Iridescence of a marine bacterium

and classification of prokaryotic structural colours

Running title: C. lytica and bacterial iridescences

Betty Kientz,1* Peter Vukusic,2 Stephen Luke,2 and Eric Rosenfeld1*

UMR 7266 CNRS - ULR LIENSs, UFR Sciences, Bâtiment Marie Curie, Université de La Rochelle, Avenue Michel Crépeau, 17042, La Rochelle, France.1

School of Physics, University of Exeter, Exeter EX4 4QL, United Kingdom 2

* Corresponding authors. Mailing address: UMR 7266 CNRS - ULR LIENSs, UFR Sciences, Bâtiment Marie Curie, Université de La Rochelle, Avenue Michel Crépeau, 17042, La Rochelle, France.

Phone: 33(0)5 46 45 82 28. Fax: 33(0)5 46 45 82 65.

Emails: eric.rosenfeld@univ-lr.fr, betty.kientz@gmail.com.

Abstract

Iridescence is a property of structural colour that is occasionally encountered in higher eukaryotes but which 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, colour 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 colours 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 principle appearance characteristics of structural colour 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 is 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 endeavours.

**Keywords:** structural colour, bacterial iridescence, glitter-like iridescence, *Cellulophaga lytica*
The use of light and colour are fundamentally important in biological systems. While the majority of animal and plant coloured appearances are generated through pigmentary processes (20), some employ micron- and sub-micron-sized physical structures as a means to generate colour appearance effects (71). These structures can interact with incident light to create preferential scattering of specific spectral colours which can generate the most intense and often functionally-targeted optical effects. If the structures are spatially arranged with very periodic geometry, then the colour-appearance of the animal or plant takes on an often strongly angle-dependent character and the coloured appearance is defined as iridescent (17, 35, 36, 78, 41, 71). Animals exhibiting iridescence, or more generally structural colour, have been the subject of keen interest to both biology and to physics fields. Structural colour has been particularly well studied in Insecta (22, 48, 65, 70, 72, 73), in fishes (34, 40) and in Aves (16, 25, 58, 67). One virus system has also been linked to structural coloration (79). In the marine environment, iridescence has been reported in crabs (50), 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 only observed 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 colours, luster, glow, glisten or rainbow-like” were employed to describe the visual effects observed (14, 19, 28). Confusion has also been made with fluorescence (8, 11, 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, 13, 14, 15, 28, 31, 77, 80) and Haemophilus influenzae (8, 19, 42, 44, 55). Two distinct observation methods were used, direct epi-illumination and trans-illumination respectively for P. aeruginosa and H.
The type of iridescence observed in certain strains of *P. aeruginosa* was described as a metallic iridescence and has been linked to cell lysis (15, 28, 80) and/or to the production of quinoline derivatives (9, 18, 77). A silvery appearance was also recently mentioned in *Aneurinibacillus migulanus* type III (2). In *H. influenzae*, colonies of capsulated cells display all spectral colours from red to blue under oblique transmitted light (trans-illumination). 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 either to 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 colour 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 coloured colonies that are spectrally brilliant in reflection has been isolated and described. Furthermore, in order to clarify the state of the art, we have compared the optical effects in a broad range of bacteria using both epi-illumination and trans-illumination. Special attention was given to the strains previously described as “iridescent”.

*C. lytica* and bacterial iridescences
C. lytica and bacterial iridescences

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. Tissue from the marine organisms were washed thoroughly with sterile artificial seawater (Instant Ocean®) in order to remove loosely attached epibionts. Two centimeter square tissues were then imprinted on Marine agar (MA) purchased from Dutscher (Laboratorios Conda, S.A. Pronadisa®) (64). Plates were examined visually after aerobic incubation of 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 rRNA 23S and ITS2 area were 23SF (5′-AACCCGTTGACGTTGAAAAG-3′), 23SR (5′-CTTGCTTTTCTCGGAGGATG-3′), ITSF (5′-TAGAGGTCCGGCAGTTCGAGT-3′) and ITSIR (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 its phylogenetic identity.

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

Bacterial strains and culture media used for iridescence comparison. Bacterial strains with their respective culture conditions are detailed in Supplemental Table S1.

(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, 13, 30, 77, 80), Alcanivorax baeleareicus (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 bacteria groups such as in coli-typoid (46, 53), cocci (52) or bacilli (19, 56), the following strains were included: Staphylococcus sp., Bacillus sp., Pseudomonas stutzeri, Salmonella sp., Yersinia sp., Proteus vulgaris, Serratia marcerens, Klebsiella pneumonia, and Escherichia coli. Control bacteria, not described as iridescent, were: Micrococcus luteus, Lactococcus lactis, Stenotrophomonas maltophilia, Streptococcus pyogenes, and Enterobacter cloacae, and two marine strains, Vibrio anguillarum and Vibrio lestus. 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 LIM-21¹ strain for which the complete genomic sequence has recently been published (47).

(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), Tryptic soy agar (TSA) for Aneurinibacillus
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migulanus (2), Luria Bertani (LB) for Pseudomonas culture (9) and MA for marine strains as

Vibrio sp. and C. lytica. Prepared media were Tryptose agar (TrypA) with 20 g tryptose, 1 g glucose, 5 g NaCl and 15 g of agar per liter for Listeria sp. (39), and TPA with 10 g tween 20, 10 g peptone, 5 g NaCl, 0.1 g CaCl$_2$H$_2$O and 15 g of agar per liter for Alcanivorax balearicus (63).

For Haemophilus influenza cultures, Levinthal’s XV media (Lev XV) was prepared by mixing 10 g peptone A, 10 g meet extract, 5 g 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 trans- illumination methods. Iridescence of bacterial colonies was observed with the aid of a streaking procedure. One colony from a 24h-old plate was subcultured in duplicate plates drawing thin 5 cm-linear streaks. After 24h-incubation, cultures were photographed in a dark room using two experimental arrangements of oblique epi-illumination and trans-illumination (Supplemental Figure S1). The camera was a Canon Powershot A650 IS image stabilizer AiAF on the Av program. The lens was a macro, large size (12.1 Mega pixels) used in superfin mode. Illumination was with E14 220-240 V, 11 W bulb (532 lumen at 2700 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 $\alpha$ of 67.5° from the plate. For trans-illumination 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 colour changes. In order to observe the colour changes as a function of the illumination angle, the epi-illumination set up was employed. Pictures were taken alternatively at five different angles of incident light. Angle $\alpha$ 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 colours. Detailed observations of coloured colonies were performed under epi-illumination by using the numeric Keyence Microscope VHX-1000E. A VHX-1100 camera was used with a VH-Z20R/Z20W objective lens with adjustable magnification of x20, x30, x50, x100, x200 and x400, the last one with a specific tool doubling magnification. 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 D.F.D (Depth From Defocus) 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 angle.

Physical measurement of *C. lytica* (microspectrophotometry). Illumination was directed onto the sample through an Ocean Optics UV-Vis-NIR optical fibre 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 fibre that was itself connected to an Ocean Optics USB4000-UV-VIS spectrometer (Supplemental Fig. S2). The angle 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 fibre 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 colour with angle, and hence the extent of each sample’s iridescence, could be measured and assessed (72).
RESULTS

Isolation and identification of a marine bacterium with a glitters-like color appearance.

While searching for new cultivable epibiontic bacteria in the marine environment, we isolated a gram-negative bacterium from the surface of a red anemone Actinia equina (Fig. 1A). Colonies exhibited bright iridescent reflected colour when grown on marine agar (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’ colour appearance comprised sub-millimetre sized centres of colour of varying brightness distributed across the iridescent region. This gave the colonies’ colour reflection and intensity a glitter-like character.

The marine strain was taxonomically identified by performing both 16S rRNA and 16S to 23S (16S-23S) internally transcribed spacer (ITS) sequence analyses. The strain was phylogenetically affiliated to the Cytophaga-Flavobacterium-Bacteroides group and the Flavobacteriacea family, and was identified as Cellulophaga lytica (CP002534, DSM 7489) (33, 37) with 16S, 23S 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 C. lytica type-strain ATCC 23178T (DSM7489 = 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 (47).

Coloration of C. lytica colonies on different culture media. Agaroysis, mucous colonies with gliding motility and the bright glitter-like colour centres effect were common characteristics for all media (Fig. 2 a1, b1, c1). On MA, colonies’ colour appearances comprised yellow pigmentation and principally a green structural colour. At the peripheral growth zones, yellow,
red, and then violet were observed (Fig. 2 a1). This colour gradation was confirmed using the
Keyence microscope (Fig.2 a2/3 and video movie #1 as supplemental data). Colonies grown on
CYT were larger and less pigmented (Fig. 2 b1). Blue was observed in the inner zone of the
colony and green, yellow, red and violet were also visible (Fig. 2 b2/3). Less growth occurred on
the LN medium (Fig.2 c1). Colonies were translucent in this medium and only green iridescence
was discernable (Fig. 2 c2/3 and video movie #2).

Angle dependence of \textit{C. lytica} colonies' colouration. 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 non-iridescent (Fig.2 D). This angle-dependence of reflected colour,
defined as iridescence, was the first direct evidence for a structural mechanism as the origin of the
colour. Colour changes were also examined microscopically using the Keyence microscope (Fig.
2 E and video movie #3). Pictures demonstrated that bright green iridescence was predominant
when illumination was close to grazing incidence (Fig. 2 e3.h and e4.h). Violet to red colour
changes were observed at colony edges (Fig. 2 e3.h/l). Different iridescent colour centers
appeared and disappeared when the illumination position was modified from high to low
incidence (Black arrows in Fig. 2 e4.h/l). The image associated with an intermediate angle of
illumination exhibited colour centers which overlapped (Fig. 2 e3/4.i).

Physical evidence of \textit{C. lytica} iridescence. The data presented in Fig.3 show optical reflection
bands that unequivocally represent the iridescence of \textit{C. lytica} bacteria colonies by the change in
their colour with angle. For instance, under illumination at an angle of -70°, the principle reflected
colour 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 colour extends still further towards higher positive angles, the peak
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wavelength of which decreases to approximately 410 nm at a scattered angle of +70°. Three other reflected bands of colour are shown on this map of reflectance data: each of these show peak wavelengths that are also angle-dependent; two at near-UV wavelengths and one in the near-IR.

Overall comparison of bacterial iridescence. For a better understanding of bacterial structural colour effects, we examined the iridescence of a broad range of bacterial strains. The extended classification of these bacterial optical effects is presented in Supplemental Table S1, 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 trans-illumination and metallic (M) and glitter-like (G) appearances under epi-illumination.

The rainbow-diffuse (D) category comprises bacterial colonies that exhibited all spectral colours, only under the condition of trans-illumination, ranging from red to blue (Fig.4). Various colour intensities were observed within this category. A large number of bacterial strains also displayed this visual effect (Supplemental Table S1). The rainbow-edge (R) iridescence was only visible on colonies’ edges. This phenomenon does not appear previously to have been described in literature. Few strains displayed rainbow-edge iridescence, namely Bacillus cereus (Fig. 4), Stenotrophomonas maltophilia, Klebsiella pneumoniae and Aneurinibacillus migulanus, these four expressing a common characteristic of thick and opaque colonies.

The metallic (M) category comprised colonies exhibiting a silvery appearance under epi-illumination. As described in literature (9), 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 (Supplemental Table S1).

C. lytica strains were not iridescent under trans-illumination (Fig.4). Their glitter-like (G) iridescence is characterized principally by an intense green iridescent reflection. This novel iridescence is significantly higher in intensity when compared to the bacterial structural coloration.
C. lytica and bacterial iridescences of all other three categories (Fig. 4). The iridescence of C. lytica was also found in another strain - DSM2040 - but not in the two strains CIP103822 and DSM2039. Moreover the genome-sequenced strain DSM 7489 only displayed very low intensity iridescent colour.
A marine bacterium exhibiting a bright iridescently coloured 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, trans-illumination 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 been used previously as an easily-observed criterion to discriminate between capsulated and non-capsulated strains of H. influenzae (26, 54, 55). 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 (ATCC27853 and a clinical mucous strain) were both rainbow-diffuse (under trans-illumination) and metallic (under epi-illumination) iridescents. Metallic reflections in P. aeruginosa 14 ΔlasRΔpqsh have been linked to the accumulation of the 4-hydroxy-2-heptylquinoline molecule (9, 18, 77). However, no explanation has yet been presented as to how the accumulated molecule creates metallic-looking reflection. Since “metallic appearance” is not associated with a change of colour 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 colour and to construct the map of wavelength-dispersive reflection bands. These represent a clear iridescence effect, namely a change of reflected colour with angle.

Interestingly, certain C. lytica strains appeared non-iridescent. The sequenced strain LIM-21T (ATCC 23178T = DSM 7489) (47), exhibited low-intensity iridescence. Described as identical
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in the bacterial collection banks, the strains DSM 7489 and CIP 103822 were found to have
different colony morphologies; possibly this has led to their different iridescent characteristics.
The isolated C. lytica 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 were evoked with “metallic tinge” (33). The term “iridescent” was only
used once in an algicidal bioactivity study of C. lytica ASM 21 (68). “Greenish metallic
iridescence” was mentioned in the Cellulophaga genus in the Bergey’s manual of systematic
bacteriology (5). It is noteworthy that Cellulophaga [Cytophaga] lytica has first been 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
only common yellow-pigmented colonies. Another picture of Cytophaga sp. showed a very weak
red colour 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 the 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 at a
more extend 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 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,
10). 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 trans-illumination and may also indicate potential eco-biological roles for the phenomenon.

In many higher organisms, structural colours have been strongly linked to biological functions associated with conspecific and interspecific communication purposes. However, these same structures can also serve non-communication functions such as those related to thermoregulation, UV protection, light filtering, water repellency, mechanical friction reduction or desiccation prevention (17). In lower organisms such as diatoms, the strong light manipulation associated with the periodic nanostructure on diatom frustules walls might influence the collection of more light into the photoreceptors for more optimized photosynthetic efficiencies (23, 45, 49).

In contrast to these examples, 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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REFERENCES


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**Figures legends**

**FIG. 1.** Observations of the marine isolated *Cellulophaga lytica*. The first isolation plate shows coloured *C. lytica* colonies together with agarolytic and white bacterial colonies (A). Pure culture of *C. lytica* observed under direct epi-illumination allowing examination of the intense structural colour is shown in (B). In both cases, *C. lytica* was aerobically grown at 25°C on Marine Agar (MA).

**FIG. 2.** Macroscopic and microscopic observation of *C. lytica* colonies’ coloration. Colonies were pictured after 24h growth on MA (A, D and E), CYT (B) and LN (C). The inoculation was a thin 5cm-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 (1) or using the Keyence microscope examination with 60° light incidence using a x30 objective lens (2) and a x100 objective lens (3). Evaluation of colour changes at the macroscopic-level was performed at diverse illumination angles (D). At the microscopic-level (E), examination was performed at high angle (h), intermediate (i) and low angle (l) of incidence light with a x100 objective lens (3) and a x400 (4). Arrows indicate position of colour center appearing and disappearing as glitters.

**FIG. 3.** Colour-map showing the angle-dependent spectral reflectance of *Cellulophaga lytica* and which confirms 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 colour-scale indicates the relative intensity of reflectance. Emitted colours are given
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by the following wavelengths value as, ultraviolet (< 400 nm), violet (400 - 435 nm), blue (435 - 490 nm), cyan (490 - 520 nm), green (520 - 560 nm), yellow (560 - 590 nm), orange (590–620 nm), red (620–700 nm) and Infrared (>700 nm).

FIG. 4. Examples of bacterial colonies belonging to different structural colour categories.

Observations were processed on epi- and trans-illumination. Iridescence categories are Rainbow-diffuse (D): diffuse colours of the light spectrum, Rainbow-edge (E): shining light spectrum colour only on edges, Metallic (M): silvery luster and glitter-like (G): iridescent green in the middle and red and violet on the colony edges. Culture conditions are informed in Supplemental Table S1.