Role is in the eye of the beholder—the multiple functions of the antibacterial compound tropodithietic acid produced by marine Rhodobacteraceae

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Abstract

Many microbial secondary metabolites have been studied for decades primarily because of their antimicrobial properties. However, several of these metabolites also possess nonantimicrobial functions, both influencing the physiology of the producer and their ecological neighbors. An example of a versatile bacterial secondary metabolite with multiple functions is the tropone derivative tropodithietic acid (TDA). TDA is a broad-spectrum antimicrobial compound produced by several members of the Rhodobacteraceae family, a major marine bacterial lineage, within the genera Phaeobacter, Tritonibacter, and Pseudovibrio. The production of TDA is governed by the mode of growth and influenced by the availability of nutrient sources. The antibacterial effect of TDA is caused by disruption of the proton motive force of target microorganisms and, potentially, by its iron-chelating properties. TDA also acts as a signaling molecule, affecting gene expression in other bacteria, and altering phenotypic traits such as motility, biofilm formation, and antibiotic production in the producer. In microbial communities, TDA-producing bacteria cause a reduction of the relative abundance of closely related species and some fast-growing heterotrophic bacteria. Here, we summarize the current understanding of the chemical ecology of TDA, including the environmental niches of TDA-producing bacteria, and the molecular mechanisms governing the function and regulation of TDA.

Keywords: antimicrobials, secondary metabolites, Rhodobacteraceae, tropodithietic acid, marine microbiomes

Introduction

Since the discovery of penicillin by Alexander Fleming in 1929 (Fleming 1929), humanity has benefited from its antimicrobial effects and an array of similar bioactive compounds produced by microorganisms. These molecules, commonly referred to as secondary metabolites, have been studied for decades because of their antimicrobial properties and their use as anti-infective agents in the clinic. It has been the perception that the antibiotic properties also define their predominant function and role in natural microbial niches—as weapons to kill off competitors (Traxler and Kolter 2015). However, sublethal concentrations of antibiotics can influence gene expression in exposed microorganisms and result in changes in phenotypes such as biofilm formation or motility (Goh et al. 2002; Linares et al. 2006; Straight, Willey and Kolter 2006; Liu et al. 2013). These effects have predominantly been observed in bacteria exposed to ‘external’ antimicrobials, but it is not known if these effects are caused indirectly by induction of a stress response or by a direct effect of the compounds on cellular targets (Romero et al. 2011; Foster and Bell 2012; Cornforth and Foster 2013; Yoon and Nodwell 2014; Dittmann et al. 2019a; Li et al. 2021). Some antimicrobial secondary metabolites can also modulate gene expression in the producer itself, acting as signaling molecules mediating quorum sensing (QS, Romero et al. 2011; Beyersmann et al. 2017). Other secondary metabolites have nonantibiotic functions serving as iron scavengers (siderophores), in predator defense, as antivirulence compounds, or as promoters of horizontal gene transfer (Mansson et al. 2011; Seyedsayamdost et al. 2011b; Briand et al. 2016; Zhang et al. 2016, 2021; Danevič et al. 2021). Thus, this challenges the original perception of antibiotic secondary metabolites being predominantly involved in direct interference competition between microbes, whilst not being required for growth and metabolism (Linares et al. 2006; Yim, Huim, and Kolter 2017). Other secondary metabolites have nonantibiotic functions serving as iron scavengers (siderophores), in predator defense, as antivirulence compounds, or as promoters of horizontal gene transfer (Mansson et al. 2011; Seyedsayamdost et al. 2011b; Briand et al. 2016; Zhang et al. 2016, 2021; Danevič et al. 2021). Thus, this challenges the original perception of antibiotic secondary metabolites being predominantly involved in direct interference competition between microbes, whilst not being required for growth and metabolism (Linares et al. 2006; Yim, Huim, and Kolter 2017).
in situ chemical detection of metabolites, for instance by mass spectrometry imaging, allows direct, high resolution analyses of secondary metabolites not hampered by bulk extraction, and enabling analysis of spatial metabolomes (Moree et al. 2012; Geier et al. 2020).

An example of an antimicrobial secondary metabolite with multiple functions and roles is the redox active metalloprotein ocyanin, produced by *Pseudomonas aeruginosa*. It serves as an antimicrobial, but also as a respiratory pigment and a quorum-related molecule (Hernandez and Newman 2001). Similarly, in *Streptomyces coelicolor*, the antimicrobial red pigment prodiginin can induce programmed cell death in subpopulations, and thereby provide nutrients to the surviving kin, suggesting an additional ecological role besides being an antibiotic (Tenconi et al. 2018). Expanding our holistic understanding of secondary metabolites demands in-depth studies of their facets, from chemistry to ecology. Here, we present a case study of tropodithiatic acid (TDA; Fig. 1), a sulfur-containing trope derivative with several functions.

TDA can act as antimicrobial and is produced by marine members of the *Rhodobacteraceae* family (class of Alphaproteobacteria; Brinkhoff et al. 2004; Bruhn et al. 2005; Geng et al. 2008; Harrington et al. 2014; Sonnenschein et al. 2017a, 2018; Duan et al. 2020). In addition to the antimicrobial properties of TDA, the compound exhibits other activities as a signaling molecule, anti-cancer agent, and weak iron chelator (Geng and Belas 2010; Wichmann et al. 2015; D’Alvise et al. 2016; Wilson et al. 2016; Beyersmann et al. 2017). TDA-producing bacteria have been detected in oceanic metagenomic data sets (Segev et al. 2016; Sonnenschein et al. 2017a) and have also been isolated from marine aquaculture and oceanic environments (Hjelm et al. 2004a; Lauzon et al. 2008; Porsby, Nielsen and Gram 2008; Grotkjær et al. 2016b). They can inhibit, or kill, fish pathogenic bacteria when co-cultivated in aquaculture live feed (microalgae, *Artemia*, rotifers, and copepods) or fish larvae, sparking a commercial interest in TDA-producing bacteria as aquaculture probiotics (D’Alvise et al. 2012; Grotkjær et al. 2016a; Dittmann et al. 2017; Rasmussen et al. 2018; Sonnenschein et al. 2021).

The purpose of this review is to provide an overview of the different functions of TDA, thus also addressing its possible broader physiological and ecological roles, with perspectives to other secondary metabolites. We focus on TDA-producing bacteria belonging to the marine *Rhodobacteraceae* family, but it should be noted that TDA-producing bacteria outside of this group have been isolated (Kintaka et al. 1984; Tsubotani et al. 1984; Kawano et al. 1997). The term ‘function’ will cover direct molecular or chemical responses attributed to TDA, whilst ‘roles’ will comprise the more holistic possible ecological effects of the compound (Fig. 1).

Given the potential broader functions of antimicrobial secondary metabolites, their definition and terminology have been debated (Bérdy 2005; Price-Whelan, Dietrich and Newman 2006; Davies 2013; Chevrette et al. 2020). ‘Secondary metabolites’ was introduced by the Nobel Prize laureate Albrecht Kossel in 1891 (Kossel 1891), and adopted by the botanist Friedrich Czapek, who in the 1920s coined the term ‘secondary modifications’ in work on plant nitrogen metabolism (Czapek 1922), with the purpose of distinguishing the compounds from growth-related primary metabolites. More recently, the term ‘specialized metabolites’ has gained traction emphasizing functions broader than merely secondary (Price-Whelan, Dietrich and Newman 2006; Davies 2013). We will, however, use the term ‘secondary metabolites’ in this review as this remains the term most commonly used.

**TDA-producing bacteria and their environmental niches**

The tautomere of TDA, thiotropolatin, was discovered in 1984 in a *Pseudomonas* species isolated from soil (Kintaka et al. 1984; Tsubotani et al. 1984), and TDA was later detected in a marine bacterium, *Roseobacter gallaeciensis* (now: *Phaeobacter* inhibens; Brinkhoff et al. 2004). Since then, TDA has only been detected in a subset of members belonging to the *Rhodobacteraceae* family. This includes strains belonging to the three genera *Phaeobacter* (formerly *Roseobacter*; Ruiz-Ponte et al. 1998; Brinkhoff et al. 2004; Martens et al. 2006; Porsby, Nielsen and Gram 2008; Geng and Belas 2010; Berger et al. 2011; Breider et al. 2014; Sonnenschein et al. 2017b), *Tritonibacter* (formerly *Epibacterium*, Ruegeria, or Silicibacter; Hjelm et al. 2004a; Bruhn, Gram and Belas 2007; Muramatsu et al. 2007; Geng et al. 2008), and *Pseudouvibrio* (Enticknap et al. 2006; Geng and Belas 2010; Penesyan et al. 2011; Bondarev et al. 2013; Harrington et al. 2014).

TDA is produced by strains belonging to four of the six described *Phaeobacter* species (Sonnenschein et al. 2018), namely *Phaeobacter gallaeciensis* (Martens et al. 2006), *P. inhibens* (Martens et al. 2006), *Phaeobacter piscinae* (Sonnenschein et al. 2017b), and *Phaeobacter porticola* (Breider et al. 2017). Production of TDA has so far not been detected in the species *Phaeobacter italicus* (Wirth and Whitman 2018) and the proposed species *Phaeobacter marinus* (Lee et al. 2015), nor has the biosynthetic gene cluster of TDA been detected through genome mining (NCBI accession numbers NZ_VOGO01000001.1 (P. marinintestinus UB-M7) and FO0Z00000000 1 (P. italicus DSM26436)) using antiSMASH 5.0 in these species (Blin et al. 2019). Several strains of *Tritonibacter* mobilis (formerly *Epibacterium mobilis*) produce TDA, while strains belonging to other species in the genera, e.g. *Tritonibacter scottomollicae* (formerly *Epibacterium scottomollicae*), do not (Geng et al. 2008; Wang and Seyedsayamdost 2017; Sonnenschein et al. 2017a). In the *Pseudouvibrio* genus, TDA-producing strains (unclassified at species level) have repeatedly been isolated (Enticknap et al. 2006; Geng and Belas 2010; Penesyan et al. 2011; Bondarev et al. 2013; Harrington et al. 2014) with *Pseudouvibrio ascidiaeola* being the closest relative to the TDA-producing *Pseudouvibrio* isolates (Penesyan et al. 2011). However, a number of *Pseudouvibrio* strains do not harbor the tdu genes, suggesting that TDA production is not a widely distributed trait within this genus (Crowley et al. 2014; Romano 2018).

Since TDA production is not a conserved trait within the three genera, and since *Phaeobacter*, *Tritonibacter*, and *Pseudouvibrio* are not close phylogenetic neighbors within the *Rhodobacteraceae* family, this could suggest that TDA genes and the ability to produce the compound have been distributed by horizontal gene transfer (Sonnenschein et al. 2018). However, short-term, noncompetitive biofilm cultivation of *P. inhibens* 2.10 induced single nucleotide polymorphisms in genes responsible for TDA production, leading to TDA deficiency (Majzoub et al. 2021). This points toward strong selection for the loss of TDA production in *P. inhibens*, presenting another possible explanation for the nonconserved pattern of TDA genes observed within the genera. To this day, the evolutionary history of TDA production is not fully understood, but would be important for unravelling the ecological role(s) of TDA. For most secondary metabolites the evolutionary route responsible for the chemical diversification remains poorly understood. Horizontal gene transfer has been identified to be an integral driver of secondary metabolite evolution (Fischbach, Walsh and Clardy 2008; Medema et al. 2014), but it is evident that vertical inheritance also influences the evolution of secondary metabolite
gene clusters (Lind et al. 2017; Adamek et al. 2018; Chase et al. 2021; Undabarrena et al. 2021). Genetic diversification of the salinosporamides A biosynthetic gene cluster, found in the marine Salinispora genus, had direct consequences on the secondary metabolite production (Chase et al. 2021). This highlights that long-term evolutionary processes can lead to genetic and chemical diversification of secondary metabolites within closely related species, potentially generating new chemical diversity.

The genera Phaeobacter and Tritonibacter belong to the Roseobacter group (‘roseobacters’), which is a paraphyletic subgroup within the family Rhodobacteraeae (Simon et al. 2017). The Roseobacter group contains multiple branching clades (Newton et al. 2010; Simon et al. 2017), where clade 1 contains strains that produce TDA (Brinkhoff et al. 2004; Newton et al. 2010). In the environment, the abundance of roseobacters is highest near the surface of temperate coastal waters and polar oceans (Buchan, González and Moran 2005), globally averaging 3.8% of bacterial populations (Wietz et al. 2010). The abundance of roseobacters correlates positively with chlorophyll a concentrations (Wietz et al. 2010), and roseobacters can constitute as much as 30% of the bacterial population in microalgal blooms (González and Moran 1997; Gonzalez et al. 2000; West et al. 2008).

In global marine metagenomic data, the Phaeobacter genus represents approximately 0.03% of all bacterial sequences in surface waters (Sunagawa et al. 2015; Sonnenschein et al. 2021), and while Phaeobacter species have not been isolated from open ocean water (Gram, Melchiorsen and Bruhn 2010), they have frequently been recovered from aquaculture facilities (Hjelm et al. 2004a; Porsby, Nielsen and Gram 2008; Grotkjær et al. 2016b) and solid surfaces in harbors (Ruiz-Ponte et al. 1998; Bernboem et al. 2011; Gram et al. 2015; Breider et al. 2017). This is in concordance with comparative genomic analyses of the Phaeobacter genus suggesting an
adaptation to a surface-associated lifestyle (Dang and Lovell 2000; Hjelm et al. 2004a; Rao, Webb and Kjelleberg 2006; Porsby, Nielsen and Gram 2008; Thole et al. 2012; Gram et al. 2015; Freese et al. 2017). Antagonistic interactions are believed to be more frequent in particle- and between surface-associated bacteria due to the high cell densities in structured microenvironments (Long and Azam 2001; Gram, Melchiorsen and Bruhn 2010). This could indicate that production of TDA provides a competitive advantage for the producer in surface colonization. TDA-producing Tritonibacter strains have also been isolated from aquaculture environments (Suchan, González and Moran 2005; Porsby, Nielsen and Gram 2008; Alsmark et al. 2013), as well from open ocean waters (Gram, Melchiorsen and Bruhn 2010; Sonnenschein et al. 2017a). The Tritonibacter genus represents 0.2% of the bacterial population in the surface ocean, and is thus more abundant in oceanic surface waters than Phaeobacter (Sunagawa et al. 2015). However, like Phaeobacter, Tritonibacter is adapted to a surface-attached lifestyle and occurs rather in the particle-associated than the free-living fraction of seawater (Sonnenschein et al. 2017a). Consequently, it has been proposed that T. mobilis could be the open-water equivalent to the coastal, macrosurface-associated Phaeobacter.

TDA-producing bacteria are also found in association with a range of marine eukaryotes, such as zooplankton (Freese et al. ), sponges (Harrington et al. 2014), molluscs (Ruiz-Ponte et al. 1999; Prado et al. 2009), and algae (Rao, Webb and Kjelleberg 2006; Nappi, Soldi and Egan 2019) and it has been suggested that up to one-third of TDA-producing bacteria are host-associated (Nappi, Soldi and Egan 2019). This is specifically seen with TDA-producing Pseudovibrio strains that are present on, and genetically adapted to, a symbiotic lifestyle with marine invertebrates such as corals and macroalgae (Enticknap et al. 2006; Penesyan et al. 2011; Bondarev et al. 2013; Crowley et al. 2014; Raina et al. 2016; Romano 2018). Pseudovibrio represent 0.04% of all bacteria in oceanic surface waters (Sunagawa et al. 2015) and TDA-producing Pseudovibrio species harbor genes associated with a free-living lifestyle (Enticknap et al. 2006; Bondarev et al. 2013).

Like other members of the Rhodobacteraceae family, TDA-producing bacteria are characterized by a high versatility of metabolic pathways (Newton et al. 2010; Bondarev et al. 2013; Zech et al. 2013; Sonnenschein et al. 2017a). TDA-producing Phaeobacter and Tritonibacter can catabolize several algal osmylates, such as dimethylsulfoniopropionate (DMSP; Miller and Belas 2004; Newton et al. 2010; Thole et al. 2012), which has been suggested to act as a chemo-attractant for TDA-producing bacteria (Miller and Belas 2004; Miller et al. 2004) capable of utilizing DMSP in their primary metabolism (Curson et al. 2011). Furthermore, DMSP may provide sulfur to be incorporated into TDA (Geng et al. 2008), and TDA-producing bacteria, thus benefit from living in association with microalgae and corals (Raina et al. 2009, 2016; Harrington et al. 2014; Segev et al. 2016). However, other studies have not been able to detect the incorporation of DMSP sulfur in TDA in either P. inhibens DSM17395 or in P. gallaeciensis DSM26640, and instead suggested that cysteine serves as the sulfur precursor (Dickshat et al. 2017).

**Biosynthesis, tautomers, and analogues of TDA**

TDA is a sulfur-containing tropone derivative (Fig. 2). It can tautomerize into two other known sulfur-containing tropones; thiotropocin and troposulfinin (Fig. 2). The structures of these compounds are characterized by an aromatic cyclohepta-2,4,6-trienone moiety. Their biosynthesis draws on the central carbon and sulfur metabolism, as well as a range of enzymes encoded by a cluster of dedicated so-called tda genes (Fig. 3A; Duan et al. 2020). For an in-depth review of the chemistry underlying the biosynthesis of TDA as well as other tropones, we refer to the review by Duan et al. (2020).

The basic skeleton of TDA and other tropone natural products arise from phenylacetic acid (1, PAA) catabolism (Fig. 3A), where PaaK, PaaABC(D)E (E. coli nomenclature, also referred to as PaaGHI(J)K in *Pseudomonas putida*), PaaG, as well as PaaZ are required to form a highly reactive intermediate, 3-oxo-5,6-dehydrosulburely-CoA semialdehyde (3, Fig. 3B; Teufel et al. 2010, 2011; Berger et al. 2012; Brock, Nikolay and Dickshat 2014). Interestingly, in TDA-producers, a homologue of PaaZ, PaaZZ, is encoded on a megaplasmid along with the core enzymes of TDA biosynthesis, TdaABCDEF (Fig. 3B; Brock, Nikolay and Dickshat 2014). PaaZ contains two domains, a C-terminal enoyl-CoA hydratase (ECH) and a N-terminal aldehyde dehydrogenase (ALDH) domain (Brock, Nikolay and Dickshat 2014). In PaaZZ only, the C-terminal ECH is conserved (Brock, Nikolay and Dickshat 2014). In the absence of the ADHl domain, the intermediate 3 cyclizes to a seven-membered ring 2-hydroxycyclohepta-1,4,6-triene-1-formyl-CoA (4), the proposed universal tropone precursor, via a spontaneous intramolecular condensation (Teufel et al. 2011). A recent study pointed to the acyl-CoA dehydrogenase-like flavoenzyme TdAE being the linker between primary metabolism and TDA biosynthesis (Duan et al. 2021). TdAE converts the PAA catabolism shunt product 4 and carries out a series of reactions, including dehydrogenation, CoA-ester oxygenolysis, and ring epoxidation to form 7 (Duan et al. 2021), which then presumably can be converted to TdaF, TdaB, and PatB to form TDA (5; Duan et al. 2021). This biosynthetic pathway therefore serves as an example where the bacteria ‘direct’ primary metabolism toward secondary metabolism.

Different analogues of TDA have been detected from TDA-producing bacteria (Choudhary et al. 2018; Phippen et al. 2019). The first analogue was tentatively characterized as TDA-methyl ester, which was isolated from a TDA-producing Pseudovibrio sp. (Choudhary et al. 2018; Fig. 2). Methyl-troposulfinin, an S-methylated congenor of TDA, was identified as a natural analogue of TDA from a TDA-producing *P. inhibens* (Phippen et al. 2019; Fig. 2). Notably, the antimicrobial activity of methyl-troposulfinin is lower than that of TDA (Phippen et al. 2019). A comparison of the MS fragmentation patterns of methyl-troposulfinin (Phippen et al. 2019) and the proposed TDA-methyl ester (Choudhary et al. 2018) show a very high level of similarity (Choudhary et al. 2018; Phippen et al. 2019), and due to the lack of NMR experiments in the characterization of TDA-methyl ester, it cannot be ruled out that TDA-methyl ester is a misassignment of methyl-troposulfinin. For other secondary metabolites, the production of analogues may serve to broaden the chemical (antimicrobial) repertoire of the producing organism or may serve as a detoxification and self-protection of the producer (Li et al. 2013; Gallagher et al. 2017).

Total synthesis of TDA has not been reported thus far. However, owing to its bioactivity, a series of TDA inspired analogues have been synthesized to elucidate structure activity relationship (Rabe et al. 2014). One particular synthetic analogue, tropone-2-carboxylic acid (Fig. 2B), a nonsulfur variant of TDA, had a stronger antibacterial activity against *Staphylococcus aureus* and *Vibrio anguillarum* than TDA itself, suggesting that the sulfur atoms are not necessary for the antimicrobial effect of TDA (Rabe et al. 2014).
Collectively, these observations support the notion that conditions facilitate biofilm formation and TDA production in many species. This has prompted the hypothesis that TDA production is influenced by a multitude of factors including mode of growth, nutrients, and cell density.

**Factors influencing TDA production**

As previously mentioned, TDA biosynthesis draws on central carbon metabolism as well as sulfur metabolism (Geng et al. 2008). The cascading production of TDA across different metabolic processes may explain why TDA production is influenced by a multitude of factors including mode of growth, nutrients, and cell density.

**Mode of growth**

When *P. piscinae* 24–7 is cultured in Marine Broth (MB), stagnant as compared to shaken conditions increase brown pigmentation (Bruhn et al. 2005), which is a TDA–iron complex, and thus a proxy for TDA production (D’Alvise et al. 2016). Similarly, TDA production by *Tritonibacter* is facilitated by stagnant growth conditions, whereas several *Phaeobacter* strains also produce TDA in aerated cultures (Bruhn et al. 2005, 2007; Porsby, Nielsen and Gram 2008; Belas et al. 2009; Geng and Belas 2010; Berger et al. 2011; D’Alvise et al. 2014). A distinct phenotype associated with stagnant growth of TDA-producing bacteria is the formation of a thick biofilm at the liquid–air interface (Bruhn, Gram and Belas 2007; Gram, Melchior and Bruhn 2010) and the appearance of the brown TDA–iron complex in this biofilm. This observation has prompted the hypothesis that TDA production and biofilm formation could be linked (Bruhn et al. 2005). In *Tritonibacter* sp. TM1040, deficiency of the swimming regulator *flaC* is associated with a shift toward the motile phase, a reduction in biofilm formation as well as decreased antibiotic activity (Belas et al. 2009). The secondary messenger cyclic dimeric guanosin-monophosphate (c-di-GMP), is likely involved in the interconnection of these phenotypic traits as increased production of c-di-GMP induces both biofilm formation and TDA production in *T. mobilis* F1926 (D’Alvise et al. 2014). However, attachment, biofilm formation, and TDA biosynthesis are not universally linked across TDA-producing genera (Prol García et al. 2014; Zhao et al. 2016; Majzoub et al. 2018). While stagnant growth conditions facilitate biofilm formation and TDA production in many *Tritonibacter* strains, this is not the case in most *Phaeobacter* species. Collectively, these observations support the notion that *Phaeobacter* and *Tritonibacter* occupy separate niches (Sonnenschein et al. 2017a), where *Tritonibacter* species predominantly reside in open waters, and requires a tighter regulation of TDA production as not to waste metabolic energy during dispersed planktonic growth.

**Nutrients**

Variation of carbon, nitrogen, sulfur, phosphorus, or iron sources affect production of TDA. In *P. inhibens* DSM17395, production of TDA increases dramatically when phenylalanine is used as the primary carbon source instead of glucose, and more TDA is produced in general, when aromatic compounds are utilized as a carbon source (Berger et al. 2012). As the backbone of TDA originates from the phenylacetic acid catabolon, this increase in TDA production is likely a result of increased precursor availability (Berger et al. 2012; Brock, Nikolay and Dickschat 2014). TDA is a sulfur-containing compound and in *Tritonibacter* sp. TM1040, growth on DMSP is associated with an increase in TDA concentrations as compared to growth on sulfate-containing substrates (Geng and Belas 2010). DMSP is produced by phytoplankton and roseobacters preferentially metabolize DMSP over the more readily available sulfate (Kiene et al. 1999), likely pointing to a niche-specific adaptation. In *Pseudoalvibrio* sp. FO-BEG1, phosphate limitation induces TDA production but this is likely attributed to a global change in sulfur metabolism rather than a specific phosphate effect (Romano et al. 2015). In laboratory cultures, production of bioactive TDA is dependent on iron in concentrations that far exceed those observed in natural marine systems (D’Alvise et al. 2016). Despite this, *Phaeobacter* exhibits TDA-dependent antagonism against vibrios in low-iron artificial seawater as well as in systems mimicking multihabitat level seawater systems (D’Alvise et al. 2010, 2012). A nonantibacterial (inactive) form of TDA (‘pre-TDA’) is produced when iron is not available, and this ‘pre-TDA’ can be converted to TDA by acidification (D’Alvise et al. 2016). Thus, the biosynthesis of TDA does not appear to be regulated by iron at the transcriptional level, yet bioactive TDA only forms in its presence. This is in contrast to other iron-chelating secondary metabolites, siderophores, which are typically upregulated in the absence of iron. The weak iron-chelating properties of TDA indicate that iron sequestration is not its main function, but potentially relate to its mode of action (Wilson et al. 2016), or to symbiosis where the TDA–iron complex...
Figure 3. TDA biosynthesis and genes responsible for TDA production. (A) Biosynthetic genes involved in TDA biosynthesis in *P. inhibens* DSM17395. *paaABCD* and *patB* (in green) are located on the chromosome, whilst *tda* and *paaZ2* (in blue) are located on a 262-kb megaplasmid. (B) TDA biosynthesis draws on primary metabolism for formation of the carbon backbone. Phenylacetic acid, 1, is converted to 2 by PaaK, PaaABC(D)E, and PaaG. PaaZ-ECH or PaaZ2 catalyzes hydrolytic ring cleavage to form 3, which is then either converted to 9 by the ALDH domain of PaaZ or spontaneously cyclized to 4. TdaE then connects primary and secondary metabolism through a series of reactions: dehydrogenation to 5, CoA-ester oxygenolysis to 6, and ring epoxidation to form 7. TdaBCDF subsequently forms TDA, 8. Stipled lines indicate multiple reactions taking place. Created with ChemDraw Professional (PerkinElmer Informatics) and Biorender.com.

Quorum sensing and autoinduction
TDA production is measurable in late exponential or early stationary growth phase (Geng and Belas 2010; Berger et al. 2011; Harrington et al. 2014; Romano et al. 2015), which is correlated with high cell densities. Since many roseobacters also produce the QS molecules acyl-homoserine lactones (AHLs), it has been suggested that TDA production is QS regulated (Gram et al. 2002; Bruhn et al. 2005, 2006, 2007; Martens et al. 2007; Berger et al. 2011; Zan et al. 2014). Transposon insertions in either the AHL synthase gene, *pgaI*, or the gene encoding the response regulator, *pgaR*, of *P. inhibens* DSM17395 results in a reduction in TDA production (Berger et al. 2011). However, the effect is temporary, indicating that TDA biosynthesis is not fully dependent on this QS system (Prol García, D’Alvise and Gram 2013). Furthermore, the effect is reversible through supplementation with phenylalanine, suggesting a hierarchical regulation of TDA biosynthesis (Berger et al. 2012). AHLs are not universally involved in the regulation of TDA production since *Tritonibacter* sp. TM1040 does not produce any known AHLs and no AHL synthase genes have been identified in their genomes (Sonnenschein et al. 2017a). Other compounds than AHLs may be involved in QS, and using a tdac::lacZ reporter plasmid in several TDA-deficient *Tritonibacter* sp. TM1040 strains, it was discovered that tdac was not expressed in the absence of TDA (Geng and Belas 2010). Subsequently, tdac expression was restored by cross-feeding with the wildtype, demonstrating that TDA acts as an autoinducer of its own biosynthesis in a manner similar to AHL signaling TDA (Geng and Belas 2010). Addition of both the AHL produced by *pgaI*, 3-OH-C10-homoserine lactone (HSL), and TDA restored TDA production in the *pgaI*– mutant, but not in the *pgaR*– mutant, indicating that TDA could potentially act as an autoinducer through the same response regulator as 3-OH-C10-HSL (Berger et al. 2011). This provides a possible link between autoin-
duction and AHL-mediated regulation of TDA biosynthesis (Berger et al. 2011).

QS regulation of TDA production has only been demonstrated in the strains TM1040 and DSM17395. Adding TDA to a final concentration of 1 μM to the TDA-producing Pseudovibrio sp. W74 did not result in earlier onset or higher production of TDA, indicating that autoinduction did not take place (Harrington et al. 2014; D’Alvise et al. 2016). However, since W74 produces TDA, TDA would also be present in the control cultures and testing TDA addition to a TDA-deficient mutant of W74 would be required to determine any possible Qs function.

The exact regulatory network controlling TDA biosynthesis has not been elucidated, however, using a tdac::lacZ reporter fusion, it has been demonstrated that TdaA is necessary for the transcriptional activation of tdac expression in Tritonibacter sp. TM1040, independent of TDA (Geng and Belas 2011). TdaA also acts as a transcriptional activator in P. inhibens DSM17395, where expression of tdas, tdaf, and tdaE is strongly downregulated in tdaA- mutants (Berger et al. 2011). A putative binding site of TdaA near the tdac promoter has been identified in Tritonibacter sp. TM1040, and binding of TdaA to the tdac promoter has been confirmed using an electrophoretic mobility shift assay (Geng and Belas 2011). Understanding the regulatory switches governing TDA biosynthesis, and how these differ between genera, may provide an important clue as to the ecological role of this compound.

**Effect of TDA on the producing bacteria**

Deletion of the 262-kb megaplasmid encoding the last part of the TDA biosynthetic pathway leads to an increase in growth rate and yield, demonstrating a significant burden of this plasmid (Trautwein et al. 2016; Wünsch et al. 2020). Similarly, transposon insertions in any of the tdas, tdaf, tdac, or tdaE genes on the plasmid result in TDA-deficient mutants exhibiting increased growth rates and yields (Will et al. 2017). The negative effects of TDA on the producer is, however, not due to the metabolic cost associated with TDA biosynthesis as supplementation with TDA-containing supernatant reverts the growth of the TDA-deficient mutant to wildtype levels (Will et al. 2017) indicating an autoantibacterial activity. Thus, as TDA production impairs growth of the producer, the compound must represent a significant ecological advantage in natural marine systems.

The fact that TDA interacts with AHL response regulators in Tritonibacter sp. TM1040 has major implications for global gene expression in the producing organism (Berger et al. 2011; Beyersmann et al. 2017). With the exception of 15 genes, TDA regulates the same genetic circuitry as the AHL 3-OH-C10-HSL in P. inhibens DSM17395, including genes involved in chemotaxis, motility, attachment, and biofilm integrity (Beyersmann et al. 2017). In effect, the QS signaling molecule and TDA in conjunction likely facilitate a swim-and-stick lifestyle through the induction of biofilm dispersion. While similar mechanisms have been observed for P. inhibens 2.10 (Majzoub et al. 2018), the evident connection between TDA and the biofilm mode of growth is likely different among producers with different niche adaptations, and more studies are necessary to fully resolve the effect of TDA on the producing organism. Several other antimicrobial secondary metabolites are known to function as QS signals in the producing strain, e.g. surfactin in Bacillus subtilis and Bacillus amyloliquefaciens (López et al. 2009; Chen et al. 2020), and pyocyanin in P. aeruginosa (Dietrich et al. 2006). Similarly to TDA, these compounds also affect biofilm formation and motility (Das and Manefield 2012). These molecules vary widely in chemical structure and are produced by bacteria that are taxonomically distant; hence, it seems plausible that this may be a somewhat common function of antimicrobial secondary metabolites which is likely facilitated by many different mechanisms.

**TDA-mediated inhibition of other microorganisms**

TDA can inhibit or kill a wide range of Gram-positive and Gram-negative bacteria, including both fish and human pathogens (Ruiz-Ponte et al. 1998, 1999; Hjelm et al. 2004a; Planas et al. 2006; Porsby, Nielsen and Gram 2008; Prado et al. 2009; Porsby et al. 2011; Porsby and Gram 2016; Zhao et al. 2016; Grotkjær et al. 2016b; Sonnenchein et al. 2021). Also eukaryotes such as the fungal pathogens Rizoctonia solani, Candida albicans, and some microalgal strains of the genera Chlorella and Scenedesmus are negatively affected by TDA (Kintaka et al. 1984; Liang 2003). Pure TDA can be lethal to some mammalian cell lines, including neuronal and cancer cells, and it has been suggested that TDA may lead to disruption of the mitochondrial membrane potential and activation of oxidative stress responses (Wichmann et al. 2015). The algal compound DMSP is to some extent protective against TDA-induced cytotoxicity, as preincubation with DMSP of mammalian neural cells exerted protective effects against TDA, potentially due to DMSP acting as an antioxidant (Wichmann et al. 2016). This could suggest that DMSP could have a role in the interplay between marine eukaryotes and TDA-producing bacteria, thus acting as a protectant against TDA, although this is highly speculative and requires further studies (Duan et al. 2020). Despite the negative effects of TDA on eukaryotic cells, no adverse effect of TDA producers has so far been observed on higher organisms (Sonnenchein et al. 2021) such as microalgae, Artemia, rotifers, or nauplii (D’Alvise et al. 2012; Prol García, D’Alvise and Gram 2013; Rasmussen et al. 2018, 2019; Sonnenchein et al. 2018). In fact, TDA producers may be important symbionts for microalgae and corals, and TDA has been proposed to act as algal and coral protectant (Seyedsayamdost et al. 2015b; Raina et al. 2016). Some TDA-producers, i.e. P. inhibens, P. gallei, and P. piscinae, also produce the algicidal troponoids roseobacticides (Seyedsayamdost et al. 2011a, Sonnenchein et al. 2018). These secondary metabolites are synthesized in response to p-coumaric acid, a potential senescence signal produced by algae (Seyedsayamdost et al. 2011a). In P. inhibens DSM17395, TDA and roseobacticides share parts of the same biosynthetic pathway, and the metabolites probably share the same precursor (Wang, Gallant and Seyedsayamdost 2016). This, thus, challenges the one-cluster–one-compound paradigm. These findings highlight that secondary metabolites may also be involved in beneficial interkingdom cross-talk. A well-studied example of this is found in Bacillus–plant interactions, where production of the secondary metabolite surfactin is stimulated in response to plant host cues and in turn triggers plant immunity against pathogens in Bacillus–plant interactions (Hoff et al. 2021).

In the proposed mechanism of antibacterial action, TDA disrupts the proton motive force by binding extracellular protons to the carboxyl group and transporting them across the cell membrane to the cytosol (Wilson et al. 2016). Here, the proton is exchanged for a metal ion, i.e. transported back to the extracellular space (Wilson et al. 2016). This destroys the transmembrane protons gradient whilst maintaining the membrane potential, making TDA an electroneutral proton antiporter (Wilson et al. 2016). In concordance with this, exposure of a Vibrio vulnificus to sublethal concentrations of pure TDA lead to upregulation of genes involved
in iron-uptake, oxidative stress, and regeneration of the cell envelope (Dittmann et al. 2019a).

The antibacterial effect of TDA against fish pathogenic bacteria has been extensively studied, due to the interest in using TDA-producing bacteria as probiotics in marine aquaculture (Ruiz-Ponte et al. 1998, 1999; Bruhn, Gram and Belas 2007; D’Alvise et al. 2012; Porsby and Gram 2016; Zhao et al. 2016; Grotkjær et al. 2016b; Rasmussen et al. 2018, 2019; Dittmann et al. 2019a; Ringø 2020). TDA-producing Phaeobacter and Tritonibacter are antibacterial against Vibrio species, such as V. anguillarum, V. vulnificus, and Vibrio coralliilyticus (D’Alvise et al. 2010; Porsby and Gram 2016; Zhao et al. 2016). Extracts from TDA-producing Pseudovibrio sp. P12 strongly inhibited the growth of V. coralliilyticus and V. owensii, two coral pathogens causing white syndrome in scleractinian corals (Raima et al. 2016). Growth of V. coralliilyticus is not suppressed by common coral-associated bacteria and the bacterium exhibits antibiotic resistance to a wide range of commercial antibiotics, greater than that of other vibrios such as V. vulnificus (Shnit-Orland and Kushmaro 2009; Rypien, Ward and Azam 2010; Vizcaino et al. 2010), emphasizing the antibiotic potential of TDA. TDA also inhibits other fish pathogens such as Aeromonas and Tenacibaculum spp. (Porsby and Gram 2016; Grotkjær et al. 2016b; Tesdorp et al. 2022).

The antagonistic effect of TDA-producing strains has primarily been determined using agar-based assays (Brinkhoff et al. 2004; Hjelm et al. 2004a; Bruhn et al. 2005; Rao et al. 2007; Porsby, Nielsen and Gram 2008; Prado et al. 2009) and different broth and/or biofilm-based co-culture setups (Hjelm et al. 2004b; Porsby, Nielsen and Gram 2008; Prado et al. 2009; Grotkjær et al. 2016b). Exposure of a V. anguillarum to surface-attached Phaeobacter or Tritonibacter resulted in significant reduction or complete elimination of V. anguillarum (D’Alvise et al. 2010). Furthermore, P. inhibens DSM17395 successfully inhibited V. vulnificus in co-culture experiments, keeping it at inoculum level, whereas monocultures of V. vulnificus were 1000-fold higher (Porsby and Gram 2016). TDA is most likely responsible for this inhibition, since TDA-negative mutants did not inhibit V. anguillarum (D’Alvise et al. 2010).

The activity of TDA-producing roseobacters against vibrios has also been assessed in more complex, nonaxenic, and microcosm experiments including marine organisms of multiple trophic levels. As pathogenic Vibrio strains may enter the aquaculture unit via live feed, many experiments have been conducted in cultures of microalgae, rotifers, brine shrimps, and copepods (D’Alvise et al. 2012; Prol García, D’Alvise and Gram 2013; Porsby and Gram 2016; Grotkjær et al. 2016a; Rasmussen et al. 2018). In these laboratory experiments, TDA-producing Phaeobacter can reduce the number of vibrios and other fast-growing heterotrophic bacteria (D’Alvise et al. 2012; Grotkjær et al. 2016a; Rasmussen et al. 2018, 2019).

Resistance and tolerance to TDA

Phaeobacter inhibited DSM17395 carries three genes, tdaR123, conferring its self-resistance to TDA (Wilson et al. 2016). tdaR1 and tdaR2 are predicted to encode transmembrane proteins, while tdaR3 is predicted to encode a γ-glutamyl-cyclotransferase, which in Escherichia coli is involved in cation–proton exchange (Wilson et al. 2016). All three genes are co-located to the TDA gene cluster on the megaplasmid, which to this day has not proven transmissible or to encode transmission genes (Petersen et al. 2013). Sensitivity to TDA in E. coli is reduced when the tdaR123 genes are transferred and heterologously expressed (Wilson et al. 2016). However, the tdaR123 genes have not been found in bacteria not producing TDA, and it has not been possible to develop resistance to TDA in target bacteria in vitro (Porsby et al. 2011; Rasmussen et al. 2016). Different in vitro approaches have been used to induce mutations or adaptations conferring TDA resistance in the non-TDA-producer species P. aeruginosa, S. aureus, Salmonella typhimurium, and E. coli, which are all species susceptible to TDA (Porsby et al. 2011). Using adaptive laboratory evolution experiments, V. anguillarum strains capable of tolerating two times the minimum inhibitory concentration were evolved, however, the tolerance was transient and vanished after one passage in medium free of TDA (Rasmussen et al. 2016). The difficulty in developing TDA resistance or tolerance could suggest that TDA has multiple additional targets besides the disruption of the proton motive force. Since only a few TDA resistant bacteria have been found, and resistance is difficult to develop, the use of TDA as an antibiotic and of TDA producers as probiotics will not add to the risk of antimicrobial resistance (Sonnenschein et al. 2021).

TDA tolerance has been observed in natural microbial communities containing indigenous TDA-producing Pseudovibrio. Here, 126 out of 136 isolated non-TDA-producing bacteria were tolerant to TDA (Harrington et al. 2014). Among the TDA-tolerant isolates were Psychrobacter, Alteromonas, Salmilbacter, Alcanivorax, Flavobacterium, and Micrococcus strains, whilst TDA-sensitive isolates included Staphylococcus, Idiomarina, and Rhodococcus strains (Harrington et al. 2014). The mechanisms enabling this tolerance are not known.

Influence of TDA or TDA producers on marine microbial communities

The potential use of TDA-producing bacteria as probiotics in marine aquaculture has prompted studies determining how TDA or TDA-producing bacteria affect natural marine microbiomes. The possible effect of TDA or bacteria producing TDA on taxonomic composition of natural microbiomes has been determined, typically by 16S rRNA gene amplicon sequencing (Table 1).

Adding pure TDA to the microalgae Nannochloropsis salina colonized by a seawater microbiome caused a decrease in relative abundance of bacteria belonging to Rhodobacteraceae, Flavobacteria, and Alteromonadaceae after 24 h, while bacteria of unclassified families within the Alteromonadales order increased in relative abundance (Geng et al. 2016). The changes were more rapidly seen in communities exposed to high concentrations (500 nM as opposed to 21 nM) of TDA, indicating a dose-dependent effect of TDA. The addition of TDA accelerated the development of the microbial community that after 3 h had a composition similar to the one reached in the nontreated community after 24 h (Geng et al. 2016). It should be noted that the concentration of TDA found in natural systems is not known. Addition of pure TDA to the microalgae Tetraselmis suecica could be detected to a lower limit of 50 nM, but TDA was not detectable when TDA-producing bacteria were cultured in the system (D’Alvise et al. 2012). Thus, it remains uncertain if the concentrations used in the N. salina study (Geng et al. 2016) were representative of the TDA concentrations in natural communities. Subsequent studies have typically studied changes in the bacterial community in the presence of TDA-producing bacteria.

The TDA-producing P. inhibens DSM17395 was added to the microbiome of the marine microalgae Emiliania huaxleyi in concentrations reflecting the in situ abundances of roseobacters during algal blooms (Amin et al. 2015; Segev et al. 2016; Sonnenschein et al. 2018; Dittmann et al. 2019b). The addition of 10⁶ CFU/ml of DSM17395 caused a decrease in the relative abundance of bacteria belonging to Rhodobacterales, with Lutunella and Marinella...
**Table 1.** Model systems used to study the effect of TDA-producing bacteria and TDA on microbial communities.

| In vivo model                  | TDA dose                              | Controls            | Duration (days) | Bacteria that increase                                                                 | Bacteria that decrease                                                                 | Reference                      |
|-------------------------------|---------------------------------------|---------------------|-----------------|----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|--------------------------------|
| *Nannochloropsis salina* (microalga) | Pure TDA (31–500 nM)                  | Untreated glucose   | 0–1             | Alteromonadaceae, Flavobacteria, Vibrio sp., Neptuniibacter sp.                         | Alteromonadales (Unclassified families) Rhodobacteraceae                               | Geng et al. (2016)              |
| *Emiliania huxleyi* (microalga) | *P. inhibens* DSM17395 (10⁴ and 10⁶ cells/ml) | Untreated medium    | 0–4             | Colwellia sp., Winogradskyella sp., Neptuniibacter sp. (absent in controls)            | Alteromonadaceae, Vibrio sp., Pseudooalteromonas sp., Alteromonadales Marisita           | Dittmann et al. (2019b)         |
| *Ostrea edulis* (oyster)       | *P. inhibens* DSM17395 (10⁴ and 10⁶ cells/ml) | Untreated medium    | 0–4             | Mycoplasma sp.                                                                         | Vibrionaceae, Mycoplasma sp., Pseudooalteromonas sp., Skewanella sp.                    | Dittmann et al. (2019b)         |
| *Tetraselmis suecica* (microalga) | *P. inhibens* DSM17395 (10⁴ cells/ml)  | Untreated medium    | 0–4             | Rhodobacteraceae: Ruegeria, Celeribacter, Pseudophaeobacter                             |                                                                                          | Dittmann et al. (2020)          |
| *Acartia tonsa* (copepod)      | *P. inhibens* DSM17395 (10⁴ cells/ml)  | Untreated medium    | 0–4             | Rhodobacteraceae: Ruegeria, Celeribacter, Pseudophaeobacter                             |                                                                                          | Dittmann et al. (2020)          |
| *Scophthalmus maximus* (turbot larvae) | *P. inhibens* DSM17395 (10⁴ cells/ml)  | Untreated medium    | 0–4             | Rhodobacteraceae: Ruegeria, Celeribacter, Pseudophaeobacter                             |                                                                                          | Dittmann et al. (2020)          |
| *Thalassiosira rotula* (microalga) | *P. inhibens* 2.10                   | TDA-deficient mutant | 0–8             |                                                                                         | Sulfitobacter, Phaeobacter, Pelagicola, Lortauana                                       | Majzoub et al. (2019)           |
being the most affected genera (Dittmann et al. 2019b). This is similar to the changes observed in the N. salina study, where pure TDA caused a decrease in relative abundance of Rhodobacterales (Geng et al. 2016). When adding DSM17395 to the European flat oyster, Ostrea edulis, the relative abundance of the Vibrioales decreased markedly compared to untreated microbiomes. In the E. huxleyi microbiome, individual amplicon sequence variants (ASVs) assigned as Vibrio sp. also decreased when treated with DSM17395, although no changes were seen at the order level. Also, the relative abundance of Alteromonadales in the O. edulis microbiome was higher in the DSM17395-treated microbiomes (up to 70%) compared to the untreated microbiomes (up to 47%). However, specific ASVs assigned as Pseudoalteromonas sp. (belonging to the Alteromonadales) decreased upon the addition of DSM17395, which was also observed in the E. huxleyi microbiome treated with DSM17395. Once again, this is similar to the N. salina study using pure TDA, where both increases and decreases were found for bacteria belonging to the Alteromonadales when treated with TDA (Geng et al. 2016).

The addition of DSM17395 to three common aquaculture livefeeds—the microalgae (T. suecica), copepod nauplii (Acartia tonsa), and turbot eggs/larvae (Scophthalmus maximus)—caused a decrease in relative abundance of closely related taxa particularly of the Rhodobacterales in the microbiomes (Dittmann et al. 2020). Specifically, species of Ruegeria, Celeribacter, and Pseudophaeobacter decreased in relative abundance. However, in contrast to the E. huxleyi microbiome (Dittmann et al. 2019b), bacteria belonging to the Vibrioales and Alteromonadales were not affected by DSM17395 in any of the microbiomes, despite the S. maximus microbiome having a high relative abundance of ASVs assigned as Vibrio sp. This is surprising since several studies have demonstrated an anti-Vibrio effect of TDA-producing Phaeobacter compared to a TDA-deficient mutants (D’Alvise et al. 2010, 2012), indicating that the effect of DSM17395 toward vibrios depend on the commensal microbiome composition or TDA being species-specific. Future studies should include a TDA-deficient mutant to specifically address the role of TDA in the microbiome development. Such a comparison of the effect of TDA-producing P. inhibens 2.10 and its TDA-deficient mutant was conducted on the microbiome assembly of the microalgae Thalassiosira rotula (Majzoub et al. 2019). Strain 2.10 demonstrated strain-specific effects in the microbiome, in concordance with the previous microbiome studies (Geng et al. 2016, Majzoub et al. 2019; Dittmann et al. 2019b, 2020). Furthermore, closely related strains belonging to the Sulfitobacter, Phaeobacter, Pelagiocola, and Loktanella genera were reduced or eliminated by the TDA-producing wildtype but not by the TDA-deficient mutant (Majzoub et al. 2019).

Overall, the addition of a TDA-producing P. inhibens has only minor effects on the taxonomic composition of marine microbial communities. Changes due to the presence of TDA-producing P. inhibens strains or TDA appear to be species, if not strain-specific, particularly decreasing the relative abundance of closely related taxa. This is in line with the competition-relatedness concept (Russel et al. 2017), where closely related species more often compete for the same metabolic and environmental niches. Niche competition has been suggested to be one of the evolutionary explanations for the selection of antimicrobial compounds—a concept known as competition sensing (Cornforth and Foster 2013). In several studies (Geng et al. 2016; Dittmann et al. 2019b), the relative abundance of bacteria belonging to the fast-growing heterotrophs of Vibrioales or Alteromonadales were affected by the addition of TDA or the TDA-producing strains, in particular the genus Pseudoalteromonas, which generally decreased in relative abundance (Geng et al. 2016; Dittmann et al. 2019b). Since TDA can act as an antipporter (Wilson et al. 2016), the compound may be particularly effective in antagonizing fast-growing bacteria depending on a high metabolic turnover. Several species of Pseudoalteromonas are potent secondary metabolite producers (Paulsen et al. 2019), and it has also been suggested that TDA-producing bacteria antagonize specifically potent secondary metabolite producing bacteria found in the same ecological niches (Lutz et al. 2016; Dittmann et al. 2019b), potentially due to competition sensing (Cornforth and Foster 2013). In fact, the ability of P. inhibens to produce TDA has been suggested to be maintained by interspecies competition with Pseudoalteromonas tunicata in biofilms (Lutz et al. 2016; Majzoub et al. 2018). However, the specific mechanism driving this pattern between TDA and Pseudoalteromonas species is not fully explored.

Conclusions

TDA is a molecule with multiple functions: antibiotic (disruption of the proton motive force in target bacteria), QS signal, and iron chelation (Fig. 1). The (weak) extracellular iron chelation by TDA could indicate that TDA also can act as an iron provider (reservoir) for other organisms, similar to other weak iron chelators, such as vibrioferin, suggested to promote bacterial–algal mutualism (Amin et al. 2009; Yarimizu et al. 2019).

In microbial communities, TDA has predominantly been studied as an antimicrobial compound. It reduces the relative abundance of bacteria closely related to the TDA producer, and sometimes fast-growing, potential secondary metabolite producers, such as vibrios and members of the Pseudoalteromonas genus. These observations point toward TDA playing a role in niche competition. The natural concentrations of TDA are not known and we speculate that most of the studies addressing the (antimicrobial) effect of TDA, or its producer are using concentrations of the compound or producer higher than the natural levels. Thus, there is a need for tractable model systems that reflect the natural environment in order to study secondary metabolism and community dynamics (Pessotti, Hansen and Traxler 2018, Grajka et al. 2020).

The effects of TDA on gene expression patterns in TDA-producing bacteria indicate that TDA can serve as a QS signal, affecting biofilm formation and motility in the TDA producer. A putative receptor, psaR, is also present in P. inhibens, but more studies are necessary to fully understand the molecular mechanism by which TDA regulates gene expression. TDA production may be part of adaptation to a surface-associated lifestyle, possibly in association with eukaryotic host organisms. TDA production in Tritonibacter occurs primarily during stagnant growth, whilst in Phaeobacter, TDA is also produced in aerated cultures, perhaps reflecting a tighter regulation of TDA biosynthesis in the open water Tritonibacter than in its coastal relative Phaeobacter.

In conclusion, TDA is indeed an antimicrobial secondary metabolite and as such serves multiple ecological roles such as an algal or coral protectant. However, TDA has other less explored functions being involved in QS regulation. Other antimicrobial secondary metabolites, such as surfactin, also serve multiple functions and this may be the case for several other secondary metabolites. Most studies of antimicrobial secondary metabolites has predominantly been motivated by their antibacterial activity and their effects studied on pure cultures of bacteria, mainly pathogens, using concentrations that are likely higher than those found in natural settings. To fully unravel the roles of antimicrobial secondary metabolites can and may play in natural commu-
nities, we must study the producing organisms and the compound in situ in natural systems.

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