The ISME Journal (2013) 7, 1065–1068

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SHORT COMMUNICATION

Host-released dimethylsulphide activates the dinoflagellate parasitoid *Parvilucifera sinerae*

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Parasitoids are a major top-down cause of mortality of coastal harmful algae, but the mechanisms and strategies they have evolved to efficiently infect ephemeral blooms are largely unknown. Here, we show that the generalist dinoflagellate parasitoid *Parvilucifera sinerae* (Perkinsozoa, Alveolata) is activated from dormancy, not only by *Alexandrium minutum* cells but also by culture filtrates. We unequivocally identified the algal metabolite dimethylsulphide (DMS) as the density-dependent cue of the presence of potential host. This allows the parasitoid to alternate between a sporangium-hosted dormant stage and a chemically-activated, free-living virulent stage. DMS-rich exudates of resistant dinoflagellates also induced parasitoid activation, which we interpret as an example of coevolutionary arms race between parasitoid and host. These results further expand the involvement of dimethylated sulphur compounds in marine chemical ecology, where they have been described as foraging cues and chemoattractants for mammals, turtles, birds, fish, invertebrates and plankton microbes.
The ISME Journal (2013) **7,** 1065–1068; doi:10.1038/ismej.2012.173; published online 24 January 2013
Subject Category: microbe-microbe and microbe-host interactions
Keywords: *Alexandrium*; dimethylsulphide; dinoflagellates; infochemistry; parasitoid; *Parvilucifera*

Marine harmful algal blooms are dense ephemeral proliferations typically of dinoflagellates, cyanobacteria or diatoms, that can directly cause illness and death in humans and marine life through the production of toxins, or cause ecosystem alterations affecting food provision and recreational activities (Zingone *et al.*, 2000; *MEA*, 2005). Even though marine harmful algal blooms have been recognized as a major environmental challenge (*MEA*, 2005), little is known about what makes them thrive and wane. In the case of dinoflagellates, which account for 75% of marine harmful algal bloom-forming phytoplankton species, bottom-up factors (including man-enhanced eutrophication, climate shifts and species dispersal) are usually invoked as triggers (Zingone *et al.*, 2000; Heisler *et al.*, 2008; Anderson, 2009), but the causes and mechanisms of termination remain obscure.

Parasitoids have been identified as a main cause of mortality of harmful dinoflagellates (Taylor, 1968; Chambouvet *et al.*, 2008), to the extent that their deliberate use has been suggested as a biological mitigation of marine harmful algal blooms (Taylor, 1968), in the same manner it is done in agricultural applications on land. The suggestion has faced opposition on the basis of the lack of knowledge on their specificity, the mechanisms of infection and the potential side effects (Anderson, 2009). The debate has prompted the need for a better understanding of parasitoid – host interactions, along the same lines as the increasing interest in chemical ecology and interspecific communication in oceanic plankton (Ianora *et al.*, 2011).

Parvilucifera sinerae (Perkinsozoa, Alveolata) is a flagellate parasitoid that efficiently infects and kills a taxonomically broad variety of dinoflagellates, including harmful bloom forming species within the genera *Alexandrium*, *Dinophysis*, *Gambierdiscus*, *Gymnodinium*, *Ostreopsis* and *Protoceratium* (Garcés *et al.*, 2012). The infection cycle proceeds as follows (Figure 1a): a flagellate zoospore penetrates the host cell, destroys its content, forms a spherical sporangium the size of the host, and divides to fill up the sporangium with dormant zoospores. They remain dormant until a wakeup call signals the presence of a sufficient density of host cells; then the zoospores activate into quick tumbling motion and eventually leave the sporangium through one or several opercula opened in the wall (Supplementary Movie S1 in the online Supplementary Material). The nature and origin of the signal were hitherto unknown.

When studying the infection of the toxic dinoflagellate *Alexandrium minutum* by *P. sinerae*, we

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Received 26 September 2012; revised 27 November 2012; accepted 29 November 2012; published online 24 January 2013

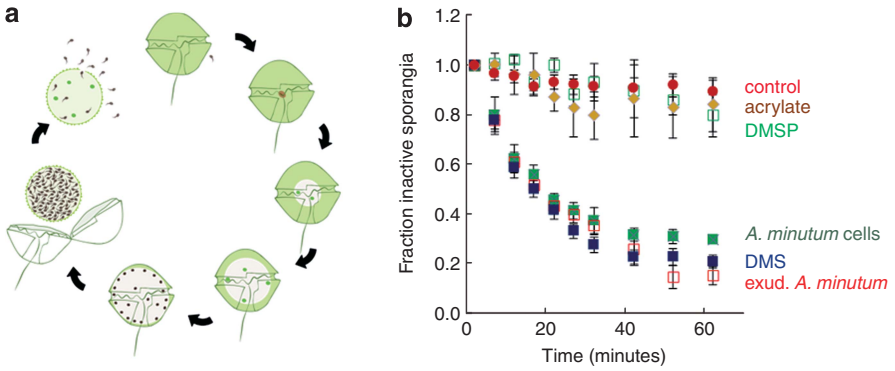

Figure 1 Response of *P. sinerae* sporangia to chemical cues. (**a**) The infection cycle of *A. minutum*: a zoospore enters the host, destroys its content, forms and sporangium and fills it with dormant zoospores; host-signalling cues cause the activation of *P. sinerae* zoospores and their release from the sporangium. (**b**) Decay in the number of inactive sporangia upon exposure to L1 medium (control), *A. minutum* AMP4 cells and exudates (which contain 300 nM of DMS), 270 nM DMSP in L1, 270 nM acrylate in L1, and 270 nM DMS in L1. Data are expressed as mean ± s.e. of 3–11 replicates.

observed that the presence of filtered exudates of the dinoflagellate was enough to activate the dormant zoospores into motion and induce their release from the sporangia as efficiently as the presence of host cells did (Figure 1b), pointing towards the involvement of a chemical signal. *A. minutum* is a strong producer of the osmolyte dimethylsulphoniopropionate (DMSP), which occurs at intracellular concentrations as high as 0.3 M, or 7% of total cell carbon (Berdalet *et al.*, 2011; Caruana *et al.*, 2012). Considering that *A. minutum* exudates contained DMSP concentrations in the order of 100–800 nM, and that this compound has been shown to induce positive chemotaxis in a variety of plankton microorganisms (Seymour *et al.*, 2010), it stood as a good candidate for the activation of the parasitoid. Additions of a lab-prepared solution of 270 nM DMSP, however, did not give any activation response significantly different from the negative control (Figure 1b).

A. minutum also harbours high activity of DMSP lyases (Caruana *et al.*, 2012), the enzymes that cleave DMSP into equimolar amounts of acrylate and dimethylsulphide (DMS). Indeed, *A. minutum* blooms and cultures have the characteristic seafood smell of DMS, and exudates used in this study had DMS concentrations of ca. 300 nM. Additions of an acrylate solution did not induce any response, but additions of DMS at a concentration similar to that in the exudates did (Figure 1b). Moreover, the response rate was proportional to the added DMS concentration down to a threshold close to 30 nM (Figure 2a).

We showed that DMS alone was enough for parasitoid activation, that is, for the necessary step before infection. As many dinoflagellates produce DMS (Stefels *et al.*, 2007; Caruana *et al.*, 2012), including susceptible and resistant strains, how host-specific is this mechanism? Could it be that DMS acts in concert with other chemicals contained only in the exudates of the susceptible dinoflagellates? Assays with culture filtrates of nonsusceptible *A. minutum*, *Amphidinium carterae* and *Karlodinium veneficum* strains showed parasitoid activation rates proportional to their DMS content (Figure 2b). In other words, exudates of dinoflagellates caused parasitoid activation as long as they had enough DMS, independently of the strains' susceptibility to infection.

Our experiments demonstrated that *P. sinerae* perceives DMS as the wakeup call for activation. In the coastal ocean, background DMS concentrations are typically 0.5–10 nM (Lana *et al.*, 2011), while a confined coastal bloom of *A. minutum* (ca. 1000 cells per ml^{-1}) on 7 March 2012 had a DMS concentration of 217 nM. As DMS is a short-lived substance in seawater (Pinhassi *et al.*, 2005), where it is consumed by bacteria and photolysis, the threshold found here for the activation of the dormant zoospores (a few tens of nM) allows the parasitoid to activate only in the presence of relatively high densities of potential host cells, and do it more rapidly within denser blooms. This alternation between a sporangium-hosted dormant stage and a chemically-activated, free-living virulent stage stands as an efficient strategy for success in the maintenance of the parasitoid population. How do they survive between host blooms, either by serially infecting a sequence of dinoflagellate hosts, as shown for other parasites (Chambouvet *et al.*, 2008), or by sporangia sinking to the sediments along with host cysts and remaining dormant therein until favourable conditions for resuspension and activation occur, remains to be solved.

DMS is a by-product of both algal physiology (Stefels *et al.*, 2007) and food web interactions, including herbivore grazing and bacterial catabolism (Simó, 2001). In dense monospecific microalgal blooms under some degree of physiological stress due to nutrient scarcity or high sunlight exposure, DMS leakage from the algal cell is suggested to occur as part of an overflow of excess of energy and

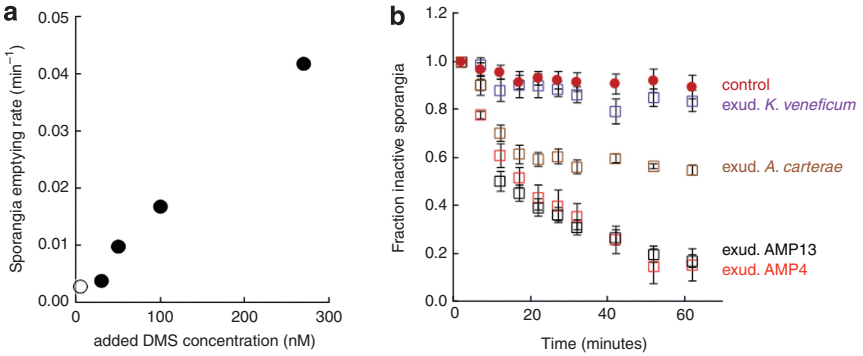

Figure 2 DMS-dependent parasitoid activation. (**a**) Rate constants of the emptying of *P. sinerae* sporangia upon exposure to L1 medium (open circle) and increasing concentrations of DMS in medium (filled circles). (**b**) Decay in the number of inactive *P. sinerae* sporangia upon exposure to L1 medium (control) and exudates of *A. minutum* AMP4 (susceptible host, 300 nM DMS), *A. minutum* AMP13 (resistant host, 270 nM DMS), *Amphidinium carterae* (resistant host, 140 nM DMS), and *Karlodinium veneficum* (resistant host, 1 nM DMS). Data are expressed as mean ± s.e. of 3–11 replicates.

sulphur (Stefels *et al.*, 2007), and/or as part of a protection mechanism against oxidative stress (Sunda *et al.*, 2002). *P. sinerae* has evolved a sensory response to this by-product and, because of its chemotactic characteristics for protists (Seymour *et al.*, 2010), it is conceivable that the zoospores further use DMS gradients for an oriented swimming towards the potential host. However, the occurrence of an eventual infection depends on host resistance mechanisms that are still unknown.

Alexandrium species are known to produce allelochemicals with deleterious (lytic) effects on autotrophic and heterotrophic protists (Tillman *et al.*, 2008) as a mechanism to overcome competition and grazing. Rather, we show that DMS behaves as a 'kairomone', that is, a chemical signal released by the dinoflagellate, which mediates an interspecific interaction that benefits the receiving organism (the parasitoid) without benefiting the producer (Pohnert *et al.*, 2007). In this case, the kairomone is even disadvantageous to the producer, as it induces infection and subsequent death. Therefore, its release must be unavoidable or its costs must be outweighed by the aforementioned physiological benefits. In any case, this stands as one of the scarce examples of a chemically-mediated arms race in the coevolution of plankton microbes (Smetacek, 2001; Ianora *et al.*, 2011).

Our study further expands the importance of ubiquitous dimethylated sulphur compounds (DMSP and DMS) in the chemical ecology of the oceans. These compounds have been described as foraging cues for seals, turtles, penguins, procellariiform birds, fishes, some macroinvertebrates and copepods (van Alstyne, 2008; Nevitt, 2011; Endres and Lohmann, 2012; and refs. therein), and chemotactic attractants for protists, microalgae and bacteria (Seymour *et al.*, 2010, and refs. therein), in what possibly stands as a unique case amidst the infochemical landscape of the biosphere. Here, we discover their involvement in planktonic host–parasitoid interactions.

Acknowledgements

Support was provided by the (former) Spanish Ministry of Science and Innovation through projects PARAL (to E.G.) and SUMMER (to R.S.). K.P. acknowledges a grant from Australian Endeavour Foundation (Australian Government). We thank Beatriz Garriz for the drawing of the infection cycle.

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Supplementary Information accompanies the paper on The ISME Journal website (http://www.nature.com/ismej)