

## COMMENTARY

# *Tritagonist* as a new term for uncharacterised microorganisms in environmental systems

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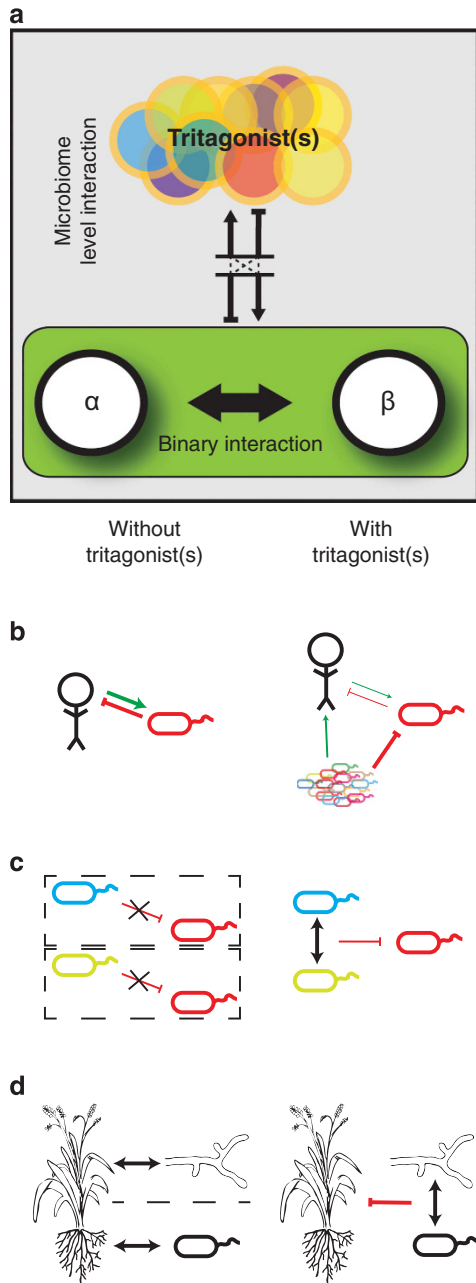
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‘Commensal’ originates from the Latin word ‘commensalis’ (‘eating at the same table’) and was introduced by Pierre–Joseph van Beneden in 1876. In ecology, the term defines a relationship between two organisms, where one, the commensal, benefits without affecting the other. Thus, commensalism defines a one-way interaction. Originally, the term was used in a host-centric view for interactions where a ‘small’ species (for example, a microorganism) is provided with a habitat (for example, skin) or waste products (for example, methanol released by plants during their cell-wall metabolism (Fall and Benson, 1996)) as food by another, usually much larger host (for example, a mammal or a plant). However, the term commensal has now started to rather imply an unknown, benign function than a truly proven commensal, mutualistic, or antagonistic lifestyle (Casadevall and Pirofski, 2014). In particular when considering interactions among microorganisms, it seems unlikely that a true commensal microorganism exists. It may thus be prudent to assume a function for any kind of microorganism and to reserve commensal for organisms that have been shown not to influence their interaction partner. To emphasise such a microbe-centric view and to imply functionality, we suggest the term *tritagonist*.

The term *tritagonist* is adopted from classic drama. In early forms of ancient Greek dramas, only two actors, the protagonist and the deuteragonist, played on stage. The inclusion of a third actor, the *tritagonist*, allowed for more lifelike and intricate characters and stories. Here, we propose *tritagonist* as an ecological term for all organisms that influences the interaction between two organisms in a system. Just as in drama, the ecological concept of the *tritagonist* is meant to promote a better understanding of natural systems. In research, it implies the need to extend oversimplified binary interactions (for example, host–pathogen and pathogen–antagonist pairs) by including additional interaction partners, *tritagonists*, to obtain a more realistic view of microbial systems (Figure 1a). We advocate the term *tritagonist* in particular with regard to the recent sequencing efforts in microbiome research,

which have revealed a plethora of uncharacterised species and thus laid the foundation to link the evolutionary and genetic diversity with biological functions (Wu *et al.*, 2009; Bulgarelli *et al.*, 2013; Rinke *et al.*, 2013; Panke-Buisse *et al.*, 2015). The in-depth sequence-based knowledge about the microbial species composition of various habitats and of individual strains is in stark contrast to the sparse experimental proof about the biological roles of the identified organisms. In most cases, only a few pathogenic, beneficial or antagonistic interactions are known, while the ecosystem services of the majority of species are unknown. We propose the *tritagonist* concept to define species with uncharacterised ecological functions to incentivise research on these organisms. The term implies biologically relevant, but yet uncharacterised functions of an organism or groups of organisms, while at the same time leaving room for the exact nature of these functions.

*Tritagonists* interfere with any other kind of interspecific interaction that may for example be mediated by the following: direct physical interaction, exchange of signalling molecules, nutrient competition, metabolising of by-products, environmental changes (for example, pH) or modulation of host-immune responses (Peleg *et al.*, 2010). Interactions via the environment also take place in time when *tritagonists* change their resource availability or alter environmental stresses during microbial succession (Fierer *et al.*, 2010). Well-known and medically relevant *tritagonists* are individual strains used as probiotics or faecal transplantation communities for the treatment of *Clostridium difficile* infections or other gastrointestinal tract diseases (Gareau *et al.*, 2010; Borody and Khoruts, 2012; Figure 1b). A more specific example for host protection by *tritagonists* is *Bacillus subtilis* GS67 that protects *Caenorhabditis elegans* against Gram-positive pathogens by producing the antibiotic-like substance fengycin in the intestine of the nematode (Iatsenko *et al.*, 2014). A case where *tritagonists* might reveal new antibiotics are combinations of bacterial species that do not suppress *Escherichia coli* or *Staphylococcus aureus* when tested alone, but effectively suppress the human pathogens when co-cultured (Tyc *et al.*, 2014; Figure 1c). Similar concepts as in medical microbiology are pursued in the realm of agricultural microbiology, where single



**Figure 1** Tritagonists regulate microbial interactions. (a) Tritagonists stimulate or inhibit individual organisms ( $\alpha$  or  $\beta$ ) or impact on their interaction and thereby determine the interaction's outcome. A given interaction can be adjusted by many different tritagonists at once. (b) *Clostridium difficile* interaction with a human host: in the absence of tritagonists, the bacterium diminishes the fitness of the host, whereas the introduction of tritagonists (for example, through faecal transplantation) controls the pathogenic bacterium (either directly or indirectly via the host) and the fitness disadvantage for the host is alleviated. (c) Tritagonistic interaction between environmental bacteria (blue/green) with *Escherichia coli* (red). Individual species do not antagonise *E. coli*, whereas co-culture of the tritagonists results in strong antagonism against *E. coli*. (d) *Rhizopus microsporus* harbouring the tritagonist *Burkholderia rhizoxinica* intracellularly leads to rhizoxin production and rice seedling blight.

species or combinations of microorganisms are studied and used to control plant pathogens and postharvest diseases, improve the nutrition and resistance of crop plants, or manage the soil, root and plant microbiome (Janisiewicz and Korsten, 2002; Lugtenberg and Kamilova, 2009; Andrews *et al.*, 2012). In a benign type of interaction, yeast species that are associated with arbuscular mycorrhizal fungi improve mycorrhization and benefit plant growth (Boby *et al.*, 2008). However, tritagonists do not always function synergistically, or at least additively, and the biological functions of individual species may alter when the microbial system or the environment changes. For example, combinations of *Pseudomonas fluorescens* A506 and *Pantoea vagans* C9-1, which are both individually used as antagonists against *Erwinia amylovora*, the causal agent of fire blight on *Rosaceae* (for example, apples, pears), result in reduced fire blight control when co-applied (Stockwell *et al.*, 2011). An intricate tritagonistic interaction between the zygomycete fungus *Rhizopus microsporus* and *Burkholderia rhizoxinica* endosymbionts, which produce the potent toxin rhizoxin, results in rice seedling blight (Partida-Martinez and Hertweck, 2005; Figure 1d). These specific examples document the applicability of the tritagonist concept and the value of studying multi-species interactions to broaden our understanding of biological systems.

In summary, we are convinced that describing and studying yet uncharacterised microorganisms as tritagonists will greatly benefit medicine and human physiology, basic research in microbial ecology and applications in agriculture. The term tritagonist shifts the emphasis from the fact that no function is yet known to the *a priori* assumption that a function must exist and may thus help restoring the proper usage of the term commensal. Tritagonist serves as a name for all the microbial species of a biological system we could ever want to identify and characterise: those that regulate, balance and interfere with microbial interactions without being the most noteworthy players themselves. The concept of the tritagonist will thus, hopefully, stimulate hypothesis-driven research and introduce a systems-biological view of the functional microbiological networks at play in the environment and in hosts.

## Conflict of Interest

The authors declare no conflict of interest.

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