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1 **Isolation and distribution of iridescent *Cellulophaga* and**
2 **further iridescent marine bacteria in the Charente Maritime**
3 **coast, French Atlantic coast**

4

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13

14

15 Running title: Distribution of iridescent marine bacteria

16 **Abstract**

17

18 An intense colored marine bacterium, identified as *Cellulophaga lytica*, has been previously
19 isolated from a sea anemone surface on the Charente Maritime rocky shore (Atlantic Coast,
20 France). Iridescence of the colonies under direct light was recently described and proved
21 physically. Iridescence intensities were found to strongly differ among *C. lytica* strains from
22 culture collections. Importantly, the occurrence and distribution of iridescent bacteria in the
23 marine environment were still unknown. In this study, we searched for marine iridescent
24 bacterial strains in different biotopes of the Charente Maritime coast. Various marine
25 samples (water, sediments, macroalgae, others macroorganisms and detritus) were collected
26 in seven biotopes using a direct plate inoculation method. Thirty-four iridescent strains
27 related to the genus *Cellulophaga* were isolated. Interestingly, sixty-three iridescent strains
28 affiliated to *Tenacibaculum* and *Aquimarina* genera were additionally isolated. Iridescence
29 colors were different through genera. Iridescent marine bacteria were widely distributed.
30 However, a majority was isolated from rocky shore and in particular on red seaweed surfaces
31 and mollusks. Our data suggest that isolates with iridescent properties are well conserved in
32 stressful environments such as coastal shore. This origin may provide insight into the
33 ecological and biological functions of iridescence.

34

35 **Keywords:** Iridescence, iridescent bacteria, marine bacteria, *Bacteroidetes*,
36 *Flavobacteriaceae*, *Cellulophaga*, *Tenacibaculum*, *Aquimarina*, culturable bacteria, epibiont,
37 seaweed, coastal shore, rocky shore

38 Introduction

39

40 Different color processes are present in the natural environment. Pigmentation, a
41 biochemical coloration, can be generated by molecular absorption of specific wavelengths.
42 The exhibited color is uniform. A very different coloration process corresponding to structural
43 colorations is referred as iridescence. An intense coloration with angle-dependent color
44 changes is then produced due to micron- and sub-micron-sized periodic structures interfering
45 with light [8, 33]. This appearance has been well studied in higher organisms particularly in
46 insects [10, 29] and birds [28]. Iridescence is also encountered in marine organisms such as
47 fishes [21], ctenophores [35], macroalgae [5, 9] and diatoms [24].

48 Bacterial iridescence has been a vague and poorly documented concept. However, a marine
49 bacterium -*Cellulophaga lytica* CECT 8139- has been recently described to exhibit, on solid
50 media, a unique intense iridescence in reflection [17]. Colonies have colored centers of
51 varying brightness distributed across the biofilm giving a glitter-like character. Iridescent
52 green is the dominant color, but red and blue-violet are also observed at the colonies'
53 peripheral edges. Iridescence of *C. lytica* CECT 8139's colonies was proved with physical
54 methods and the phenomenon was named glitter-like iridescence. Since this type of
55 iridescence occurred under direct natural light exposure it was described as a more natural
56 coloration effect compared to the other bacterial iridescences.

57

58 We recently demonstrated that the iridescent "*Cellulophaga lytica*" CECT 8139 belongs to
59 the species *Cellulophaga lytica* by a DNA-DNA hybridization against *C. lytica* LIM-21^T (88.9%
60 similarity, unpublished data obtained from DSMZ company). The *C. lytica* species is adapted
61 to a wide range of marine biotopes in free-living, biofilm and associated forms [2, 19].
62 Diverse intensities of iridescence were found within the *C. lytica* strains [17]. Non-iridescent
63 or less iridescent *C. lytica* strains Cy I2 (DSM 2039), LIM-21^T (CIP 103822 and DSM 7489)
64 were all isolated from muddy sediment of Costa Rica beaches [13, 19]. In contrast, iridescent

65 strains *C. lytica* WFB 21 (DSM 2040) and ACEM 21 were isolated from seawater in an
66 aquarium outflow and an estuary, respectively [19, 30]. The iridescent *C. lytica* CECT 8139
67 was isolated from a red anemone *Actinia equine* surface at Oléron island rocky shore on the
68 Charente maritime coast in France [17]. Therefore, different ecotypes exist and unlike the
69 benthic origin, the epibiotic and planktonic origins seemed favorable for the iridescent trait.
70 However, an extended study on origins and biotopes favoring the iridescence trait has not
71 yet been performed. In addition, even if bacterial iridescence has not been yet directly
72 observed in the marine environment, investigating the specific distribution of iridescent
73 strains could be of major importance to determine the possible roles of marine bacterial
74 iridescence.

75 In this work, we studied the occurrence and distribution of iridescent bacterial strains in the
76 Charente maritime coast in France, with special attention paid to the *C. lytica* species. A
77 specific observation device was employed to detect iridescent colonies. Isolated strains were
78 affiliated to a genus by sequence analysis of the 16S rRNA gene.

79

80 **Materials and methods**

81 *Sample collection and bacterial isolation*

82 The sampling was performed between October and December 2011. The seven sites were
83 located on the Charente maritime coast in France (Fig. 1). Diverse biotopes as rocky shore,
84 mudflat and rocky-sandy shores were included (Table 1). A total of 139 samples were
85 collected at low tide for bacterial isolation: seawater (11), sediments (20), red macroalgae
86 (Rhodophyta) (16), green macroalgae (Chlorophyta) (11), brown macroalgae (Phaeophyta)
87 (20), decomposed macroalgae (7), marine plants (4), sponges (5), cnidarians (8), worms (3),
88 crustaceans (7), mollusks (19), echinoderms (6) and detritus (2).

Fig. 1

89 A “fresh” inoculation protocol was defined in order to isolate environmental strains and to
90 avoid population changes during transport. For seawater samples, a side of the agar plate
91 was immersed directly in the water and the Petri dish was rapidly closed. For solid samples,
92 a fragment was rinsed in artificial sterile seawater (ASW) Instant Ocean[®] (30 g L⁻¹ in pure
93 water) in order to remove loosely attached epibionts and then scraped onto the agar plate
94 [17]. For sediment samples, about 1 ml was directly added onto the agar. Three days of
95 incubation at 20°C were needed to observe iridescent colonies. Picked colonies were then
96 subcultured, purified and stored at -80°C in Cryovials[®] (Dutscher).

97

98 *Culture conditions of iridescent marine bacteria*

99 The solid medium Cytophaga (CYT) was used to isolate iridescent marine bacteria [13]. CYT
100 medium contained 1 g tryptone, 0.5 g yeast extract, 0.5 g CaCl₂·2H₂O, 0.5 g MgSO₄·7H₂O,
101 and 15 g agar in 1 L of artificial seawater (ASW) Instant Ocean[®] (30 g L⁻¹ in pure water).
102 Incubation temperature was fixed at 20°C.

103

104 *Iridescence observation*

105 Plates from the collection were observed using a direct illumination set up (see below) to
106 elicit iridescent colonies. Additionally, iridescence of the pure strains was observed using a

107 streaking procedure. One colony from a 24 h-old plate was subcultured in triplicate plates
108 drawing thin 5 cm-linear streaks. Cultures were photographed after 24 h and 72 h of growth
109 in a dark room using an experimental arrangement of oblique epi-illumination at a fixed
110 illumination angle of 60 °C [17]. The light source was a lamp (Kaiser RB 218N HF copy
111 lighting unit) of 18 watts, 5400 Kelvin, the operating voltage correspond to AC 220-240 V, 50
112 Hz with an operating frequency of 40 kHz. The camera was a Nikon D1500 18-55VR on Av
113 program with f22, the lens was a macro, large size (12.1 Mega pixels) used in superfin mode.

114

115 *Molecular characterization of the strains*

116 Identification of each iridescent isolate was determined by 16S rRNA gene sequencing. The
117 DNA suspension for the PCR consisted of colonies picked on agar plates and resuspended
118 in 500 µl of sterile water. Before the PCR, 3 heat shocks (incubation at 60°C during 2 min)
119 and cold shocks (incubation in liquid nitrogen during 2 min) were applied on the bacterial
120 suspension in order to lyse the cells and liberate the DNA. Primers used for 16S RNA full
121 length gene sequencing were 27F (5'-AGA GTT TGA TCC TGG CTC AG-3'), and 1492R (5'-
122 GGT TAC CTT GTT ACG ACT T-3') [18, 22]. Reaction mixtures of 50 µl contained 20 µl of
123 PCR master mix (DyNAzyme II, Finnzymes), 100 pmol of each primer, 2 µl of washed cells
124 and MilliQ water to the 50 µl volume. PCR reaction was carried out in a Labcycler
125 SensoQuest. The thermal PCR profile was as follows: initial denaturation at 94°C for 5 min
126 followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min,
127 and elongation at 72°C for 1 min. The final elongation step was 9 min at 72°C. The 16S
128 rDNA products were analysed by electrophoresis in 1% agarose gels. The 16S rDNA
129 products were then sequenced with an automatic DNA analysis system (Genoscreen, Lille,
130 France and GATC, Konstanz, Germany). Sequences were compared with sequences
131 available in the GenBank database by using the BLAST (Basic Local Alignment Search Tool)
132 service to determine their approximate phylogenetic affiliations [1]. Sequences of
133 representative strains were deposited in the GenBank database under accession numbers
134 JX454520 to JX454530.

135

136 *Phylogeny*

137 Sequences have been analyzed using the ARB software package (November 2011 version)
138 [20] and the corresponding SILVA 16S RNA-based LTP released 106 SSU database [36].
139 After importing, all sequences were automatically aligned according to the SILVA SSU [7]
140 reference alignment thanks to the online tool SINA. Manual refinement of the alignment was
141 carried out taking into account the secondary structure information of the rRNA in ARB. Tree
142 reconstruction was using the Neighbour Joining (ARB), Maximum Parsimony (PHYLIP DNA-
143 Parsimony v 3.6a3 [7]) and Maximum Likelihood (PHYML v2.4.5 [12]) methods. Tree
144 topology was further tested by the application bacterial variables positional filters and by the
145 application of 30%, 40% and 50% positional conservatory filters. The final tree was
146 calculated with 43 sequences based on 1274 valid columns (50 % filtering) with PHYML
147 (model: GTRGAMMA). Partial sequences have been added to the tree using the ARB
148 parsimony tool.

149

150 **Results**

151

152 *Collection and isolation of iridescent marine bacteria*

153 For isolation of iridescent marine bacteria, the CYT medium was preferred to rich media such
154 as marine agar to avoid too rapid growth. Using our collection and isolation procedures, we
155 could easily observe several iridescent marine strains from diverse origins from the Charente
156 maritime coast. Diverse bacterial colonies grew on CYT plates but iridescent colonies were
157 only visible after three days of incubation. Interestingly, various visual effects were observed
158 under direct illumination (Fig. 2, arrows). Mostly large green or red iridescent colonies were
159 present. Small red pigmented colonies with red iridescence were also observed.

Fig. 2

160

161 *Iridescence and distribution of the novel strains of Cellulophaga*

162 Iridescence in the *Cellulophaga* strains is characterized by a green bright effect with
163 red/violet edges. This type of iridescence was observed on several plates of diverse origins
164 (Fig. 2) and colonies resembling to *C. lytica* were isolated and then identified by molecular
165 analysis. The 34 strains belonging to the genus *Cellulophaga* were grouped in OTU 1 (Table
166 2). Different iridescent intensities and color variations were visible. Some colonies exhibited
167 yellow or blue colors (Fig. 3). Most of the strains were positive for agarolysis and gliding
168 motility. The rocky shore biotope was found to contain a lot of iridescent *Cellulophaga*
169 species (Fig. 4). These strains were isolated from a wide range of samples but mostly from

Fig. 3

Fig. 4

170 macroalgae, and in particular from Rhodophyta and Phaeophyta, and from mollusks. Most
171 intense iridescent strains were isolated from the surface of Rhodophyta and mollusks (Fig.
172 4).

173

174 *Genotypic diversity of other iridescent bacteria*

175 While searching for *Cellulophaga* iridescent strains, we observed other colonies with a bright
176 effect but with different types of iridescence (Fig. 2). A total of 97 strains (including strains of

177 *Cellulophaga*) were isolated and affiliated to genus by sequencing of the of 16S rRNA gene
178 (Table 2). Similarity values for the 16S rRNA gene sequences with previously reported
179 isolated species in databases are presented in Table 2. Overall, 11 different OTUs were
180 found. For 3 OTUs (5, 7 and 9), the isolated strains showed sequence similarities lower than
181 97% to previously reported species, suggesting the possible presence of new species. All
182 isolates belonged to the phylum of *Bacteroidetes* and formed two clusters (Fig. 5). The
183 *Tenacibaculum* isolates named OTU 3, 4, 5, 6, 7, 8, 9, 10 and 11 clustered with the genus
184 *Tenacibaculum* exhibiting between 96 and 99% 16S rRNA sequence similarity (Table 2). In
185 the other cluster, isolates belonging to the OTU 1 had 99% similarities with *Cellulophaga*
186 *lytica* and isolates belonging to the OTU 2 had 98% similarities with *Aquimarina latercula*
187 (Table 2).

Fig. 5

188 The iridescent strains belonging to the genus *Cellulophaga* (OTU 1) were the most
189 representative strains with 35.1% (Table 2). Next, 59.8% of isolated strains (OTUs 3, 4, 5, 6,
190 7, 8, 9, 10 and 11) belong to the genus *Tenacibaculum*. Finally, 5.2% of isolated strains
191 belong to the genus *Aquimarina*.

192

193 *Phenotypic diversity of the others iridescent strains*

194 Different iridescent colonies were visible on the original plates (Fig. 2). Identified strains
195 showed diversity in iridescence coloration and intensity when cultivated by the streaking
196 method. Green or red iridescence was often observed (Fig. 6). The majority of
197 *Tenacibaculum* strains expressed bright angle-dependent green iridescence, except for OTU
198 5, 7 and a few strains of OTU 8, 9 expressing a red iridescence. The red iridescence of OTU
199 5 could be observed on colony edges only after three days of incubation. OTU 3 and 4
200 strains expressed a particular iridescence resembling to holograms with multicolor effects
201 observed by eyes but difficult to photograph (Fig. 6 c,d). Strains affiliated to *Aquimarina* sp.
202 (OTU 2) were red-pigmented with red iridescence. Most isolated strains were motile by
203 gliding, especially for the bright iridescent isolates.

Fig. 6

204

205 *Source of isolation of iridescent bacteria*

206 Iridescent bacteria were isolated from all types of marine biotope and samples except from
207 the mudflat environment where no iridescent strain was isolated. As for *Cellulophaga*
208 species, the rocky shore ecosystem contained most iridescent bacteria (Fig. 4). Efficient
209 sources for the isolation of iridescent bacteria were mollusks, Rhodophyta, Phaeophyta,
210 seawater and cnidarians. Rhodophyta species possessing at their surface iridescent bacteria
211 were, for example, *Palmaria palmata*, *Chondrus crispus* or *Corallina officinalis*. Mollusk
212 samples and, in particular, gastropods could contain a wide diversity of iridescent species
213 within the same sample. For example, six different OTUs (OTU 1, 3, 4, 6, 10, 11) within three
214 genera were isolated from the same decomposed oyster shell. The original source of the
215 isolated species is informed in the phylogenic tree (Fig. 5). All isolates related to the genus
216 *Aquimarina* (OTU 2) were from the surface of red macroalgae. The strains belonging to OTU
217 5 were isolated from the same brown macroalgae *Fucus vesiculosus*. The 2 isolates of OTU
218 6 were isolated from the surface of different gastropods (winkle).

219

220 Discussion

221

222 Bacterial iridescence has been ambiguously described or ignored in the literature. We have
223 recently isolated a marine bacterium intensively iridescent, *C. lytica* CECT 8139, in a rocky
224 shore of the Charente-Maritime [17]. This discovery raised the question whether iridescent
225 marine bacteria are widely distributed in coastal biotopes.

226 In this study, iridescent strains belonging to the genus *Cellulophaga* were isolated for a
227 second time in the Charente Maritime coast. These strains were the most representative
228 iridescent isolates in the collection but diverse bacterial strains belonging to others genera
229 were also isolated. In total, iridescent strains were affiliated to 4 different genera. All isolated
230 strains were belonging to the *Bacteroidetes* phylum. Three groups of isolates (OTU 5, 7 and
231 9) had 16S rRNA sequence similarity equal or lower to 97%. Therefore, we found putative
232 new species in the genus *Tenacibaculum* that were never detected before through both
233 cultivation and culture independent studies and that could have interest for future research.
234 However, resolution of 16S rRNA sequence analysis is insufficient to distinguish closely
235 related organisms [4, 31]. More fine phylogenetic analyses on the DNA gyrase B subunit
236 gene or the ITS region and the determination of the DNA-DNA hybridization values should
237 confirm the novelty [31, 32]. These iridescent bacterial strains must be further characterized
238 by a polyphasic approach including genetic, phenotypic and biochemical studies.

239 This study permitted to illustrate and describe precisely colonies' coloration effects on agar
240 plates. Different iridescence effects were observed among the new isolates of *Cellulophaga*.
241 This data confirms that these strains can be differentiated phenotypically regarding
242 iridescence. Different iridescence appearances were also identified in the other isolates
243 belonging to the *Bacteroidetes* phylum. For few species, vague or ambiguous descriptions of
244 the phenomenon were found in the literature but the occurrence of a true iridescent trait
245 remained unclear. The red pigmentation was used to differentiate the *Aquimarina* spp. but
246 any information described iridescence [23]. To our knowledge, the only description of

247 iridescence was given for *Tenacibaculum discolor* isolated from a sole surface: “On marine
248 agar 2216 (Difco), colonies also appear bright yellow from the front, but appear bright green
249 when viewed at an angle of 30–45°C” [26]. Mentions of optical effects were available for
250 other *Tenacibaculum* strains: greenish glistening for *T. litoreum* [3] and *T. aestuarii* [14] and
251 glistening and pale yellow for *T. lutimaris* [37]. No description was given for the other species
252 [26, 27, 34]. The iridescence phenomenon in marine bacteria, and more generally in
253 prokaryotes, was generally omitted and it remains therefore still poorly known from a
254 taxonomic point of view. Our data suggest that iridescence is potentially widespread and
255 represents an interesting criterion for new microorganisms.

256

257 Gliding motility was observed for most iridescent strains isolated. Moreover, fast gliding (fast
258 spreading edges) was typical of intensely iridescent colonies. A strong link between gliding
259 motility and iridescence could therefore exist in several members of the *Bacteroidetes*
260 phylum. As recently proposed [15], gliding motility may be involved in the establishment, in
261 time and space, of the necessary periodic structures responsible for the iridescent
262 appearance. Such structures are still uncharacterized but they are under investigation by
263 electron microscopy in our laboratory. Since mostly green and red iridescences were
264 observed for the isolated strains, similar periodic structures, with minor variations of
265 dimensions and periodicity, are likely inferred to be responsible for the iridescent
266 appearances observed.

267

268 *Flavobacteriales* are common in the marine environment [2]. We found that iridescent strains
269 were particularly abundant on rocky shore (53%). This biotope comprises a higher
270 biodiversity of organisms than muddy environment. The presence of iridescent bacteria in
271 rocky shore ecosystem that combined stressful conditions from solar exposure, desiccation,
272 and great fluctuations in salinity, may give preliminary indications on the possible biological
273 roles of the iridescence (thermoregulation, UV protection, light filtering and desiccation
274 prevention [6, 29]). Moreover, we recently showed that iridescence of *C. lytica* CECT 8139

275 was conserved under environmental stress conditions such as low temperature and high
276 salinity [16]. Interestingly, the majority of isolated species were in association with the marine
277 flora (macroalgae) and fauna (mollusks, cnidarians). For several macroorganisms,
278 recruitment of an epibiontal community with protection activities has been proved [11, 25].
279 Iridescence may therefore provide selective advantages for the bacterial population itself
280 and/or their hosts. Moreover, several strains and especially strains of *Cellulophaga* sp. were
281 isolated from the surfaces of red macroalgae. For the isolates belonging to the genus
282 *Aquimarina* and showing a red pigmentation, the source was only the surface of
283 Rhodophytae (*i.e.* red macroalgae). These observations might be of a major importance
284 since a few red macroalgae have been previously described to exhibit iridescence. Indeed,
285 *Chondrus crispus* and *Irideae* spp. possess iridescent multilayered fronds [5, 9]. Moreover,
286 iridescence of *Irideae* has been supposed to prevent dessication [9]. It can therefore be
287 hypothesized that iridescent epibiontic bacteria might play beneficial roles in some red
288 malcroalgae. More generally, iridescent marine *Bacteroidetes* might have biological or
289 ecological functions and play important roles in rocky shore ecosystems. These hypotheses
290 have to be examined in the future.

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426 **Figures legends**

427

428 **Fig. 1.** Maps of the Charente Maritime coast in France and of the different sampling sites
429 (see details in the Table 1)

430

431 **Fig. 2.** Observation under direct illumination of CYT isolation plates incubated for three days
432 at 20°C. Origins of collected samples were (a) *Palmaria palmata* rhodophyta at Whales
433 lighthouse (b) holdfast of an unattached *Saccorhiza polyschides* phaeophyta at Chassiron
434 lighthouse, (c) plastic detritus at Smoke peak and (d) limpet at Smoke peak. White arrows
435 indicate red or green iridescent colonies.

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438 affiliated isolates (OTU 1) after one day (up line) and four days (bottom line) of incubation at
439 20°C on CYT medium. Strains were isolated from (a) dry sand at Ronce les bains, (b)
440 rhodophyta *Palmaria palmata* at Whales lighthouse, (c) phaeophyta *Fucus vesiculosus* at
441 Ronce les bains, (d) chlorophyta *Ectocarpus siliculosus* at Chassiron lighthouse.

442 Gliding motility can be identified as the spreading zone from the colony center.

443 Agarolysis corresponds to the dark halo visible on colony edges.

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445 **Fig. 4.** Distribution of all isolated iridescent strains within the diverse biotopes and samples.

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447 **Fig. 5.** Phylogenetic tree constructed 16S rDNA genes of isolates and related species of
448 CFB group. The tree was built with the maximum likelihood method. Bar indicated 1%
449 dissimilarity sequence. Selected sequences from the *Rikenellaceae* family of CFB bacteria
450 were used to root the tree. Bootstrap percentages are obtained using 1000 replicates, and

451 values >50% indicated at the nodes. * indicated branches that were also found using the
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455 culture at 20°C on CYT medium. Both OTU 2 (a,b) were isolated from *Chondrus crispus* red
456 macroalgae. Strains belonging to OTU 3 were isolated from (c) *Pterocladia* sp. red
457 macroalgae, (d) an unknown hydroid and (e) a seastar *Asterina gibosa*. Strains belonging to
458 OTU 4 were from (f) a decomposed oyster, (g) seawater sample and (h) a limpet. OTU 5, 6
459 and 7 were respectively isolated from *Fucus vesiculosus* (i), a winkle (j), and from seawater
460 (k). Isolated grouped in OTU 8 were isolated from (l) *Pterocladia* sp. red macroalgae, (m) an
461 unattached laminaria, (n) sand, (o) *Corallina officinalis* red macroalgae, (p) worms sand
462 formation, (q) seawater and (r) crab. OTU 9, 10 and 11 were respectively isolated from a
463 *Zostera marina* (s), a decomposed oyster (t) and an unattached *Saccharina* sp. brown
464 macroalgae.

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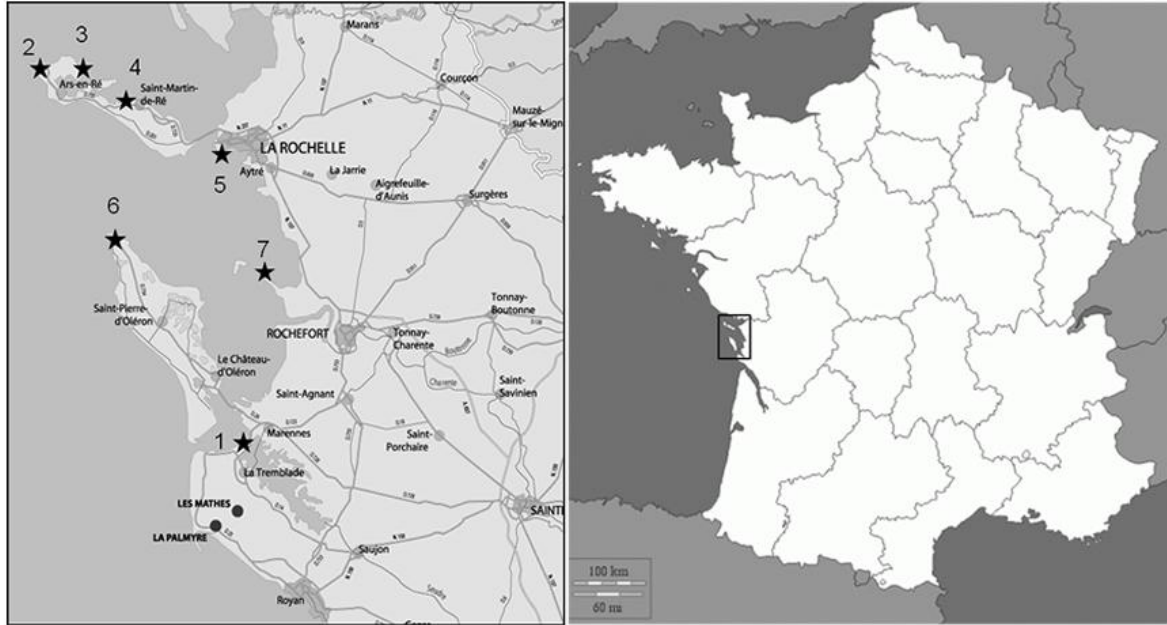
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469 **Figures**

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★ Collection sites

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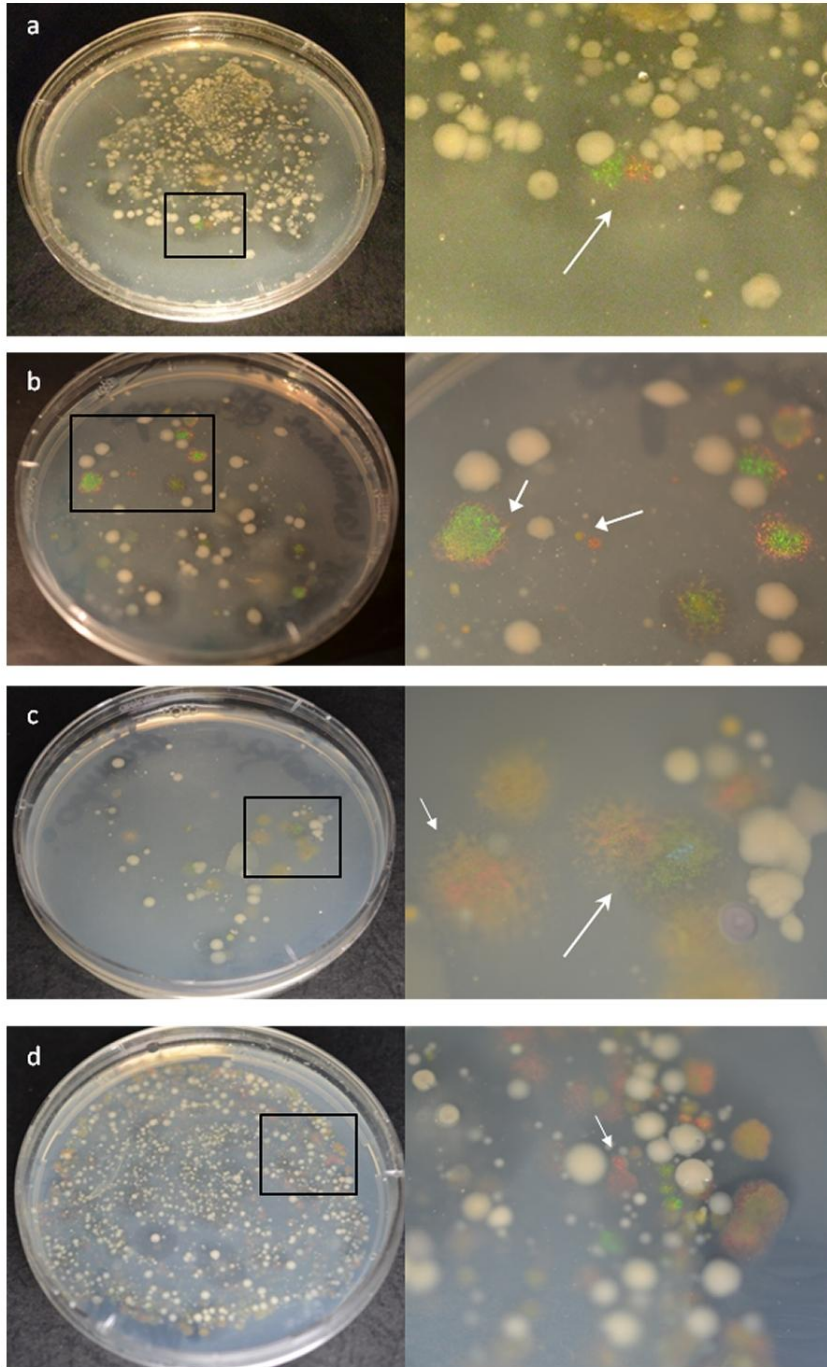
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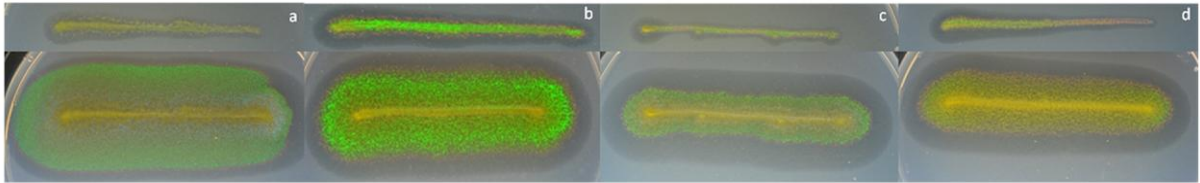
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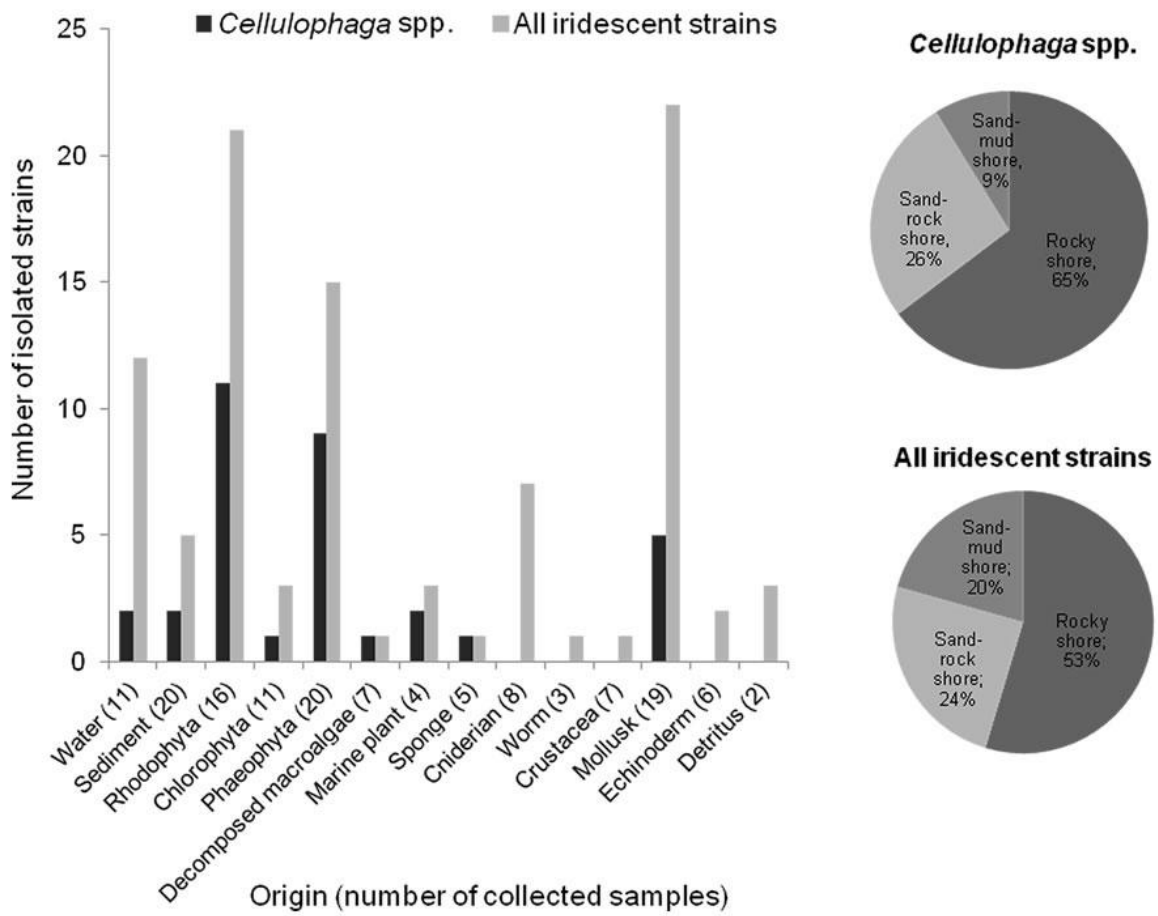
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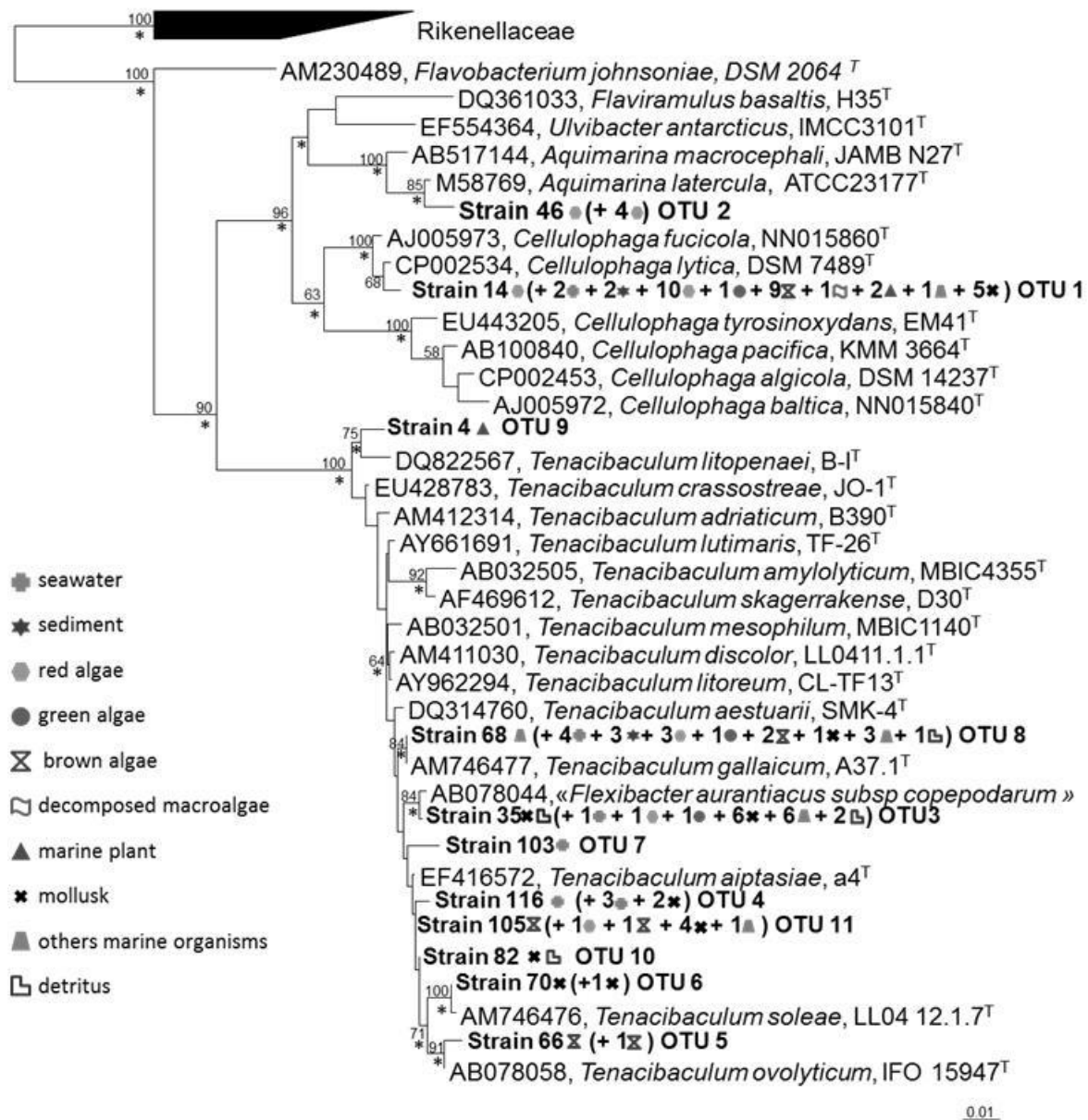
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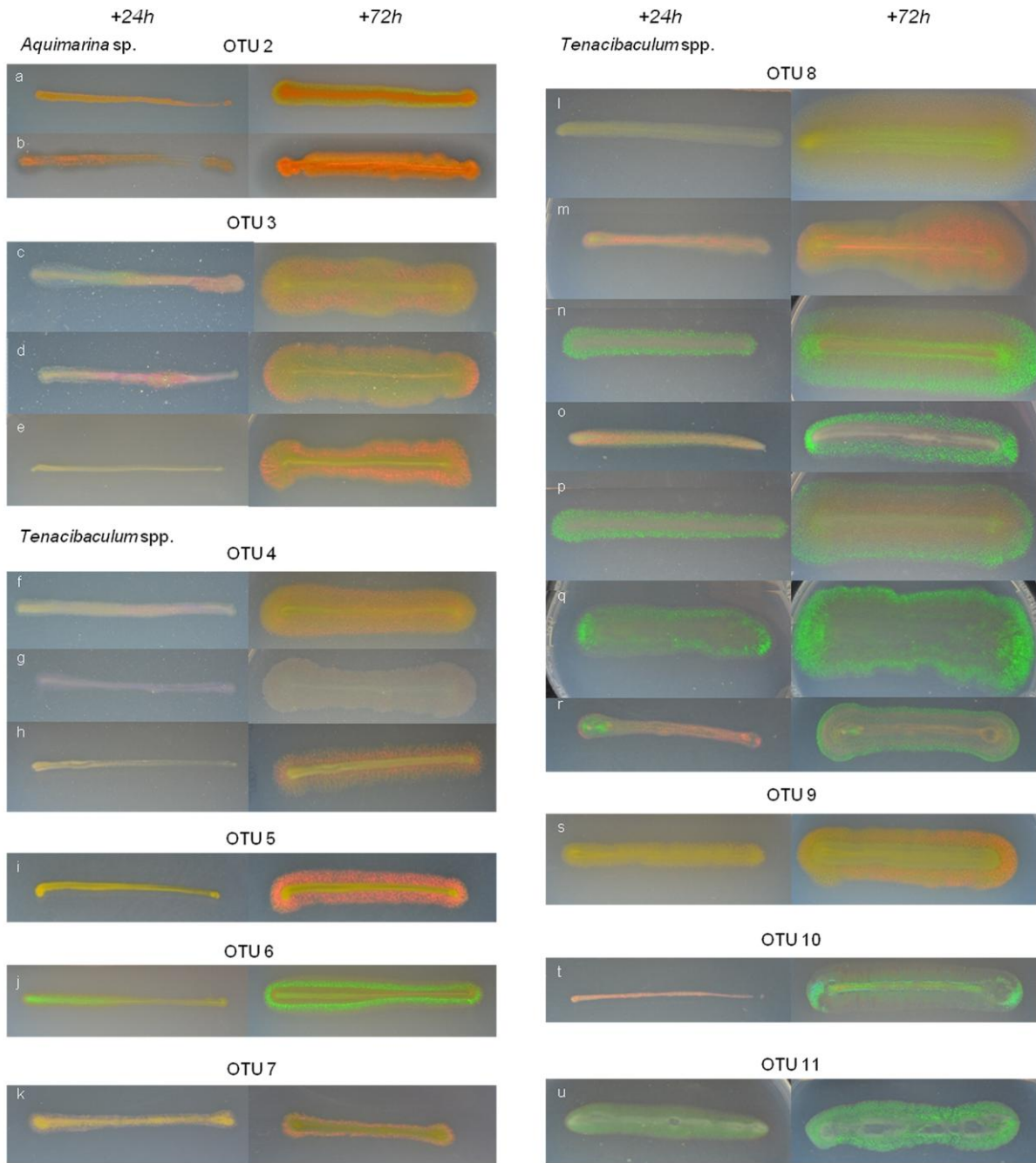
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 554 respectively isolated from a *Zostera marina* (s), a decomposed oyster (t) and an unattached
 555 *Saccharina* sp. brown macroalgae.

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557 **Tables**

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Table 1. Sampling sites with specified biotopes.

Site	Location	Sampling date	Nature of biotope	Number of samples *
1 Mus du loup beach	Ronce les bains, La Tremblade	29.09.11	Sand and rocks	6
2 Whales lighthouse	St Clément des Baleines, Ré Island	24.10.11	Rocks	51
3 Eelgrass park	Loix, Ré Island	3.11.11	Silt with eelgrass	10
4 Arnérault Beach	La Flotte, Ré Island	3.11.11	Sand and rocks	7
5 Belvédère	Chef de Baie, La Rochelle	3.11.11	Sand and rocks	9
6 Chassiron lighthouse	Chassiron, Oléron Island	15.11.11	Rocks	31
7 Smoke peak	Fouras	29.11.11	Silt and sand with oysters	25

* Total number of samples: 139

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Table 2. Affiliation of isolated iridescent strains.

OTU	Representative strain	Accession number	Number (%) of isolates	-Closest relative strain -Closest relative Type Strain (T)	% sequence similarity	Accession number of the closest relative
OTU 1	Strain 14	JX454529	34 (35.1%)	- <i>Cellulophaga lytica</i>	99 %	AB032512
				- <i>Cellulophaga lytica</i> DSM7489 ^T	98%	CP002534
OTU 2	Strain 46	JX454530	5 (5.2%)	- <i>Aquimarina latercula</i>	98 %	AB681002
				- <i>Aquimarina latercula</i>	97%	M58769
				ATCC23177 ^T		
OTU 3	Strain 35	JX454522	18 (18.6 %)	-« <i>Flexibacter aurantiacus</i> subsp. <i>Copepodarum</i> »	99 %	AB078044
				- <i>Tenacibaculum aiptasiae</i> a4 ^T	98%	EF416572
OTU 4	Strain 116	JX454523	6 (6.2 %)	-« <i>Flexibacter aurantiacus</i> subsp. <i>Copepodarum</i> »	98 %	AB078044
				- <i>Tenacibaculum aiptasiae</i> a4 ^T	97%	EF416572
OTU 5	Strain 66	JX454528	2 (2.1%)	- <i>Tenacibaculum ovolyticum</i>	97 %	AB681005
				- <i>Tenacibaculum ovolyticum</i> IFO 15947 ^T	97%	AB078058
OTU 6	Strain 70	JX454527	2 (2.1%)	- <i>Tenacibaculum soleae</i>	99 %	AM989479
				- <i>Tenacibaculum soleae</i> LL04 12.1.7 ^T	99%	AM746476
OTU 7	Strain 103	JX454524	1 (1.0 %)	- <i>Tenacibaculum discolor</i>	97 %	JQ231117
				- <i>Tenacibaculum aiptasiae</i> a4 ^T	96%	EF416572
OTU 8	Strain 68	JX454521	19 (19.6 %)	- <i>Tenacibaculum gallaicum</i> A37.1 ^T	99 %	AM746477
OTU 9	Strain 4	JX454520	1 (1.0 %)	-« <i>Flexibacter aurantiacus</i> subsp. <i>Copepodarum</i> »	96 %	AB078044
				- <i>Tencibaculum litopenaei</i> B-I ^T	95%	DQ822567
OTU 10	Strain 82	JX454526	1 (1.0 %)	- <i>Tenacibaculum aiptasiae</i>	98 %	NR044202
				- <i>Tenacibaculum aiptasiae</i> a4 ^T	98%	EF416572
OTU 11	Strain 105	JX454525	8 (8.2 %)	- <i>Tenacibaculum aestuarii</i> SMK-4 ^T	99 %	DQ314760

OTU: Operational Taxonomic Unit.