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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 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

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).

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 immersed 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 CaCl₂·2H₂O, 0.5 g MgSO₄·7H₂O, 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

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

Fig. 5

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

Phenotypic diversity of the others iridescent strains

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

Fig. 6

Source of isolation of iridescent bacteria

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

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

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

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

Flavobacteriales are common in the marine environment [2]. We found that iridescent strains were particularly abundant on rocky shore (53%). This biotope comprises a higher biodiversity of organisms than muddy environment. The presence of iridescent bacteria in rocky shore ecosystem that combined stressful conditions from solar exposure, desiccation, and great fluctuations in salinity, may give preliminary indications on the possible biological roles of the iridescence (thermoregulation, UV protection, light filtering and desiccation 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.

References

- [1] Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. (1990) Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410.
- [2] Bernardet, J.-F., Nakagawa, Y. (2006) An introduction to the family *Flavobacteriaceae*. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E. (Eds.), *The Prokaryotes*. Third edition, Volume 7, Springer, New York, pp. 455–480.
- [3] Choi, D.H., Kim, Y.-G., Hwang, C.Y., Yi, H., Chun, J., Cho, B.C. (2006) *Tenacibaculum litoreum* sp. nov., isolated from tidal flat sediment. *Int. J. Syst. Bacteriol.* 56, 635–640.
- [4] Clayton, R.A., Sutton, G., Hinkle, P.S., Bult, C., Fields, C. (1995) Intraspecific variation in small-subunit rRNA sequences in GenBank: why single sequences may not adequately represent prokaryotic taxa. *Int. J. Syst. Bacteriol.* 45, 595–599.
- [5] Craigie, J.S., Correa, J.A., Gordon, M.E. (1992) Cuticules from *Chondrus crispus* (Rhodophyta). *J. Phycol.* 28, 777–786.
- [6] Doucet, S.M., Meadows, M.G. (2009) Iridescence: a functional perspective. *J. R. Soc. Interface* 6(Suppl. 2), S115–S132.
- [7] Felsenstein, J. (2004) *Inferring Phylogenies*, Sunderland, Massachusetts: Sinauer Associates, Inc. Publishers.

- 316 [8] Fox, D.L. (1976) Animal biochromes and structural colors: physical, chemical,
317 distributional & physiological features of colored bodies in the animal world. Second edition.
318 University of California Press, Berkeley, CA.
- 319
- 320 [9] Gerwick, W.H., Lang, N.J. (1977) Structural, chemical and ecological studies on
321 iridescence in *Iridaea* (Rhodophyta). J. Phycol. 13, 121–127.
- 322
- 323 [10] Ghiradella, H. (1991) Light and color on the wing: structural colors in butterflies and
324 moths. Appl. Optics 30, 3492–3500.
- 325
- 326 [11] Goecke, F., Labes, A., Wiese, J., Imhoff, J.F. (2010) Chemical interactions between
327 marine macroalgae and bacteria. Mar. Ecol. Prog. Ser. 409, 267–300.
- 328
- 329 [12] Guindon, S., Gascuel, O. (2003) A Simple, Fast, and Accurate Algorithm to Estimate
330 Large Phylogenies by Maximum Likelihood. Syst. Biol. 52, 696–704
- 331
- 332 [13] Johansen, J.E., Nielsen, P., Sjøholm, C. (1999) Description of *Cellulophaga baltica* gen.
333 nov., sp. nov. and *Cellulophaga fucicola* gen. nov., sp. nov. and reclassification of
334 [*Cytophaga*] *lytica* to *Cellulophaga lytica* gen. nov., comb. nov. Int. J. Syst. Evol. Micr. 49,
335 1231–1240.
- 336
- 337 [14] Jung, S.Y., Oh, T.K., Yoon, J.H. (2006) *Tenacibaculum aestuarii* sp. nov., isolated from a
338 tidal flat sediment in Korea. Int. J. Syst. Evol. Micr. 56, 1577–1581.
- 339
- 340 [15] Kientz, B., Ducret, A., Luke, S., Vukusic, P., Mignot, T. Rosenfeld, E. (2012) Glitter-like
341 iridescence within the *Bacteroidetes* especially *Cellulophaga* spp.: optical properties and
342 correlation with gliding motility. PLoS ONE 7(12): e52900. doi:10.1371/journal.pone.0052900

- 343 [16] Kientz, B., Marié, P., Rosenfeld, E. (2012b) Effect of abiotic factors on the unique glitter-
344 like iridescence of *Cellulophaga lytica*. FEMS Microbiol. Lett. 333, 101-108.
345
- 346 [17] Kientz, B., Vukusic, P., Luke, S., Rosenfeld, E. (2012a) Iridescence of a marine
347 bacterium and classification of prokaryotic structural colors. Appl. Environ. Microbiol. 78,
348 2092–2099
349
- 350 [18] Lane, D.J. (1991) 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M.
351 (Eds.), Nucleic acid techniques in bacterial systematics. John Wiley and Sons, New York,
352 NY, pp. 115-175.
353
- 354 [19] Lewin, R.A., Lounsbery, D.M. (1969) Isolation, cultivation and characterization of
355 Flexibacteria. J. Gen. Microbiol. 58, 145–170.
356
- 357 [20] Ludwig, W., Strunk, O., Westram, R. *et al.* (2004) ARB: a software environment for
358 sequence data. Nucleic Acids Research 32, 1363–1371
359
- 360 [21] Lythgoe, J.N., Shand, J. (1989) The structural basis for iridescent color changes in
361 dermal and corneal iridophores in fish. J. Exp. Biol. 141, 313–325.
362
- 363 [22] Muyzer, G., De Waal, E.C., Uitterlinden, A.G. (1993) Profiling of complex microbial
364 populations by denaturing gradient gel electrophoresis analysis of polymerase chain
365 reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695–700.
366
- 367 [23] Nedashkovskaya, O.I., Vancanneyt, M., Christiaens, L., Kalinovskaya, N.I., Mikhailov,
368 V.V., Swings, J. (2006) *Aquimarina intermedia* sp. nov., reclassification of *Stanierella*
369 *latercula* (Lewin 1969) as *Aquimarina latercula* comb. nov. and *Gaetbulimicrobium brevivitae*

- 370 (Yoon et al. 2006) as *Aquimarina brevivittae* comb. nov. and emended description of the
 371 genus *Aquimarina*. Int. J. Syst. Evol. Micr. 56, 2037–2041.
- 372
- 373 [24] Noyes, J., Sumper, M., Vukusic, P. (2008) Light manipulation in a marine diatom. J.
 374 Mater. Res. 23, 3229–3232.
- 375
- 376 [25] Patel, P., Callow, M.E., Joint, I., Callow, J.A. (2003) Specificity in the settlement-
 377 modifying response of bacterial biofilms towards zoospores of the marine alga
 378 *Enteromorpha*. Environ. Microbiol. 5, 338–349.
- 379
- 380 [26] Piñeiro-Vidal, M., Riaza, A., Santos, Y. (2008a) *Tenacibaculum discolor* sp. nov. and
 381 *Tenacibaculum gallaicum* sp. nov., isolated from sole (*Solea senegalensis*) and turbot
 382 (*Psetta maxima*) culture systems. Int. J. Syst. Evol. Micr. 58, 21–25.
- 383
- 384 [27] Piñeiro-Vidal, M., Carballas, C.G., Gómez-Barreiro, O., Riaza, A., Santos, Y. (2008b)
 385 *Tenacibaculum soleae* sp. nov., isolated from diseased sole (*Solea senegalensis* Kaup). Int.
 386 J. Syst. Evol. Micr. 58, 881–885.
- 387
- 388 [28] Prum, R.O., Torres, R. (2003) Structural coloration of avian skin: convergent evolution of
 389 coherently scattering dermal collagen arrays. J. Exp. Biol. 206, 2409–2429.
- 390
- 391 [29] Seago, A.E., Brady, P., Vigneron, J.-P., Schultz, T.D. (2009) Gold bugs and beyond: a
 392 review of iridescence and structural color mechanisms in beetles (Coleoptera). J. R. Soc.
 393 Interface 6(Suppl 2), S165–S184.
- 394
- 395 [30] Skerratt, J.H., Bowman, J.P., Hallegraeff, G., James, S., Nichols, P.D. (2002) Algicidal
 396 bacteria associated with blooms of a toxic dinoflagellate in a temperate Australian estuary.
 397 Mar. Ecol. Prog. Ser. 244, 1–15.

398

399 [31] Stackebrandt, E. (2006) Molecular identification, systematics, and population structures
400 of prokaryotes. Erko stackebrandt edition, Springer-Verlag Berlin.

401

402 [32] Suzuki, M., Nakagawa, Y., Harayama, S., Yamamoto, S. (2001) Phylogenetic analysis
403 and taxonomic study of marine Cytophaga-like bacteria: proposal for *Tenacibaculum* gen.
404 nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov.,
405 and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp.
406 nov. Int. J. Syst. Evol. Microbiol. 51(), 1639–1652.

407

408 [33] Vukusic, P., Sambles, J.R., Lawrence, C.R., Wootton, R.J. (2001) Structural color: now
409 you see it-now you don't. Nature 410, 36.

410

411 [34] Wang, J.-T., Chou, Y.-J., Chou, J.-H., Chen, C.A., Chen, W.-M. (2008) *Tenacibaculum*
412 *aiptasiae* sp. nov., isolated from a sea anemone *Aiptasia pulchella*. Int. J. Syst. Evol.
413 Microbiol. 58(Pt 4), 761-766.

414

415 [35] Welch, V., Vigneron, J.-P., Lousse, V., Parker, A. (2006) Optical properties of the
416 iridescent organ of the comb-jellyfish *Beroë cucumis* (Ctenophora). Phys. Rev. E 73,
417 0419161–0419167.

418

419 [36] Yarza, P., Richter, M., Peplies, J., Euzéby, J., Amann, R., Schleifer, K.-H., Ludwig, W.,
420 Glöckner, F.O., Rosselló-Móra, R. (2008) The All-Species Living Tree project: A 16S rRNA-
421 based phylogenetic tree of all sequenced type strains. Syst. Appl. Microbiol. 31, 241–250

422

423 [37] Yoon, J.-H., Kang, S.-J., Oh, T.-K. (2005) *Tenacibaculum lutimaris* sp. nov., isolated
424 from a tidal flat in the Yellow Sea, Korea. Int. J. Syst. Evol. Microbiol. 55(), 793-798.

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Figures legends

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

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

Fig. 3. Glitter-like iridescence appearance under direct illumination of different *Cellulophaga* affiliated isolates (OTU 1) after one day (up line) and four days (bottom line) of incubation at 20°C on CYT medium. Strains were isolated from (a) dry sand at Ronce les bains, (b) rhodophyta *Palmaria palmata* at Whales lighthouse, (c) phaeophyta *Fucus vesiculosus* at Ronce les bains, (d) chlorophyta *Ectocarpus siliculosus* at Chassiron lighthouse. Gliding motility can be identified as the spreading zone from the colony center. Agarolysis corresponds to the dark halo visible on colony edges.

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

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

values >50% indicated at the nodes. * indicated branches that were also found using the neighbor-joining and the maximum parsimony methods

Fig. 6. Iridescence of the isolated marine bacteria after 1 day (left) and 3 days (right) of culture at 20°C on CYT medium. Both OTU 2 (a,b) were isolated from *Chondrus crispus* red macroalgae. Strains belonging to OTU 3 were isolated from (c) *Pterocladia* sp. red macroalgae, (d) an unknown hydroid and (e) a seastar *Asterina gibbosa*. Strains belonging to OTU 4 were from (f) a decomposed oyster, (g) seawater sample and (h) a limpet. OTU 5, 6 and 7 were respectively isolated from *Fucus vesiculosus* (i), a wrinkle (j), and from seawater (k). Isolated grouped in OTU 8 were isolated from (l) *Pterocladia* sp. red macroalgae, (m) an unattached laminaria, (n) sand, (o) *Corallina officinalis* red macroalgae, (p) worms sand formation, (q) seawater and (r) crab. OTU 9, 10 and 11 were respectively isolated from a *Zostera marina* (s), a decomposed oyster (t) and an unattached *Saccharina* sp. brown macroalgae.

Figures

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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 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> - <i>Cellulophaga lytica</i> DSM7489 ^T	99 % 98%	AB032512 CP002534
OTU 2	Strain 46	JX454530	5 (5.2%)	- <i>Aquimarina latercula</i> - <i>Aquimarina latercula</i> ATCC23177 ^T	98 % 97%	AB681002 M58769
OTU 3	Strain 35	JX454522	18 (18.6 %)	-« <i>Flexibacter aurantiacus</i> subsp. <i>Copepodarum</i> » - <i>Tenacibaculum aiptasiae</i> a4 ^T	99 % 98%	AB078044 EF416572
OTU 4	Strain 116	JX454523	6 (6.2 %)	-« <i>Flexibacter aurantiacus</i> subsp. <i>Copepodarum</i> » - <i>Tenacibaculum aiptasiae</i> a4 ^T	98 % 97%	AB078044 EF416572
OTU 5	Strain 66	JX454528	2 (2.1%)	- <i>Tenacibaculum ovolyticum</i> - <i>Tenacibaculum ovolyticum</i> IFO 15947 ^T	97 % 97%	AB681005 AB078058
OTU 6	Strain 70	JX454527	2 (2.1%)	- <i>Tenacibaculum soleae</i> - <i>Tenacibaculum soleae</i> LL04 12.1.7 ^T	99 % 99%	AM989479 AM746476
OTU 7	Strain 103	JX454524	1 (1.0 %)	- <i>Tenacibaculum discolor</i> - <i>Tenacibaculum aiptasiae</i> a4 ^T	97 % 96%	JQ231117 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> » - <i>Tenacibaculum litopenaei</i> B-I ^T	96 % 95%	AB078044 DQ822567
OTU 10	Strain 82	JX454526	1 (1.0 %)	- <i>Tenacibaculum aiptasiae</i> - <i>Tenacibaculum aiptasiae</i> a4 ^T	98 % 98%	NR044202 EF416572
OTU 11	Strain 105	JX454525	8 (8.2 %)	- <i>Tenacibaculum aestuarii</i> SMK-4 ^T	99 %	DQ314760

OTU: Operational Taxonomic Unit.

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