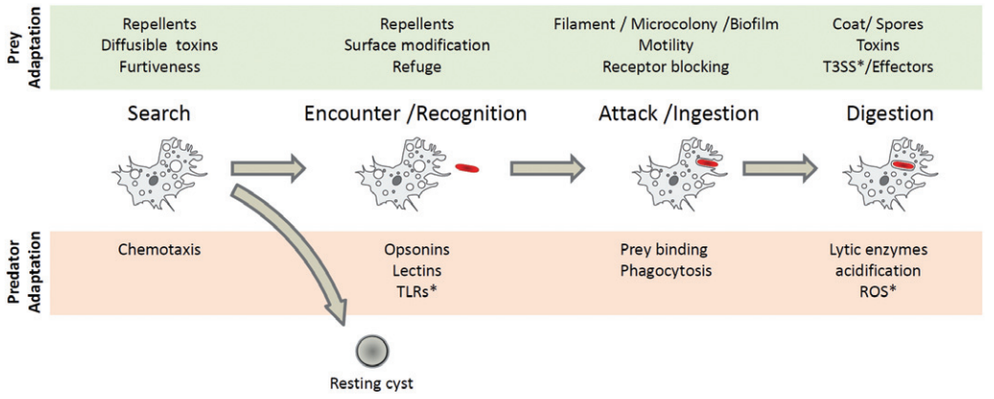


**Fig. 2.** Schematic view of the different steps involved during the predation process of a bacterial prey (red) by a protozoan predator (symbolized by the amoeba). The adaptation mechanisms increasing predator efficiency (red) and prey resistance (green) are highlighted for each step. Abbreviations (*): TLR, Toll-like receptor; T3SS, type three secretion system; ROS, reactive oxygen species.

the search process. This may be achieved by chemical furtiveness, for example by abandoning the production of quorum sensing signals. Bacteria escaping to microrefuges inaccessible to the predator also impede efficient search by protozoa and nematodes (Postma *et al*., 1990; Yeates *et al*., 2002), and the production of repellents may be used to deter chemotactic predators (Hilliard *et al*., 2002; Pradel *et al*., 2007). Many bacterial strains further produce toxic secondary metabolites that inhibit predators before encounter. Lytic enzymes such as proteases contribute to the toxicity of *Pseudomonas fluorescens* CHA0 for various protozoa (Jousset *et al*., 2006; Vaitkevicius *et al*., 2006; Niu *et al*., 2010), and the pathogenic bacterium *Vibrio cholerae* uses extracellular protease to kill bacterivorous nematodes and protozoa (Vaitkevicius *et al*., 2006). Other bacteria like *Enterobacter intermedium* repel predators by acidifying their environment (Gomez *et al*., 2010), and numerous strains produce secondary metabolites (Andersen and Winding, 2004; Jousset *et al*., 2006; Mazzola *et al*., 2009) that inhibit or deter the predator before encounter. In an extreme case, these compounds induce the formation of resting cysts by protozoa, or even kill the predator (Schlimme *et al*., 1999; Andersen and Winding, 2004; Jousset *et al*., 2006).

### *Encounter/recognition*

When encountering potential prey, predators must recognize it and prevent it from escaping. Eukaryotes use conserved receptors such as the Toll-like receptors (TLRs) to recognize molecular patterns associated with Gram-positive and -negative bacteria (Underhill, 2004; Cosson and Soldati, 2008). Protozoa select their prey based on surface properties, such as flagella, cell wall components (Wootton *et al*., 2007), lipopolysaccharide envelope compositions of Gram-negative bacteria (Wildschutte *et al*., 2004) and hydrophobicity (Matz *et al*., 2002). Few data are available on prey recognition by nematodes, but recent studies indicate that they can discriminate between mixed bacteria, partly based on their toxicity (Jousset *et al*., 2009; Freyth *et al*., 2010). A number of mechanisms are employed by bacteria to impair prey encounter and recognition thereby reducing the attack rate and the predation pressure at low prey density (Fig. 1). For example, high motility prevents capture (Matz and Jurgens, 2005), probably by allowing prey retraction upon contact with enemies (Matz *et al*., 2002). Further, diversification of molecular patterns provides a protection against protozoan predators (Matz *et al*., 2005). This likely occurs through masking of chemical cues used for prey recognition (Wildschutte *et al*., 2004), a technique also used to evade the immune system (Trent *et al*., 2006) and plant defences (Boller and Felix, 2009). Some pathogenic bacteria with a modified flagellum structure are not recognized by macrophages (Galkin *et al*., 2008), and the conserved nature of TLRs suggests that the same mechanism may help bacteria to evade recognition by microfaunal predators. Morphological adaptations such as the production of capsule (exopolysaccharide, EPS) also reduce ingestion, either through size effect or by masking prey surface (Hahn *et al*., 2004). The production of toxic or repelling metabolites may also play a role at this phase of predation, as flagellates reject prey after 'tasting' them (Montagnes *et al*., 2008). Protists also select their prey according to their size (Jürgens and Simek, 2000; Pfandl *et al*., 2004), and organisms out of the prey spectrum of the predator get selective advantage (Pernthaler, 2005). For example, bacteria producing filaments and aggregates, but also very small bacteria, are less consumed (Corno and Jurgens, 2008), an effect discussed more in detail in the next chapter.

*Attack/ingestion*

Prey ingestion by protozoa usually occurs by phagocytosis, a conserved mechanism present in most eukaryotes (Stuart and Ezekowitz, 2008). Phagocytosis is a complex process involving prey binding to specific receptors and engulfment in a digestive vacuole. Morphological adaptations that can prevent or reduce ingestion are widespread in bacteria (Fig. 1, Table 1). Flagellates cannot ingest large prey (Matz *et al*., 2002), and filament formation help prevent ingestion (Hahn *et al*., 1999; Corno and Jurgens, 2006). However, some flagellates can grow on filamentous bacteria, suggesting that this defence mechanism only provides partial protection against predators (Wu *et al*., 2004). Similarly, the formation of microcolonies (Matz *et al*., 2004a) or aggregates (Blom *et al*., 2010), reduces ingestion by flagellates and ciliates, but provide inefficient protection against amoebae (Matz *et al*., 2004a). Another morphological defence results from the formation of biofilms (Matz *et al*., 2005), where cell aggregation also favours intercellular communication, potentially increasing the production of secondary metabolites active against predators (Queck *et al*., 2006). Again, this adaptation only provides limited protection as indicated by the high diversity of amoebae growing on bacterial biofilms (Thomas *et al*., 2008). Pathogenic bacteria can prevent phagocytosis by inhibiting the restructuration of the actin cytoskeleton, or by blocking the receptors responsible for prey binding and initiation of phagocytosis (Ernst, 2000). Since phagocytosis is conserved among most eukaryotes, similar mechanisms probably help to escape predation.

*Digestion*

Once the prey has been taken up, the digestion process begins with engulfment of the prey and the formation of a digestive vacuole, the phagosome. The phagosome is subsequently acidified, and fused to lysosomes that provide lytic enzymes and reactive oxygen species (ROS) that will degrade the prey. The resulting phagolysosome is a dual place for the interaction between predator and prey. On the one hand it is the cell compartment where prey will be killed and digested. On the other hand it is a place of intricate contact between predator and prey that allows for complex signalling, luring and poisoning from both sides (Stuart and Ezekowitz, 2008). Secreted bacterial metabolites may be more effective after prey ingestion (Table 1). as they can target directly against the predator without diffusion into the environment (Matz *et al*., 2004b; 2008a; Deines *et al*., 2009). The vulnerability of the predator to ingested bacteria is also apparent in nematodes. *Burkholderia cepacia* and *Pseudomonas aeruginosa* can rapidly kill *C. elegans* after ingestion (Kothe *et al*., 2003; Zaborin *et al*., 2009), and secreted toxins from *V. cholerae* inhibit digestion, delay development and eventually kill *C. elegans* (Cinar *et al*., 2010). Shiga toxin-producing strains of *Escherichia coli* can sense phagosome-like conditions and kill ciliate predators by expressing the toxin after ingestion (Lainhart *et al*., 2009). The opportunistic human pathogen *P. aeruginosa* use the type III secretion system to deliver effectors and toxins into the predator's cytosol (Matz *et al*., 2008b), and this process likely continues after ingestion. Other contact-dependant delivery systems like the recently discovered type VI secretion system (Hayes *et al*., 2010) play a role in predator–prey interactions and contribute to inhibit predators (Jani and Cotter, 2010). This is a new and dynamic research field, and further studies will help elucidate the role of secretion systems as predator defence. Adaptations that delay or prevent digestion may reduce predation pressure even without predator inhibition: at high prey density, predators are limited by the time needed to digest their prey, and saturated predators stop feeding until having digested the prey already consumed (Jeschke, 2006). Prey adaptations extending digestion time will delay the next attack, and contribute to prey protection (Fig. 1) even if the predator survives and if the ingested prey is eventually digested (Jeschke, 2006). The protein coat of *Bacillus* spores increases the resistance against digestion by ciliates (Klobutcher *et al*., 2006), and the S-layer of actinobacteria has been suggested to impair digestion by flagellates (Tarao *et al*., 2009). Such adaptations likely favour Gram-positive bacteria and contributes to their dominance in natural communities consumed by protozoa (Rønn *et al*., 2002). Other bacteria such as *B. cepacia* survive in the digestive vacuoles of *Acanthamoeba polyphaga* (Lamothe *et al*., 2004), and intracellular survival is considered to be the first step in the evolution of endosymbionts (Horn and Wagner, 2004) or intracellular parasites such as *Legionella* (Molmeret *et al*., 2005).

The presented defence mechanisms are not mutually exclusive and may even act in concert. The same defence adaptation can function at various steps of the predation process (Fig. 2, Table 1). For example, the volatile compound hydrogen cyanide is an efficient deterrent of various eukaryotes, and at the same time a potent inhibitor of cytochrome oxydases (Blumer and Haas, 2000) and causing paralytic death of nematodes (Gallagher and Manoil, 2001). The S-layer of actinobateria is another case of adaptation affecting different predation steps, putatively affecting both ingestion and digestion (Tarao *et al*., 2009). Additionally, the efficiency of different defence mechanisms varies with predator species or functional group. Biofilm formation by *P. aeruginosa* inhibits its consumption by amoebae and flagellates, but not by ciliated protozoa (Weitere *et al*., 2005). Diverging impact of predators on bacterial communities is correlated with

their phylogenetic distance (Glucksman *et al*., 2010), and this may be due to differences in the resistance to bacterial toxins. Microfaunal predators of different phylogenetic affiliation show very different sensitivity to bacterial secondary metabolites (Jousset *et al*., 2006; Pedersen *et al*., 2011), and the variety of strategies employed to resist predation presumably contributes to maintaining diverse bacterial communities.

## Regulation of defence strategies

### *Predator mediated induction of bacterial defences*

Defence mechanisms increase the fitness of bacterial prey in presence of predators but their production is costly (Leibold, 1996; Callahan *et al*., 2008). Their expression either distracts resources, as is the case in the production of secondary metabolites, or alter resource uptake, as do morphological adaptations, such as size changes or filament formation. It is thus advantageous for the prey to only express defence traits when predator defence indeed results in fitness gain, i.e. if predators are present. Induction of defence traits in presence of predators is a common strategy to optimize the investment in predator defence strategies, and it is not surprising that many bacteria are able to modify their defence strategies in presence of predators. Bacteria can sense diffusible chemical cues secreted by protozoan predators and respond by forming inedible filaments or microcolonies (Corno and Jurgens, 2006; Blom *et al*., 2010). Similarly, *P. fluorescens* responds to diffusible predator cues by upregulating the production of toxic secondary metabolites such as membrane disrupting biosurfactants (Mazzola *et al*., 2009) or mitochondrial inhibitors such as the polyketid 2,4-diacetylphloroglucinol (DAPG) (Jousset and Bonkowski, 2010; Jousset *et al*., 2010). Chemical communication is common in the rhizosphere (Dubuis *et al*., 2007) and perception of predator associated chemical cues (kairomones) is widespread in planktonic microorganisms (Pohnert *et al*., 2007). Further studies are needed to better understand the nature of protozoan associated cues, and the regulatory cascades required for induced defences and the transcriptome-level response of bacteria to predators.

### *Density-dependent expression of bacterial defences*

Another efficient strategy for predator defence is the use of density-dependent regulation of defence traits. Many Gram-positive and -negative bacteria present cooperative multicellular behaviour regulated by cell-to-cell signalling. Typically, each cell produces and senses autoinducers, small diffusible molecules that function as proxy for cell density and diffusion gradients (Keller and Surette, 2006). Once a given population density is achieved, all cells activate social behaviours that would be inefficient at low cell density. Interestingly, many defence traits protecting bacteria against microfaunal predators are activated by quorum sensing. Fluorescent pseudomonads regulate their secondary metabolism via the Gac/Rsm cascade in a density-dependent way (Lapouge *et al*., 2008), and many of these secondary metabolites are toxic for protozoa (Jousset *et al*., 2006) and nematodes (Bjornlund *et al*., 2009; Romanowski *et al*., 2010). Violacein production by *Chromobacter violaceum* is regulated by AHLs (Matz *et al*., 2004b), and quorum sensing-derived traits are also important for the resistance of *P. aeruginosa* (Matz *et al*., 2004a) and *Serratia marcescens* (Queck *et al*., 2006) biofilms against predation by protozoa. Cell signalling is also required for *B. cepacia* and *P. aeruginosa* to kill bacterivorous nematodes (Gallagher and Manoil, 2001; Kothe *et al*., 2003).

## Ecological and evolutive implications

Bacterial defences against microfaunal predators can have trophic and non-trophic effects on various other ecological and evolutive processes (summarized in Fig. 3). In this section, I will present some mechanisms causing bacterial defences to influence other processes, and discuss the environmental conditions likely to favour these interactions.

### *Food web structure and stability*

Prey defence is an increasingly recognized determinant of predator–prey relationships (Altwegg *et al*., 2006). This is also the case for bacterial communities, which is of particular importance considering the fundamental role of bacteria for ecosystem functioning.

Trade-offs between prey defence and performance are common, and the outcome of competition between resistant and sensitive prey species depends on both resource supply and predation pressure (Leibold, 1996). Prey selection by predators strongly varies with prey size (Jürgens and Simek, 2000; Salinas *et al*., 2007) and, in particular at high resource availability, large bacteria are less consumed (Corno and Jurgens, 2008). Larger organisms have lower surface-to-volume ratios, and size is negatively correlated to resource uptake by algae (Sunda and Hardison, 2010). The spread of larger phenotypes under predation pressure can therefore lead to a decreased resource uptake at the community level. This effect may be further accentuated if morphological adaptations such as filaments or microcolonies are induced by chemical cues from predators (Corno and Jurgens, 2006; Blom *et al*., 2010).

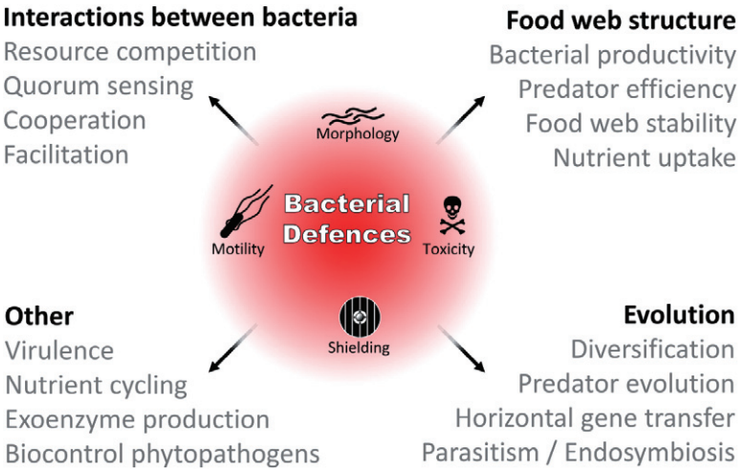


**Fig. 3.** Summary of some ecological and evolutive processes altered by bacterial defences against microfaunal predators. Bacterial defence mechanisms such as morphological adaptations (microcolonies, filaments), toxicity, motility or surface modifications ('shielding' with surface molecules impairing detection or digestion) can interfere directly or indirectly with various ecological and evolutive processes, which are discussed in detail in the text.

Bacterial defences may have both stabilizing and destabilizing effects on population dynamics, and a case by case analysis is necessary to understand the possible outcomes. Any adaptation causing alterations in the predator functional response curve can potentially affect the stability and strength of the interaction. Especially, adaptations causing a shift from a type II to a type III functional response (Fig. 1) may have far reaching consequences. In a type II functional response, predation efficiency steadily increases as prey density declines, until eventually causing prey extinction. In contrast, in a type III functional response predators are inefficient capturing prey at low prey density, resulting in more stable predator–prey systems (Rall *et al.*, 2008). Body size ratio between predators and prey is another predictor of food web stability (see Brose, 2010), and the skewed size distribution of bacterial communities resulting from selective prey consumption by protozoa (Pernthaler, 2005) may destabilize food webs via allometric effects: if smaller prey become dominant, the interaction may remain stable albeit weaker, but dominance of larger prey may destabilize food web structure (Brose, 2010). This is likely the case in productive aquatic systems, where large phenotypes, microcolonies and filamentous bacteria dominate under predation pressure (Corno, 2006). The presence of resistant phenotypes within the population causes prolongation of cycles in predator–prey oscillations (Yoshida *et al.*, 2003). At the same time, toxic secondary metabolites like 2,4-DAPG, hydrogen cyanide or proteases can cause protozoa to produce dormant cysts (Jousset *et al.*, 2006). In soil the 'cyst bank' of dormant predators reaches densities orders of magnitude above the active population (Ekelund *et al.*, 2002), and encysted protozoa can rapidly resume their activity as soon as palatable prey becomes available, thereby reducing predator–prey oscillations.

Toxic secondary metabolites can also affect different trophic links at the same time. Secondary metabolites of *P. fluorescens* DSS73 reduce predation by bacterivorous nematodes, but also suppresses omnivorous prey consumption by flagellates feeding on both the bacteria and the nematodes (Bjornlund *et al.*, 2009), with to date unpredicted effects on food web stability. Future studies combining quantitative measurements of secondary metabolite production and modelling approaches are needed to improve our understanding of the various consequences of bacterial defence mechanisms in complex communities.

Bacterial defences can also affect the link between community diversity and functioning. Different bacterial species consume complementary resources, and diverse bacterial communities consume complex resources more efficiently (Gravel *et al.*, 2011; Jousset *et al.*, 2011). Substrate availability also influences the structure of bacterial communities, and diversity peaks at intermediate productivity (Kassen *et al.*, 2000). Protozoa preferentially feeding on dominant or undefended prey species contribute to sustain diversity in productive systems (Corno and Jurgens, 2008; Bell *et al.*, 2010), e.g. by promoting the spread of less competitive or inedible species/strains. Because of trade-offs between predation resistance and growth rate, bacteria investing in predator defence likely are less productive (Leibold, 1996), and predators can cause negative relationships between bacterial diversity and productivity via negative selection effects, i.e. the dominance of predation resistant, albeit unproductive species (Jiang *et al.*, 2008). While indigestible or toxic prey may be indiscriminately consumed by protozoa (Lekfeldt and Ronn, 2008), many protozoa show a marked food selectivity (Weekers *et al.*, 1993), and prey selection by flagellates increases with prey density (Boenigk *et al.*, 2002). High resource availability reduces the relative cost of adaptations to predation less costly, and resistant phenotypes are favoured at high resource supply (Leibold, 1996; Corno and Jurgens, 2008; Friman

*et al*., 2008). Prey adaptations leading to unpalatability thus are likely to be more advantageous at high prey density and the effects of prey defence mentioned above are likely to be more prevalent in high resources environments, but marginal in oligotrophic systems where resource acquisition is the main limiting factor.

### *Cooperation and cheating*

Defence strategies affecting handling time or digestion reduce the consumption of neighbouring bacteria by predators (Fig. 1), even if they are not related to the organisms investing in defence (Jousset *et al*., 2008). Defence mechanisms therefore are open to cheating: palatable phenotypes can hide within a toxic community, therefore gaining benefit from its protection without investing in this shared function (Jousset *et al*., 2009). The production of shared goods such as defence products is more stable if cooperation is conditional, i.e. organisms only start cooperating when the partners are likely to also do so (West *et al*., 2007). Many bacteria coordinate shared behaviours by quorum sensing: they produce diffusible signals with the concentration increasing with bacterial abundance. Above a threshold signal concentration indicating a large population of cooperators bacteria activate social behaviours (Keller and Surette, 2006). Various defence traits are elicited by quorum sensing (Matz *et al*., 2004b; Jousset *et al*., 2006), with bacteria more intensively investing in antipredator defences in populations of highly related individuals maintaining defence evolutionary stable despite of the associated costs (Keller and Surette, 2006). Shared defence mechanisms also are reinforced by prey selectivity of predators. Palatable phenotypes cheating by not producing secondary metabolites toxic to the predators benefit from the investment of faithfully cooperating phenotypes in their vicinity are restricted from spreading over a threshold frequency by predators (Jousset *et al*., 2009). If predator defence is coupled to other bacterial functions (see below), predators may therefore enforce cooperation under conditions otherwise advantageous for cheaters.

### *Facilitation*

The protection of neighbouring bacteria by toxic organisms may also function as a facilitation mechanism (Bruno *et al*., 2003). Toxicity against protozoa not only reduce predation pressure on the producer, but also on the remaining community (Jousset *et al*., 2008). As discussed above this may put constraints on cooperation, but also facilitate the establishment of undefended bacteria in the vicinity of secondary metabolite producers. This local increase of bacterial diversity may introduce new functionalities into the community (Peter *et al*., 2011). Antipredator defences may thus play an important role in promoting the taxonomical and functional diversity of microbial communities. The actual functions indirectly favoured by toxic bacteria likely vary with ecological systems and microsites in ecological systems; future studies have to shed light on the implications of this type of interaction for ecosystem functioning.

### *Diversification and prey evolution*

According to the type of defence mechanisms, predator resistance may contribute to diversification or be a side-effect of it. In a multi-prey system, toxic and palatable prey species/strains will coexist. Predators with a type III functional response underconsume rare phenotypes (Kalinkat *et al*., 2011). As prey recognition is based on morphological and surface parameters (Montagnes *et al*., 2008), the evolution of surface structures, such as modifications of the lipopolysaccharides (LPS) composition, reduce consumption (Wildschutte *et al*., 2004) and may contribute to the observed diversification of bacteria in presence of predators (Meyer and Kassen, 2007). Intense predation pressure may favour rapidly evolving bacteria, for example those with high mutation or recombination rate (Vos, 2009), accelerating the evolution of bacterial communities. Evolution may be further increased by horizontal gene transfer between bacteria. Predator resistance genes can be passed from undefended to defended bacteria. The pyrrolnitrin biosynthetic operon is a well-described example for such horizontal transfers of defence mechanisms (Costa *et al*., 2009). Pyrrolnitrin is an efficient secondary metabolite active against protozoan predators (Jousset *et al*., 2010), and very similar versions of the biosynthetic operon are present in many bacterial phyla, suggesting high mobility of this genetic element (Costa *et al*., 2009). Interestingly, vacuoles of protozoa are hot spots of horizontal gene transfer (Schlimme *et al*., 1997). Bacteria able to survive passage through protozoa may thus have the double advantage of reduced mortality and increased gene shuffling.

### *Virulence*

The evolution of resistance mechanism against predation has often been put in parallel with pathogenicity and virulence (Molmeret *et al*., 2005; Adiba *et al*., 2010). Indeed, prey recognition and digestion follow conserved mechanisms that form the basis of innate immunity by animals (Stuart and Ezekowitz, 2008). The mechanisms responsible for survival within the phagosome of protozoa and macrophages present also striking similarities, and various genes responsible for pathogenesis by *Salmonella enterica* are upregulated during the passage through the phagosome of *Tetrahymena* (Rehfuss *et al*., 2011).

*Acanthamoeba* stimulates the growth of the opportunistic *Acinetobacter* pathogen, while other pathogens such as *Legionella* survive within its amoebal host, converting its predator into a vector (Marciano-Cabral and Cabral, 2003). Shiga toxin-producing *E. coli* O157:H7 may also have evolved as protozoan-resistant strain, or at least may have been favoured by protozoan predators (Steinberg and Levin, 2007). Additionally, pathogens challenged with protozoa show an increased virulence. Co-incubation of *Streptomyces californicum* with *A. polyphaga* increases its cytotoxicity against macrophages, and *S. enterica* exposed to rumen protozoa of ruminants show an increased pathogenicity (Rasmussen *et al*., 2005). Bacterial defence mechanisms appear therefore to have evolved parallel to or in synergy with pathogenesis. However, Friman and colleagues (2009) report an opposite trend, and show that predation by *T. thermophila* selects for lower virulence in *Serratia liquefaciens*, suggesting that negative trade-offs between predator resistance and virulence also occur.

### *Other ecosystem processes*

If defence mechanisms overlap with other functions, they may also indirectly affect other ecosystem processes. Cyclic lipopeptides, a category of biosurfactants inhibiting protozoan predators (Andersen and Winding, 2004; Mazzola *et al*., 2009), are also involved in various other physiological processes like biofilm formation or bacterial motility (Raaijmakers *et al*., 2010). Other bacterial secondary metabolites primarily known for their antifungal activity have been reported to be essential for reducing predation. For example, cyclic lipopeptides, hydrogen cyanide or DAPG reduce the survival of bacterivorous protozoa and nematodes (Jousset *et al*., 2006; Mazzola *et al*., 2009; Meyer *et al*., 2009; Neidig *et al*., 2011), and the promotion of toxic bacteria under predation pressure (Jousset *et al*., 2008) may contribute to the inhibition of plant pathogens. This effect may be further increased if secondary metabolite production is upregulated in presence of microfaunal predators, as demonstrated by pseudomonads (Mazzola *et al*., 2009; Jousset and Bonkowski, 2010; Jousset *et al*., 2010). Predators may therefore contribute to increase the ability of soil bacterial communities to control plant pathogens and understanding the interaction between microfaunal predators and their bacterial prey may be central for improving the use of biocontrol bacteria in agricultural systems. Various bacterial compounds active against predators, such as DAPG, also activate induced plant defences, improving plant health (Iavicoli *et al*., 2003; Raaijmakers *et al*., 2010). DAPG moreover increase the exudation of labile nutrients by plant roots (Phillips *et al*., 2004), and its overproduction in presence of protozoa may lead indirectly to an increased activity of rhizosphere bacteria and an enhanced nutrient supply to plants (Bonkowski, 2004). The often reported, but as yet unexplained impact of microfaunal predators on plant architecture and hormone balance (Bonkowski, 2004; Krome *et al*., 2010), may be related to bacterial traits involved in predator defence which indirectly also affect plant performance.

The interplay between bacterial defence mechanisms and predators could also play a role in decomposition processes and the biodegradation of complex organic compounds such as polycyclic aromatic hydrocarbons. This class of recalcitrant pollutants usually degrades slowly because of their hydrophobicity. Biosurfactants produced by bacteria increase the bioavailability of polycyclic aromatic hydrocarbons in soil, thereby accelerating the bioremediation process (Tecon and van der Meer, 2010). Promoting biosurfactant producers by manipulating predation pressure might be thus an interesting strategy to enhance biodegradation processes.

Bacterial defence mechanisms also overlap with functions involved in nutrient cycling. Proteases, such as the alkaline protease AprA of *P. fluorescens*, contribute to soil matrix degradation, but are also an efficient defence mechanism against protozoa (Jousset *et al*., 2006) and protects the host plant against root-knot nematodes (Siddiqui *et al*., 2005). Gluconic acid is another interesting case of all-purpose molecule. This compound is involved in the solubilization of phosphorus in soil and improves plant nutrition (Rodriguez and Fraga, 1999). Recently, it has been discovered that gluconic acid also efficiently deters and inhibits microfaunal predators (Gomez *et al*., 2010). These overlaps between traits involved in defence and other bacterial services suggest that conditions favouring prey defence may also have a direct and indirect impact on a range of processes including nutrient cycling, pollutant degradation and plant productivity.

### *Predator evolution*

In the same way as bacteria adapted to predators, predators evolved adaptations to bacterial defence mechanisms. Predators and prey rapidly co-evolve in experimental microcosms, and this mutual adaptation is a major driver of predator–prey dynamics (Yoshida *et al*., 2007). Adaptations to prey defences are common, but vary markedly between predator species. Protozoa from distinct high level taxonomic groups show strong differences in their sensitivity to bacterial toxic metabolites (Pedersen *et al*., 2011), and the broad spectrum toxin violacein distinctly impact taxonomically related protozoan species (Deines *et al*., 2009). The amoeba *Acanthamobea castellanii* is tolerant to high cyanide concentrations (Jousset *et al*., 2010), a feature that many eukaryotes acquired by expressing alternative cyto-

chrome oxidases (McDonald, 2008). At the community level, soil inoculation with *P. fluorescens* DR54 producing the biosurfactant viscosinamide selects for protozoan populations resistant to this toxin (Johansen *et al*., 2005), and some filamentous flagellates evolved the ability to consume filamentous bacteria (Wu *et al*., 2004), an otherwise efficient and widespread defence strategy (Hahn *et al*., 1999). Other predators evolved more subtile interference mechanisms. *Caenorhabditis elegans* disrupts quorum sensing in a number of Gram-negative bacteria (Kaplan *et al*., 2009), and amoeba can inhibit the production of defence toxins by bacteria (Jousset *et al*., 2010). As a consequence, bacterial populations investing in antipredator defences may still be consumed by adapted predators. Predicting the outcome of this arms race is a complex and rapidly evolving research field, and the next years will certainly uncover novel and exciting aspects of this battleground.

## Future perspectives

The study of bacterial defence against predation has been gaining momentum during the last decades, and emerged as a fascinating topic crossing boundaries of various research fields (Jessup *et al*., 2005). It provides an ideal field for interdisciplinary research, and is likely to contribute significantly to join research activities in distantly related fields such as microbiology, ecology and immunology. Bacteria are ideal model organisms for ecological research; they are easy to modify, allowing switching on and off traits of interest and monitoring effect of these modifications on predator–prey interactions (Yoshida *et al*., 2007). The short doubling time allows setting up evolutionary experiments in the laboratory (Elena and Lenski, 2003), and using high-throughput analytical tools enable to test predictions with up to now unrealized levels of precision (Brockhurst *et al*., 2011). The use of predators as model organisms for infection and immunological research uncovered a number of novel molecular mechanisms involved in interactions between bacteria and eukaryotes (Adiba *et al*., 2010). Our understanding of bacterial defence in complex bacterial communities is likely to benefit from recent technical advances in high-throughput sequencing. Mutagenesis and transcriptome analysis may help to understand the pathways involved in the regulation of defence mechanisms, and deep sequencing allows following population fluctuations in high number of replicates and with a sufficient temporal resolution to leap from descriptive to predictive studies (Brockhurst *et al*., 2011) or to investigate complex environmental systems in detail. These new techniques, combined with new modelling approaches and the use of model predators, form a solid basis that will certainly help understand better the mechanisms and implications of bacterial defences.

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