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Iridescence of a Marine Bacterium and Classification of Prokaryotic Structural Colors

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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 faintly iridescent traits have previously been attributed, we identify four principal appearance characteristics of structural color in prokaryotes. A new general classification of bacterial iridescence is therefore proposed in this study. Furthermore, a specific separate class is described for iridescent *C. lytica* strains because they exhibit what is so far a unique intense glitter-like iridescence in reflection. *C. lytica* is the first prokaryote discovered to produce the same sort of intense iridescence under direct illumination as that associated with higher eukaryotes, like some insects and birds. Due to the nature of bacterial biology, cultivation, and ubiquity, this discovery may be of significant interest for both ecological and nanoscience endeavors.

The use of light and color is fundamentally important in biological systems. While the majority of animal and plant colored appearances are generated through pigmentary processes (20), some employ micron- and submicron-sized physical structures as a means to generate color appearance effects (71). These structures can interact with incident light to create preferential scattering of specific spectral colors which can generate the most intense and often functionally targeted optical effects. If the structures are spatially arranged with very periodic geometry, then the color appearance of the animal or plant takes on an often strongly angle-dependent character and the colored appearance is defined as iridescent (18, 35, 36, 78, 41, 71). Animals exhibiting iridescence or, more generally, structural color have been the subject of keen interest to both the biology and physics fields. Structural color has been particularly well studied in Insecta (22, 47, 65, 70, 72, 73), in fishes (34, 40), and in Aves (17, 25, 58, 67). One virus system has also been linked to structural coloration (79). In the marine environment, iridescence has been reported in crabs (49), seashells (6, 38), squid (68), ctenophores (76), macroalgae (21, 51), and diatoms (23, 45).

Iridescence in prokaryotes has been poorly documented since its first observation, made in 1904 by Preisz (57). Until now, the phenomenon has been observed only on colonies or concentrated cell suspensions (53, 54, 57), and detailed illustrations are limited. Various general terms such as "shine," "sheen," "glistening," "metallic effect," "bright colors," "luster," "glow," "glisten," or "rainbow-like" were employed to describe the visual effects observed (15, 19, 28). Confusion with fluorescence has also been made (8, 13, 29). The lack of such precision has created difficulty in the accurate description of the visual characteristics of bacterial iridescence.

The two most described iridescent bacteria are *Pseudomonas aeruginosa* (9, 14, 15, 16, 28, 31, 77, 80) and *Haemophilus influenzae* (8, 19, 42, 44, 55). Two distinct observation methods were used, direct epi-illumination and transillumination, respectively, for *P. aeruginosa* and *H. influenzae*. The type of iridescence observed in certain strains of *P. aeruginosa* was described as a metallic iridescence and has been linked to cell lysis (16, 28, 80) and/or to the production of quinoline derivatives (9, 10, 77). A silvery appearance was also recently mentioned in *Aneurinibacillus migulanus* type III (2). In *H. influenzae*, colonies of capsulated cells display all spectral colors from red to blue under oblique transmitted light (transillumination). This type of iridescence was reported in several bacteria, including *Listeria marthii* (24), *Pasteurella multocida* (7, 29), coli-typhoid group bacteria (53), *Listeria monocytogenes* (39), and *Alcanivorax balearicus* (63). Several attempts were made to explain the transmitted iridescence, notably, by using spectral observations (19, 26, 44, 53, 55, 56). The phenomenon has been ascribed to either orderly arranged cells (19, 52, 53) or randomly arranged cells (27, 56). In these older works, both diffraction grating (19, 44, 52, 53, 56) and film effect (27) theories were proposed, but none could be confirmed.

Taken all together, the literature data suggest that bacterial iridescence is at best a loosely defined phenomenon that lacks rigorous description and understanding. Moreover, an intense structural color similar to some insect and vertebrate iridescence has not yet been documented in the bacterial kingdom. In this study, a marine bacterium forming intense structurally colored colonies that are spectrally brilliant in reflection has been isolated and described. Furthermore, in order to clarify the state of the art,

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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 Oléron Island, west Atlantic coast of France (46°02′48″N, 1°24′37″W) in December 2009. Various marine organisms (macroalgae, sponges, anemones, crustaceans, mollusks, starfishes, and fishes) were collected with plastic gloves, 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 epibionts. Two-centimeter-square tissue specimens were then imprinted on marine agar (MA) purchased from Dutscher (Laboratorios Conda, S.A. Pronadisa) (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′-AGAGTTTGATCCTGGCTCAG-3′), R1 (5′-GTATTACCGCGGCTGCTGGCAC-3′), F2 (5′-CTCCTACGGGAGGCAG-3′), and R2 (5′-GACACGAGCTGACGACA-3′) (75). Primers used for the 23S rRNA and the internal transcribed spacer 2 (ITS2) area were 23SF (5′-AACCCGTTGACGTTGAAAAG-3′), 23SR (5′-CTTGCTTTTCTCGGAGGATG-3′), ITSF (5′-TAGAGGTCGGCAGTTCGAGT-3′), and ITSR (5′-ATCTTCAATATGCCGGGTTG-3′). The sequences were compared with the sequences available in 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 (CYT) 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 $CaCl_2 \cdot H_2O$, 0.5 g of $MgSO_4 \cdot H_2O$, 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 comparison. Bacterial strains with their respective culture conditions are detailed in Table S1 in the supplemental material.

(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), *Aneurinibacillus migulanus* (2), *Listeria marthii* (24), *Listeria monocytogenes* (39), *Bordetella trematum* (69), *Salmonella typhi* (43), *Mannheimia haemolytica* (*Pasteurella mastitidis*) (31), and *Pasteurella multocida* (7, 12, 29). Since iridescence was mentioned in bacterial groups such as coli-typhoid (46, 53), cocci (52), or bacilli (19, 56), strains of the following species were included: *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas stutzeri*, *Salmonella* spp., *Yersinia* spp., *Proteus vulgaris*, *Serratia marcescens*, *Klebsiella pneumoniae*, and *Escherichia coli*. Control bacteria, described as not being iridescent, were *Micrococcus luteus*, *Lactococcus lactis*, *Stenotrophomonas maltophilia*, *Streptococcus pyogenes*, *Enterobacter cloacae*, and two marine strains, *Vibrio anguillarum* and *Vibrio lentus*. Among the tested bacteria, several clinical strains were selected since bacterial iridescence has been associated with pathogenicity (30, 44, 55). Finally, four *Cellulophaga lytica* strains were compared, including the DSM 7489 strain corresponding to the LIM-21^T strain, for which the complete genomic sequence has recently been published (50).

(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 *Aneurinibacillus migulanus* (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 (TrypA) 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 (TPA) with 10 g of Tween 20, 10 g of peptone, 5 g of NaCl, 0.1 g of $CaCl_2 \cdot H_2O$, 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 superfin 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 25°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 camera with a VH-Z20R/Z20W objective lens with adjustable magnifications of ×20, ×30, ×50, ×100 ×200, and ×400, the last one with a specific tool doubling the magnification, was used. To avoid specular reflections, the VH-S30 supporting mount of the camera was oriented at a 60° angle from the plate. The DEPTH UP/3D tool corresponding to the depth-from-defocus (DFD) process was employed at high magnification to focus on all optical fields and to improve image quality. In order to observe transitory colorations, the Keyence device was equipped with a VH-K20 lens ring. The support of the camera was oriented at a 90° angle. By moving the ring from right to left, three positions of illumination were used, namely, high, intermediate, and low light incidence angles.

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 HPX-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*

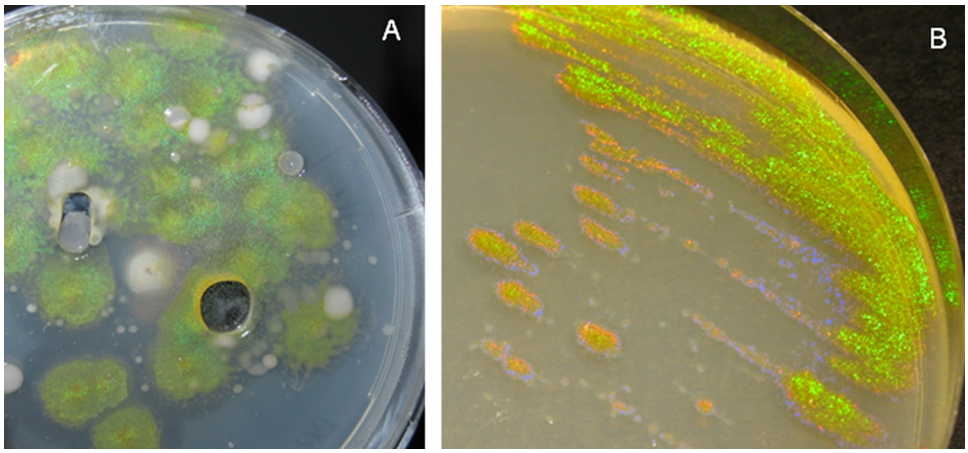

FIG 1 Observations of the marine environment-isolated *Cellulophaga lytica.* (A) The first isolation plate shows colored *C. lytica* colonies together with agarolytic and white bacterial colonies; (B) the second shows a pure culture of *C. lytica* observed under direct epi-illumination allowing examination of the intense structural color. In both cases, *C. lytica* was grown aerobically at 25°C on MA.

equine) (Fig. 1A). Colonies 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 23178^T (DSM 7489 = CIP 103822 = LIM-21^T) (33). The relative strain LIM-21^T 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). On MA, colonies' color appearances comprised yellow pigmentation and principally a green structural color. At the peripheral growth zones, yellow, red, and then violet were observed (Fig. 2a1). This color gradation was confirmed using the Keyence microscope (Fig. 2a2 and a3; see Movie S1 in the supplemental material). 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. On MA, 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.

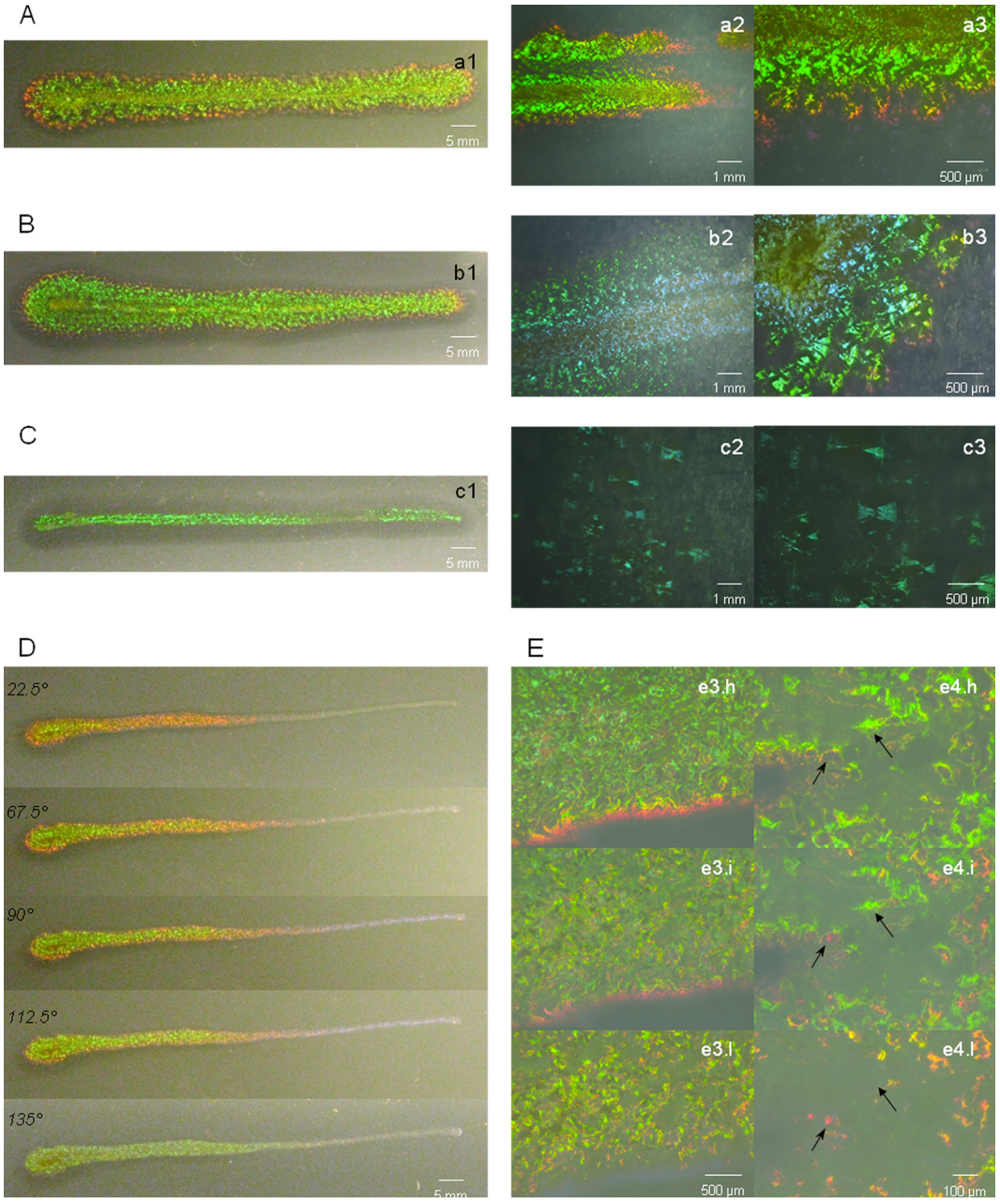

FIG 2 Macroscopic and microscopic observation of *C. lytica* colonies' coloration. Colonies were pictured after 24 h growth on MA (A, D, and E), CYT (B), and LN (C). The inoculation was a thin 5-cm linear streak. Gliding motility can be identified as the spreading zone from the colony center. Bacterial agarolysis corresponds to the dark halo visible on colony edges. Pictures were taken under epi-illumination with a light angle of 67.5° at the macroscopic level (a1 to c1) or using Keyence microscope examination with 60° light incidence using a ×30 objective lens (a2 to c2) and a ×100 objective lens (a3 to c3). Evaluation of color changes at the macroscopic level was performed at diverse illumination angles (D). At the microscopic level (E), examination was performed at high (h), intermediate (i), and low (l) angles of incidence light with a ×100 (e3) and a ×400 (e4) objective lens. Arrows indicate positions of the color center appearing and disappearing as glitters.

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 Δ*lasR* mutant of *P. aeruginosa* exhibited a faintly silver appearance (Fig. 4). The metallic appearance of *Aneurinibacillus*

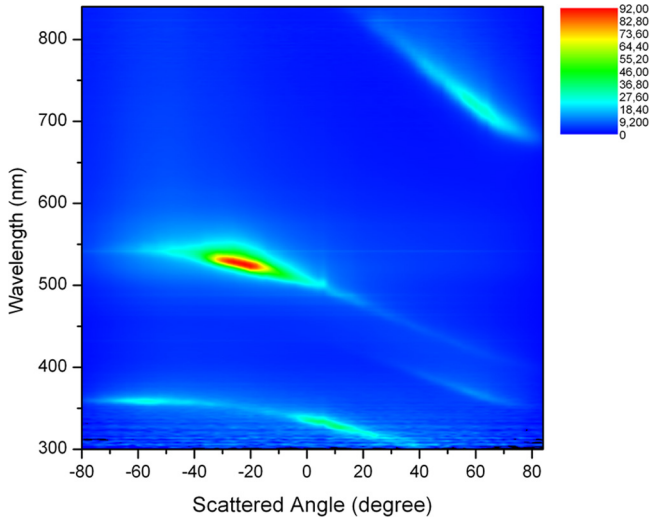

FIG 3 Color map showing the angle-dependent spectral reflectance of *Cellulophaga lytica* and confirming its iridescent appearance. The *C. lytica* sample was illuminated at a fixed light angle of −70°. Scattered wavelengths from 300 nm to 850 nm were recorded at different detection angles from −80° 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.

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).

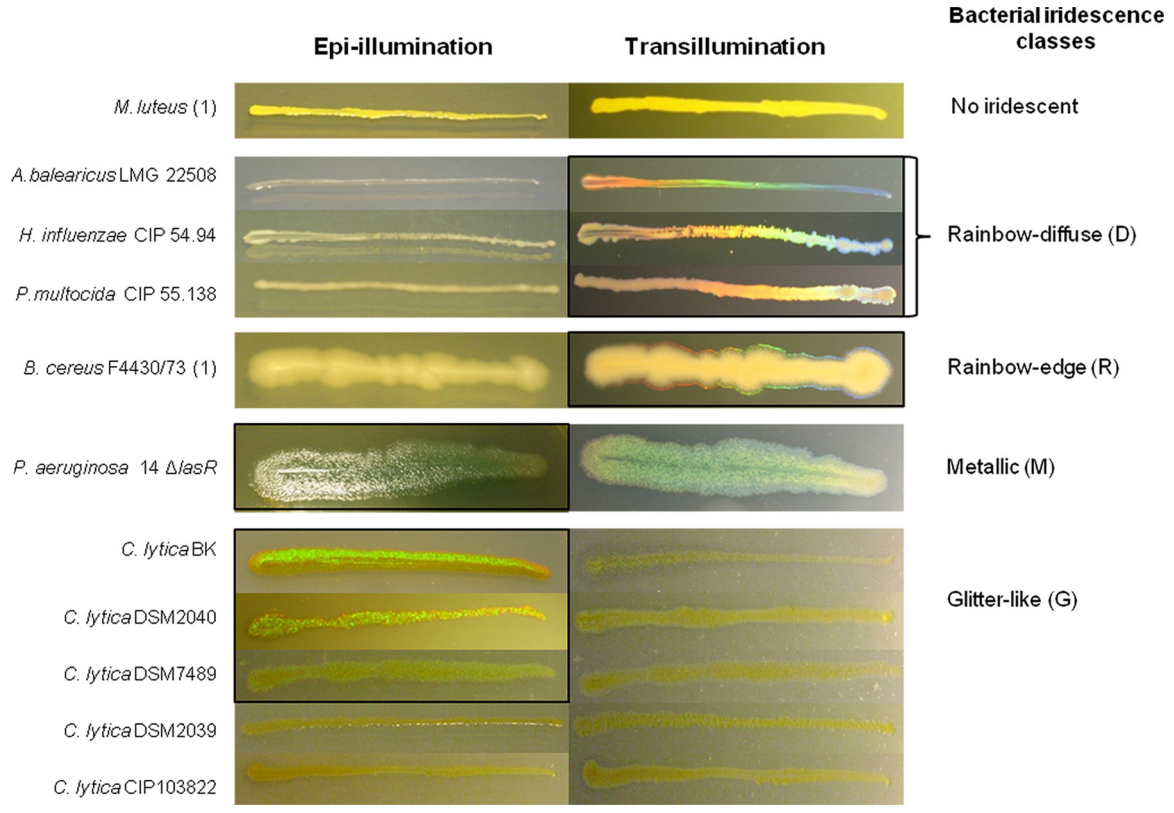

FIG 4 Examples of bacterial colonies belonging to different structural color categories. Observations were processed on epi- and transillumination. Iridescence categories are rainbow-diffuse (D), consisting of diffuse colors of the light spectrum; rainbow-edge (E), consisting of shining light spectrum 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 Δ*lasR* Δ*pqsh* 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-21^T (ATCC 23178^T = 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 23178^T with "metallic tinge" were evoked (33). The term "iridescent" was used only once in an algicidal 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, "reddish-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 micron- and submicron-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.

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