




Sensitivity of *Xylella fastidiosa* subsp. *pauca* Salento-1 to light at 410 nm

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Abstract

All over the world, from America to the Mediterranean Sea, the plant pathogen *Xylella fastidiosa* represents one of the most difficult challenges with many implications at ecological, agricultural, and economic levels. *X. fastidiosa* is a rod-shaped Gram-negative bacterium belonging to the family of *Xanthomonadaceae*. It grows at very low rates and infects a wide range of plants thanks to different vectors. Insects, through their stylets, suck a sap rich in nutrients and inject bacteria into xylem vessels. Since, until now, no antimicrobial treatment has been successfully applied to kill *X. fastidiosa* and/or prevent its diffusion, in this study, antimicrobial blue light (aBL) was explored as a potential anti-*Xylella* tool. *Xylella fastidiosa* subsp. *pauca* Salento-1, chosen as a model strain, showed a certain degree of sensitivity to light at 410 nm. The killing effect was light dose dependent and bacterial concentration dependent. These preliminary results support the potential of blue light in decontamination of agricultural equipment and/or plant surface; however, further investigations are needed for in vivo applications.

Keywords *Xylella fastidiosa* · aBL (Antimicrobial blue light) · Light at 410 nm

1 Introduction

Xylella fastidiosa is one of the most harmful alien pathogens originating from the American continent affecting grapevine, citrus, coffee, and olive trees [1]. It is transmitted by insects belonging to families of Cicadellidae, Cicadellinae, and Cercopoidae that assimilate amino acids, organic acids, and sugars from sucking xylem sap [2]. The bacterium is injected

into the xylem vessels of leaf petioles after the penetration of the insect vector's stylet [3]. *X. fastidiosa* is a Gram-negative rod-shaped bacterium belonging to the *Xanthomonas* family. It is a slow-growing and fastidious bacterium that colonizes the xylem of its host plants moving against water flow, forms biofilms, and occludes the vessels, killing the plants [4].

In 2013, *X. fastidiosa* was detected the first time in Europe affecting olive orchards of the Salento Peninsula in the region of Apulia, causing olive quick decline syndrome (OQDS) [5]. Although complex abiotic and biotic factors were strongly related to this syndrome, the fulfillment of Koch's postulates found *X. fastidiosa* as the etiological agent of OQDS [6]. Among the hosts of *X. fastidiosa*, many endemic Mediterranean species in addition to oil tree have been found: both ornamental and crop species such as cherry (*Prunus avium*), oleander (*Nerium oleander*), and almond (*Prunus dulcis*) [5]. In 2015, *X. fastidiosa* has been detected in the island of Corsica and in continental France [7], and in 2016 in the Balearic Islands in Spain with a big delay compared to its hypothesized introduction 23 years before [8]. Further outbreaks in Israel, Tuscany, and Portugal have been more recently reported [8]. On the basis of 16S-23S rRNA internal transcribed spacer (ITS) sequencing, different

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subspecies have been proposed and among these the most harmful are *fastidiosa*, *pauca*, *multiplex*, and *sandyi* [9].

Nowadays, the European Community is facing the consequences of such enemy that are devastating from ecological, agricultural, and economic point of view. Until now, no cure has been found for this pest, but different strategies have been proposed and authorized. In particular, the decision (EU) 2015/789 required the elimination of plants in infected areas, within 100 m radius around *X. fastidiosa*-positive plant, regardless of their phytosanitary status. In addition, the vector control can be achieved through the use of insecticides for the treatment of olive trees, such as Kaolin 95% WP p/p, Deltamethrin 10% (EC) p/v, and Lambda-Cyhalothrin 10% (CS) p/v [8]. Additional controls are based on propagation of plant material pathogen-free or resistant plant cultivars, territory demarcation, and bactericidal treatments [10].

Although, in literature, several studies report antimicrobial treatments aimed at killing *X. fastidiosa*, new efforts are undoubtedly needed to manage this enemy. Furthermore, there is evidence of development of resistance to copper-based antimicrobial compounds often used for crop protection, especially in *Xanthomonas* family. The discovery of novel bactericidal compounds or treatments is essential to counteract the spread of resistant strains. Promising results have been obtained by da Silva et al. that injected in vivo, using a syringe, ruthenium and magnesium complexes that drastically reduced the detection, through real-time quantitative PCR, of *X. fastidiosa* [11]. The same culture-independent method was exploited by Barò et al. to verify the anti-*Xylella* potential of peptide BP100 and fragments of cecropin, magainin, or melittin that showed to be good candidates. Indeed, antimicrobial peptides from plants represent promising compounds with low cytotoxicity and low probability to induce resistance mechanisms in microbial targets [12]. The screening of synthetic peptides from libraries could represent a good reservoir of putative antimicrobials [13]. The blocking of the afimbrial adhesin XadA2 of *X. fastidiosa* successfully inhibited biofilm formation. The combination of chitosan nano-materials combined with fosetyl-Al crystals showed antibiofilm activity in *X. fastidiosa* subsp. *pauca* and subsp. *fastidiosa* [14].

In this context, the antimicrobial blue light (aBL) could represent a novel strategy to be considered. This approach exploits the ability of visible light alone, particularly in the range of blue light from 400 to 500 nm, in damaging microbial pathogens, and leading to cell death. Many microbial species demonstrated to be sensitive to blue light treatment, especially those causing human infections such as ESKAPE pathogens including *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, and *A. baumannii* [15]. It has been hypothesized that aBL mechanism of action is based on

the generation of photooxidative stress upon the excitation of endogenous molecules that behave as photosensitizing agents [16]. In different bacterial and fungal species, putative endogenous molecules acting as photosensitizers (PSs) have been recognized, belonging to chemical classes of porphyrins and/or flavins. Indeed, the main types of endogenous compounds found in bacteria and yeasts are protoporphyrin IX, coproporphyrin I, and coproporphyrin III [15, 17]. In the last years, a wider literature reports the antimicrobial power of blue light in inhibiting the growth of bacteria, with many potential applications in agri-food field, livestock, and industrial sectors, for the treatment of plant and animal pathogens, and for disinfection purposes [15, 18]. Many studies suggested that no evidence of microbial resistance toward aBL was found in *P. aeruginosa*, *A. baumannii*, and *C. albicans* after sublethal doses of blue light [19–21]. However, a certain degree of tolerance to aBL was found in *S. aureus*. [22].

The authors previously showed that light at 410 nm was efficient in inhibiting the growth of an opportunistic pathogen such as *Pseudomonas aeruginosa* [23]. In this study, the sensitivity of *X. fastidiosa* to light at 410 nm was investigated.

2 Materials and methods

2.1 Bacterial strains and culture conditions

Xylella fastidiosa subsp. *pauca* Salento-1 isolate (NCCPB No. 4595 LMG 29352) [24, 25] was grown in Buffered Charcoal Yeast Extract (BCYE) Agar medium (LaBM) for 15–20 days at 28 °C. For long-term storage, glycerol stocks of *X. fastidiosa* cells were prepared in 1X PBS buffer (KH₂PO₄/K₂HPO₄ 10 mM, NaCl 137 mM, KCl 2.7 mM, pH 7.4) with a final glycerol concentration of 15% (v/v) and stored at – 80 °C.

2.2 Light sources

The lighting unit device used to photo-inactivate bacteria is equipped with a head composed by 25 high-power LEDs with maximum emission peak at 410 nm and allows the uniform irradiation of a square area of 75 mm × 75 mm (Fig. 1A, and C, black line). The source was placed 10 cm above the Petri dishes to be treated. At this distance, the irradiance was 100 mW/cm², measured with the spectral light meter MSC15 (Gigahertz Optik GmbH). A 50 W incandescent lamp emitting white light ($T = 2900$ K) with negligible emission in the blue region was used for irradiation control experiments to assess the sensitivity of *X. fastidiosa* to wavelengths other than that under investigation. The source was placed 8 cm above the Petri dishes (Fig. 1B)

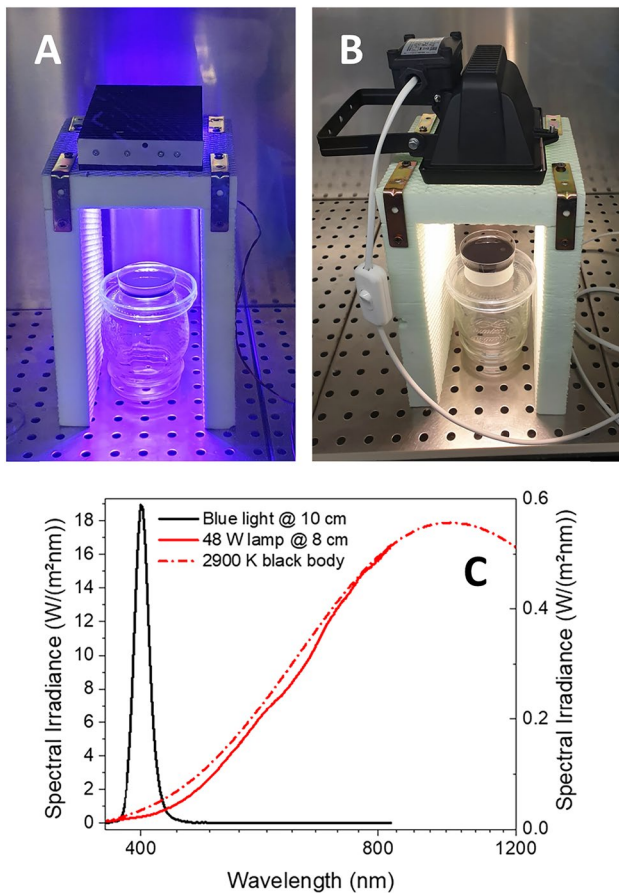


Fig. 1 Experimental setups used for blue light irradiation (A) and white light irradiation (B), and relevant emission spectra (C), where left scale refers to the blue light and right scale refers to the white one

to obtain the same heating effect as the blue light (see later). Also in this case, the geometry of the system allows uniform illumination of an area larger than the Petri dishes used for the experiments. The relevant emission spectrum is shown in Fig. 1C (red solid line) for the interval 350–830 nm where the MSC15 spectral light meter is sensitive, with a measured irradiance of 11 mW/cm². Figure 1C (red dash-dotted line) shows the calculated emission spectrum of a black body with a temperature of 2900 K, rescaled to match the measured emission of the 50 W incandescent lamp. The good agreement between measured and calculated values indicates the quasi-ideal behavior of our white light source. The calculated peak wavelength and total irradiance are 1000 nm and 84 mW/cm², respectively.

2.3 Photoinactivation assays

Isolated colonies of *Xylella fastidiosa* were suspended in 1X PBS and adjusted to a final optical density at 600 nm of ~ 1.0 by a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan), corresponding to a concentration of ~ 10⁶ CFU/mL,

as previously assessed by bacterial serial dilutions on BCYE Agar and plate counting.

Photoinactivation assay in liquid medium. Effect of exposure to blue light was investigated with bacterial cells inoculated in phosphate buffer. Aliquots of 2 mL of 1X PBS with initial cell count of 7 × 10⁵ CFU/mL (evaluated by plate counting of bacterial serial dilutions on BCYE Agar) were placed in a 6-well plate and dark incubated or illuminated with blue light for 16 min, reaching a total fluence of 100 J/cm². At the end of the treatment, a viable count technique was performed on the samples and bacterial viability was expressed as CFU/mL by plating the samples in serial dilutions on BCYE Agar. The plates were incubated at 28 °C for at least 15–20 days. Experiments were performed at least in triplicate.

Untreated samples of *X. fastidiosa* suspended in 1X PBS were observed with the optical microscope AxioSkop-2 Microscope (Zeiss, Oberkochen, Germany) at the magnification of 100x. Digital images were acquired connecting the optics to an Optikam B5 camera.

Photoinactivation assays on solidified medium. Two experimental setups were performed in solidified medium. In the former one, herein called “photo-spot test” and previously optimized for microbial models by authors, samples of *X. fastidiosa* at ~ 10⁶ CFU/mL were serially tenfold diluted and drops of 14 μL were inoculated on BCYE Agar. In the latter setup (“spread test”), volumes of 100 μL of cell suspension (~ 10⁶ CFU/mL) were inoculated and spread on BCYE Agar. In both setups, inoculated cells were irradiated under 410 nm light at increasing times, 8, 16, and 32 min, to reach fluence values of 50, 100, and 200 J/cm², respectively. For each experiment, a dark control was considered. Since irradiation caused the rise of water vapor droplets under the cover, it was necessary to leave the Petri dish uncovered to not decrease bacterial irradiation. To minimize sample contamination, no heat dissipation fan was used. This is a very crucial step since cell growth is very slow (at least 15–20 days) and even small contaminations can compromise the experiment. Furthermore, due to the black color of the growth medium, heating of the Petri dish was unavoidable. After irradiation or dark incubation, Petri dishes were sealed with top cap and parafilm and incubated at 28 °C in the dark. After 20–30 days, the bacterial growth was checked. The irradiation under wide spectrum white light was also performed for cells inoculated as previously described for “photo-spot test” for 8 and 16 min. The experiments have been performed at least three times.

2.4 Recovery of potential dormant bacteria

The following procedure was performed to search for potential dormant cells, to verify the bactericidal/bacteriostatic activity of the treatment. We evaluated whether

X. fastidiosa cells incubated for 20 days at 28 °C after dark incubation or irradiation from previously described “photo-spot test” could grow on fresh medium after re-inoculum. The procedure was performed for all samples that give or not a visible bacterial growth. The bacterial biomass, visible or not at naked eye, was collected and inoculated in fresh BCYE Agar. The three samples corresponding to undiluted (ND), 10^{-1} , and 10^{-2} diluted samples were recovered from cells dark incubated or irradiated at increasing fluence (50, 100, and 200 J/cm²) and incubated at 28 °C. After incubation for further 20–30 days at 28 °C, the growth was checked.

2.5 Heating curves

BCYE Agar is black due to its charcoal content. The heating effect caused by the prolonged irradiation with blue light placed 10 cm above the Petri dish was hence determined. A thermocouple was inserted just below the surface of the solid medium to record its temperature during the 32-min illumination period. Temperature was finally plotted against time to obtain the heating curve. The white light source was placed at different heights above the Petri dishes to find the distance leading to the same heating effect, and the eight of 8 cm was chosen.

2.6 Statistical analysis

Statistical analysis was assessed by one-way ANOVA in conjunction with Tukey’s post hoc test for CFU/mL values obtained with photoinactivation experiments of suspended bacteria.

3 Results and discussion

3.1 Sensitivity of *Xylella fastidiosa* subsp. *pauca* Salento-1 to light at 410 nm

Xylella fastidiosa subsp. *pauca* Salento-1 was isolated from an olive plant suffering from OQDS in Apulia (South Italy) and characterized by authors [24, 26]. This subspecies is known to invade olive trees through the world (California, Argentina, Brazil) [25].

With the aim of searching for anti-*Xylella* treatments, in this study, the potential of aBL was evaluated. LED emitting at 410 nm was preferred to 455 nm because the former showed to be more efficient in photoinactivation of *Pseudomonas aeruginosa*, a bacterial species belonging to γ -Proteobacteria and phylogenetically close to *X. fastidiosa* [23].

To observe the effect of blue light, bacterial suspensions of *X. fastidiosa* in phosphate buffer at a concentration $\sim 10^5$ CFU/mL were irradiated under fluence of 100 J/cm². A

significant reduction ($p = 1.97 \times 10^{-6}$) of ~ 2 log units was observed (Fig. 2).

In the case of *X. fastidiosa* Salento-1, the culturable fraction measured as CFU on agar plates could not originate from a single cell but from a multicellular aggregate that was not completely separated by mixing with vortex and micropipette [36], as shown in Fig. 3. Since aggregation could mask the real anti-*Xylella* effect of blue light, further approaches were performed to verify a photoinactivation activity. With the so-called photo-spot test, undiluted and tenfold serially diluted suspensions of *X. fastidiosa* Salento-1 were inoculated on BCYE Agar and dark incubated or irradiated at increasing light doses. The dark incubated samples, after at least 20 days of incubation at 28 °C, showed growth spots with decreasing dimensions, thickness and colony densities, and numbers compatible with corresponding bacterial sample dilutions (Fig. 4A). An anti-*Xylella* effect was clear upon irradiation: no growth spot was visible for most dilutions (Fig. 4B–D). Furthermore, blue light irradiation affected the growth of undiluted samples (ND) that appeared less thick and smooth than dark controls.

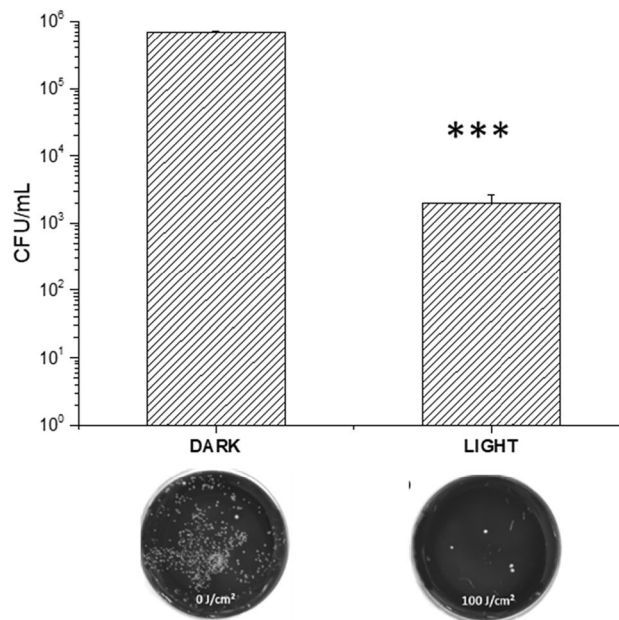


Fig. 2 Effect of irradiation on *Xylella fastidiosa* viability. *Xylella fastidiosa* subsp. *pauca* Salento-1 cells were suspended in PBS 1X at concentration $\sim 10^5$ CFU/mL and incubated in the dark or irradiated under light at 410 nm (100 J/cm²). The cell viability is expressed as CFU/mL (mean \pm standard deviation). The experiments were carried out at least three times with independent cultures. ANOVA analysis was performed to evaluate the significant differences between irradiated and dark incubated samples ($***p < 10^{-6}$). Representative images of bacterial growth deriving from plating 100 μ L of the 1:100 dilution of the starting bacterial preparations are shown below histogram bars

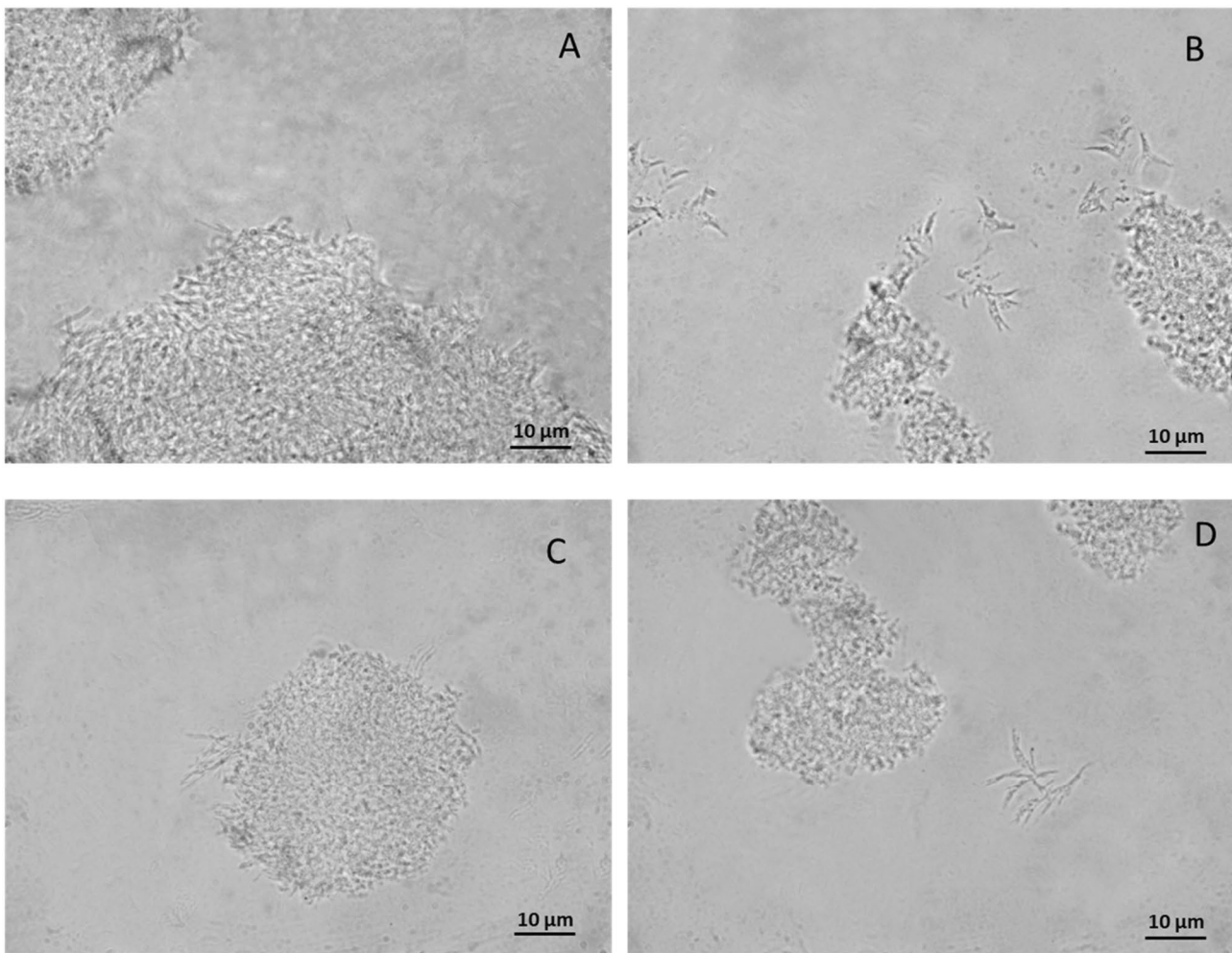


Fig. 3 Cell aggregates formed by *Xylella fastidiosa* subsp. *pauca* Salento-1 at $\times 100$ magnification. **A–D** Examples of bacterial aggregates observed in different slide preparations

Indeed, under the tested conditions, the samples with the highest bacterial density were the most difficult to be inactivated. The antimicrobial effect of blue light showed a certain degree of cellular density dependence, as previously observed in *P. aeruginosa* PAO1 [23]. On the contrary, the highest tested fluence ($\sim 200 \text{ J/cm}^2$) reached after 32 min of irradiation did not fully inhibit the growth of *X. fastidiosa* Salento-1 strain, as observed in PAO1 strain. It has been previously cited the hypothesis that consider the bactericidal effect of blue light as a result of the excitation of endogenous photosensitizers involved in metabolic pathways [15, 16]. Thus, the lower sensitivity of *X. fastidiosa* that is a slow-growing bacterium could be ascribable to a lower content of putative photosensitizers compared to that of *P. aeruginosa* PAO1. To the best of our knowledge, this study shows for the first time the susceptibility of *X. fastidiosa* to photoinactivation through light at 410 nm. Thus, endogenous compounds

excitable by light at 410 nm could mediate the arising of a photooxidative stress as hypothesized for other microbial species. Indeed, porphyrin-based electron transporters could play the role of putative PSs [16]. Since it is known that *X. fastidiosa* is strictly aerobic and the low growth rate could be ascribable to a low rate of aerobic respiration, the content of porphyrin-based compounds could be poor [3, 27]. This could be in accordance with the lowest sensitivity observed in a fastidious growing bacterium such as *X. fastidiosa* compared to higher growing *P. aeruginosa*.

A second experimental setup (“spread test”) was performed inoculating samples of 100 μL of *X. fastidiosa*, at $\sim 10^6$ CFU/mL, on the whole surface of BCYE Agar. As can be observed in Fig. 5, dark incubated cells after at least 20 days of incubation at 28 $^\circ\text{C}$ showed a confluent growth, while a drastic reduction of the bacterial growth was already induced after irradiation under the lowest

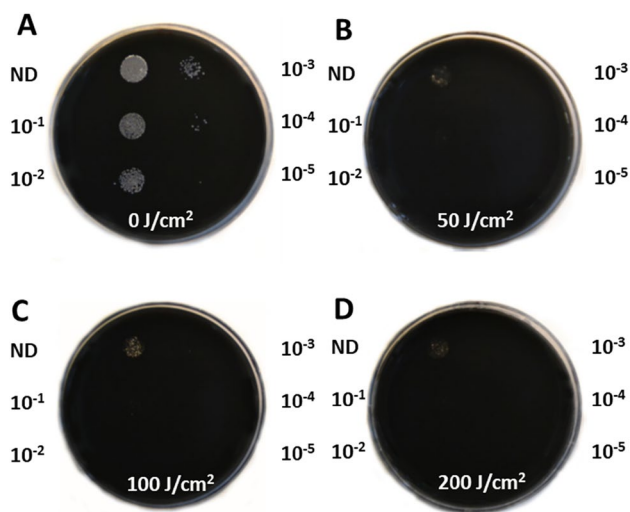


Fig. 4 Representative images of “photo-spot test.” *Xylella fastidiosa* subsp. *pauca* Salento-1 was inoculated on BCYE Agar at decreasing bacterial concentrations. Samples of 14 μL of 10^6 CFU/mL bacterial suspension and decimal following dilutions were spotted and irradiated under light at 410 nm at increasing fluence rates. After 20 days of incubation at 28 °C, growth spots of dark incubated bacteria (A) were considered as control to be compared with irradiated samples (B–D). The experiments have been performed at least three times with independent cultures

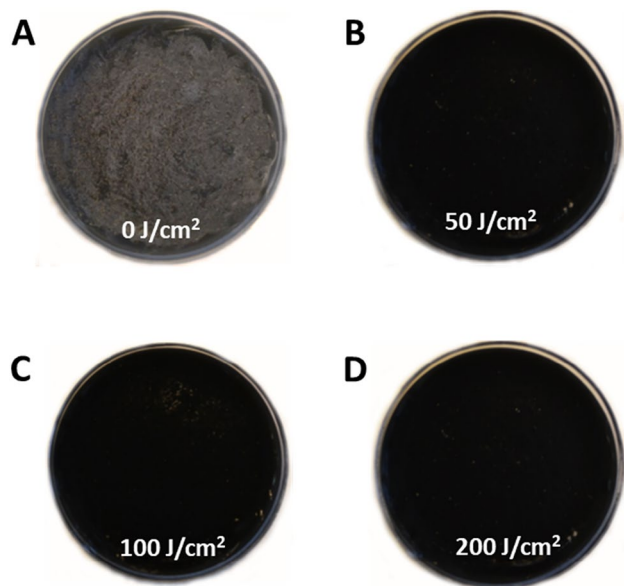


Fig. 5 Representative images of “spread test.” A sample of 100 μL *Xylella fastidiosa* subsp. *pauca* Salento-1 at 10^6 CFU/mL was inoculated on BCYE agar, dark incubated or irradiated under light at 410 nm at increasing times (8, 16, and 32 min). After 20 days of incubation at 28 °C, bacterial growth of dark incubated cells (A) was compared to irradiated samples (B–D). The experiments have been performed at least three times with independent cultures

fluence (50 J/cm^2). Higher fluences, 100 and 200 J/cm^2 , respectively, showed a photoinactivation effect. Few colonies were visible close to the borders of the Petri dishes, and this could be ascribable to the geometry of irradiation from the irradiating device.

Taken as a whole, the data support that blue light impairs the viability of *X. fastidiosa* Salento-1. Since *X. fastidiosa* forms clusters and cellular aggregations before adhering and forming biofilm communities in natural environments, this technique could be applied to prevent biofilm formation. This common strategy used by plant-associated bacteria is essential for the development of mutualistic, commensal, and pathogenic host–microbe interactions [28]. Biofilm formation is likely a major virulence factor in diseases related to *X. fastidiosa* for establishing within the mouthparts of insect vectors [29] and enhancing the likelihood of bacterial transmission by insects [30] and for colonizing plant vessels developing into large colonies to cell aggregates similar to those visible in Fig. 3 [31–33].

3.2 Further investigations on the bactericidal effect of photoinactivation

The slow-growing rate of *X. fastidiosa* makes more difficult to evaluate the efficiency of any antimicrobial technique, including that of blue light. If bacteria grown after irradiation are not able to furtherly grow, an underestimation of the antimicrobial effect could occur. In addition, if after irradiation dormant and viable cells are present and not detectable through cultivation technique, an overestimation of antimicrobial effect could occur. This last effect could be also due to *X. fastidiosa*'s natural tendency to form biofilms and cell aggregates that can affect cell count measurement of the colony-forming units by both bacterial plating and optical density (OD) [34, 35].

To assess risks of bias, “recovery” experiments were performed with cells collected from the “photo-spot test.” After 20 days of incubation at 28 °C, Petri dishes from “photo-spot tests” were evaluated and both biomasses visible and not visible from spots at the lowest dilutions (undiluted “ND,” and 10^{-1} and 10^{-2} diluted samples) were collected and inoculated in fresh BCYE Agar for further 20 days at 28 °C. This procedure did not compromise the growth of the dark controls as can be appreciated in Fig. 6. Interestingly, bacterial growth was only observed with bacteria recovered after irradiation with the lowest fluence rate (50 J/cm^2 , 8 min of irradiation) and the highest bacterial density (ND). Thus, light at 410 nm showed a bactericidal effect at the highest fluences ($\geq 100 \text{ J/cm}^2$) irrespective of bacterial density. Indeed, these experiments confirmed that the photoinactivation of *X. fastidiosa* with light at 410 nm is both light-dose and bacterial density dependent.

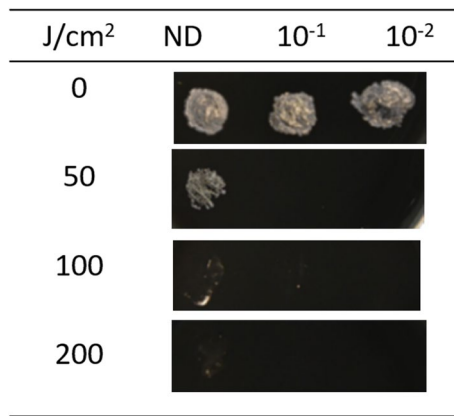


Fig. 6 Representative images of “recovery” experiments. Samples of *Xylella fastidiosa* subsp. *pauca* Salento-1 were photoinactivated through “photo-spot test” as depicted in Fig. 4. Unirradiated and irradiated (50, 100, and 200 J/cm²) cells were recovered from corresponding growth spots (ND, 10⁻¹, and 10⁻²), and further incubated for at least 20 days at 28 °C on fresh medium

3.3 Effect of heating on *Xylella fastidiosa* growth

Since the optimal temperature for *X. fastidiosa* is 28 °C, it was necessary to rule out any detrimental effect caused by increased temperature during treatment. Furthermore, the growth medium used for *X. fastidiosa* is black due to the presence of charcoal among the components and prolonged illumination could be supposed to enhance a heating effect. Thus, we therefore build the heating curves of Petri dishes containing BCYE agar and irradiated under either blue or white light. Both light sources showed a similar thermogram, with temperature rising from 28 to 40 °C within the first 15 min illumination time and then remaining constant (Fig. 7A). Thus, we evaluated the effect of incubating tenfold diluted samples at 40 °C for 8 and 16 min (Fig. 7B). A certain degree of toxicity was observed after 16 min of incubation at 40 °C for the samples at the highest dilutions. However, even if the contribution of the thermal effect cannot be ruled out, the sensitivity of *X. fastidiosa* to light at 410 nm is clearly shown (Figs. 4, 7 B).

To assess whether the photoinactivation of *X. fastidiosa* could be ascribable to a blue light-specific effect, we carried out the photo-spot test irradiating bacteria under the extended white light spectrum of a 50-W incandescent lamp. This light source was placed 8 cm above the Petri dishes, the distance at which the same thermal effect of the blue light is obtained (Fig. 7A). The bacterial growth of samples irradiated for 8 and 16 min under this light source was comparable to that of dark control, irrespective of cellular density (Fig. 7C). The observed selective activity of blue light (Fig. 7D) is in accordance with the hypothetical action mechanism proposed for aBL. The absorption of energy of visible

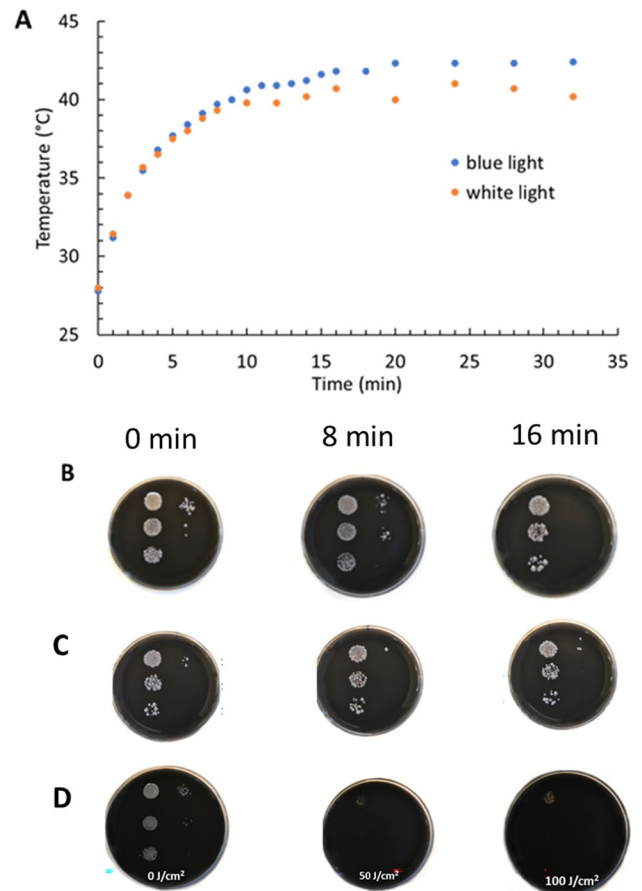


Fig. 7 A Heating curves for BCYE Agar Petri dishes irradiated under blue light (blue dots) and white light (orange dots). B Representative pictures of *Xylella fastidiosa* subsp. *pauca* Salento-1 strain untreated control and after heating at 40 °C for 8 and 16 min, respectively. All plates were inoculated by spotting 14 µL of ~ 10⁶ CFU/mL bacterial suspension and tenfold following dilutions. C Effect of white light irradiation on *X. fastidiosa* for 8 min and 16 min. After 20 days of incubation at 28 °C, the growth spots were checked. The experiments have been performed at least three times with independent cultures. The comparison with effect of blue light from Fig. 4 is shown in D

light at a specific wavelength could induce the passage of putative endogenous photosensitizers from a ground state to an excited one. The following photochemical reactions could generate oxidizing species. In accordance with photodynamic process, type I reaction could involve direct electron or hydrogen transfer to a biomolecule, producing superoxide anion and other reactive oxygen species (ROS). While, in type II reaction, PS transfers energy to molecular oxygen, eliciting the production of singlet oxygen (¹O₂), characterized by extremely oxidant power and a very short lifetime [36]. The threshold of energy and/or electron transfer induced by blue light, especially at the highest fluences, seems to cause permanent damage to *X. fastidiosa* cells, likely at cytoplasmic and/or membrane levels.

Although *X. fastidiosa* is a mesophilic microorganism characterized by the optimal growth temperature 28–30 °C and the continuous incubation at 36 and 40 °C for 7-day period has been demonstrated to be lethal for the bacterial cells [37], the sunlight irradiation in Apulia Region did not prevent the diffusion of this pathogen that is or is becoming endemic. Indeed, the growth and survival of *X. fastidiosa* in Apulia Region both in trees and insects is probably ensured by the daily alternation of light and darkness that is a condition producing a temperature change mitigating the effects of extreme temperature during the warmer hours of the day. Moreover, it is conceivable that the anatomy and the tissue structure, as well as some physiological/adaptation strategies of the tree branches and trunks, and the insect vectors should maintain the internal temperature to conditions suitable for the life, enzyme, and cell activities, by avoiding the excessive increase of the temperature in inner compartments.

4 Conclusions

In this work, for the first time, preliminary evidences on the activity of aBL against *X. fastidiosa* were produced, suggesting that aBL deserves attention as a technique potentially useful to control the dissemination of *X. fastidiosa*. The photokilling effect is ascribable most to blue light than heating effect. Further investigations should be aimed at evaluating whether irradiation at 410 nm contributes to decrease the possible quantity of pathogens on the surface of pruning residual plant material, a phytosanitation approach for reducing potential *X. fastidiosa* reservoir. The combination of blue light with exogenous photosensitizers belonging to porphyrins family, eventually formulated as porphyrin-based nanomaterial and/or dyes [38], could enhance the antimicrobial effect especially against dormant forms and resistance structures, such as biofilm, reducing the risk of tolerance development and producing low environmental impact. Indeed, the visible light alone or together with natural compounds could be used for disinfection of agricultural equipment used for dissemination control.

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Data availability The data will be made available upon request.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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