



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

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

3

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13

14 **Summary**

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

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

34

### 35 **Originality-significance statement**

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

## 47 **Introduction**

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

71 TDA is a small acid comprising sulfur and a 7-membered carbon ring (Kintaka *et al.*, 1984;  
72 Brock *et al.*, 2013; D'Alvise *et al.*, 2016). The roseobacticide family was discovered in 2011  
73 and contains roseobacticide A to K with roseobacticide A and B generally being the  
74 dominant forms (Seyedsayamdost, Carr, *et al.*, 2011; Seyedsayamdost, Case, *et al.*,  
75 2011). Like TDA, the roseobacticides also contain at least one sulfur atom and a tropone  
76 ring. Furthermore, they contain additional aromatic side chains derived from aromatic  
77 amino acids. It has been hypothesized that under nutrient-rich conditions, *P. inhibens*  
78 produces TDA to protect *E. huxleyi* from pathogens and, when *E. huxleyi* senesces, the  
79 algal degradation product would induce production of roseobacticides in *P. inhibens*,  
80 enhancing the algal decay. While the metabolic pathways of both compound groups are  
81 not fully elucidated (Geng *et al.*, 2008; Brock *et al.*, 2014; Seyedsayamdost *et al.*, 2014;  
82 Wang *et al.*, 2016), it has been shown for *P. inhibens* DSM 17395 that the production of  
83 TDA and roseobacticides are interlinked, i.e. that the same genes are essential for the  
84 biosynthesis of both compounds and that the metabolites probably share the same  
85 precursor (Wang *et al.*, 2016).

86 TDA-producing roseobacters are also of interest in biotechnological application as  
87 potential probiotic bacteria in marine aquaculture (Bruhn *et al.*, 2005; D'Alvise *et al.*, 2012,  
88 2013; Bentzon-Tilia *et al.*, 2016). Especially, fish larvae are prone to bacterial infections  
89 due to their undeveloped immune system and probiotics are a promising alternative to the  
90 use of antibiotics that can result in development and spread of antibiotic resistance  
91 (Cabello, 2006). The fish larvae are fed with live feed such as rotifers and *Artemia* that are  
92 themselves fed with live microalgae. Pathogenic bacteria may proliferate in these feed  
93 cultures due to high levels of nutrients (Verdonck *et al.*, 1997). *P. inhibens* is an excellent  
94 antagonist of fish pathogenic Vibrionaceae due to the production of TDA and can kill or

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

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

107

## 108 **Results and discussion**

### 109 *Phylogenetic distribution of roseobacticides*

110 Thirty-three *Roseobacter*-group bacteria, including 27 TDA producers, were analyzed for  
111 the ability to biosynthesize roseobacticides upon induction with *p*-coumaric acid (see  
112 Supplementary materials & methods for details). If the dominant roseobacticide,  
113 roseobacticide B, was detected in the ethyl acetate extract of the culture by mass  
114 spectrometry, the strain was considered positive for production of roseobacticides (Table  
115 1, Fig. S1, Table S1). With the exception of *P. piscinae* 27-4 and *P. porticola* P97, all  
116 tested *Phaeobacter* strains produced both, TDA and roseobacticide B (Fig. 1).

117 Interestingly, the roseobacticide producer 8-1 was even collected in the same location and  
118 year as 27-4 underlining the high phenotypic diversity among genetically similar

119 *Roseobacter*-group strains (Table 1) (Sonnenschein *et al.*, 2017). Strains from the closely  
120 related genera *Pseudophaeobacter* and *Leisingera* as well as TDA-producers from the  
121 genera *Ruegeria* and *Pseudovibrio* did not produce roseobacticides under the tested  
122 conditions. This confirmed previous roseobacticide analyses of the strains DSM 17395  
123 (BS107), 2.10, 27-4 and TM1040, and adds DSM 16374 as a roseobacticide producer  
124 although not detected previously (Seyedsayamdost, Carr, *et al.*, 2011; Wang and  
125 Seyedsayamdost, 2017). Thus, the ability of roseobacticide production appears to be  
126 limited to the phylogenetic neighbours *P. inhibens*, *P. gallaeciensis* and *P. piscinae*. In  
127 contrast, TDA is produced by several *Roseobacter*-group bacteria, which are not closely  
128 related. Evolution of the TDA genes is in agreement with the phylogenetic clustering of the  
129 strains (Fig. S2). We propose that a common ancestor of *P. inhibens*, *P. gallaeciensis* and  
130 *P. piscinae* obtained the TDA biosynthetic genes e.g. by horizontal gene transfer and  
131 subsequently, additionally developed the ability to produce roseobacticides. This  
132 hypothesis is supported by the findings by Wang *et al.*, who demonstrated a tight genetic and  
133 metabolic dependency of roseobacticides to TDA in *P. inhibens* DSM 17395 (Wang *et al.*,  
134 2016).

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

143 protein clusters unique to the producer strains (Fig. S3, see <sup>a</sup>; Table 2A) and five unique to  
144 the non-producer (Fig. S3, see <sup>b</sup>; 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



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

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

177

#### 178 *Bioactivity of roseobacticides against microalgae*

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

185 The algae were treated with a roseobacticide extract 10-fold diluted with respect to the  
186 original bacterial culture (i.e. for roseobacticide-containing extract, the bacterial strains  
187 were cultivated in 25 mL ½YTSS with *p*-coumaric acid. The culture was extracted twice  
188 with 1:1 ethylacetate, dried and resuspended in 2.5 mL methanol. 50 µl of this  
189 roseobacticide-containing extract was added to 5 mL of algal culture for the bioassay). Cell  
190 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

214

215 *Bacterial-microalgal co-cultivation*

216 The roseobacticide producer DSM 17395 or the non-producer 27-4 were co-cultivated with  
217 the microalgae *E. huxleyi* for 27 days and cell numbers were assessed (Fig. 3). The algae  
218 reached the maximum cell concentrations on days 12 (*E. huxleyi* + DSM 17395), 15 (*E.*  
219 *huxleyi* + 27-4) and 18 (*E. huxleyi*). The algal cell concentrations of the axenic cultures  
220 compared to those in the co-cultivation setup with DSM 17395 were significantly lower on  
221 days 9 ( $p \leq 0.01$ ) and 12 ( $p \leq 0.05$ ) and significantly higher on days 21 ( $p \leq 0.0001$ ), 24  
222 ( $p \leq 0.001$ ), 27 ( $p \leq 0.0001$ ). There was no difference between the axenic cultures and those  
223 incubated with 27-4. Accordingly, co-cultivation setups of DSM 17395 and 27-4 differed in  
224 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  
225 bacterial cell concentrations of both strains were significantly higher in the co-cultivation  
226 setups in comparison to the axenic samples of DSM 17395 and 27-4 from day 6 ( $p \leq 0.01$ )  
227 and 3 ( $p \leq 0.001$ ) onwards, respectively. There was no significant difference of the bacterial  
228 numbers in the co-cultivations between DSM 17395 and 27-4, but on day 0 ( $p \leq 0.001$ ).

229 Thus, we demonstrated the different direct effects on microalgae of two closely related  
230 TDA-producers; a roseobacticide producer and a non-producer. The roseobacticide  
231 producer promoted algal growth and enhanced their decay, an observation that was also  
232 found by a recent study on the metabolic dynamics of the interaction of *E. huxleyi* and *P.*  
233 *inhibens* (Segev *et al.*, 2016b). While the enhanced decay might be attributed to the  
234 production of roseobacticides, the growth promotion would indicate that there is more to  
235 the beneficial effect of *P. inhibens* than TDA only. In contrast, the roseobacticide non-  
236 producer had no effect on the microalgae. The switch between mutualism and parasitism  
237 in algae-bacteria interactions has also been found in the interaction between the  
238 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.

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

267

## 268 **Conclusions**

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

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

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

288

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299

## 300 **Supplementary information**

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

303

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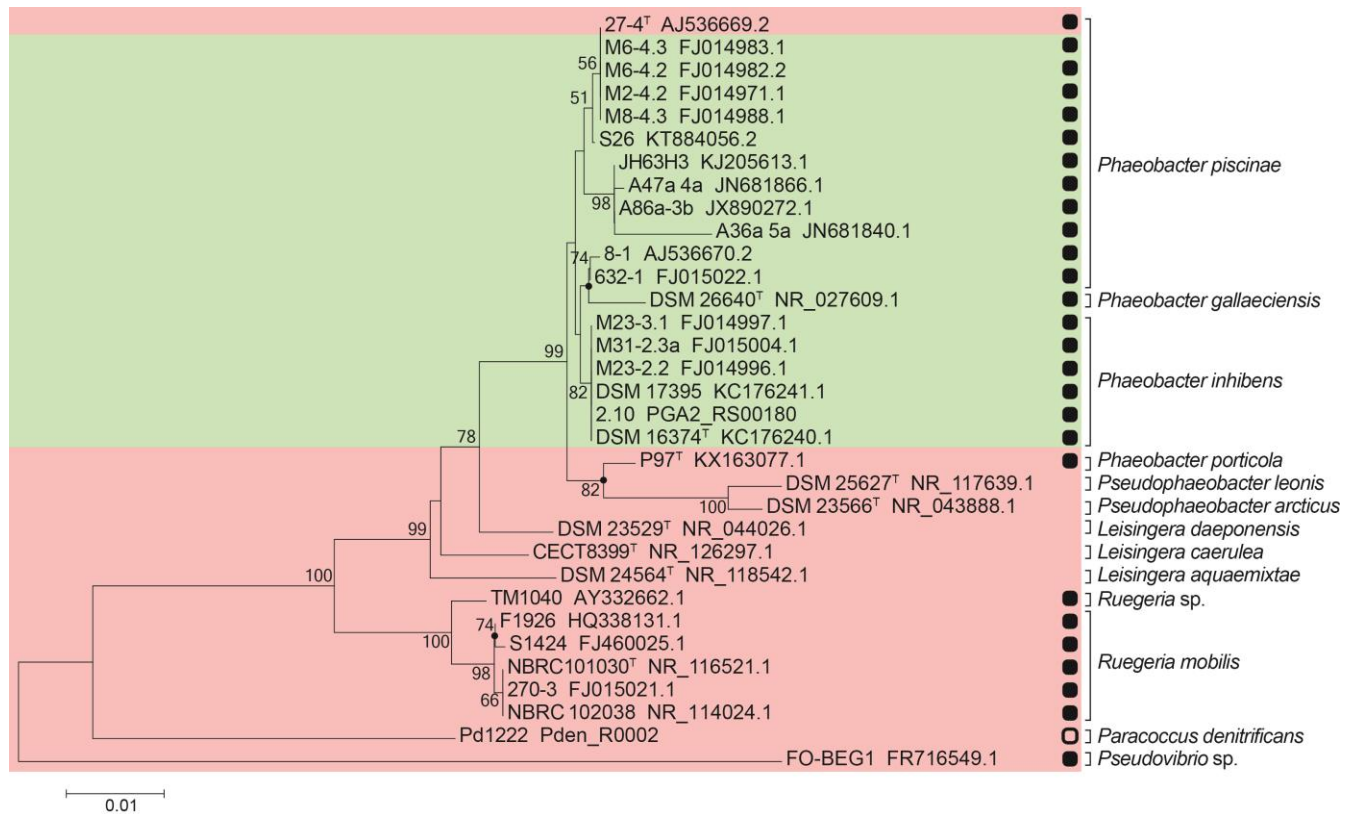
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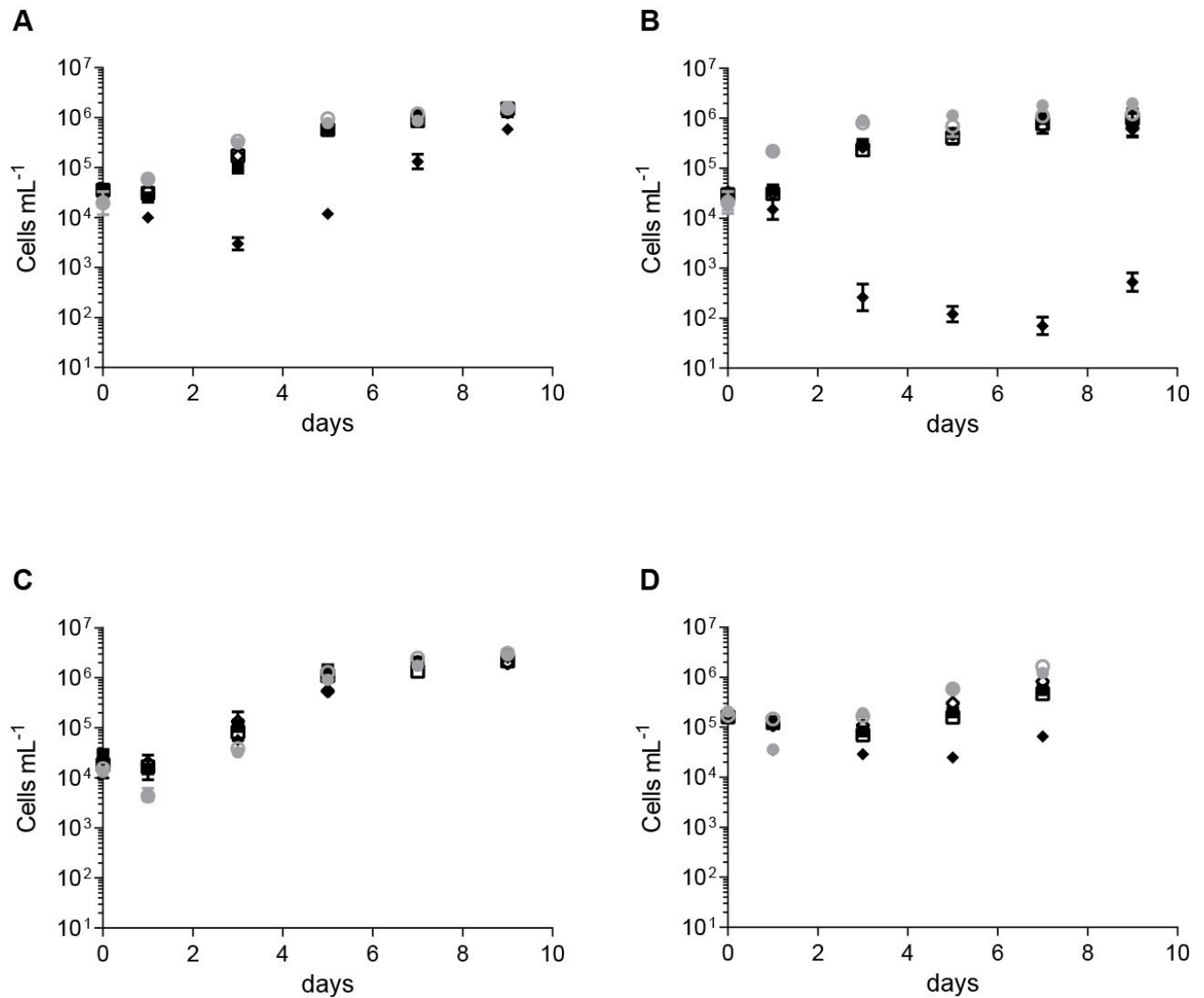
519 **Figures and legends**



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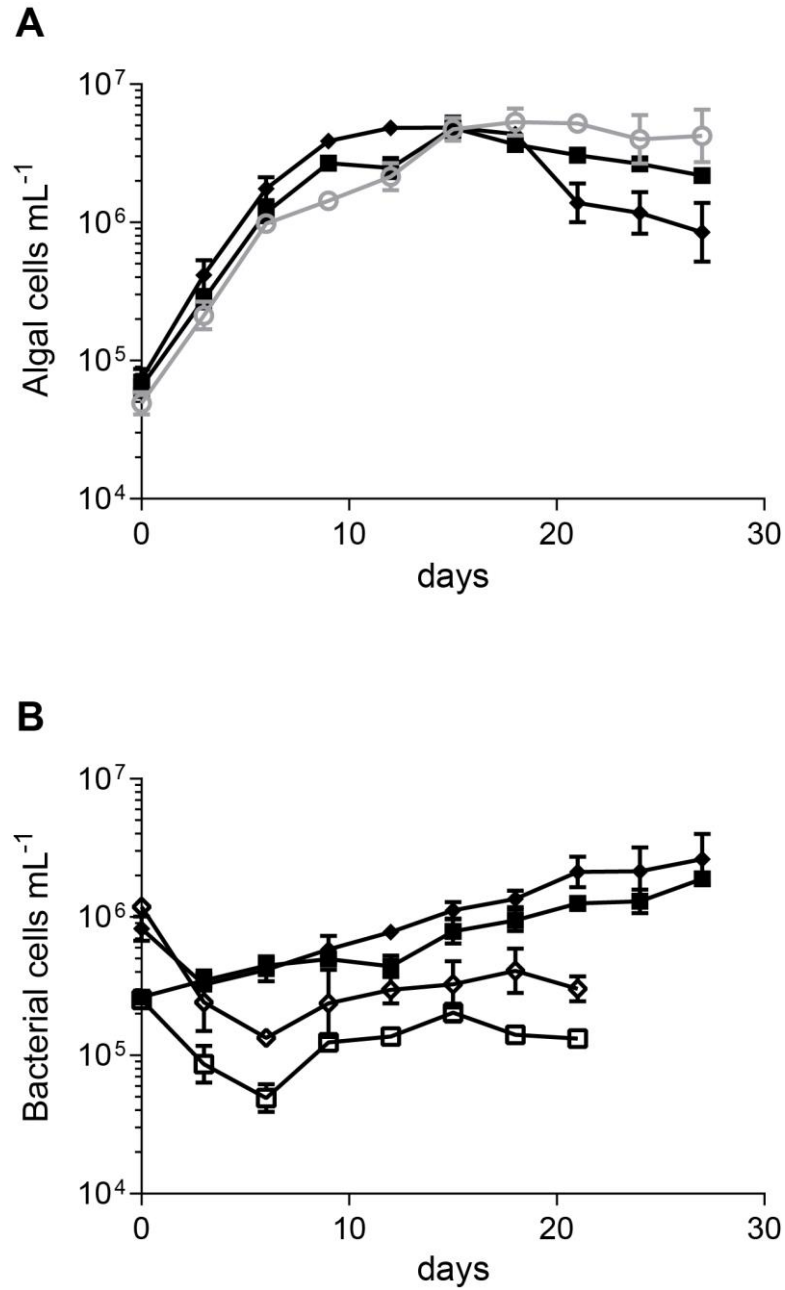
521 **Figure 1.** Neighbor-joining (NJ) tree of 16S rRNA gene sequences of *Roseobacter* group  
 522 strains tested for production of roseobacticide B. The NJ analysis was performed using the  
 523 bootstrap test with 1000 replicates. Support values (percentages) from 1000 bootstrap  
 524 replicates 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.





529

530 **Figure 2.** Bioactivity of *Phaeobacter* extracts against the microalgae A) *Rhodomonas*  
531 *salina*, B) *Thalassiosira pseudonana*, C) *Tetraselmis suecica* and D) *Emiliana huxleyi*. The  
532 extracts had a final concentration of 10% in A)-C) and 1% in D); percentage is given in  
533 regard to the original bacterial culture. ●: no addition, ○: methanol, ◆: DSM 17395 wildtype,  
534 ◇: DSM 17395 *tdaB::Tn5*, ■: 27-4 wildtype, □: 27-4 *tdaB::Tn5*. Points are means of three  
535 replicates and error bars are standard deviations of the mean, except for D), which is one  
536 replicate.



537

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

539 *Emiliana 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.

542

543

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

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

<i>Pseudophaeobacter leonis</i>	DSM 25627 <sup>T</sup>	sediment, Mediterranean Sea	42.6933	3.8416	2008	-	-	na	(Gaboyer <i>et al.</i> , 2013)
<i>Pseudovibrio</i> sp.	FO-BEG1	coral reef, Florida, USA	na	na	<2011	-	+	CP003147.1-48.1	(Brock and Schulz-Vogt, 2011)
<i>Ruegeria mobilis</i>	270-3	turbot farm, Spain	na	na	<2004	-	+	LNXY01	(Hjelm, Bergh, <i>et al.</i> , 2004)
<i>Ruegeria mobilis</i>	F1926	Indian Ocean	-31.4061	91.1776	2006	-	+	CP015230.1-34.1	(Gram <i>et al.</i> , 2010)
<i>Ruegeria mobilis</i>	NBRC101030 <sup>T</sup>	glass slide exposed to seawater, Ishigaki Island, Japan	na	na	1990	-	+	LNWY01	(Muramatsu <i>et al.</i> , 2007)
<i>Ruegeria mobilis</i>	NBRC102038	seawater, Sargasso sea	na	na	<2007	-	+	LNWZ01	(Lee <i>et al.</i> , 2007)
<i>Ruegeria mobilis</i>	S1424	seawater, South Atlantic Ocean	-27.6162	15.1324	2006	-	+	LNXB01	(Gram <i>et al.</i> , 2010)
<i>Ruegeria</i> sp.	TM1040*	dinoflagellate culture	na	na	<2004	-	+	CP000375.1-77.1	(Miller <i>et al.</i> , 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

	<b>Locus tag in DSM 17395</b>	<b>Description</b>	<b>GO classification</b>
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**

	<b>Locus tag in 27-4</b>	<b>Description</b>	<b>GO classification</b>
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	-