Iridescence is a property of structural color that is occasionally encountered in higher eukaryotes but that has been poorly documented in the prokaryotic kingdom. In the present work, we describe a marine bacterium, identified as Cellulophaga lytica, isolated from the surface of an anemone, that exhibits bright green iridescent colonies under direct epi-illumination. This phenomenon has not previously been investigated in detail. In this study, color changes of C. lytica colonies were observed at various angles of direct illumination or observation. Its iridescent green appearance was dominant on various growth media. Red and violet colors were also discerned on colony edges. Remarkable C. lytica bacterial iridescence was revealed and characterized using high-resolution optical spectrometry. In addition to this, by culturing other bacterial strains to which various forms of violet colors were also discerned on colony edges. Remarkable C. lytica bacterial iridescence was revealed and characterized using high-resolution optical spectrometry. In addition to this, by culturing other bacterial strains to which various forms of violet colors were also discerned on colony edges. Remarkable C. lytica bacterial iridescence was revealed and characterized using high-resolution optical spectrometry. In addition to this, by culturing other bacterial strains to which various forms of violet colors were also discerned on colony edges. Remarkable C. lytica bacterial iridescence was revealed and characterized using high-resolution optical spectrometry. In addition to this, by culturing other bacterial strains to which various forms of violet colors were also discerned on colony edges.
we have compared the optical effects in a broad range of bacteria using both epi-illumination and transillumination. Special attention was given to the strains previously described as “iridescent.”

MATERIALS AND METHODS

Sample collection and bacterial isolation. Collection of samples was performed on Chassiron lighthouse rocky shore at Oleron Island, west Atlantic coast of France (46°02’48”N, 1°24’57”W) in December 2009. Various marine organisms (macroalgae, sponges, anemones, crustaceans, mollusks, starfishes, and fishes) were collected with plastic bags, transported in sterile plastic bags (to avoid terrestrial contamination), and processed immediately for microbiological studies. Tissues from the marine organisms were washed thoroughly with sterile artificial seawater (ASW; Instant Ocean) in order to remove loosely attached epibions. Two-centimeter-square tissue specimens were then imprinted on marine agar (MA) purchased from Dutscher (Laboratoires Conda, S.A. Prona- disa) (64). Plates were examined visually after aerobic incubation for 24 h at 20°C or 30°C.

Taxonomic identification. Genetic sequencing identified the isolated bacterial strain. Primers used for rRNA 16S gene sequencing were F1 (5’-AGAATTGCTGCTGGTCT-TG3’), R1 (5’-AGGTTTGAACCTGAGGAG-TG3’), and R2 (5’-GA CAGGACGGTACGACA3’-3’) (75). Primers used for the 16S rDNA and the internal transcribed spacer 2 (ITS2) were 23SF (5’-AACCCTGTTGA CGTTGAAAAG-3’) and 23SR (5’-ATTCCAGGTTTCTTCGAGG-3’), respectively. The sequences were compared with the NCBI database and Lebibi database (http://umr5558-sud-str1.univ-lyon1.fr/lebibi/lebibi.cgi) by using the BLAST service to determine their phylogenetic identity.

Culture of C. lytica. The isolated marine bacterium was cultivated at 20°C or 25°C on three solid media. MA medium was employed preferentially for analysis of iridescence. Cytophaga agar (CTY) and low-nutrient (LN) media were made with ASW (30 g · liter−1; Instant Ocean). CYT medium contained 1 g of tryptone, 0.5 g of yeast extract, 0.5 g of CaCl2 · H2O, 0.5 g of MgSO4 · H2O, and 15 g of agar in 1 liter of ASW. In this medium, casein was replaced by tryptone because C. lytica does not degrade casein (33). LN medium contained only 15 g of agar in 1 liter of ASW (32).

Bacterial strains and culture media used for iridescence compari-

(i) Bacterial strains. A total of 74 strains were compared. Bacteria described as iridescent in previous literature were Haemophilus influenzae (8, 19, 42, 44, 55), Pseudomonas aeruginosa (9, 14, 30, 77, 80), Alcanivorax balearicus (63), Anaerobic bacilli (2), Listeria monocytogenes (24), Listeria innocua (39), Bordetella pertussis (69), Salmonella enterica (43), Mannheimia haemolytica (Pasturella multocida) (31), and Pasteurella multocida (7, 12, 29). Since iridescence was mentioned in bacterial groups, Stenotrophomonas maltophilia, described as iridescent in previous literature were Vibrio cholerae, Pseudo-

(ii) Culture media. Appropriate media for iridescence observations were selected from literature data or were defined by experimental assays. Ready-to-use media (Dutscher) were nutrient agar (NA) (53), brain heart agar (BHA), and tryptic soy agar (TSA) for Anaerobic bacilli (2), Luria-Bertani (LB) for Pseudomonas culture (9), and MA for marine strains such as Vibrio spp. and C. lytica. Prepared media were tryptose agar (TryptA) with 20 g of tryptose, 1 g of glucose, 5 g of NaCl, and 15 g of agar per liter for Listeria spp. (39) and Tween-peptone agar (TGA) with 10 g of Tween 20, 10 g of peptone, 5 g of NaCl, 0.1 g of CaCl2 · H2O, and 15 g of agar per liter for Alcanivorax balearicus (63). For Haemophilus influenzae cultures, Levinthal’s XV medium (Lev XV) was prepared by mixing 10 g of peptone A, 10 g of meat extract, 5 g of NaCl, and 20 g of agar per liter, with a supplement of 15 mg of X (hemin) and V (NAD+ ) factors added after autoclaving (19, 55).

Macroscopic examination of bacterial iridescence. (i) Epi- and transillumination methods. Iridescence of bacterial colonies was observed with the aid of a streaking procedure. One colony from a 24-h-old plate was subcultured in duplicate plates by drawing thin 5-cm linear streaks. After 24 h incubation, cultures were photographed in a dark room using two experimental arrangements of oblique epi-illumination and transillumination (see Fig. S1 in the supplemental material). The camera was a Canon Powershot A650 IS image stabilizer AIAF on the Av program. The lens was a macro, large size (12.1 megapixels) used in superfine mode. Illumination was with an E14 220- to 240-V, 11-W bulb (532 lumen at 2,700 K). For oblique epi-illumination, the plate was placed on a black backing. The optical axis of the camera formed an angle of 45° with the center of the plate. The light was fixed obliquely with an angle α of 67.5° from the plate. For transillumination measurements, samples were photographed from an angle of 45° above the petri dish with the light source directly behind it (i.e., normal incidence illumination in transmission).

(ii) Examination of C. lytica color changes. In order to observe the color changes as a function of the illumination angle, the epi-illumination setup was employed. Pictures were taken alternatively at five different angles of incident light. Angle α values were 22.5°, 67.5°, 90°, 112.5°, or 135°. For these experiments, C. lytica was grown at 20°C (instead of 23°C) to observe all colorations more effectively.

Microscopic examination of C. lytica colony colors. Detailed observations of colored colonies were performed under epi-illumination by using a numeric Keyence microscope (VHX-1000E). A VHX-1100 cam-

Physical measurement of C. lytica (microspectrophotometry). Illumination was directed onto the sample through an Ocean Optics UV-visible-near infrared optical fiber that was connected to an Ocean Optics HPI-2000 light source that spans approximately 300 nm to 850 nm. The reflected light was collected using a similar optical fiber that was itself connected to an Ocean Optics USB4000-UV-visible spectrometer (see Fig. S2 in the supplemental material). The angles of illumination and of detection could be separately set and controlled to a resolution of 0.5°. For a series of chosen fixed illumination angles, the collection fiber was stepped in 2° angle steps in an arc over the sample, and reflection spectra were recorded at each angular position. In this way, the dependence of reflected color with angle and, hence, the extent of each sample’s iridescence could be measured and assessed (72, 74).

RESULTS

Isolation and identification of a marine bacterium with a glitter-like color appearance. While searching for new cultivable epibiotic bacteria in the marine environment, we isolated a Gram-negative bacterium from the surface of a red anemone (Actinia
strain LIM-21T was recently genome sequenced, but no shiny structural color. In both cases, and white bacterial colonies; (B) the second shows a pure culture of C. lytica observed under direct epi-illumination allowing examination of the intense structural color. In both cases, C. lytica was grown aerobically at 25°C on MA.

Colonies of C. lytica exhibited bright iridescent reflected color when grown on MA plates and viewed under epi-illumination (Fig. 1B). The iridescence was not visible when colonies were resuspended or cells were grown in liquid media (data not shown). Iridescent green was the dominant color, but red and blue-violet were also observed at the colonies’ peripheral edges. The MA-grown colonies’ color appearance comprised submillimeter-sized centers of color of varying brightness distributed across the iridescent region. This gave the colonies’ color reflection and intensity a glitter-like character.

The marine strain was taxonomically identified by performing both 16S rRNA and 16S to 23S (16S-23S) ITS sequence analyses. The strain was phylogenetically affiliated with the Cytophaga-Flavobacterium-Bacteroides (CFB) group and the Flavobacteriaceae family and was identified as Cellulophaga lytica (CP002534, DSM 7489) (33, 37) with 16S rRNA, 23S rRNA, and ITS sequence similarities of 100%, 100%, and 99%, respectively. A thorough analysis of literature data showed that a “metallic tinge” of the colonies was previously mentioned for the affiliated strain C. lytica ATCC 23178T (DSM 7489 = CIP 103822 = LIM-21T) (33). The relative strain LIM-21T was recently genome sequenced, but no shiny effect was detailed in the description of its morphological appearance (50).

**Coloration of C. lytica colonies on different culture media.**

Agarolytic, mucous colonies with gliding motility and the bright glitter-like color centers effect were common characteristics for all media (Fig. 2a1, b1, and c1). Colonies grown on CYT were larger and less pigmented (Fig. 2b1). Blue was observed in the inner zone of the colony, and green, yellow, red, and violet were also visible (Fig. 2b2 and b3). Less growth occurred on the LN medium (Fig. 2c1). Colonies were translucent in this medium, and only green iridescence was discernible (Fig. 2c2 and c3; see Movie S2 in the supplemental material).

**Angle dependence of C. lytica colonies’ coloration.**

By changing the illumination angle from 22.5° to 135°, red and violet zones of the colony became green, while the central bright green region became blue or noniridescent (Fig. 2D). This angle dependence of reflected color, defined as iridescence, was the first direct evidence for a structural mechanism as the origin of the color. Color changes were also examined microscopically using the Keyence microscope (Fig. 2E; see Movie S3 in the supplemental material). Pictures demonstrated that bright green iridescence was predominant when illumination was close to grazing incidence (Fig. 2e3.h and e4.h). Violet-to-red color changes were observed at colony edges (Fig. 2e3.h and e3.l). Different iridescent color centers appeared and disappeared when the illumination position was modified from high to low incidence (black arrows in Fig. 2e4.h and e4.l). The image associated with an intermediate angle of illumination exhibited color centers which overlapped (Fig. 2e3.i and e4.i).

**Physical evidence of C. lytica iridescence.**

The data presented in Fig. 3 show optical reflection bands that unequivocally represent the iridescence of C. lytica bacterial colonies by the change in their color with angle. For instance, under illumination at an angle of −70°, the principal reflected color is green over a 70° angle range (−60° to +10°). However, within this angle range, the peak reflection wavelength changes continuously from approximately 550 nm to approximately 500 nm. This band of reflected color extends still further toward higher positive angles, the peak wavelength of which decreases to approximately 410 nm at a scattered angle of +70°. Three other reflected bands of color are shown on this map of reflectance data; each of these shows peak wavelengths that are also angle dependent: two at near-UV wavelengths and one in the near infrared.

**Overall comparison of bacterial iridescence.**

For a better understanding of bacterial structural color effects, we examined the iridescence of a broad range of bacterial strains. The extended classification of these bacterial optical effects is presented in Table S1 in the supplemental material, with a selection of images presented in Fig. 4. We propose a model of four separate bacterial iridescence categories: rainbow-diffuse (D) and rainbow-edge (R) appearances under transillumination and metallic (M) and glitter-like (G) appearances under epi-illumination.
The rainbow-diffuse category comprises bacterial colonies that exhibited all spectral colors ranging from red to blue only under the condition of transillumination (Fig. 4). Various color intensities were observed within this category. A large number of bacterial strains also displayed this visual effect (see Table S1 in the supplemental material). The rainbow-edge iridescence was visible only on colonies’ edges. This phenomenon does not appear to have previously been described in literature. A few strains, namely, Bacillus cereus (Fig. 4), Stenotrophomonas maltophilia, Klebsiella pneumoniae, and Aneurinibacillus migulanus, displayed rainbow-edge iridescence, with these four expressing a common characteristic of thick and opaque colonies.

The metallic category comprised colonies exhibiting a silvery appearance under epi-illumination. As described in the literature (9), the ΔlasR mutant of P. aeruginosa exhibited a faintly silver appearance (Fig. 4). The metallic appearance of Aneurinibacillus...
migulanus type III previously described (2) could not be reproduced (see Table S1 in the supplemental material).

C. lytica strains were not iridescent under transillumination (Fig. 4). Their glitter-like iridescence is characterized principally by an intense green iridescent reflection. This novel iridescence is significantly higher in intensity than that of the bacterial structural coloration of all other three categories (Fig. 4). The iridescence of C. lytica was also found in another strain, DSM 2040, but not in the two strains CIP 103822 and DSM 2039. Moreover, the genome-sequenced strain DSM 7489 displayed only very-low-intensity iridescent color.

DISCUSSION

A marine bacterium exhibiting a bright iridescently colored colony appearance has been isolated in this study. Although other forms of bacterial iridescence have been described in selected literature, the phenomenon has never been comprehensively investigated or discussed.

The comparison of diverse bacteria by two illumination protocols, transillumination and epi-illumination, enabled the classification of four categories of bacterial iridescence. The rainbow-diffuse iridescence was common in particular in smooth colonies and was present in mucous, capsulated, and pathogenic bacteria. This iridescence, which has never been explained, has previously been used as an easily observed criterion to discriminate between capsulated and noncapsulated strains of H. influenzae (26, 54, 55).

FIG 3 Color map showing the angle-dependent spectral reflectance of Cellulophaga lytica and confirming its iridescent appearance. The C. lytica sample was illuminated at a fixed light angle of −70°. Scattered wavelengths from 300 nm to 850 nm were recorded at different detection angles from −80° to 85° with 2° angle step resolution (the illumination plane and the detection plane were offset from each other by 3° to enable unobstructed detection over the full angle range). The color scale indicates the relative intensity of reflectance. The following emitted colors are given by the indicated wavelength value: UV, <400 nm; violet, 400 to 435 nm; blue, 435 to 490 nm; cyan, 490 to 520 nm; green, 520 to 560 nm; yellow, 560 to 590 nm; orange, 590 to 620 nm; red, 620 to 700 nm; and infrared, >700 nm.

FIG 4 Examples of bacterial colonies belonging to different structural color categories. Observations were processed on epi- and transillumination. Iridescence categories are rainbow-diffuse (D), consisting of diffuse colors of the light spectrum; rainbow-edge (E), consisting of shining light spectrum color only on edges; metallic (M), consisting of silvery luster; and glitter-like (G), consisting of iridescent green in the middle and red and violet on the colony edges. Culture conditions are informed in Table S1 in the supplemental material.
The rainbow-edge iridescence was less common and might occur only at specific thicknesses of the colonies. Only a few *P. aeruginosa* strains exhibited the metallic appearance. Surprisingly, two *P. aeruginosa* strains (ATCC 27853 and a clinical mucous strain) had both rainbow-diffuse (under transillumination) and metallic (under epi-illumination) iridescence. Metallic reflections in *P. aeruginosa* 14 ΔlasR Δpqsh have been linked to the accumulation of the 4-hydroxy-2-heptylquinoline molecule (9, 10, 77). However, no explanation as to how the accumulated molecule creates a metallic-looking reflection has yet been presented. Since metallic appearance is not associated with a change of color with angle, the term “metallic iridescence” should not be used.

A novel iridescence category for the appearance of isolated *C. lytica* was discovered and termed “glitter-like” iridescence. The practical measurement of a broad range of spectrophotometric reflection data on *C. lytica* colonies has enabled us to prove this structural color and to construct the map of wavelength-dispersive reflection bands. These represent a clear iridescence effect, namely, a change of reflected color with angle.

Interestingly, certain *C. lytica* strains appeared noniridescent. The sequenced strain LIM-21T (ATCC 23178T = DSM 7489) (50) exhibited low-intensity iridescence. Described to be identical in the bacterial collection banks, the strains DSM 7489 and CIP 103822 were found to have different colony morphologies; this has possibly led to their different iridescent characteristics. The *C. lytica* organism isolated in this study has the most intense glitter-like appearance.

The iridescence of *C. lytica* was mentioned only superficially in two studies. Colonies of *C. lytica* ATCC 23178T with “metallic tinge” were evoked (33). The term “iridescent” was used only once in an algalic bioactivity study of *C. lytica* ASM 21 (66). “Greenish metallic iridescence” was mentioned in the *Cellulophaga* genus in Bergey’s *Manual of Systematic Bacteriology* (5). It is noteworthy that *Cellulophaga* (*Cytophaga*) *lytica* was first related to the group *Bacteroides* and the order *Cytophagales* before its reclassification within the *Cytophaga-Flavobacterium-Bacteroides* (CFB) group and the order *Flavobacteriales* (33). The unique illustration of *C. lytica* colony in the book *The Prokaryotes* does not show iridescence but shows only common yellow-pigmented colonies. Another picture of a *Cytophaga* species showed a very weak red color appearance described as iridescence (62). Since that date, “redish-greenish iridescence” has been employed as a descriptor for strains belonging to the order *Cytophagales* in the second edition of *The Prokaryotes* or in Bergey’s manual but without additional explanations (37, 59, 60). Moreover, no mention or illustration of iridescence was found in the most recent editions (3, 4, 61). Glitter-like iridescence within the genus *Cellulophaga* and in the family *Flavobacteriaceae* is under investigation.

Structures responsible for the coherent scattering that creates the *C. lytica* iridescence are under investigation by electron microscopy; however, specialized preparation protocols are needed and under development for observation of the micro- and sub-micron-scale biofilm structures in their original state. However, since iridescence involves periodicity, then intercellular communication mechanisms may be involved in the multicellular organization (1, 11). Although these mechanisms are still unknown, it is possible that iridescence implies associated biological roles for spatial organization that offer advantage for the ensemble population. In addition, the iridescence of *C. lytica* colonies was observed under epi-illumination. This manner of illumination is more natural and ubiquitous than transillumination and may also indicate potential ecobiological roles for the phenomenon.

In many higher organisms, structural colors have been strongly linked to biological functions associated with conspecific and interspecific communication purposes. However, these same structures can also serve noncommunication functions such as those related to thermoregulation, UV protection, light filtering, water repellency, mechanical friction reduction, or desiccation prevention (18). In lower organisms such as diatoms, the strong light manipulation associated with the periodic nanostructure on diatom frustule walls might influence the collection of more light into the photoreceptors for more optimized photosynthetic efficiencies (23, 45, 48). In contrast to these examples, the functional roles of iridescence in bacteria have never been explored. Also unanswered is whether bacterial iridescence occurs in natural habitats. *C. lytica*’s iridescence might provide a selective advantage under the relatively extreme conditions (high salinity, temperature variation, desiccation, and light exposure) of its habitats.

**ACKNOWLEDGMENTS**

Betty Kientz was a Ph.D. student with a grant from the Ministère de la Recherche et de l’Enseignement Supérieur. Peter Vukusic acknowledges the support of AFOSR grant FA9550-10-1-0020.

We are indebted to Deborah Hogan (Dartmouth Medical School), Olivier Gaillot (CHU Lille, Lille, France), and Jocelyne Caillon (CHU Nantes, Nantes, France) for providing bacterial strains. H. Agouge’s expert technical assistance with preliminary 16S rRNA gene sequence analysis is gratefully acknowledged.

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