Phylogenetic distribution of roseobacticides in the *Roseobacter* group and their effect on microalgae

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Summary

The *Roseobacter*-group species *Phaeobacter inhibens* produces the antibacterial tropodithietic acid (TDA) and the algaecidal roseobacticides with both compound classes sharing part of the same biosynthetic pathway. The purpose of this study was to investigate the production of roseobacticides more broadly in TDA-producing roseobacters and to compare the effect of producers and non-producers on microalgae. Of 33 roseobacters analyzed, roseobacticide production was a unique feature of TDA-producing *P. inhibens*, *P. gallaeciensis* and *P. piscinae* strains. One TDA-producing *Phaeobacter* strain, 27-4, was unable to produce roseobacticides, possibly due to a transposable element. TDA-producing *Ruegeria mobilis* and *Pseudovibrio* did not produce roseobacticides. Addition of roseobacticide-containing bacterial extracts affected the growth of the microalgae *Rhodomonas salina*, *Thalassiosira pseudonana* and *Emiliania*

huxleyi, while growth of *Tetraselmis suecica* was unaffected. During co-cultivation, growth of *E. huxleyi* was initially stimulated by the roseobacticide producer DSM 17395, while the subsequent decline in algal cell numbers during senescence was enhanced. Strain 27-4 that does not produce roseobacticides had no effect on algal growth. Both bacterial strains, DSM 17395 and 27-4, grew during co-cultivation presumably utilizing algal exudates. Furthermore, TDA-producing roseobacters have potential as probiotics in marine larviculture and it is promising that the live feed *Tetraselmis* was unaffected by roseobacticides-containing extracts.

Originality-significance statement

Some *Roseobacter*-group bacteria produce the antibacterial compound tropodithetic acid (TDA) and have potential as probiotics in marine aquaculture. However, a few of these strains additionally produce algaecidal compounds, the roseobacticides, which would restrict their use in marine larviculture where algae are used as live feed for fish larvae. We herein found that roseobacticides are limited to TDA-producing *Phaeobacter* strains and were not biosynthesized by TDA-producers outside this genus. Roseobacticides affected several strains of microalgae, but not the chlorophyte that is used as live feed in the aquaculture industry. Thus, the application of *Roseobacter* strains as probiotics is not hampered. Furthermore, these results demonstrate how *Roseobacter*-group strains act as gardeners of microalgae and thereby would be involved in environmental processes on a larger scale.

Introduction

Microalgae are responsible for half of the global primary production and are the basis of the marine food web (Field *et al.*, 1998). The phycosphere surrounding an algal cell is inhabited by bacteria that benefit from increased nutrient concentrations (Cole, 1982; Azam and Malfatti, 2007; Amin *et al.*, 2012). In return, these bacteria may provide the algae with supplements such as vitamins (Croft *et al.*, 2005; Cooper and Smith, 2015) and protect them from infections or grazing (Seyedsayamdost, Case, *et al.*, 2011). The bacteria may, however, also act as pathogens or parasites causing lysis and death of the algal cells (Seyedsayamdost, Case, *et al.*, 2011; Riclea *et al.*, 2012; Wang *et al.*, 2014). Possibly, these modes are interlinked genetically, or metabolically, and depend on exterior factors such as nutrient supply. This scenario was proposed for the interaction between *Emiliania huxleyi*, a globally important, bloom-forming haptophyte (Read *et al.*, 2013) and the alphaproteobacterium *Phaeobacter inhibens* (Seyedsayamdost, Case, *et al.*, 2011; Segev *et al.*, 2016b; Wang *et al.*, 2016; Wang and Seyedsayamdost, 2017). The bacterium belongs to the *Roseobacter* group, one of the most common and widespread marine bacterial groups and a group often co-appearing with microalgae (Sapp *et al.*, 2007; Luo and Moran, 2014; Tan *et al.*, 2015; Eva C Sonnenschein *et al.*, 2017). *Roseobacter*-group bacteria can be involved in the degradation of the algae-produced dimethylsulfoniopropionate into dimethylsulfide, which is released into the atmosphere and serves as cloud nuclei (Miller and Belas, 2004; Dickschat *et al.*, 2010). *P. inhibens* produces at least two groups of bioactive molecules: the antibacterial compound tropodithietic acid (TDA), and the algaecidal roseobacticides, the production of the latter being induced by *p*-coumaric acid, an algal degradation product (Brinkhoff *et al.*, 2004; Seyedsayamdost, Carr, *et al.*, 2011). First identified as its tautomer thiotropocin in 1984,

TDA is a small acid comprising sulfur and a 7-membered carbon ring (Kintaka *et al.*, 1984; Brock *et al.*, 2013; D'Alvise *et al.*, 2016). The roseobacticide family was discovered in 2011 and contains roseobacticide A to K with roseobacticide A and B generally being the dominant forms (Seyedsayamdost, Carr, *et al.*, 2011; Seyedsayamdost, Case, *et al.*, 2011). Like TDA, the roseobacticides also contain at least one sulfur atom and a tropone ring. Furthermore, they contain additional aromatic side chains derived from aromatic amino acids. It has been hypothesized that under nutrient-rich conditions, *P. inhibens* produces TDA to protect *E. huxleyi* from pathogens and, when *E. huxleyi* senesces, the algal degradation product would induce production of roseobacticides in *P. inhibens*, enhancing the algal decay. While the metabolic pathways of both compound groups are not fully elucidated (Geng *et al.*, 2008; Brock *et al.*, 2014; Seyedsayamdost *et al.*, 2014; Wang *et al.*, 2016), it has been shown for *P. inhibens* DSM 17395 that the production of TDA and roseobacticides are interlinked, i.e. that the same genes are essential for the biosynthesis of both compounds and that the metabolites probably share the same precursor (Wang *et al.*, 2016).

TDA-producing roseobacters are also of interest in biotechnological application as potential probiotic bacteria in marine aquaculture (Bruhn *et al.*, 2005; D'Alvise *et al.*, 2012, 2013; Bentzon-Tilia *et al.*, 2016). Especially, fish larvae are prone to bacterial infections due to their undeveloped immune system and probiotics are a promising alternative to the use of antibiotics that can result in development and spread of antibiotic resistance (Cabello, 2006). The fish larvae are fed with live feed such as rotifers and *Artemia* that are themselves fed with live microalgae. Pathogenic bacteria may proliferate in these feed cultures due to high levels of nutrients (Verdonck *et al.*, 1997). *P. inhibens* is an excellent antagonist of fish pathogenic Vibrionaceae due to the production of TDA and can kill or

inhibit fish pathogens in the live feed and reduce mortality caused by vibriosis in fish larvae (D'Alvise *et al.*, 2012, 2013; Grotkjær *et al.*, 2016). So far, no negative effect of *P. inhibens* was found on the aquaculture organisms themselves (Neu *et al.*, 2014), but pure TDA was able to change the natural microbiota of the feed algae *Nannochloropsis salina* (Geng *et al.*, 2016). However, it is obviously of concern that the bacterium potentially can harm the microalgae used for feeding.

The aim of this study was to analyze the distribution of roseobacticides within the *Roseobacter* group and determine which TDA-producing organisms also produce roseobacticides. We compared the genomes of producers and non-producers to identify genes likely contributing to the biosynthesis of roseobacticides. Furthermore, we investigated the impact of a roseobacticide producer and a non-producer on microalgae using bacterial extracts as well as co-cultivation.

Results and discussion

Phylogenetic distribution of roseobacticides

Thirty-three *Roseobacter*-group bacteria, including 27 TDA producers, were analyzed for the ability to biosynthesize roseobacticides upon induction with *p*-coumaric acid (see Supplementary materials & methods for details). If the dominant roseobacticide, roseobacticide B, was detected in the ethyl acetate extract of the culture by mass spectrometry, the strain was considered positive for production of roseobacticides (Table 1, Fig. S1, Table S1). With the exception of *P. piscinae* 27-4 and *P. porticola* P97, all tested *Phaeobacter* strains produced both, TDA and roseobacticide B (Fig. 1). Interestingly, the roseobacticide producer 8-1 was even collected in the same location and year as 27-4 underlining the high phenotypic diversity among genetically similar

Roseobacter-group strains (Table 1) (Sonnenschein *et al.*, 2017). Strains from the closely related genera *Pseudophaeobacter* and *Leisingera* as well as TDA-producers from the genera *Ruegeria* and *Pseudovibrio* did not produce roseobacticides under the tested conditions. This confirmed previous roseobacticide analyses of the strains DSM 17395 (BS107), 2.10, 27-4 and TM1040, and adds DSM 16374 as a roseobacticide producer although not detected previously (Seyedsayamdost, Carr, *et al.*, 2011; Wang and Seyedsayamdost, 2017). Thus, the ability of roseobacticide production appears to be limited to the phylogenetic neighbours *P. inhibens*, *P. gallaeciensis* and *P. piscinae*. In contrast, TDA is produced by several *Roseobacter*-group bacteria, which are not closely related. Evolution of the TDA genes is in agreement with the phylogenetic clustering of the strains (Fig. S2). We propose that a common ancestor of *P. inhibens*, *P. gallaeciensis* and *P. piscinae* obtained the TDA biosynthetic genes e.g. by horizontal gene transfer and subsequently, additionally developed the ability to produce roseobacticides. This hypothesis is support by the findings by Wang *et al.*, who demonstrated a tight genetic and metabolic dependency of roseobacticides to TDA in *P. inhibens* DSM 17395 (Wang *et al.*, 2016).

P. *piscinae* 27-4 produced TDA, while roseobacticides were not detected. The species *P. piscinae* is phylogenetically distinct from the species *P. inhibens* and *P. gallaeciensis* (Sonnenschein *et al.*, 2017), but based on whole genome comparison, 27-4 is very similar to at least three other *P. piscinae* strains (average nucleotide identity (ANI) to M6-4.2, S26, and 8-1 is 98.5, 98.3, 95.9%, respectively). To investigate the genetic background of roseobacticide production, the predicted protein sequences of the roseobacticide producer strains M6-4.2, 8-1, DSM 17395 and DSM 26640 and the non-producer 27-4 were compared using OrthoVenn (Wang *et al.*, 2015). This revealed fourteen orthologous

143 protein clusters unique to the producer strains (Fig. S3, see [a]; Table 2A) and five unique to
144 the non-producer (Fig. S3, see [b]; Table 2B). The proteins unique to the producer strains
145 differ from those found by Wang *et al.* (Wang *et al.*, 2016) as being essential for the
146 production of roseobacticides in DSM 17395 using a transposon library; however, some lie
147 within close proximity (distance of < 20 genes) (Fig. S4). Since being identified by two
148 independent approaches, the genetic loci are likely to be involved in the production. The
149 proteins herein identified as unique to the producer strains include a sulfurase and
150 glutathione S-transferase that could be involved in the biosynthesis of roseobacticides
151 (Table 2). Sulfur is a key component of both TDA and roseobacticides (Wang *et al.*, 2016)
152 and glutathione was proposed to be involved in the TDA resistance mechanism by *P.*
153 *inhibens* (Wilson *et al.*, 2016).
154 Four of the orthologous proteins unique to the non-producer 27-4 represent a
155 transposable element (Fig. S5). This element appears three times in the genome of 27-4
156 on the plasmids F, E and C. It covers the majority of plasmid F (13 kb) and thus, the
157 transposable element could have entered the strain via this plasmid. The transposon
158 located on plasmid C disrupts a gene cluster that contains four genes encoding a
159 transcriptional regulator, an endonuclease, an oxidoreductase and an aldehyde
160 dehydrogenase, with the transposable element inserted in the gene of the oxidoreductase
161 (Fig. S5). Using this bioinformatic approach, we can only speculate that disruption of this
162 gene cluster could have led to loss of the ability to produce roseobacticides and further
163 experimentation by e.g. site-directed deletion is required.
164 The transposable element itself contains seven genes encoding a transposase, a
165 resolvase, a RNA polymerase sigma factor, an anti-sigma E protein, two secreted surface
166 proteins, and a peptide methionine sulfoxide reductase. The peptide methionine sulfoxide

reductase is also present in the non-roseobactide producing TDA-producer *Ruegeria* sp. strain TM1040 (Fig. S6), but not in other *Ruegeria* or *Pseudovibrio* strains, making it unlikely as a shared reason for the non-production of roseobacticides.

The ability to biosynthesize roseobacticides appears to have been developed in a common ancestor of *P. inhibens*, *P. gallaeciensis* and *P. piscinae* in contrast to TDA production. The TDA-producer *Phaeobacter*, *Ruegeria* and *Pseudovibrio* are not phylogenetic neighbours, thus this feature possibly 'jumped' by horizontal gene transfer between different *Roseobacter*-group species. Furthermore, the strains selected for the analysis herein were obtained from different locations and time points demonstrating how conserved the phenotype of roseobacticide production is within those species.

Bioactivity of roseobacticides against microalgae

Ethyl acetate extracts of DSM 17395 and 27-4 and their corresponding TDA-negative mutants were prepared from cultures grown under roseobacticide-inducing conditions. Only the extract of DSM 17395 contained roseobacticides as determined by HPLC-HRMS (Fig. S7). The strains were grown under iron limited conditions, which generally diminishes the production of TDA (D'Alvise *et al.*, 2016) and correspondingly TDA was not present in detectable quantities.

The algae were treated with a roseobacticide extract 10-fold diluted with respect to the original bacterial culture (i.e. for roseobacticide-containing extract, the bacterial strains were cultivated in 25 mL ½YTSS with *p*-coumaric acid. The culture was extracted twice with 1:1 ethylacectate, dried and resuspended in 2.5 mL methanol. 50 µl of this roseobacticide-containing extract was added to 5 mL of algal culture for the bioassay). Cell numbers were assessed over 9 days. Growth of the cryptophyte *R. salina* (Fig. 2A) and

191 the diatom *T. pseudonana* (Fig. 2B) was only affected by the roseobacticide-containing
192 extract of the DSM 17395 wildtype. While the cell numbers initially dropped, the algae had
193 recovered until the end of the experiment. The chlorophyte *T. suecica* (Fig. 2C) was not
194 affected by any extract. Roseobacticides might also have an effect on *T. suecica* at higher
195 concentrations, however, this could not be evaluated due to non-availability of pure
196 standards. Initially, no growth of the haptophyte *E. huxleyi* was observed in cultures
197 supplemented with any of the four extracts at the given concentration (Fig. S8). However,
198 when the extract was diluted to a 100-fold, an inhibitory effect of the DSM 17395 wildtype
199 extract was observed (Fig. 2D). Growth of the eukaryotic organisms was unaffected by the
200 addition of diluent (methanol) (Fig. 2).
201 In comparison to previous bioassays (Seyedsayamdost, Case, *et al.*, 2011), we
202 monitored microalgae representing four different groups (haptophyte, cryptophyte,
203 heterokont, and chlorophyte) over nine days. We found the same impact on the
204 cryptophyte *R. salina* and the haptophyte *E. huxleyi.* While the heterokont studied
205 previously, *Chaetoceros muelleri*, was only weakly affected by roseobacticides, the
206 heterokont *T. pseudonana* was highly affected in our experiments. The effect on any algae
207 was temporary and the algae were able to recover, possibly due to the degradation of
208 roseobacticides. Using the high concentration of *Phaeobacter* extracts, both the
209 roseobacticide-containing and non-containing extracts had a high lytic effect on the
210 haptophyte *E. huxleyi* (Fig. S8) suggesting that other so far undescribed metabolites play a
211 role in the interaction with the *Roseobacter*-group bacteria.
212
213
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Bacterial-microalgal co-cultivation

The roseobacticide producer DSM 17395 or the non-producer 27-4 were co-cultivated with the microalgae *E. huxleyi* for 27 days and cell numbers were assessed (Fig. 3). The algae reached the maximum cell concentrations on days 12 (*E. huxleyi* + DSM 17395), 15 (*E. huxleyi* + 27-4) and 18 (*E. huxleyi*). The algal cell concentrations of the axenic cultures compared to those in the co-cultivation setup with DSM 17395 were significantly lower on days 9 ($p \leq 0.01$) and 12 ($p \leq 0.05$) and significantly higher on days 21 ($p \leq 0.0001$), 24 ($p \leq 0.001$), 27 ($p \leq 0.0001$). There was no difference between the axenic cultures and those incubated with 27-4. Accordingly, co-cultivation setups of DSM 17395 and 27-4 differed in algal cell counts on day 12 ($p \leq 0.05$), 21 ($p \leq 0.05$), 24 ($p \leq 0.05$) and 27 ($p \leq 0.01$). The bacterial cell concentrations of both strains were significantly higher in the co-cultivation setups in comparison to the axenic samples of DSM 17395 and 27-4 from day 6 ($p \leq 0.01$) and 3 ($p \leq 0.001$) onwards, respectively. There was no significant difference of the bacterial numbers in the co-cultivations between DSM 17395 and 27-4, but on day 0 ($p \leq 0.001$).

Thus, we demonstrated the different direct effects on microalgae of two closely related TDA-producers; a roseobacticide producer and a non-producer. The roseobacticide producer promoted algal growth and enhanced their decay, an observation that was also found by a recent study on the metabolic dynamics of the interaction of *E. huxleyi* and *P. inhibens* (Segev *et al.*, 2016b). While the enhanced decay might be attributed to the production of roseobacticides, the growth promotion would indicate that there is more to the beneficial effect of *P. inhibens* than TDA only. In contrast, the roseobacticide non-producer had no effect on the microalgae. The switch between mutualism and parasitism in algae-bacteria interactions has also been found in the interaction between the dinoflagellate *Prorocentrum minimum* and *Dinoroseobacter shibae* (Wang *et al.*, 2014; H.

239 Wang *et al.*, 2015). It was suggested that *D. shibae* obtains its energy by degradation of
240 polyhydroxyalkanoate and dimethylsulfoniopropionate (DMSP) (Wang *et al.*, 2014),
241 pathways that are also present in *P. inhibens* (Dickschat *et al.*, 2010; Kanehisa *et al.*,
242 2015) and with the latter molecule actually preventing the effect of TDA (Wichmann *et al.*,
243 2016). Additionally, *E. huxleyi* is a known producer of DMSP (Wolfe and Steinke, 1996;
244 Steinke *et al.*, 2007). The growth promoting effect of bacterial indole-3-acetic acid
245 demonstrated for the interaction between a diatom and *Sulfitobacter* appears unlikely due
246 the inability of *Phaeobacter* to produce this specific molecule (Amin *et al.*, 2015; Labeeuw
247 *et al.*, 2016). Furthermore, the establishment of these microbial interactions depend on
248 motility, chemotaxis, attachment and quorum sensing (Sonnenschein *et al.*, 2012; H.
249 Wang *et al.*, 2015), which are well-known characteristics of *Phaeobacter inhibens* (Newton
250 *et al.*, 2010; Berger *et al.*, 2011; Gram *et al.*, 2015; Wang *et al.*, 2016). Thus, while some
251 pathways appear universally important for microalgae-bacteria interactions, each species,
252 and even each strain, might facilitate unique interactions. Such is the case in the herein
253 presented phaeobacters where the interaction ranges from having no effect to detrimental
254 effects on different microalgae.
255
256 *LC-HRMS and LC-MS/MS analysis of co-cultivation*
257 Ethyl acetate extracts of upscaled DSM 17395 and *E. huxleyi* co-culture, collected at 17
258 and 27 days (early and late stationary phase of *E. huxleyi*), were examined for the
259 presence of TDA and roseobacticides by both full scan LC-HRMS (QTOF) and multiple
260 reaction monitoring (MRM) LC-MS/MS (QqQ). Neither TDA or roseobacticides were
261 detected in the co-culture at either time point.

In our experience, roseobacticides are produced in minute quantities, even in dense bacterial monocultures. Although roseobacticides were not detected in our LC-MS analysis in the co-cultures, it is plausible, if not likely, that roseobacticides are produced in quantities below our current limits of detection (LOD), even despite the approximately 5-10 fold lower LOD of the triple-quadrupole mass spectrometer (QqQ) compared to the QTOF.

Conclusions

A mutualistic-parasitic interaction between the bacterium *P. inhibens* and the microalgae *E. huxleyi* has been proposed in previous studies (Seyedsayamdost, Case, *et al.*, 2011; Segev *et al.*, 2016a). This interaction is driven by the bacterial metabolites TDA and roseobacticides. It is not, however, known if all TDA producing roseobacters also produce roseobacticides. We here demonstrated that TDA production is a more widespread feature than roseobacticide biosynthesis and that the production of both compound groups is typical of the *Phaeobacter* genus. Furthermore, the proposed conversion from mutualistic to parasitic interaction of *P. inhibens* with the microalgae *E. huxleyi* was reproduced *in vitro*, while this effect was not observed in a closely related *Phaeobacter* strain that lost the ability of roseobacticide production.

The finding of a non-roseobacticide producing *Phaeobacter* strain, in combination with the fact that the microalgae used as aquaculture live feed were unaffected by the roseobacticides, is promising for future applications of phaeobacters as biocontrol agents in aquaculture. Also, roseobacticides are produced in very small quantities even when induced in bacterial culture and thus, *in situ* concentrations are presumably much lower than those tested in this study (not detectable with current techniques). Furthermore, we demonstrated that small genetic changes, possibly due to a transposable element, caused

a different interaction phenotype with the ecologically important microalgae *E. huxleyi*, which thus may have larger environmental implications.

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Supplementary information

Supplementary information containing materials and methods, supplementary tables and figures accompanies this article.

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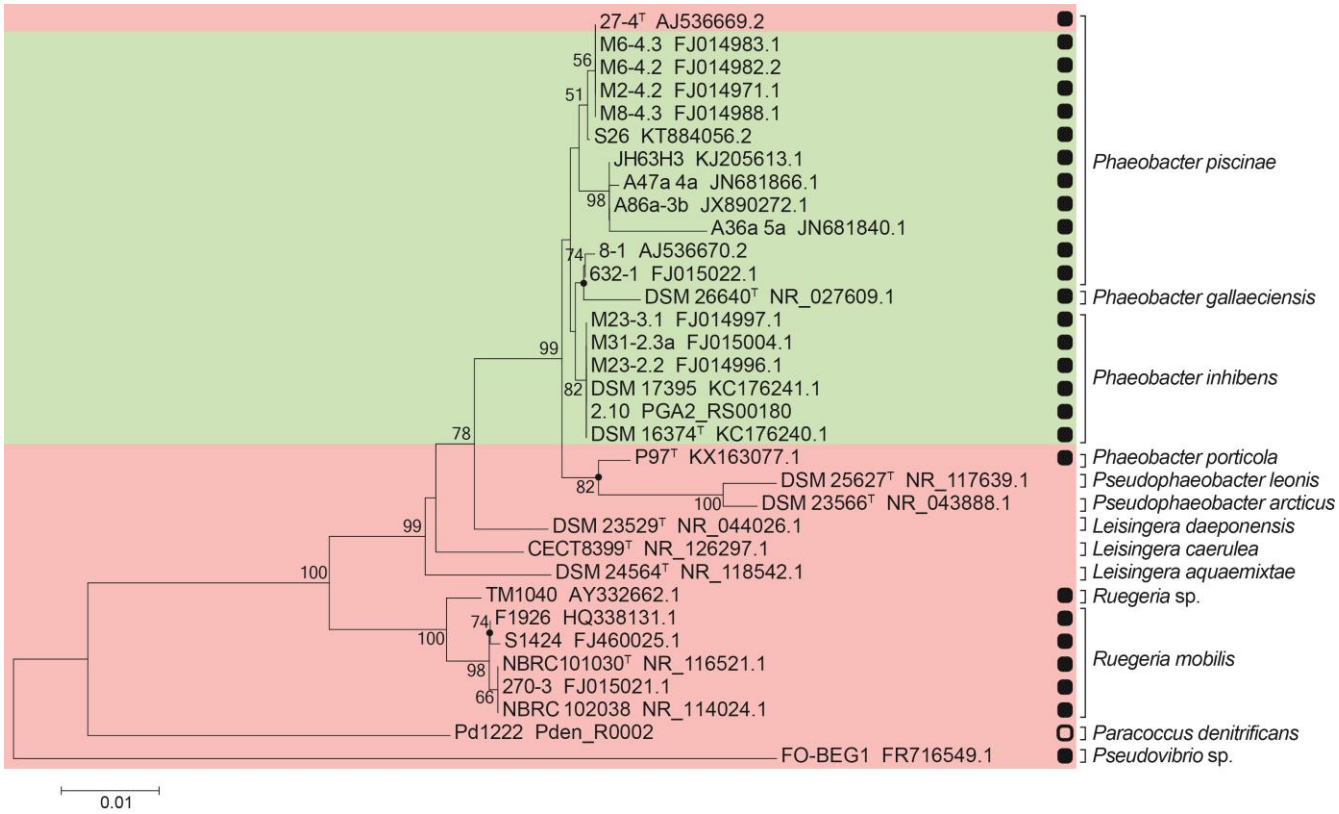
518

519 **Figures and legends**



520

521 **Figure 1.** Neighbor-joining (NJ) tree of 16S rRNA gene sequences of *Roseobacter* group
522 strains tested for production of roseobacticide B. The NJ analysis was performed using the
523 bootstrap test with 1000 replicates. Support values (percentages) from 1000 bootstrap
524 replicates are displayed above branches. Only values >50% are shown at major nodes.
525 Filled circles indicate nodes also recovered reproducibly with the maximum-likelihood
526 method. Roseobacticide B producers are marked in green, non-producers in red. TDA-
527 producers are marked with a black square and the strain carrying the disrupted TDA
528 biosynthetic gene cluster with an empty square.

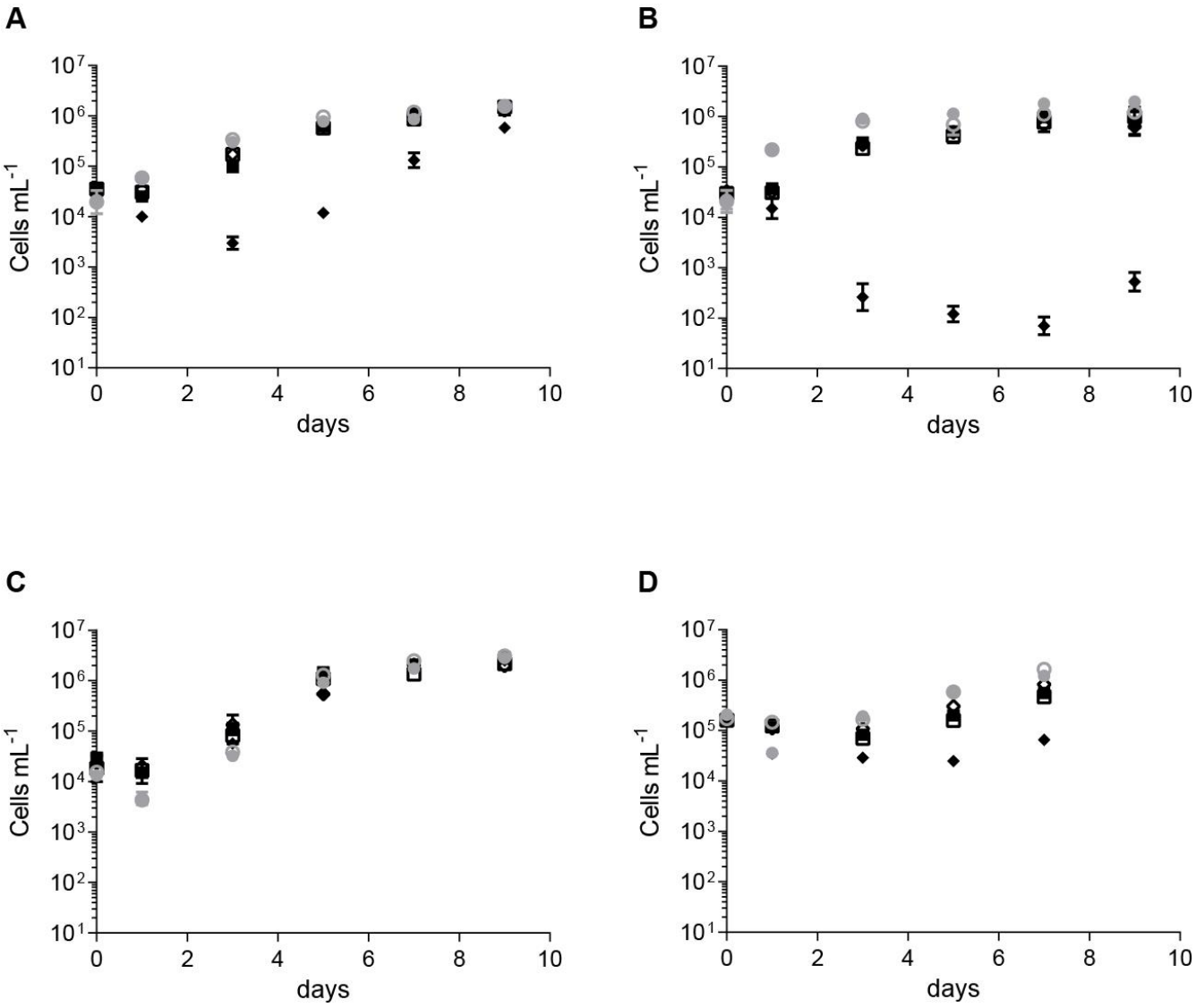
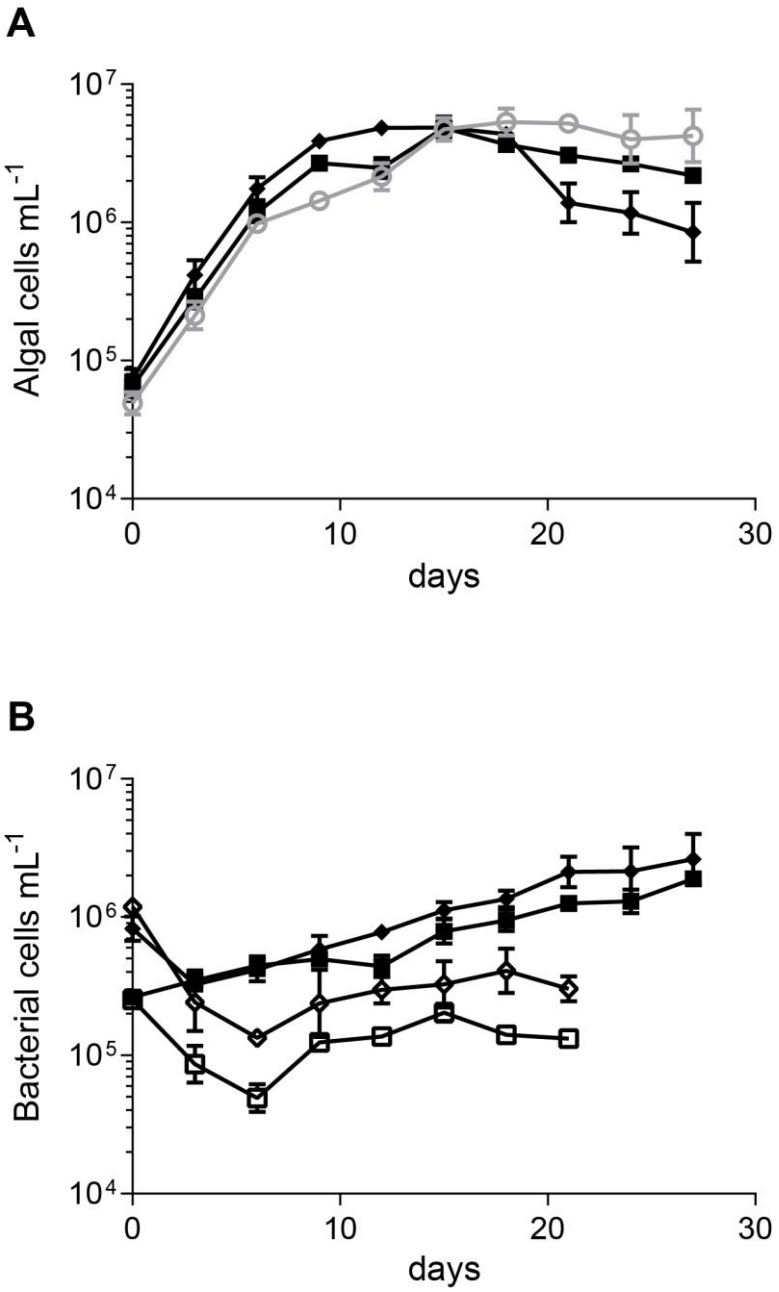


Figure 2. Bioactivity of *Phaeobacter* extracts against the microalgae A) *Rhodomonas salina*, B) *Thalassiosira pseudonana*, C) *Tetraselmis suecica* and D) *Emiliania huxleyi*. The extracts had a final concentration of 10% in A)-C) and 1% in D); percentage is given in regard to the original bacterial culture. ●: no addition, ○: methanol, ♦:DSM 17395 wildtype, ◊: DSM 17395*tdaB*::Tn*5*, ■: 27-4 wildtype, □: 27-4*tdaB*::Tn*5*. Points are means of three replicates and error bars are standard deviations of the mean, except for D), which is one replicate.



537

538 **Figure 3.** Co-cultivation of DSM 17395 wildtype and 27-4 wildtype with the microalgae

539 *Emiliania huxleyi*. A) Algal cell counts, B) bacterial cell counts. Points are means of

540 duplicates and error bars are standard deviations of the mean. ○: *E. huxleyi*, ♦: *E. huxleyi* +

541 DSM 17395, ■: *E. huxleyi* + 27-4, ◊: DSM 17395, □: 27-4.

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Tables and legends

Table 1. Strains evaluated for roseobacticide and TDA production in this study. Ros. = roseobacticides, TDA = tropodithietic acid; na = not available; unpub = unpublished. * = strains that were also previously analyzed for roseobacticide production (Seyedsayamdost, Carr, *et al.*, 2011; Wang and Seyedsayamdost, 2017)

Species	Strain	Isolation site	Latitude	Longi-tude	Year of isolation	Production of		Genome NCBI acc. no.	Reference
						Ros.	TDA		
Paracoccus denitrificans	Pd1222	na	Na	na	<1989	-	-	CP000489.1-91.1	(Vries *et al.*, 1989)
Phaeobacter sp.	632-1	turbot farm, Spain	na	na	<2004	+	+	na	(Hjelm, Riaza, *et al.*, 2004)
Phaeobacter sp.	A36a-5a	Jyllinge harbour, Denmark	55.7444	12.0958	2009	+	+	na	Bernbom unpub
Phaeobacter sp.	A47a-4a	Jyllinge harbour, Denmark	55.7444	12.0958	2009	+	+	na	Bernbom unpub
Phaeobacter sp.	A86a-3b	Jyllinge harbour, Denmark	55.7444	12.0958	2011	+	+	na	Bernbom unpub
Phaeobacter sp.	JH63H3	Jyllinge harbour, Denmark	55.7444	12.0958	2012	+	+	na	unpub
Phaeobacter sp.	M2-4.2	turbot farm, Limfjord, Denmark	56.8579	9.0973	2006	+	+	na	(Porsby *et al.*, 2008)
Phaeobacter sp.	M6-4.3	turbot farm, Limfjord, Denmark	56.8579	9.0973	2006	+	+	na	(Porsby *et al.*, 2008)
Phaeobacter sp.	M8-4.3	turbot farm, Limfjord, Denmark	56.8579	9.0973	2006	+	+	na	(Porsby *et al.*, 2008)
Phaeobacter sp.	M23-2.2	turbot farm, Limfjord, Denmark	56.8579	9.0973	2006	+	+	na	(Porsby *et al.*, 2008)

Phaeobacter sp.	M23-3.1	turbot farm, Limfjord, Denmark	56.8579	9.0973	2006	+	+	na	(Porsby *et al.*, 2008)
Phaeobacter sp.	M31-2.3a	turbot farm, Limfjord, Denmark	56.8579	9.0973	2006	+	+	na	(Porsby *et al.*, 2008)
Phaeobacter piscinae	27-4^{T}*	turbot farm, Spain	na	na	2001	-	+	CP010681-88	(Hjelm, Bergh, *et al.*, 2004)
Phaeobacter piscinae	8-1	turbot farm, Spain	na	na	2001	+	+	CP010767-75	(Hjelm, Bergh, *et al.*, 2004)
Phaeobacter piscinae	M6-4.2	turbot farm, Limfjord, Denmark	56.8579	9.0973	2006	+	+	CP010643-49	(Porsby *et al.*, 2008)
Phaeobacter piscinae	S26	sea bass farm, Greece	38.6021	23.2785	2013	+	+	JSWK01	(Grotkjær *et al.*, 2016)
Phaeobacter inhibens	2.10*	macroalgae, intertidal zone, Australia	na	na	<2005	+	+	CP002972.1-5.1	(Rao *et al.*, 2005)
Phaeobacter inhibens	DSM16374^{T}*	intertidal mud flat, Wadden Sea, Germany	53.6647	7.7620	1999	+	+	AXBB01	(Brinkhoff *et al.*, 2004)
Phaeobacter inhibens	DSM17395*	scallop farm, Spain	43.3613	-8.3740	<1998	+	+	CP002976.1-79.1	(Ruiz-Ponte and Cilia, 1998)
Phaeobacter gallaeciensis	DSM 26640^{T}	scallop farm, Spain	43.3613	-8.3740	<1998	+	+	CP006966.1-73.1	(Ruiz-Ponte and Cilia, 1998)
Phaeobacter porticola	P97^{T}	harbor, North Sea, Germany	53.7026	7.7063	2014	-	+	CP016364.1-69.1	(Breider *et al.*, 2017)
Leisingera aquaemixtae	CECT 8399^{T}	freshwater spring, Jeju Island, South Korea	33.2503	126.6171	<2014	-	-	CYSR01	(Park *et al.*, 2014)
Leisingera caerulea	DSM 24564^{T}	cathode exposed to seawater, Italy	44.3700	8.9400	<2009	-	-	AXBI01	(Vandecandelaere *et al.*, 2009)
Leisingera daeponensis	DSM 23529^{T}	tidal flat sediment, Yellow Sea, South Korea	31.6500	127.1000	<2007	-	-	AXBD01	(Yoon *et al.*, 2007)
Pseudophaeobacter arcticus	DSM 23566^{T}*	sediment, Arctic Ocean	75.0067	-169.9936	2003	-	-	AXBF01	(Zhang *et al.*, 2008)

Pseudophaeobacter leonis	DSM 25627^T	sediment, Mediterranean Sea	42.6933	3.8416	2008	-	-	na	(Gaboyer *et al.*, 2013)
Pseudovibrio sp.	FO-BEG1	coral reef, Florida, USA	na	na	<2011	-	+	CP003147.1-48.1	(Brock and Schulz-Vogt, 2011)
Ruegeria mobilis	270-3	turbot farm, Spain	na	na	<2004	-	+	LNVY01	(Hjelm, Bergh, *et al.*, 2004)
Ruegeria mobilis	F1926	Indian Ocean	−31.4061	91.1776	2006	-	+	CP015230.1-34.1	(Gram *et al.*, 2010)
Ruegeria mobilis	NBRC101030^T	glass slide exposed to seawater, Ishigaki Island, Japan	na	na	1990	-	+	LNWY01	(Muramatsu *et al.*, 2007)
Ruegeria mobilis	NBRC102038	seawater, Sargasso sea	na	na	<2007	-	+	LNWZ01	(Lee *et al.*, 2007)
Ruegeria mobilis	S1424	seawater, South Atlantic Ocean	-27.6162	15.1324	2006	-	+	LNXB01	(Gram *et al.*, 2010)
Ruegeria sp.	TM1040*	dinoflagellate culture	na	na	<2004	-	+	CP000375.1-77.1	(Miller *et al.*, 2004)

Table 2. Unique orthologous proteins to **A)** the producers M6-4.2, 8-1, DSM 17395 and DSM 26640 and **B)** the roseobacticide non-producer 27-4 .

A) Unique orthologues to the roseobacticide producers M6-4.2, 8-1, DSM 17395 and DSM 26640

	Locus tag in DSM 17395	**Description**	**GO classification**
1	PGA1_RS19085	hypothetical protein	-
2	PGA1_RS14175	transposase	transposase activity, sequence-specific DNA binding, transposition, DNA-mediated
3	PGA1_RS03520	hemolysin-type calcium-binding region	calcium ion binding, intein-mediated protein splicing
4	PGA1_RS04200	hypothetical protein	integral component of membrane
5	PGA1_RS02970	sulfurase	catalytic activity, molybdenum ion binding, pyridoxal phosphate binding, metabolic process
6	PGA1_RS03280	peptidase M50	integral component of membrane
7	PGA1_RS04870	class A beta-lactamase; EC:3.5.2.6	beta-lactamase activity, beta-lactam antibiotic catabolic process, response to antibiotic
8	PGA1_RS06640	glutathione S-transferase; EC:2.5.1.18	glutathione transferase activity, metabolic process
9	PGA1_RS09195	hypothetical protein	-
10	PGA1_RS09205	hypothetical protein	-
11	PGA1_RS09210	Lj965 prophage replication	-
12	PGA1_RS10850	hypothetical protein	-
13	PGA1_RS14180	integrase	nucleic acid binding, DNA integration
14	PGA1_RS19900	hypothetical protein	-

B) Unique orthologues to roseobacticide non-producer 27-4

	Locus tag in 27-4	Description	GO classification
1	PhaeoP14_03815, PhaeoP14_03816, PhaeoP14_03912, PhaeoP14_03913, PhaeoP14_03930, PhaeoP14_03931	uncharacterized protein	-
2	PhaeoP14_03813, PhaeoP14_03915, PhaeoP14_03928	RNA polymerase sigma factor SigK	DNA binding, transcription factor activity, sequence-specific DNA binding, sigma factor activity, DNA-templated transcription, initiation, regulation of transcription, DNA-templated
3	PhaeoP14_03814, PhaeoP14_03914, PhaeoP14_03929	hypothetical protein	membrane, integral component of membrane, plasma membrane
4	PhaeoP14_03817, PhaeoP14_03911, PhaeoP14_03932	peptide-methionine (R)-Sulfoxide reductase; EC:1.8.4, EC:1.8.4.12	Peptide-methionine (R)-sulfoxide reductase activity, response to oxidative stress, protein repair, oxidation-reduction process
5	PhaeoP14_03663, PhaeoP14_03731	hypothetical protein	-