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1 **Genetic Diversity and Phenotypic Plasticity of AHL Mediated Quorum**
2 **Sensing in Environmental Strains of *Vibrio mediterranei***

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25 **ABSTRACT**

26 *N*-Acyl-Homoserine Lactone (AHL) mediated Quorum Sensing (QS) is one of the most
27 studied social behavior among *Proteobacteria*. However, despite the current knowledge on
28 QS-associated phenotypes such as bioluminescence, biofilm formation or pathogenesis, the
29 characterization of environmental factors driving QS in realistic ecological settings remains
30 scarce. We investigated the dynamic of AHL and AHL-producing *Vibrio* among 840 strains
31 isolated fortnightly from the Salses-Leucate Mediterranean lagoon in Spring and Summer
32 2015 and 2016. *Vibrio* isolates were identified by *gyrB* gene and genome sequencing and
33 AHL production was investigated by a biosensors-based UHPLC-HRMS/MS approach. Our
34 results revealed, for the first time, a succession of *V. mediterranei* isolates with different
35 AHL-production phenotypes over time and this dynamic lies with a single genotype (Average
36 genomic Nucleotide Identity (ANI) > 99.9). A multivariate DistLM analysis revealed that the
37 temporal variation of *V. mediterranei* QS phenotypes was explained by environmental
38 variables at 83.4%. Overall, our results suggest that isolates of a single genotype are able to
39 change their QS phenotypes in response to environmental conditions, highlighting phenotypic
40 plasticity of bacterial communication in the environment.

41

42 INTRODUCTION

43 Bacterial communities inhabiting marine coastal areas are subjected to rapid and somewhat
44 unpredictable changes in their environment (Lennon and Jones, 2011). In order to cope with
45 these changes, bacteria implement flexible strategies supporting a certain degree of
46 phenotypic plasticity (Agrawal, 2001; West-Eberhard, 2003; Chevin *et al.*, 2010). *Vibrio* are
47 well-known for their capacity of rapid physiological adaptation in response to changing
48 environmental conditions, making them highly dynamic over short-term and seasonal scales
49 (Takemura *et al.*, 2014). These opportunistic bacteria are sensitive to unfavorable conditions
50 by switching from an active free living state into a “dormant” viable but not culturable
51 (VBNC) phenotype, or by colonizing viscous surfaces such as biofilms (Whitesides and
52 Oliver, 1997; Li *et al.*, 2014; Chimetto Tonon *et al.*, 2015; Vezzulli *et al.*, 2015). When
53 favorable conditions reappear their high reactivity allow them to colonize new substrate and
54 to be part of the microbial community associated with zooplankton, phytoplankton, and
55 marine vertebrates and invertebrates (Heidelberg *et al.*, 2002; Ben-Haim, 2003; Soto *et al.*,
56 2009; Wendling and Wegner, 2015).

57 In the past decades, our vision of bacterial communities has significantly changed and
58 microbial cells are no longer considered to behave individually but rather to act socially (West
59 *et al.*, 2006, 2007; Goo *et al.*, 2015). It is now clear that microorganisms perform social
60 behaviors, synchronizing the expression of functional genes at high cell-density by sensing
61 their surrounding environment. This mechanism is generally known as Quorum sensing (QS)
62 (Fuqua *et al.*, 1994) and Type 1 auto-inducers (AI-1) also called *N*-acyl-homoserine lactones
63 (AHL) are important signal molecules for the communication between close relative members
64 of *Proteobacteria*. Among *Vibrio* species, most of the studies are highlighting the processes
65 controlled by QS, i.e. QS-associated phenotypes, such as bioluminescence and symbiosis
66 (Bassler *et al.*, 1994; Schwartzman and Ruby, 2015), biofilm formation (Hammer and Bassler,

67 2003), toxin production or expression of virulence factors (Zhu *et al.*, 2002; Natrah *et al.*,
68 2011; Ha *et al.*, 2014). *In vitro* studies have demonstrated the involvement of QS in the
69 “resuscitation” of viable but non-culturable (VBNC) *Vibrio* cells (Bari *et al.*, 2013;
70 Ayrapetyan *et al.*, 2014). However, Platt and Fuqua (2010) presented the QS process itself
71 (i.e. the production of signal molecules), as impacted by multiple aspects of natural
72 environments, and in support to this idea, some studies on AHL biosynthetic pathways have
73 suggested that any environmental factor likely to affect the primary metabolism of bacteria
74 can modify his spoken "language" (Hoang *et al.*, 2002; Gould *et al.*, 2006). Finally, several
75 studies have reported heterogeneous AHL Production Phenotypes (APP) among different
76 isolates of a single *Vibrio* species, i.e. the production of different sets of AHLs, according to
77 their origin (Buchholtz *et al.*, 2006; García-Aljaro *et al.*, 2012; Purohit *et al.*, 2013;
78 Rasmussen *et al.*, 2014).

79 Despite all this previous knowledge, the question of whether AHL Production Phenotypes
80 (APP) form coherent populations in the environment, show temporal dynamics, or respond to
81 different environmental conditions, remains largely underexplored. Therefore, in the present
82 study we investigated over a two-year period, the temporal dynamics of AHL-producing
83 *Vibrio* isolates from a French Mediterranean lagoon and evaluated the possible links between
84 phenotypic plasticity of AHL production and environmental conditions.

85

86 **MATERIALS & METHODS**

87 ***Sample collection***

88 A two-years study was carried out during Spring and Summer (April to July) 2015 and
89 2016, at one sampling site in the South basin of the Salses-Leucate lagoon, on the
90 Mediterranean coast of France, (42° 50'54.6" N, 3°00'08.7" E; **Fig S1**). Surface water was

91 collected in sterile bottles and pre-filtered through a 50 µm mesh. Plankton was collected
92 using a 5 min horizontal tow at 2 knots of a 50 µm plankton net (30 cm diameter, 1 m length,
93 500 mL cod-end), and all samples were processed within three hours. For each sampling date,
94 triplicate plankton aliquots of 15 mL were fixed with 4% neutral Lugol's solution (0.68 %
95 potassium iodide and 0.34 % iodine in distilled water) and stored in the dark at 4°C.
96 Taxonomic identification and enumeration were performed within two months. Surface
97 temperature (°C), salinity (psu), dissolved oxygen (DO, % saturation), conductivity (mS/cm)
98 and pH were recorded at each sampling date using a HQ40d multiparametric profiler (Hach).
99 Physico-chemical parameters, as well as pigments were analyzed using standard methods (the
100 methodological details can be found in **Table 1**).

101 *Large phytoplankton and picoplankton counts*

102 Phytoplankton counts were carried out with an Olympus IMT inverted microscope in
103 5, 10, 25 or 50 ml plate chambers using the Utermöhl method updated by Karlson and
104 collaborators (Karlson *et al.*, 2010). Replicates of different volumes were counted according
105 to the size and the abundance of the cells. Small sized species were mainly counted in
106 diagonal transects at 300x magnification and for the large or less abundant species half or
107 whole chambers were scanned at 100x and 40x magnification. A minimum of one hundred
108 total cells were counted for each enumeration. Different settling times were applied according
109 to chamber volume in a temperature-controlled room (Karlson *et al.*, 2010). For picoplankton
110 enumeration, seawater samples were collected (1.5 ml in triplicate fixed on board with 1%
111 glutaraldehyde and then quickly frozen at -80°C). The picoplankton abundances, including
112 cyanobacteria (mainly represented by *Synechococcus*), pico- and nano-eukaryotes, and
113 cryptophytes were measured with a FACSCanto II (Becton Dickinson) flow cytometer. The
114 analyses were performed within one month after the sampling. Heterotrophic bacterial cells
115 were stained with SYBR-Green I (Molecular Probes) before flow cytometry analysis.

116 Reference beads (Fluoresbrite YG Microspheres, calibration grade 1.00 μm and 10.00 μm ,
117 PolySciences, Inc) were added to each sample before acquiring the data. Each planktonic
118 group was analysed according to Marie *et al.* (2014) with the Cell Quest Pro software (Becton
119 Dickinson), in logarithmic mode, to separate the populations based on their scattering and
120 fluorescence signals.

121 ***Vibrio* isolation and characterization**

122 For the enumeration of *Vibrio*, water samples (0.1, 1, 10 mL) were filtered onto 0.45
123 μm pore-size nitrocellulose filters (Millipore, 47 mm), plated on the selective medium
124 thiosulfate-citrate–bile–sucrose (TCBS) (Pfeffer and Oliver, 2003) and incubated 24h at 20°C.
125 *Vibrio* strains were isolated from two fractions: less than 50 μm assumed to mostly represent
126 *Vibrio* in the water column and greater than 50 μm corresponding large phytoplankton and
127 zooplankton-associated *Vibrio*. The <50 μm fraction consisted of 20 L of 50 μm prefiltered
128 water further concentrated using a hollow fiber filter HF80S (Hemoflow, Fresenius Medical
129 Care). The concentrate was back-washed with 500 mL of a wash solution (0.01% sodium
130 hexametaphosphate, and 0.5% Tween 80; Sigma-Aldrich). The >50 μm fraction consisted of a
131 plankton net tow concentrate splited into five subsamples of 15 mL and homogenized by
132 gentle sonication for 5 min in order to unbind attached bacteria. For both fractions, a ten-fold
133 serial dilution was made in artificial seawater and 100 μL of each was plated in triplicate on
134 TCBS agar. Thirty colonies by fraction and by sampling date were randomly picked using a
135 gridded Petri dish.

136 ***Isolate identification***

137 A total of 840 isolates were identified based on *gyrB* gene sequences. The PCR was
138 performed using colony cells and amplification reactions consisted of 12.5 μL of Kappa2G
139 Master Mix (KAPA2G Fast Hotstart ReadyMix PCR kit, KapaBiosystems), 6.25 μL of 10

140 mM MgCl₂ and 1.25 μL of each of universal *gyrB* primers UPIE
141 (5'GAAGTCATCATGACCGTTCTGCA YGCNGGNGGNAARTTYRA 3') and UP2AR
142 (3'AGCAGGGTACGGATGTGCGAGCCRTC NACRTCNGCRTCNGYCAT 5') (Le Roux *et*
143 *al.*, 2004). The PCR conditions were slightly optimized for the KAPA2G Fast DNA
144 Polymerase and were as following: a 5 min initial denaturation step at 95 °C, followed by 40
145 cycles at 95 °C for 15 sec, 60 °C for 30 s and 72 °C for 30 s, and a final elongation step of 2
146 min at 72 °C. PCR products were sent to Macrogen Europe for sequencing using the dideoxy-
147 termination (Sanger) reaction with the primer *gyrB* UP1S (5'GAAGTCATCATGACC-
148 GTTCTGCA3') (Yamamoto and Harayama, 1995) to obtain sequences around 800 bp length.

149 ***Detection of QS-active isolates***

150 The methodological approach for the screening of AHL producers and the
151 identification of AHL compounds by Ultra High Performance Liquid Chromatography
152 coupled with High Resolution tandem Mass spectrometry UHPLC-HRMS/MS were strictly
153 identical to those previously described in Girard *et al.*, (2017). Briefly, two biosensors were
154 used, *Pseudomonas putida* (pKR-C12) for long acyl-side chained AHLs (C8-C18) and
155 *Escherichia coli* (pJBA-132) for short acyl-side chained AHLs (C6-C10) (Girard *et al.*, 2017).
156 Isolates were tested, in triplicate, on each biosensor immediately after isolation from the
157 environmental samples and also before mass spectrometry analysis.

158 ***Genetic and genomic characterization of V. mediterranei isolates***

159 The genetic diversity of *V. mediterranei* isolates was evaluated by ERIC-PCR (Rivera
160 *et al.*, 1995). Briefly, genomic DNA from 253 isolates was obtained by cellular lysis (3 quick
161 cycles of liquid nitrogen freeze/70°C thaw) and DNA concentrations were measured
162 spectrophotometrically in order to dilute each lysate at 100 ng/μL. The ERIC-PCR reactions
163 were performed using previously described protocols employing the ERIC1 and ERIC2

164 primers (Hulton *et al.*, 1991; Khan *et al.*, 2002). PCR amplifications were performed with the
165 GoTaq G2 Flexi polymerase (Promega) and fingerprinting profiles were visualized using a
166 Typhoon FLA 9000 imager (GE Life Sciences). Gel images analysis were performed using
167 the GelJ software (Heras *et al.*, 2015). For whole genome sequencing, genomic DNA of two
168 *V. mediterranei* isolates (17 LN0615E and 21 LS0615E) was obtained with a classical CTAB-
169 β -mercaptoethanol extraction protocol and sent for DNA sequencing (Illumina MiSeq) by Mr
170 DNA (Shallowater, TX, USA) as described by Doberva *et al.*, (2014). Genomes were
171 automatically annotated using the RAST server (Aziz *et al.*, 2008).

172 ***Multivariate analyses***

173 A preliminary PERMANOVA analysis (Anderson, 2001a) was used to test for
174 significant differences among *Vibrio* assemblages and *V. mediterranei* APP between the two
175 size fractions ($<50\mu\text{m}$ and $> 50\mu\text{m}$). As the results indicated that there were no significant
176 differences between the two size fractions the remainder of the analysis was then performed
177 by pooling the counts from both fractions. The relationships between G1 *V. mediterranei*
178 assemblage (i.e. the abundance of each AHL production phenotypes over time, Square-root
179 transformed and Bray-Curtis distance matrix) and all measured environmental variables
180 (Normalization and Euclidean distance matrix) were investigated using a Distance Based
181 Linear Model (DISTLM) (McArdle and Anderson, 2001). In order to visualize if the temporal
182 variations of G1 phenotypes co-occurred with specific phytoplankton taxa, we conducted a
183 second step of analysis consisting of a Principal Component Ordination (PCO) of G1
184 phenotypes using phytoplankton counts (Square-root transformed and Bray-Curtis distance
185 matrix) as predictor variables (non-parametric Spearman rank correlation). All the
186 multivariate analyses were performed using PRIMER v.7 and its add-on package
187 PERMANOVA + (Anderson, 2001a; Anderson, 2001b; McArdle and Anderson, 2001).

188 RESULTS

189 *Phenotypic and Genetic temporal variation of V. mediterranei isolates*

190 The partitioning of *Vibrio* isolates, between the water column (<50 µm) and plankton
191 (>50 µm), was monitored during spring and summer 2015/2016 and a total of 840 isolates
192 were identified as *Vibrio* spp. We did not observe differences in *Vibrio* assemblages between
193 the fractions over time (data not shown, PERMANOVA, *p* value = 0.904; the temporal
194 variation of the total *Vibrio* spp. can be found in **Fig S2**). Isolates identification, based on *gyrB*
195 gene sequences, from both size fractions, showed a seasonal dynamic of *V. mediterranei*
196 isolates (**Figure 1**) in particular for 2016, starting from 0-28% of total *Vibrio* isolates during
197 spring and increasing gradually to up to 97% (Summer 2016). Interestingly, the phenotyping
198 of these isolates, under identical culture and screening conditions, revealed a temporal
199 variation of AHL production phenotypes (APPs) among time. APPs varied for example from
200 strains producing only long chain AHLs (F117⁺/MT102⁻) to those producing long and short
201 chain AHLs (F117⁺/MT102⁺) in 2015, or from a predominance of isolates exhibiting no
202 production (F117⁻/MT102⁻) to an exclusive production of long chain AHLs (F117⁺/MT102⁻) in
203 2016 (**Figure 1B**).

204 In order to determine if these phenotypic changes were related to a shift between
205 different genotypes of *V. mediterranei*, we used ERIC-PCR (Rivera *et al.*, 1995) to genotype
206 253 *V. mediterranei* strains. A total of 22 genotypes were identified, G1 to G22 (i.e. **Figure**
207 **1A** and **Supplementary Figure 3**) and remarkably, 83% of the *V. mediterranei* isolates, over
208 the two years period, belonged to Genotype 1 (G1). To implement the genetic characterization
209 of *V. mediterranei* G1 isolates and to better understand the genetic basis of this phenotypic
210 variations, we obtained a draft whole genome sequence for two strains with different
211 phenotypes (17LN 0615E with F117⁺/MT102⁻ phenotype and 21LN 0615E with

212 F117⁺/MT102⁺ phenotype, NCBI accession numbers NZ_NWTN00000000 and
213 NZ_NWTO00000000, respectively). A comparison between these genomes yielded an ANI
214 value of 99.96%. Since, as previously proposed, two strains can be considered identical if they
215 have a ANI value >99.9% (Snitkin *et al.*, 2012; Olm *et al.*, 2017), we consider that we
216 observed a phenotypic plasticity of AHL production within a single strain of *V. mediterranei*.
217 To summarize, we observed in 2016, the predominance of a single strain of *V. mediterranei*,
218 up to 97% of the total cultivable *Vibrio* spp., showing different APPs over time.

219 ***AHL characterization of G1 V. mediterranei APP***

220 In order to confirm that the observed APPs indeed corresponded to different AHL
221 patterns, we analyzed the produced AHLs among two representative isolates of each
222 phenotype (F117⁺/MT102⁻) and (F117⁺/MT102⁺) by UHPLC-HRMS/MS. AHL
223 characterization was carried out according to the procedure described in Girard *et al.*, 2017,
224 briefly, “anticipated” AHLs were identified by comparison with their corresponding standards
225 (**Table S8**) while “unanticipated” AHLs, without commercially available standards, were
226 identified based on their fragmentation patterns, predicted retention time and molecular weight
227 (i.e. **Table S6 and S7**). A total of 13 AHLs were detected and 8 were common to the four
228 isolates, namely OH-C10-HSL, oxo-C10-HSL, OH-C11-HSL, OH-C12-HSL, oxo-C12-HSL,
229 C12-HSL, C13-HSL and oxo-C13-HSL (**Fig. 2** and **Figure S4**). Three short chain AHLs, C6-
230 HSL, oxo-C6-HSL, and oxo-C8-HSL were putatively identified as being responsible for the
231 F117⁺ /MT102⁺ phenotype. Interestingly, we observed that two long chain AHLs, oxo-C11-
232 HSL and C14-HSL, were produced by the (F117⁺/MT102⁻) isolates but were not detected in
233 the (F117⁺/MT102⁺) isolates. Summarizing, the AHL analysis confirmed that the different
234 APPs detected by the biosensors are producing different AHLs patterns, with (F117⁺/MT102⁻)
235 producing long acyl-side chains and (F117⁺/MT102⁺) producing long plus short acyl-side
236 chains AHLs.

237 *Genetic basis of AHL production*

238 One possibility to explain these different APPs with highly similar genomes would be
239 that these differences were carried by different AHL synthase genes. Thus, we searched for
240 AHL synthase genes in the two genomes of *V. mediterranei* G1 strains and a LuxI/LuxR QS
241 system was found. However, as it has been shown that some *Vibrio* species can harbor two
242 AHL production pathways (LuxI/R and LuxM/N) we also searched for other putative *N*-
243 acetyltransferases in our two genomes. The comparison between the two strains (17 LN0615E
244 and 21 LS0615E) revealed a 100% protein identity for the AHL synthase LuxI (accession
245 numbers PRQ68803.1 and PCD89229.1, respectively) but also for the 15 putative *N*-
246 acetyltransferases (**Table S9**). Altogether these results suggest that the differences observed in
247 terms of AHL production patterns are not explained by different presumptive AHL synthases.

248 *Factors affecting the dynamic of G1 - AHL production phenotypes*

249 To investigate the relationships between the dynamic of the APPs among G1 strains
250 and the environmental variables we conducted a distance based linear modeling approach
251 (DistLM). A total of 7 variables explained 83.4% of the temporal variation of G1 APPs, and
252 among those, phosphates concentration was the variable with the highest explanatory value
253 (37.4 %) and ammonium concentration the lowest with 4.5% (**Figure 3** and sequential tests,
254 **Table 2**). Axis 1 (db RDA1) represented essentially a gradient in abundance of the genotype
255 varying from high abundances at the left-end to low abundances or no counts at the right-end
256 (July 2015 and April/May 2016). Samples with high counts of *V. mediterranei* G1 were
257 associated with higher conductivity (an integrated measure of higher temperature and salinity),
258 total dissolved nitrogen, and low phaeophytin concentrations, while an inverted trend was
259 observed for samples with low counts or where *V. mediterranei* G1 was not retrieved. Axis 2
260 (db RDA2) reflected the distribution of the different APPs. A peak of phosphates and nitrites

261 were associated with an increase of non AHL producers in the higher end of the axis. As the
262 (F117⁺/MT102⁻) phenotype was dominant at dates with high counts of G1 isolates, the same
263 parameters are linked to counts of the (F117⁺/MT102⁻) phenotype at the lower end of db
264 RDA2 axis.

265 A principal coordinates analysis (PCO) was performed in order to visualize which
266 phytoplankton species co-occurred with the different APP (**Fig 4**). As observed in the db
267 RDA1, Axis 1 (PCO1) reflected the gradient in G1 abundances among the sampling dates with
268 the lowest counts of G1 in the right-end (July 2015 and April/May 2016) and where
269 *picoeukaryotes*, *Cryptophyta*, unidentified *nanoeukaryotes*, *Dinophysis* sp. and *Prorocentrum*
270 *micans* were present in their highest abundances. In the left-end of PCO axis 1 were samples
271 with the highest G1 counts (June 2015 and June/July 2016) associated to the highest counts of
272 *Grammatophora* sp. and *Scrippsiella* sp. and bacteria abundances. Axis 2 (PCO2) mostly
273 separates dates based on the presence of *V. mediterranei* G1 AHL producers and non-
274 producers. While *Gonyaulax* sp., *Amylax* sp., seemed to be associated to dates with the highest
275 counts of non-producers (2016/06/21), *Cyanobacteria* and *Pyrophacus* sp. abundances were
276 linked to AHL producers.

277 **Discussion**

278 It is well known that the ability of *Vibrio*, as well of other gram negative bacteria, to
279 survive by making biofilms, to infect hosts, to modify their metabolism or to enter into a
280 VBNC state, is modulated by QS (Ayrapetyan *et al.*, 2014; Bondí *et al.*, 2014; Goo *et al.*,
281 2015; Persat *et al.*, 2015). In addition to the fact that QS allows bacteria to sense their number
282 and coordinate their actions, the variability of auto-inducers production might offer an
283 advantageous response to adapt their actions according to environmental fluctuations.

284 In order to survive in changing environment, bacteria have evolved to set up various
285 adaptive responses. Phenotypic plasticity is one of them and it reflects the direct influence of
286 the environment on the development of individual phenotypes, in other words, bacteria can
287 adapt to environmental selective pressures to maintain their own fitness. Phenotypic diversity
288 of QS systems has already been described for different strains of a single *Vibrio* species
289 (Buchholtz *et al.*, 2006; Joelsson *et al.*, 2006; García-Aljaro *et al.*, 2012; Purohit *et al.*, 2013)
290 and temporal changes in AHLs production during the development of natural biofilm
291 including *Vibrio* species as been shown (Huang *et al.*, 2009). However, the scope of these
292 earlier studies did not show clear evidence of QS phenotypic plasticity. Our results show a
293 clear short-term temporal variation in APPs among environmental isolates of a single *Vibrio*
294 species (*V. mediterranei*) that were remarkably grouped in a single genotype (G1) based on
295 *gyrB* sequences, ERIC-PCR and genomic analysis. This change of AHL production patterns,
296 was temporally coherent and in some cases reached the near totality of *Vibrio* isolates in a
297 sample. As the ANI value among the two sequenced genomes was very high (99.96%) but not
298 identical, it is always possible that the residual 0.04% would be responsible for the differences
299 of APPs by some unknown mechanism. Nonetheless, the *luxI* sequences, coding for the AHL
300 synthase, as well as other putative *N*- acetyltransferases sequences are identical.

301 While it appears that we have different APPs belonging to a single genotype, the
302 temporal sampling of *Vibrio* isolates in parallel to physicochemical parameters measurements
303 allowed us to evaluate the variation in the “environment” leading to the phenotypic plasticity
304 of AHL production. However, as the sampling was originally designed to evaluate the
305 diversity of total *Vibrio* spp. (and not just that of *V. mediterranei* G1) the total numbers of
306 evaluated strains are limited, and thus in the following discussion we point to major trends and
307 propose ideas to be tested by more targeted studies.

308 Based on our DistLM analysis, phosphates concentration appeared as the most
309 explanatory variable by being correlated with high abundances of non AHL producers. That
310 was particularly striking on 21 June 2016, where the near totality of the strains belonged to the
311 (F117⁻/MT102⁻) phenotype which coincided with a peak of phosphates likely associated with
312 sediment resuspension. In order to explain this potential link between non-AHL producers and
313 high phosphates concentrations, we propose a possible implication of the polyphosphate
314 kinase activity. The whole genome analysis of the G1 isolates revealed few copies of the *ppk*
315 gene and several studies have demonstrated that polyphosphate kinase (*ppk*) enhance the
316 ability of bacteria to survive under environmental stresses. Since this enzyme has a negative
317 impact on AHLs production in *P. fluorescens* and is repressed at high phosphates
318 concentrations (Rashid *et al.*, 2000; Jahid *et al.*, 2006; Silby *et al.*, 2009), it is tempting to link
319 the lack of AHL production to a repression of *ppk* by phosphates. As the peak of non AHL
320 producers, the 2016/06/21, occurred at the highest phosphates concentration in the two years
321 sampling, we hypothesized that in this context there is low expression of *ppk* gene and down
322 regulation of AHL production (Rashid *et al.*, 2000).

323 The Salses-Leucate lagoon was also characterized by high *Cyanobacteria*
324 concentrations, mainly *Synechococcus* species, ranging between 3.86×10^5 to 2.88×10^8
325 cells/L, making them an important part of the microbial community. Over the study,
326 *Cyanobacteria* counts were highly correlated to the (FF17⁺/MT102⁺) phenotype abundances
327 (Pearson correlation 77.3%; *p* value = 0.002; June/July 2015) but also to the peak of the
328 (F117⁺/MT102⁻) phenotype (July 2016, **Figure 4**). In the past, *Vibrio* species have been
329 associated to cyanobacterial blooms (Berg *et al.*, 2009), especially by benefiting from their
330 derived organic matter (Eiler *et al.*, 2007). Mutual interactions were described between
331 *Synechococcus* species and heterotrophic bacteria (Hayashi *et al.*, 2011), and co-culture
332 experiments between *Vibrio* species and *Synechococcus* revealed a deleterious effect on the

333 cyanobacteria physiology especially on iron, phosphates and nitrogen pathways (Tai *et al.*,
334 2009). In the other hand, since *Synechococcus* species harbors “orphan” *luxR* genes, possibly
335 encoding for the receptor LuxR involved in AHL signal reception, and shows quorum
336 quenching activity against *Vibrio* species, complex QS-based relationships might occur
337 between these two genera (Yoshino *et al.*, 2013; Honda *et al.*, 2014; Marsan *et al.*, 2014;
338 Santhakumari *et al.*, 2016; Shimura *et al.*, 2017). Thereby, the temporal dynamic of different
339 APPs can be related to changes in the cyanobacterial assemblages, switching between
340 commensal/consumers, probably benefitting from cyanobacterial dissolved organic matter and
341 competitors for inorganic nutrient such as phosphates, nitrogen and iron. Understanding the *in*
342 *situ* interactions between APPs of *V. mediterranei* and cyanobacterial assemblages remains an
343 interesting subject for future studies.

344 Overall, our results indicate a phenotypic plasticity of AHL production among isolates
345 of a single genotype and converges towards the fact that our strains are harboring a single
346 AHL synthase gene (*luxI*). The underlying physiological mechanisms leading to this
347 phenotypic plasticity still yet to describe. However, it is well known that AHL synthases are
348 catalyzing the reaction between S-adenosylmethionine (SAM) and acyl-ACPs to produce
349 AHLs (Keller and Surette, 2006) and that the available acyl-ACP pools in bacteria may be
350 susceptible to metabolic changes (Gould *et al.*, 2005). Considering that it has been recognized
351 that AHL production can be altered through the modulation of the fatty acid metabolic
352 pathway and that the variation of APPs was explained at 83.4% by the environmental
353 variables, it seems likely that fluctuations in the environment can change the nature of the
354 produced AHLs (Hoang *et al.*, 2002; Gould *et al.*, 2006). However, the facts that 1) a near
355 totality of the isolates were non-AHL producers at a time point and AHL-producers in a
356 subsequent time point, and 2) that these phenotypes were observed after two rounds of
357 isolation on rich medium, raises the intriguing possibility that epigenetic regulation might be

358 at play. It is well-known that the regulation of phenotypic variation is not always linked to
359 changes in DNA sequence and can be epigenetic in nature (Smits *et al.*, 2006) and
360 interestingly, Kurz *et al.* (2013) have shown that epigenetic mechanisms can be involved in
361 the regulation of AHL-based QS system. We hypothesize that *V. mediterranei* strains could
362 keep a memory of the social traits expressed in the environment. However, as any previous
363 work have been published on the QS mechanisms of *V. mediterranei*, a consequent work on
364 the description of the QS pathways and QS associated phenotypes still need to be achieved to
365 confirm or deny this possible epigenetic control. Regardless of the mechanisms leading to the
366 observed phenotypic variation, phenotypic plasticity should confer an increased fitness as
367 heterogeneous populations should be better to adapt to rapid changes in the environment
368 (Feinberg *et al.*, 2010). The fact that the studied phenotypically plastic *V. mediterranei*
369 populations thrive in a shallow lagoon subject to a relatively fast variation in physical and
370 biogeochemical conditions supports this idea. Understanding the possible mechanisms of
371 regulation of AHL-based QS in *V. mediterranei* remains a fascinating avenue for further
372 studies.

373 Finally, our work highlight the phenotypic plasticity of AHL production among
374 environmental *Vibrio* isolates. These results were obtained on strains after isolation and culture
375 on rich media which underlines the fact that *Vibrio* isolates could retain a memory of *in situ*
376 AHL production status. Considering the fact that quantification of AHL synthases expression
377 by RTqPCR is still difficult due to a very low sequence homology among vibrios (Tait *et al.*,
378 2010), the study of AHL-mediated QS by a biosensors based approach seems an interesting
379 alternative to estimate whether or not AHL synthesis occurred in the environment.

380

381 **Conclusion**

382 QS is a well-studied social trait among *Vibrio* species, however, despite the current
383 knowledge obtained by *in vitro* studies on few species, there is a lack of studies regarding QS
384 in complex ecological communities. Our study provides a first investigation on the seasonal
385 dynamic of AHL producing *Vibrio* in a changing coastal environment and open new
386 perspectives for outgoing studies regarding QS mechanisms in natural habitats and a possible
387 epigenetic regulation of QS phenotypic plasticity.

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399

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634 **Figure 1** Temporal dynamic of *V. mediterranei* isolates relative to all *Vibrio* spp. isolates. **A.**
635 G1-G22 are Genotypes based on ERIC-PCR; **B.** APP: AHL Production Phenotypes; where
636 F117+ and F117- are isolates detectable or not by the biosensor *Pseudomonas putida* (pKR-
637 C12) and MT102+ and MT102- are detectable or not by the biosensor *Escherichia coli*
638 (pJBA-132).

639 **Figure 2** Heat-Map of AHL diversity in *V. mediterranei* G1 isolates by UHPLC-HRMS/MS
640 (Ward's classification based on Jaccard index; Caraux and Pinloche, 2005). **1:** 17LN0615E
641 and **2:** 2LS0615E corresponding to the F117+/MT102+ phenotype; **3:** 21LN0615E and **4:**
642 9LS0615E corresponding to the F117+/MT102- phenotype. (*) unanticipated AHLs
643 corresponding to any analyzed AHL standards. Grey squares represent the absence of AHL.

644 **Figure 3** The dbRDA ordination representing the results of the DistLM analysis in 2D. The
645 DistLM is used to examine the relationship between the distribution and temporal dynamics
646 of *V. mediterranei* G1 APP and all measured environmental variables. Vectors represent
647 predictor variables used for the construction of the constrained ordination (the dbRDA
648 diagram), and the length of the vectors represents the explanatory percentage of each variable.
649 The analysis is detailed **Table 2**.

650 **Figure 4** Principal co-ordinate plot (PCO) of *V. mediterranei* G1 phenotypes for the first and
651 second principal co-ordinates using the Bray-Curtis similarity matrix. Phytoplankton and
652 Bacteria counts were used as predictor variables, are shown here only the variable with
653 Pearson correlation >0.45. The analysis is detailed in **Supplementary Table 4** and the
654 contribution of each variable in the construction of PCO axis (Pearson correlation) is detailed
655 in **Supplementary Table 5**.

656

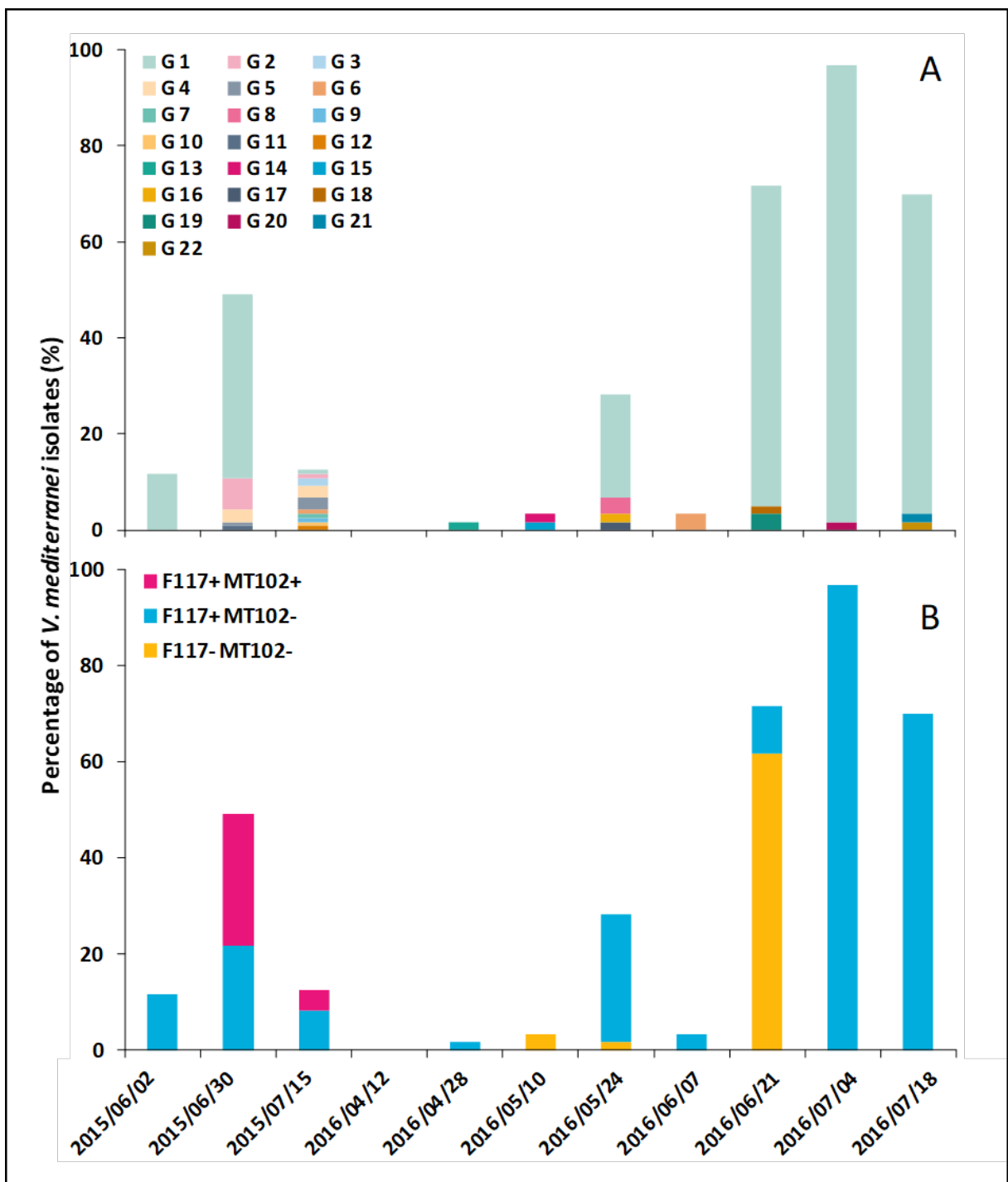


Figure 1

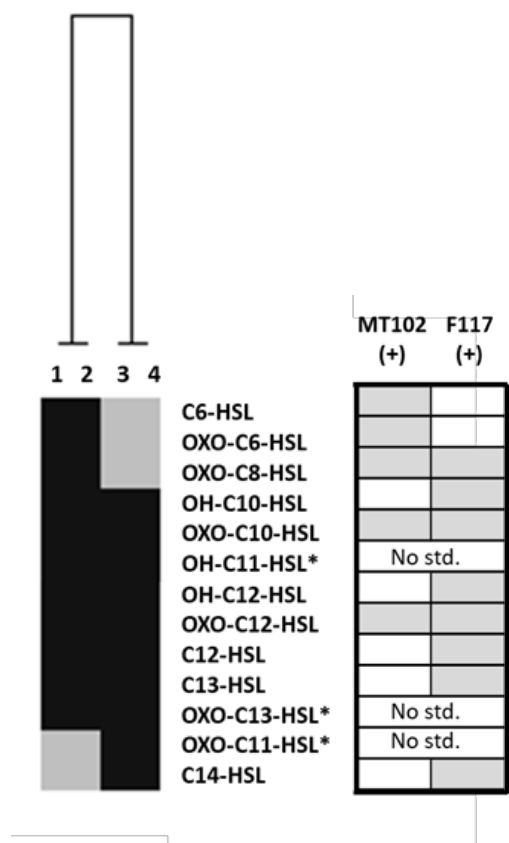


Figure 2

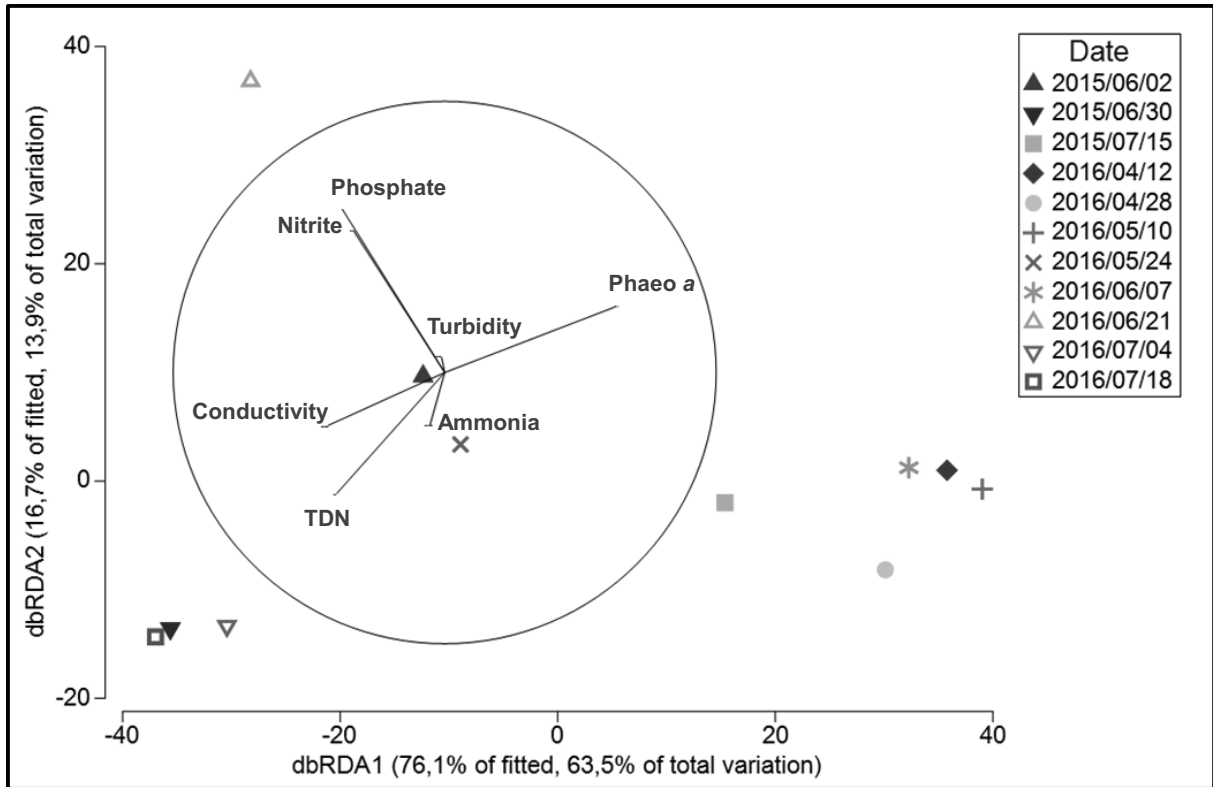


Figure 3.

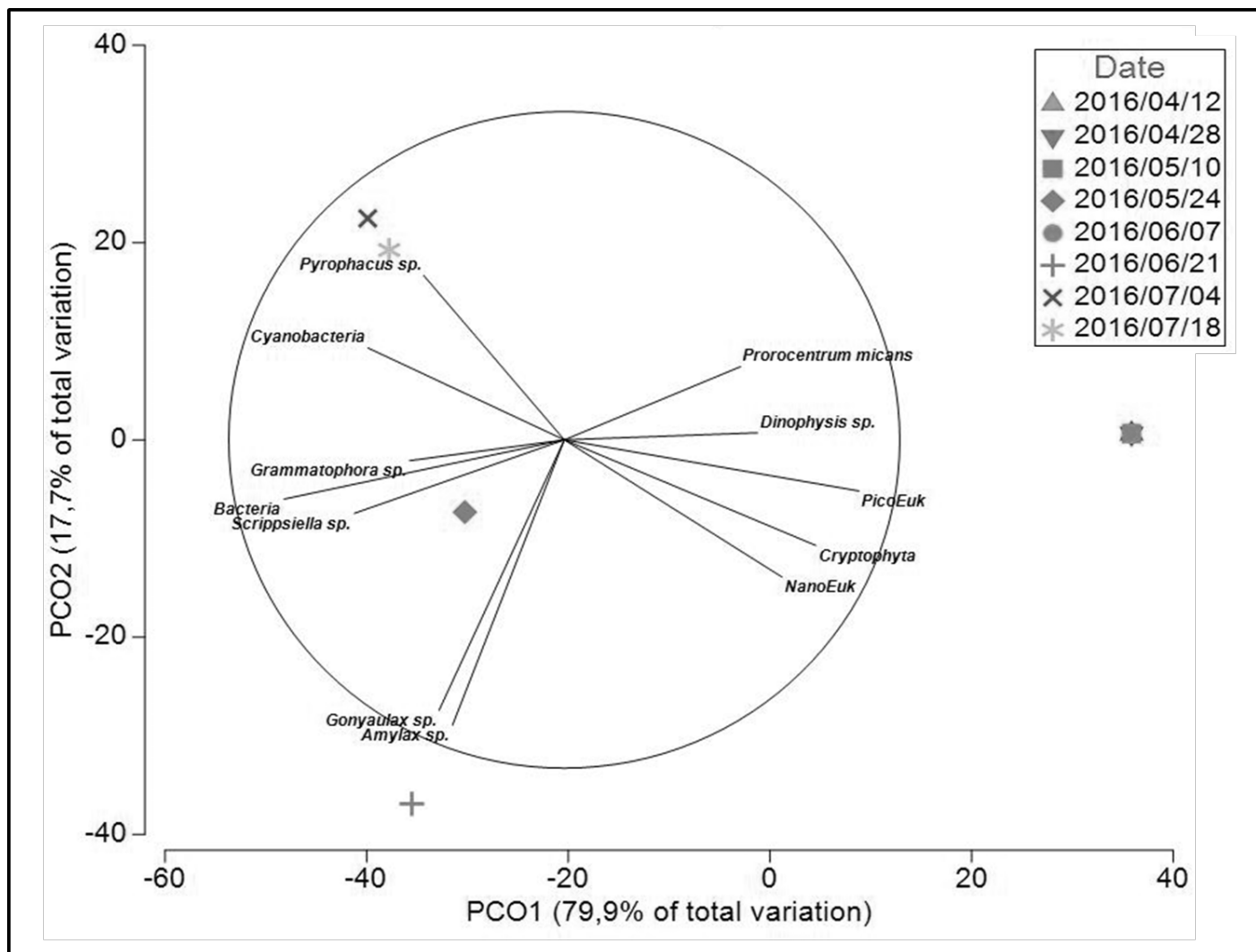


Figure 4.

Table 1 Environmental parameters measurements

Parameter	Methods/Instrumentation	Reference	Unit	Ranges	
				-	+
Temperature			° celcius	14.2	26.4
Salinity			‰	32.8	40.1
Conductivity	Hach HQ40d multi	Al-Bairuty <i>et al.</i> , 2013; Vouvé <i>et al.</i> , 2014	mS/cm	39.9	55.7
DO concentration			mg/L	7.82	10.09
DO saturation			%	91.7	116
pH			*	7.32	8.5
Nitrates			mg/L	1.89	9.26
Nitrites	Hach DR/890 Colorimeter	Aryal <i>et al.</i> , 2012; Vouvé <i>et al.</i> , 2014	mg/L	0.002	0.043
Ammonium			mg/L	<0.03	
Phosphates			mg/L	0.05	0.15
DOC	V _{CSN} /TNM-1 Shimadzu TOC/TN analyzer	Romero <i>et al.</i> , 2013; Vouvé <i>et al.</i> , 2014	mg/L	2.5	6.69
TDN			mg/L	0.15	0.59
POC	Perkin Elmer C,H,N 2400	Rembauville <i>et al.</i> , 2016	mg/L	217.635	453.27
PON			mg/L	36.99	92.40
Chlorophyll <i>a</i>	Lorenzen	Lorenzen, 1966; Lorenzen, 1967	µg/L	0.08	1.23
Phaeophytin <i>a</i>			µg/L	0.021	1.34
Cryptophyta			cells/L	6.74 x 10 ²	1.65 x 10 ⁶
Nanoeukaryote			cells/L	6.13 x 10 ⁵	1.01 x 10 ⁷
Picoeukaryote	FACSCantoII	Marie <i>et al.</i> , 2014	cells/L	1.84 x 10 ⁵	2.55 x 10 ⁷
Cyanobacteria			cells/L	3.86 x 10 ⁵	2.88 x 10 ⁸
Bacteria			cells/L	3.04 x 10 ⁹	2.14 x 10 ¹⁰

Table 2: Test statistics for Distance-based Linear Model (DISTLM) analyses marginal and sequential tests based on ‘Forward’ procedure and AIC criteria of *V. mediterranei* G1 phenotypes abundance at the 11 sampling dates. Marginal tests show how much variation each variable explains when considered alone, ignoring other variables. Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

Marginal Tests							
Variable	AIC	SS (trace)	Pseudo- <i>F</i>	P	Prop.	Cumul.	res.df
Phosphates	-	11291.0	11.953	0.002	0.3741	-	-
Conductivity	-	9146.6	8.696	0.002	0.3030	-	-
TDN	-	2788.2	2.035	0.137	0.0924	-	-
Nitrites	-	4424.8	3.435	0.039	0.1466	-	-
Phaeo <i>a</i>	-	8055.3	7.280	0.006	0.2669	-	-
Turbidity	-	1490.9	1.039	0.322	0.0494	-	-
Ammonia	-	2821.9	2.062	0.138	0.0935	-	-
Sequential tests							
+ Phosphates	152.62	11291.0	11.953	0.001	0.3741	0.3741	20
+ Conductivity	148.16	4810.1	6.490	0.008	0.1594	0.5335	19
+ TDN	145.80	2530.8	3.944	0.014	0.0838	0.6173	18
+ Nitrites	143.97	1846.0	3.234	0.057	0.0612	0.6785	17
+ Phaeo <i>a</i>	141.94	1624.1	3.216	0.052	0.0538	0.7323	16
+ Turbidity	138.66	1723.3	4.066	0.031	0.0571	0.7894	15
+ Ammonia	135.41	1350.8	3.777	0.032	0.0448	0.8341	14

SUPPLEMENTARY INFORMATION



Fig S1: Location of the sampling site within the Salses-Leucate Mediterranean lagoon.

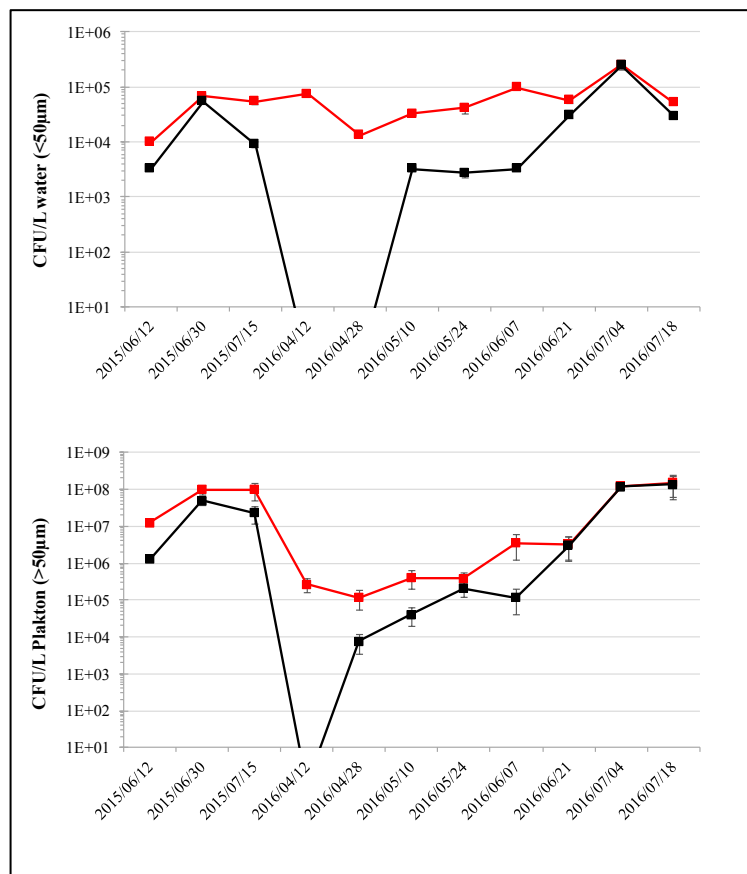
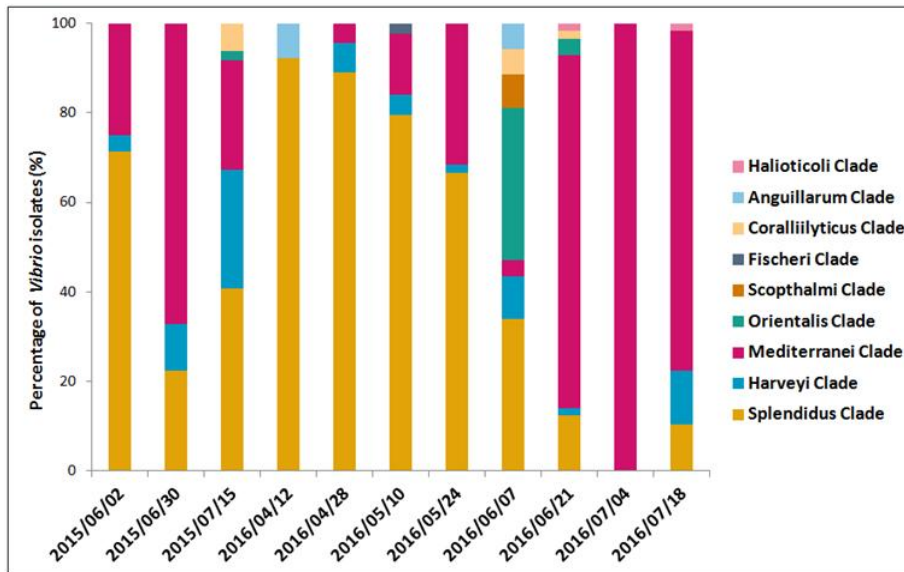


Fig S2: Temporal dynamic of *Vibrio* spp. isolates. (A). Relative proportion of isolates related – clade among the isolates identified as *Vibrio* spp. (B). Abundance in water and plankton of total culturable *Vibrio* spp. (red line) and culturable *V. mediterranei* (black line)

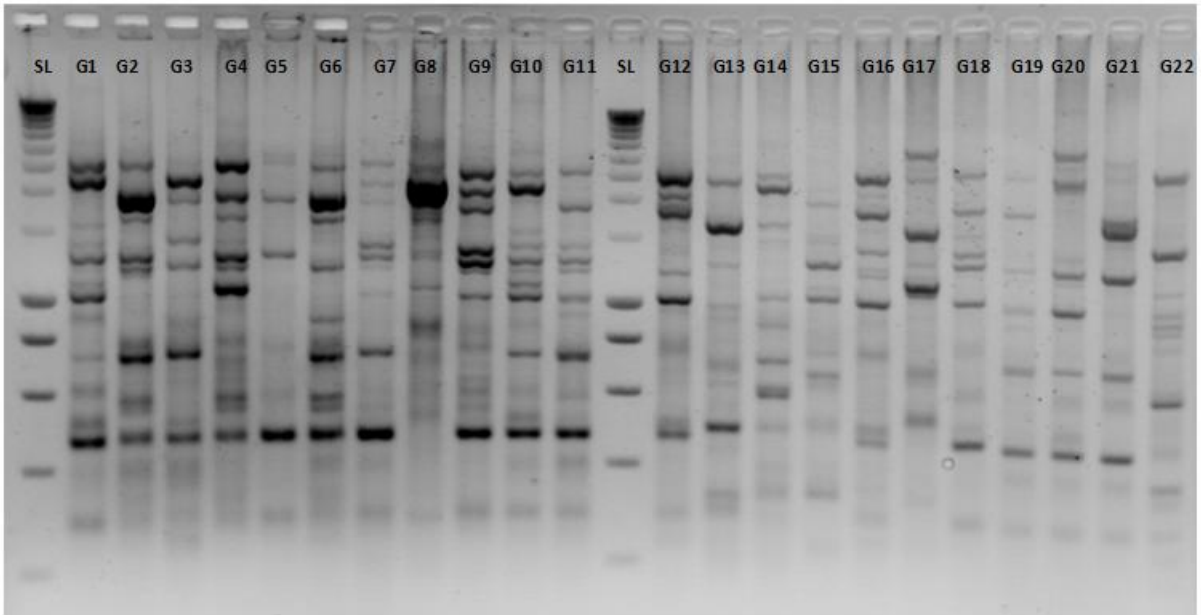


Fig S3: Agarose gel electrophoresis of *V. mediterranei* isolates using ERIC 1R and ERIC 2 primers.

SL: Smart Ladder marker, G1 to G22 : 22 Genotypes of *V. mediterranei*.

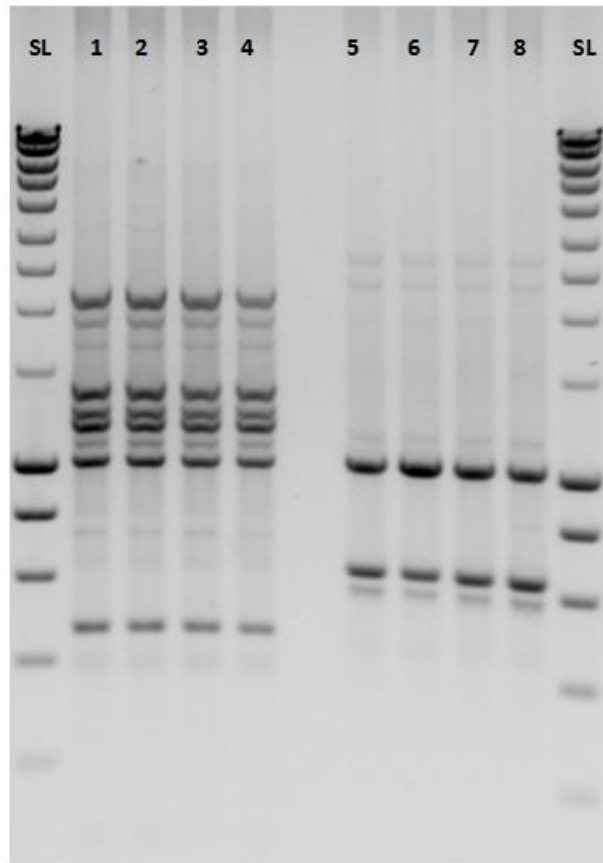


Fig S4: Agarose gel electrophoresis of Genotype 1 *V. mediterranei* isolates using: ERIC 1R and ERIC 2 (lanes 1-4); BOX A1R (5-8). **1, 5:** 2LS0615E; **2, 6:** 9LS0615E; **3, 7:** 17LN0615E; **4, 8:** 21LN0615E.

Table S1: Raw data table of *V. mediterranei* G1 phenotypes abundances.

	F117- MT102-	F117+ MT102-	F117+ MT102+	Fraction	Month	Year
2015/06/02 P	1	1	0	Particule associated	June	2015
2015/06/02 W	0	11	0	Water column	June	2015
2015/06/30 P	0	12	4	Particule associated	June	2015
2015/06/30 W	0	10	18	Water column	June	2015
2015/07/15 P	0	1	0	Particule associated	Jully	2015
2015/07/15 W	0	0	0	Water column	Jully	2015
2016/04/12 P	0	0	0	Particule associated	April	2016
2016/04/12 W	0	0	0	Water column	April	2016
2016/04/28 P	0	0	0	Particule associated	April	2016
2016/04/28 W	0	0	0	Water column	April	2016
2016/05/10 P	0	0	0	Particule associated	May	2016
2016/05/10 W	0	0	0	Water column	May	2016
2016/05/24 P	1	11	0	Particule associated	May	2016
2016/05/24 W	0	0	0	Water column	May	2016
2016/06/07 P	0	0	0	Particule associated	June	2016
2016/06/07 W	0	0	0	Water column	June	2016
2016/06/21 P	23	3	0	Particule assooiated	June	2016
2016/06/21 W	11	4	0	Water column	June	2016
2016/07/04 P	0	28	0	Particule associated	Jully	2016
2016/07/04 W	0	29	0	Water column	Jully	2016
2016/07/18 P	0	27	0	Particule associated	Jully	2016
2016/07/18 W	0	13	0	Water column	Jully	2016

Table S2: Raw data table of environmental variables.

	2015/06/02	2015/06/30	2015/07/15	2016/04/12	2016/04/28	2016/05/10	2016/05/24	2016/06/07	2016/06/21	2016/07/04	2016/07/18
Temperature	17.3	26.4	24.3	14.6	14.2	16	16.9	22.6	20.8	23.6	21.5
Salinity	33.6	35.1	36.7	32.8	35.6	36.2	36.3	35.9	37.1	38.2	40.1
Conductivity	48.6	54.2	54.6	39.9	42.5	45.2	46.1	51.4	50.9	55.4	55.7
Dissolved oxygen	10.09	8.13	8.11	9.62	9.96	9.14	9.02	9.08	8.22	7.82	9.13
pH	8.5	8.26	7.5	7.38	8.41	8.13	8.22	8.25	8.25	8.15	8.29
Turbidity	1.58	0.68	1.63	2.45	3.66	9.21	7.99	0.98	4.58	3.54	1.08
Nitrate	4.26	3.03	3.65	9.26	5.53	1.89	3.47	3.27	3.69	3.66	5.15
Nitrite	0.006	0.007	0.002	0.014	0.009	0.003	0.023	0.011	0.043	0.023	0.004
Ammonia	0.03	0.06	0.05	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Phosphate	0.12	0.13	0.07	0.07	0.05	0.07	0.09	0.05	0.15	0.06	0.12
DOC	2.74	2.96	2.54	2.50	4.72	4.98	6.12	6.69	5.27	5.71	6.66
TDN	0.28	0.55	0.15	0.47	0.40	0.43	0.44	0.27	0.40	0.33	0.59
POC	319.51	267.98	217.635	453.27	305.35	442.95	372.24	312.55	297.74	283.30	237.12
PON	49.33	41.56	36.99	92.40	59.09	63.79	56.15	49.84	43.52	48.17	41.20
<i>Chl a</i>	0.13	0.082	0.08	0.72	0.94	1.08	1.23	0.76	0.65	0.39	0.21
<i>Phaeo a</i>	0.031	0.024	0.021	1.13	1.00	1.34	0.99	0.59	0.60	0.36	0.26

Table S3: Raw data table of phytoplankton and bacteria counts.

	2016/04/12	2016/04/28	2016/05/10	2016/05/24	2016/06/07	2016/06/21	2016/07/04	2016/07/18
<i>Chaetoceros + Bacteriastrum sp.</i>	0	800	80	0	83000	960	0	0
<i>Coscinodiscus sp.</i>	0	0	3600	0	0	160	0	200
<i>Diploneis + Cocconeis sp.</i>	0	320	1040	0	0	0	800	0
<i>Grammatophora sp.</i>	0	0	2720	5100	0	960	3800	0
<i>Navicula and assimilated</i>	352	2240	2960	19360	950	3400	12100	0
<i>Nitzschia sp.</i>	50	160	2560	22000	480	1760	160	0
<i>Pseudo-Nitzschia sp.</i>	0	0	0	2200	16000	1300	1800	0
<i>Rhizosolenia sp.</i>	0	0	80	0	0	0	0	0
<i>Thalassionema nitzschioides</i>	100	0	0	0	0	0	0	0
<i>Alexandrium sp.</i>	580	480	80	100	320	640	160	640
<i>Amylax sp.</i>	0	0	0	100	160	1400	0	0
<i>Ceratium sp.</i>	0	0	0	1760	0	0	0	200
<i>Dinophysis sp.</i>	0	0	160	0	160	0	0	0
<i>Gonyaulax sp.</i>	0	0	0	0	0	160	0	0
<i>Prorocentrum micans</i>	140	1280	1520	0	2000	320	320	1000
<i>Prorocentrum minimum</i>	0	160	0	4300	41000	100	880	400
<i>Protoperidinium sp.</i>	0	0	0	0	1120	100	80	200
<i>Pyrophacus sp.</i>	0	0	0	0	0	0	480	0
<i>Scrippsiella sp.</i>	0	0	960	2900	2700	3000	1800	1600
Cryptophyta	7.56 x 10 ⁵	1.65 X 10 ⁶	4.73 X 10 ⁵	8.61 X 10 ⁵	1.05 X 10 ⁶	1.87 X 10 ⁵	9.37 X 10 ²	2.81 X 10 ³
NanoEuk	6.97 X 10 ⁶	1.01 X 10 ⁷	5.78 X 10 ⁶	7.37 X 10 ⁶	4.91 X 10 ⁶	4.42 X 10 ⁶	1.97 X 10 ⁶	1.80 X 10 ⁶
PicoEuk	1.66 X 10 ⁷	2.35 X 10 ⁷	1.53 X 10 ⁷	4.95 X 10 ⁶	6.40 X 10 ⁶	2.22 X 10 ⁶	9.58 X 10 ⁵	1.84 X 10 ⁵
Cyanobacteria	3.86 X 10 ⁵	6.41 X 10 ⁷	3.25 X 10 ⁷	2.74 X 10 ⁷	4.92 X 10 ⁷	6.81 X 10 ⁷	1.08 X 10 ⁸	1.23 X 10 ⁸
Bacteria	4.74 X 10 ⁹	7.52 X 10 ⁹	3.53 X 10 ⁹	2.14 x 10 ¹⁰	6.11 X 10 ⁹	1.34 x 10 ¹⁰	1.20 x 10 ¹⁰	1.13 x 10 ¹⁰

Table S4: PCO analysis, variation explained by individual axis.

Axis	Eigenvalue	Individual%	Cumulative%
PCO1	10339.0	79.9	79.9
PCO2	2291.7	17.7	97.6
PCO3	310.1	2.4	100.0
PCO4	6.6	0.1	100.0

Table S5: Pearson correlation between each variable and *V. mediterranei* G1 phenotypes ordination by axis.

Variable	PCO1	PCO2	PCO3	PCO4
<i>Chaetoceros</i> + <i>Bacteriastrum</i> sp.	0.3957	-0.0746	0.0727	-0.0074
<i>Coscinodiscus</i> sp.	0.2091	-0.0596	0.1201	-0.2093
<i>Diploneis</i> + <i>Cocconeis</i> sp.	0.1765	0.3734	0.2915	0.4676
<i>Grammatophora</i> sp.	-0.4613	-0.0634	-0.3167	0.6165
<i>Navicula</i> and assimilated	-0.4203	-0.1030	-0.4395	0.7537
<i>Nitzschia</i> sp.	-0.2447	-0.3751	-0.8087	0.2763
<i>Pseudo-Nitzschia</i> sp.	0.0122	-0.1159	-0.0715	0.2894
<i>Rhizosolenia</i> sp.	0.3770	0.0141	0.0226	-0.0003
<i>Thalassionema nitzschiiodes</i>	0.3770	0.0141	0.0226	-0.0003
<i>Alexandrium</i> sp.	-0.0268	-0.2175	0.4391	-0.5969
<i>Amylax</i> sp.	-0.3331	-0.8688	0.1870	-0.0066
<i>Ceratium</i> sp.	-0.4481	-0.0184	-0.8920	-0.0565
<i>Dinophysis</i> sp.	0.5759	0.0215	0.0346	-0.0004
<i>Gonyaulax</i> sp.	-0.3734	-0.8242	0.4209	-0.0642
<i>Prorocentrum micans</i>	0.5253	0.2231	0.4144	-0.3464
<i>Prorocentrum minimum</i>	0.1879	0.0392	-0.2027	0.0875
<i>Protoperdinium</i> sp.	-0.0147	0.0849	0.2631	-0.1889
<i>Pyrophacus</i> sp.	-0.4194	0.5014	0.3709	0.6596
<i>Scrippsiella</i> sp.	-0.6256	-0.2238	-0.1128	0.0574
Cryptophyta	0.7498	-0.3218	-0.4345	0.1154
NanoEuk	0.6491	-0.4180	-0.4407	0.1613
PicoEuk	0.8787	-0.1557	-0.1498	0.1512
Cyanobacteria	-0.5851	0.2801	0.3576	-0.1657
Bacteria	-0.8356	-0.1805	-0.4241	0.1272

Table S6: Retention times for C_x-HSL, oxo-C_x-HSL and OH-HSL. Predictions based on retention times curves of AHL standards. NP: Non Predictable. The retention time noted with an asterisk are used to predict the unanticipated AHLs presented in the table S7.

Acyl-side chain length (C atoms)	Retention time (Rt, min)		
	C _x -HSL	oxo-C _x -HSL	OH-C _x -HSL
5	7.85	6.97	NP
6	8.43	7.56	NP
7	8.83	8.15	8.09
8	9.27	8.69	8.55
9	9.57	9.04	8.92
10	9.9	9.43	9.25
11	10.13	9.74*	9.59*
12	10.46	10.04	9.87
13	10.63	10.33*	10.15
14	10.93	10.56	10.42
15	11.15	10.83	10.63
16	11.34	11.06	10.85
17	11.49	11.27	11.05
18	11.66	11.47	11.24

Table S7: Mass spectrometry (UHPL-HRMS/MS) data used for the identification of unanticipated AHLs in *V. mediterranei* strains. Rt: Retention Time. Theoretical mass correspond to the pseudo-molecular ion [M+H]⁺.

Strain	Rt (min)	Observed mass	Molecular Formula	Delta ppm	Fragmentation	Identification			
						Name	Molecular Formula	Molecular Weight	Theoretical Mass
17LN 0615E	9,54	286,2012	C15H28NO4	-0,055	69.071 (51.87), 74.061 (18.46) , 81.070 (81.33), 83.086 (41.72), 93.070 (33.08), 95.086 (100.00), 102.055 (52.81) , 107.086 (32.94), 109.101 (40.91), 121.101 (67.63), 149.132 (12.43), 170.057 (20.82), 287.201 (15.08)	OH-C11-HSL	C15H27NO4	285,1940	286,2013
	10,26	312,2168	C17H30NO4	-0,135	69.071 (9.79), 71.086 (6.62), 74.061 (36.96) , 81.070 (13.35), 83.086 (6.35), 95.086 (44.97), 102.055 (100.00) , 107.087 (24.97), 109.101 (10.05), 121.101 (18.36), 135.117 (17.34), 185.071 (64.95), 211.169 (59.48), 312.276 (14.58)	OXO-C13-HSL	C17H29NO4	311,2097	312,2169
21LS 0615E	9,55	286,2011	C15H28NO4	-0,145	69.071 (14.64), 74.061 (22.24) , 81.070 (17.80), 83.086 (20.68), 93.070 (11.82), 95.086 (25.37), 102.055 (100.00) , 107.086 (12.88), 109.101 (14.28), 121.101 (12.49), 149.132 (11.29), 170.057 (26.13), 287.201 (18.21)	OH-C11-HSL	C15H27NO4	285,1940	286,2013
	10,26	312,2174	C17H30NO4	1,426	69.0701 (14.76), 71.086 (16.86), 74.061 (15.64) , 81.070 (27.04), 83.086 (17.96), 95.086 (46.07), 102.055 (42.05) , 107.086 (16.36), 109.101 (20.61), 121.101 (15.26), 135.117 (16.86), 185.071 (100.00), 211.169 (21.80), 312.113 (21.79)	OXO-C13-HSL	C17H29NO4	311,2097	312,2169
211LN 0615E	9,54	286,2011	C15H28NO4	-0,145	69.071 (13.52), 74.061 (21.23) , 81.070 (14.69), 83.086 (16.68), 93.070 (9.02), 95.086 (13.95), 102.055 (100.00) , 107.086 (10.64), 109.101 (17.15), 121.101 (7.24), 149.132 (11.28), 170.057 (23.23), 287.201 (8.34)	OH-C11-HSL	C15H27NO4	285,1940	286,2013
	10,26	312,2169	C17H30NO4	-0,015	69.070 (25.41), 71.086 (12.84), 74.061 (12.12) , 81.070 (30.68), 83.086 (27.48), 95.086 (36.26), 102.055 (65.45) , 107.086 (15.44), 109.101 (20.68), 121.101 (15.93), 135.117 (11.54), 185.071 (17.43), 211.169 (48.71), 312.325 (12.16)	OXO-C13-HSL	C17H29NO4	311,2097	312,2169
	9,71	284,1859	C15H26NO4	0,275	56.050 (26.96) , 67.055 (33.74), 69.070 (34.97), 81.070 (73.61), 83.086 (48.93), 93.070 (47.13), 95.086 (75.94), 97.102 (25.44), 102.055 (17.93) , 107.086 (55.85), 109.101 (48.94), 114.962 (30.51), 119.086 (26.78), 121.101 (39.53), 143.073 (100.00), 235.632 (31.38), 244.637 (95.08), 253.643 (40.17), 283.241 (28.80), 284.295 (77.62)	OXO-C11-HSL	C15H25NO4	283,1784	284,1856
9LS 0615E	9,54	286,2012	C15H28NO4	-0,085	69.071 (14.64), 74.061 (22.24) , 81.070 (17.80), 83.086 (20.68), 93.070 (11.82), 95.086 (25.37), 102.055 (100.00) , 107.086 (12.88), 109.101 (14.28), 121.101 (12.49), 149.132 (11.29), 170.057 (26.13), 287.201 (18.21)	OH-C11-HSL	C15H27NO4	285,1940	286,2013
	10,26	312,2169	C17H30NO4	-0,015	69.070 (38.02), 71.086 (22.36), 74.061 (18.54) , 81.070 (100.00), 83.086 (36.43), 95.086 (93.55), 102.055 (18.80) , 107.086 (22.81), 109.101 (62.49), 121.101 (25.50), 135.117 (28.01), 185.071 (27.34), 211.169 (20.55), 312.276 (17.86)	OXO-C13-HSL	C17H29NO4	311,2097	312,2169
	9,7	284,1857	C15H26NO4	0,124	56.050 (15.38) , 67.055 (29.34), 69.070 (25.92), 81.070 (69.81), 83.086 (34.59), 93.070 (53.28), 95.086 (81.05), 97.102 (18.33), 102.055 (25.72) , 107.086 (58.46), 109.101 (39.87), 114.962 (33.54), 119.086 (23.16), 121.101 (42.96), 143.073 (100.00), 235.632 (19.63), 244.637 (74.43), 253.643 (36.27), 283.241 (22.64), 284.295 (73.19)	OXO-C11-HSL	C15H25NO4	283,1784	284,1856

Table S8: Mass spectrometry (UHPL-HRMS/MS) data used for the identification of anticipated AHLs in *V. mediterranei* strains. Rt: Retention Time. Theoretical mass correspond to the pseudo-molecular ion $[M+H]^+$.

AHL Standard	Molecular Formula	Theoretical Mass	Observed Mass	Rt (min)
C4-HSL	C ₈ H ₁₃ NO ₃	172.0968	172.0968	5.26
C6-HSL	C ₁₀ H ₁₇ NO ₃	200.1281	200.1281	8.43
OXO-C6-HSL	C ₁₀ H ₁₅ NO ₃	214.1074	214.1072	7.56
C7-HSL	C ₁₁ H ₁₉ NO ₃	214.1438	214.1440	8.83
C8-HSL	C ₁₂ H ₂₁ NO ₃	228.1594	228.1594	9.27
OXO-C8-HSL	C ₁₂ H ₁₉ NO ₄	242.1387	242.1381	8.69
OH-C8-HSL	C ₁₂ H ₂₁ NO ₄	244.1543	244.154	8.55
C9-HSL	C ₁₃ H ₂₃ NO ₃	242.1751	242.1748	9.57
C10-HSL	C ₁₄ H ₂₅ NO ₃	256.1907	256.1907	9.90
OXO-C10-HSL	C ₁₄ H ₂₃ NO ₄	270.1700	270.1699	9.43
OH-C10-HSL	C ₁₄ H ₂₅ NO ₄	272.1856	272.1856	9.25
C11-HSL	C ₁₅ H ₂₇ NO ₃	270.2064	270.2063	10.13
C12-HSL	C ₁₆ H ₂₉ NO ₃	284.2220	284.2220	10.46
OXO-C12-HSL	C ₁₆ H ₂₇ NO ₄	298.2013	298.2013	10.04
OH-C12-HSL	C ₁₆ H ₂₉ NO ₄	300.2169	300.2169	9.87
C13-HSL	C ₁₇ H ₃₁ NO ₃	298.2377	298.2377	10.63
C14-HSL	C ₁₈ H ₃₃ NO ₃	312.2533	312.2533	10.93
C14:1-HSL	C ₁₈ H ₃₁ NO ₃	310.2377	310.2370	10.51
OXO-C14:1-HSL	C ₁₈ H ₂₉ NO ₄	324.2169	324.2170	10.23
OXO-C14-HSL	C ₁₈ H ₃₁ NO ₄	326.2326	326.2322	10.56
OH-C14-HSL	C ₁₈ H ₃₃ NO ₄	328.2482	328.2482	10.42
C15-HSL	C ₁₉ H ₃₅ NO ₃	326.2690	326.2689	11.15
C16-HSL	C ₂₀ H ₃₇ NO ₃	340.2846	340.2846	11.34
C16:1-HSL	C ₂₀ H ₃₅ NO ₃	338.2690	338.2704	10.93
OXO-C16:1-HSL	C ₂₀ H ₃₃ NO ₄	352.2482	352.2497	10.61
C18-HSL	C ₂₂ H ₄₂ NO ₃	368.3159	368.3155	11.66
C18:1-HSL	C ₂₂ H ₃₉ NO ₃	366.3003	366.3003	11.40

Table S9: Potential candidate proteins for AHL production in G1 *V. mediterranei* isolates 17 LN0615E and 21 LS0615E with their accession numbers. The 15 proteins are showing 100% identity between the two strains.

Predicted proteins	17LN0615E	21LN0615E
N-acetyltransferase	PRQ67551.1	PCD90480.1
long-chain fatty acid--CoA ligase	PRQ66386.1	PCD88787.1
lauroyl-Kdo(2)-lipid IV(A) myristoyltransferase	PRQ66024.1	PCD86675.1
GNAT family N-acetyltransferase	PRQ67405.1	PCD87616.1
lipid A biosynthesis lauroyl acyltransferase	PRQ66025.1	PCD86676.1
lipid A biosynthesis acyltransferase	PRQ68200.1	PCD89439.1
Predicted hydrolase or acyltransferase	PRQ65458.1	PCD89625.1
Predicted hydrolase or acyltransferase	PRQ67698.1	PCD90333.1
GNAT family N-acetyltransferase	PRQ66292.1	PCD86936.1
N-acetyltransferase	PRQ66291.1	PCD86937.1
N-acetyltransferase	PRQ68639.1	PCD89064.1
GNAT family N-acetyltransferase	PRQ66253.1	PCD86975.1
N-acetyltransferase	PRQ66250.1	PCD86978.1
N-acetyltransferase	PRQ66266.1	PCD86962.1
N-acetyltransferase	PRQ68671.1	PCD89096.1