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

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Running title: Distribution of iridescent marine bacteria

## Abstract

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


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

## Introduction

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

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

We recently demonstrated that the iridescent "*Cellulophaga lytica"* CECT 8139 belongs to the species *Cellulophaga lytica* by a DNA-DNA hybridization against *C. lytica* LIM-21$^{T}$ (88.9% similarity, unpublished data obtained from DSMZ company). The *C. lytica* species is adapted to a wide range of marine biotopes in free-living, biofilm and associated forms [2, 19]. Diverse intensities of iridescence were found within the *C. lytica* strains [17]. Non-iridescent or less iridescent *C. lytica* strains Cy I2 (DSM 2039), LIM-21$^{T}$ (CIP 103822 and DSM 7489) 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 epibiontic 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

## Materials and methods

*Sample collection and bacterial isolation*

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

Fig. 1

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

*Culture conditions of iridescent marine bacteria*

The solid medium Cytophaga (CYT) was used to isolate iridescent marine bacteria [13]. CYT medium contained 1 g tryptone, 0.5 g yeast extract, 0.5 g $CaCl_2.2H_2O$, 0.5 g $MgSO_4.7H_2O$, and 15 g agar in 1 L of artificial seawater (ASW) Instant Ocean© (30 g $L^{-1}$ in pure water). Incubation temperature was fixed at 20°C.

*Iridescence observation*

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

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

*Molecular characterization of the strains*

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

# Results

*Collection and isolation of iridescent marine bacteria*

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

Fig. 2

*Iridescence and distribution of the novel strains of Cellulophaga*

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

Fig. 3

Fig. 4

*Genotypic diversity of other iridescent bacteria*

While searching for *Cellulophaga* iridescent strains, we observed other colonies with a bright 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 Fig. 5
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).
188 The irisdescent 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 Fig. 6
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.
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

## Discussion

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

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

This study permitted to illustrate and describe precisely colonies' coloration effects on agar plates. Different iridescence effects were observed among the new isolates of *Cellulophaga*. This data confirms that these strains can be differentiated phenotypically regarding iridescence. Different iridescence appearances were also identified in the other isolates belonging to the *Bacteroidetes* phylum. For few species, vague or ambiguous descriptions of the phenomenon were found in the literature but the occurrence of a true iridescent trait remained unclear. The red pigmentation was used to differentiate the *Aquimarina* spp. but 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

was conserved under environmental stress conditions such as low temperature and high salinity [16]. Interestingly, the majority of isolated species were in association with the marine flora (macroalgae) and fauna (mollusks, cnidarians). For several macroorganisms, recruitment of an epibiontal community with protection activities has been proved [11, 25]. Iridescence may therefore provide selective advantages for the bacterial population itself and/or their hosts. Moreover, several strains and especially strains of *Cellulophaga* sp. were isolated from the surfaces of red macroalgae. For the isolates belonging to the genus *Aquimarina* and showing a red pigmentation, the source was only the surface of Rhodophytae (*i.e.* red macroalgae). These observations might be of a major importance since a few red macroalgae have been previously described to exhibit iridescence. Indeed, *Chondrus crispus* and *Irideae spp.* possess iridescent multilayered fronds [5, 9]. Moreover, iridescence of *Irideae* has been supposed to prevent dessication [9]. It can therefore be hypothesized that iridescent epibiontic bacteria might play beneficial roles in some red malcroalgae. More generally, iridescent marine *Bacteroidetes* might have biological or ecological functions and play important roles in rocky shore ecosystems. These hypotheses 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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437 **Fig. 3.** Glitter-like iridescence appearance under direct illumination of different *Cellulophaga*
438 affiliated isolates (OTU 1) after one day (up line) and four days (bottom line) of incubation at
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440 rhodophyta *Palmaria palmata* at Whales lighthouse, (c) phaeophyta *Fucus vesiculosus* at
441 Ronce les bains, (d) chlorophyta *Ectocarpus siliculosis* at Chassiron lighthouse.
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443 Agarolysis corresponds to the dark halo visible on colony edges.

444

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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454 **Fig. 6.** Iridescence of the isolated marine bacteria after 1 day (left) and 3 days (right) of
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
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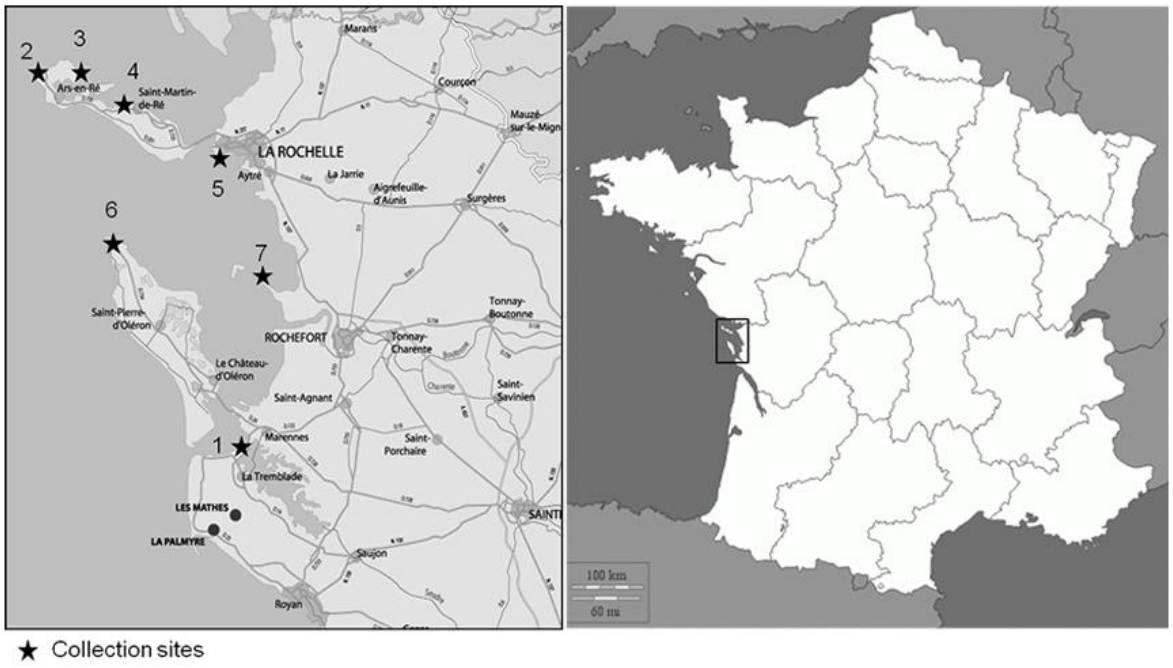
## Figures

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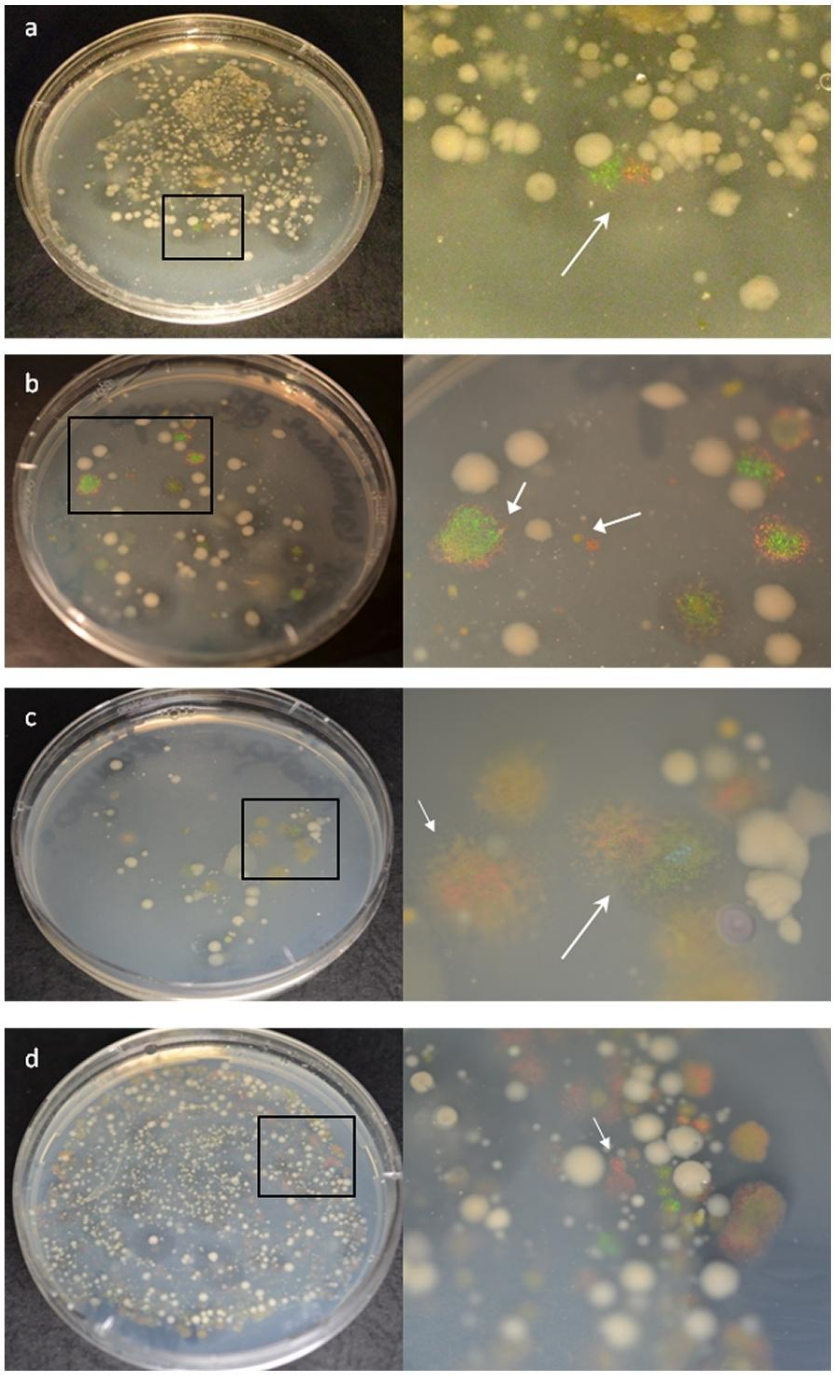


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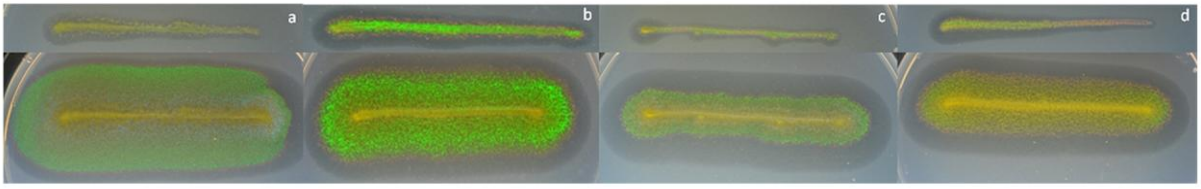


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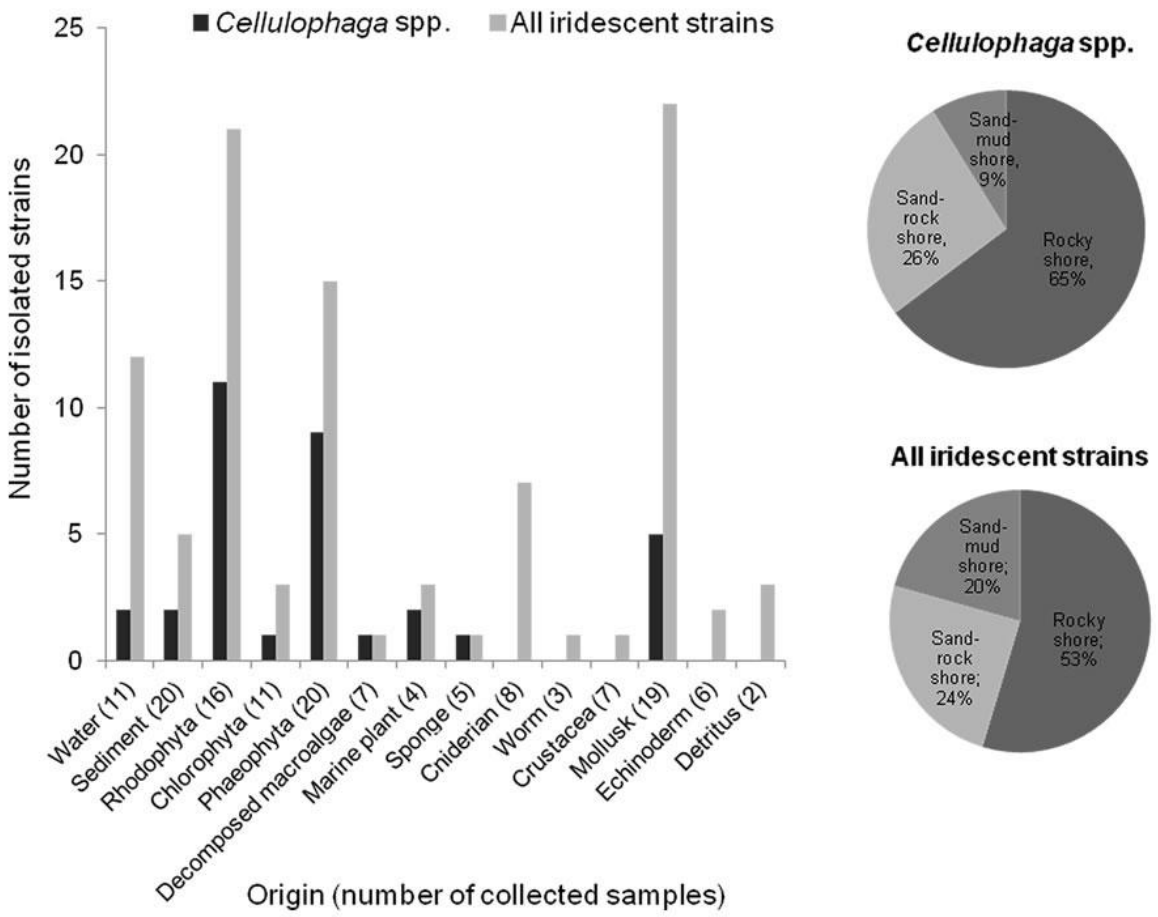


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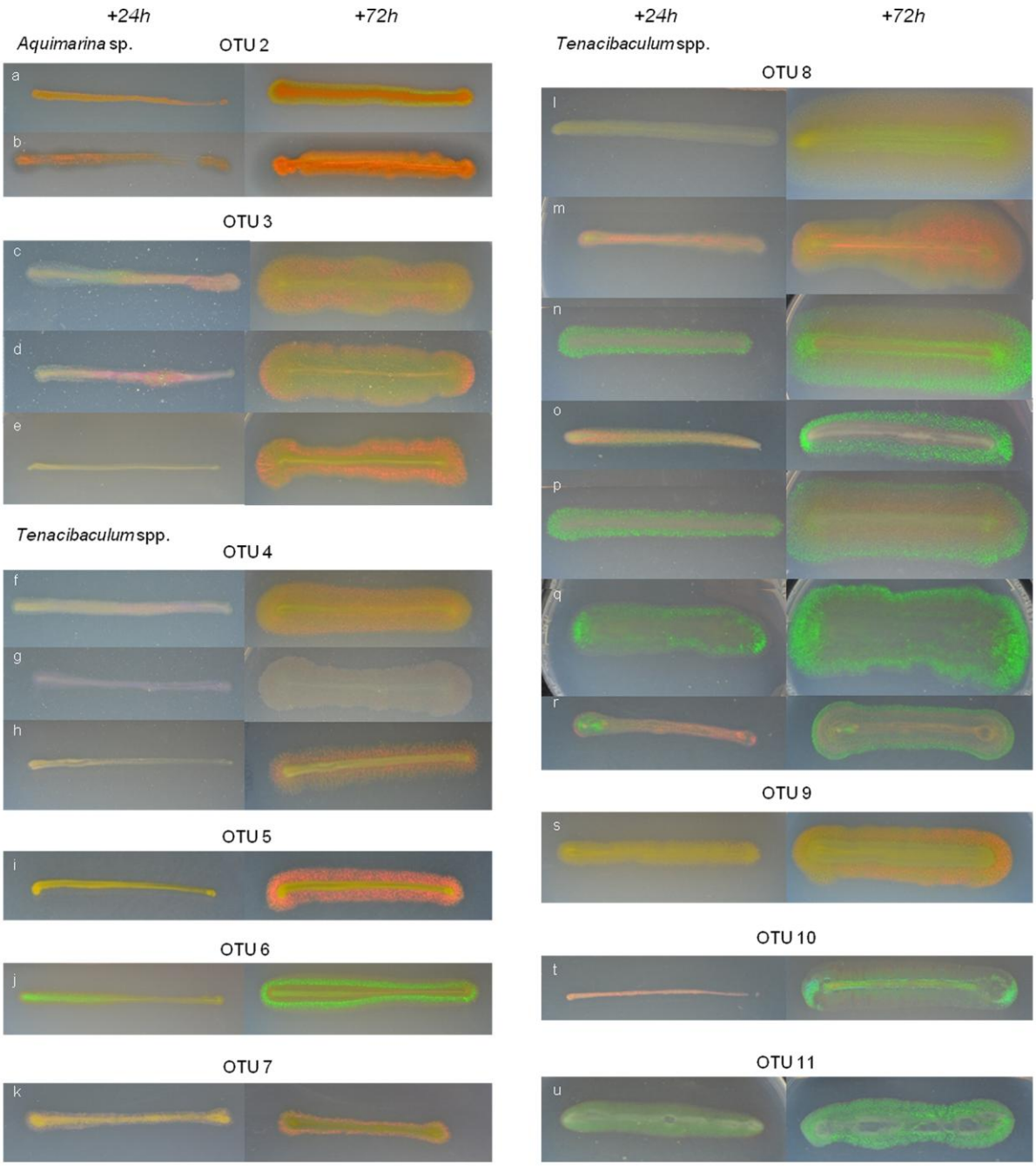


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## Tables

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

OTU: Operational Taxonomic Unit.

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