



Evaluation of growth and motility in non-photosynthetic *Azospirillum brasilense* exposed to red, blue, and white light

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Abstract

Azospirillum brasilense is a non-photosynthetic rhizobacterium that promotes the growth of plants. In this work, we evaluated the effects of different light qualities on the growth, viability, and motility in combination to other culture conditions such as temperature or composition of the culture medium. Exponential cultures of *A. brasilense* Az39 were inoculated by drop-plate method on nutritionally rich (LB) or chemically defined (MMAB) media in the presence or absence of Congo Red indicator (CR) and exposed continuously to white light (WL), blue light (BL), and red light (RL), or maintained in dark conditions (control). The exposure to BL or WL inhibited growth, mostly in LB medium at 36 °C. By contrast, the exposure to RL showed a similar behavior to the control. Swimming motility was inhibited by exposure to WL and BL, while exposure to RL caused only a slight reduction. The effects of WL and BL on plant growth-promoting rhizobacteria should be considered in the future as deleterious factors that could be manipulated to improve the functionality of foliar inoculants, as well as the bacterial effects on the leaf after inoculation.

Keywords *Azospirillum* · Non-photosynthetic rhizobacteria · Red light · Blue light · White light · Bacterial growth · Swimming motility

Introduction

Light is an essential environmental factor for the development and continuity of life. Certain organisms, known as “photosynthetic” organisms, have developed the ability to capture light and transform it into chemical energy, a transformation through which they regulate metabolic functions

(Elías-Arnanz et al. 2011). However, light can also cause photophysical and photochemical damage to the structure of nucleic acids, proteins, and lipids, and/or trigger the synthesis of highly toxic metabolites (van der Horst and Hellingwerf 2004). Particularly, the wavelengths responsible for such lethal effects (Christie et al. 1999; Hitomi et al. 2000) are found in the violet (380–450 nm) and blue (440–480 nm) parts of the visible spectrum. In presence of visible light, oxygen, and certain photosensitizing molecules, compounds known as reactive oxygen species (ROS) can appear, such as singlet oxygen, which can cause photooxidative stress (Ziegelhoffer and Donohue 2009). Several physical factors, intensity and wavelength among them, determine whether light has a beneficial or deleterious effect on a specific microorganism (Purcell et al. 2007). Thus, a very wide variety of bacteria (photosynthetic or non-photosynthetic) are able to control different motility mechanisms through the perception of light, which gives them a series of physiological advantages (Häder 1987; Armitage and Hellingwerf 2005; Wilde and Mullineaux 2017). Also there are reports based on the bactericidal effect of light on non-photosynthetic bacteria and of the interaction between light receptors and mobility-associated proteins (Lubart et al. 2011;

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Wu et al. 2013; Amin et al. 2016). *Azospirillum* is one of the best studied genera among non-photosynthetic plant growth-promoting rhizobacteria, but the effect of visible light on growth has not been elucidated, and it is still difficult to determine when this is beneficial or noxious. It is able to colonize numerous species of plant and significantly improve their growth, development and productivity under field conditions. In South America, *Azospirillum* is used as commercial inoculants in approximately 3.0 million ha cultivated, mainly with cereal crops (i.e., maize and wheat) and legumes (i.e., soybean). Specifically, *A. brasilense* Az39, which is presented as a model of beneficial bacteria for plants, is the active principle for 75% of registered products (Cassán and Diaz-Zorita 2016). In the last 2 decades, different inoculation practices have been developed under field conditions with several beneficial bacteria (i.e., farmer or industrial seed treatments, in-furrow, foliar, or soil-sprayed applications, etc.). In particular, combined inoculation of soybean seeds and leaves with rhizobia and azospirilla, respectively, has improved plant performance and crop yield under controlled and agricultural conditions (Puente et al. 2017, 2018). After foliar inoculation, *Azospirillum* spp. remains on the leaf surface for a while before colonizing the plant tissues. In such conditions, the effects of environmental factors (i.e., light, temperature, water potential, etc.) are restrictive for bacterial survival and the whole colonization process. This way, bacterial exposure to direct light on the leaf surface could modify bacterial survival and plant growth promotion capacity. Therefore, in the formulation of foliar microbial inoculants, deleterious effect of light during the spraying should be taken into account to ensure the functionality of these products by adding different protective compounds such as light absorbers (Congo Red and stilbene derivatives) or nanoparticles (zinc oxide, titanium oxide, silica, and graphene oxide) to prevent microbe deactivation by sunlight (Preininger et al. 2018). In the case of *Azospirillum*, there is only one paper in the literature, by Kumar et al. (2012), which reported that exposure of *A. brasilense* Sp7 to red light does not modify bacterial growth or motility under controlled environmental conditions.

The aim of this work was to evaluate how different wavelengths in the visible non-UV spectrum in combination with other factors such as temperature and composition of the culture medium affected the growth, survival, and motility of *A. brasilense* Az39 in controlled environmental conditions.

Materials and methods

Biological material and growth conditions

A. brasilense Az39 was obtained from the Microbiology and Zoology Institute for Agriculture (IMyZA) from the

National Institute for Agricultural Technologies (Instituto Nacional de Tecnología Agropecuaria, INTA) (Castelar, Buenos Aires, Argentina). *A. brasilense* Az39 was grown in sterile Luria–Bertani (LB medium) (Bertani 1951) in darkness until OD_{595} 1.2 at 36 °C overnight. Then, 40 μ l from this pre-inoculum were transferred into a glass tube-containing sterile LB medium, which was incubated in a dark growth chamber at 36 °C with 200 rpm shaking until exponential growth phase corresponding to $OD_{595} = 1.0$, equivalent to approximately 5×10^8 CFU/ml. Aliquots from these dilutions, containing LB as a rich growth medium or MMAB as a minimal growth medium (Vanstockem et al. 1987), were distributed on Petri dishes by the drop-plate method (Miles and Misra 1938). Briefly, from each pure culture of *A. brasilense* Az39, decimal dilutions were made in borosilicate tubes containing sterile solution C1Na 0.85% until reaching a dilution of 10^{-6} . From each tube, four aliquots of 20 μ l were taken and inoculated in triplicate in polystyrene Petri dishes containing the LB or MMAB culture media with UV radiation absorption at 280–290 nm (Deltalab, Spain). The inoculated plates were independently exposed to experimental conditions related to the presence or absence of light: white light (WL) [$56 \mu\text{W}/\text{mm}^2$], generated by a 75 W incandescent light lamp (General Electric, USA), blue light PAR 38 (BL) [$11 \mu\text{W}/\text{mm}^2$], and red light PAR 38 (RL) [$13.9 \mu\text{W}/\text{mm}^2$], generated by 450 nm and 660 nm LED lamps, respectively (OSRAM, Germany), at 36 °C for 72 h. In all cases, a control treatment was prepared by maintaining the same growth media and growth conditions in darkness with aluminum foil. After the incubation period, the typical colonies were counted in those spots (drops) containing between 5 and 50 colonies. The colony-forming units per milliliter (CFU/ml) were calculated considering dilution and inoculation factors [$\text{CFU}/\text{ml} = \text{dF} \times \text{iF} \times 50$ (dF: dilution factor; iF: inoculation factor)] and converted to \log_{10} for the figures. A Vernier, Spectro Vis-Plus spectrophotometer (USA) was used to confirm the wavelength values for each light source, and their intensity was measured using a Tenmars TM-201 lx meter (Taiwan).

Effect of light on Az39 growth at different incubation temperatures and medium culture

20 μ l aliquots were sampled and sown on Petri dishes by drop-plate method with LB agar (Bertani 1951) or LB agar modified by the addition of 15 mg/l of Congo Red indicator (LB-CR), (Molina et al. 2014) and MMAB agar (Vanstockem et al. 1987) or MMAB modified by the addition of 15 mg/l Congo Red indicator (MMAB-CR). After the inoculation, plates were incubated at either 28 °C or 36 °C for 72 h.

Effect of light on Az39 growth in modified LB and MMAB medium

20 μl aliquots from each different growth medium (LB, LB-CR, MMAB, and MMAB-CR) were sown on Petri dishes by drop-plate method (Rivera et al. 2014). Petri dishes containing LB medium modified by the addition of distilled sterile water were prepared as experimental variants, so the medium was diluted at 25% (v/v) or 50% (v/v). The same was done for MMAB medium modified by the addition of LB medium at 25%, 50%, and 75%. In all cases, plates were either incubated under dark conditions (control) or exposed to WL [56 $\mu\text{W}/\text{mm}^2$], BL [11 $\mu\text{W}/\text{mm}^2$] and RL [13.9 $\mu\text{W}/\text{mm}^2$] as previously described.

Effect of exposure time to light

Plates with LB-CR medium were used to evaluate the effect of light at different exposure times. Plates were placed in a growth chamber and covered with aluminum foil after 2, 4, 8, and 24 h of growth at 36 °C. Plates exposed from the start (time 0) to conditions of exposure to light or darkness and covered with aluminum foil were used as controls until the end of the experiment.

Effect of light intensity

From dilutions previously obtained, 20 μl aliquots were sown using the drop-plate method on Petri dishes with MMAB medium. Once inoculated, the plates were incubated under different light intensities which were regulated by adjusting the distance between the plate and the light source. These intensities were 67, 56, and 25 $\mu\text{W}/\text{mm}^2$ for WL, and 51, 11, and 8 $\mu\text{W}/\text{mm}^2$ for BL, with respective controls in conditions of darkness at 36 °C for 72 h.

Effect of light on Az39 motility

From a pure culture in LB medium, 1 μl was sown on Petri dishes with Minimal Swim medium (SM) (Atkinson et al. 2006). This medium was formulated with 0.3% agar (w/v). After sowing, plates were incubated at 36 °C under dark conditions (control) or exposed to WL [56 $\mu\text{W}/\text{mm}^2$], BL [11 $\mu\text{W}/\text{mm}^2$], and RL [13.9 $\mu\text{W}/\text{mm}^2$]. Plates were incubated for 72 h, after which bacterial displacement was measured in centimeters. Petri dishes completely covered with aluminum foil were used as controls in darkness conditions. The assay was performed in triplicate.

Statistical analyses

All assays were performed in triplicate with three repetitions for each growth condition. Data obtained were tested with

ANOVA and Honestly-Significant-Difference (HSD) Tukey, with a 95% confidence level. Infostat software (Universidad Nacional de Córdoba, Argentina) was used to carry out the analyses, and the graphs were designed with GraphPad Prism 5.0 software (GraphPad Software, USA).

Results

Growth and development in *A. brasilense* Az39 were affected by the presence of different light qualities. As can be seen in Fig. 1, both BL and WL had an inhibitory effect on growth. There were no significant differences ($P < 0.05$) neither in biomass production nor cell number per unit volume compared to the control in dark conditions when bacterium was exposed to RL.

Complexity of the culture medium increases the sensitivity of Az39 to WL and BL

BL was lethal for the microorganism growing in LB and LB 50% (v/v), but WL only inhibited growth in undiluted LB medium (Fig. S1b).

Congo Red (CR) is an indicator normally used for the recognition of *Azospirillum* colonies. Colonies appear dark red or scarlet with typical colony characteristics (rough appearance, and hardened and dry surface). In addition, this indicator is capable of absorbing light in the blue part of the spectrum. For this reason, we considered to evaluate the possible protective effect of Congo Red on the survival of Az39 exposed to light at different wavelengths. When LB modified with the addition of Congo Red indicator was used as growth medium, exposure to either BL (Fig. S1d) or WL caused differential bacterial behaviors: in LB-CR 50% medium, the bacterium continued growing after the

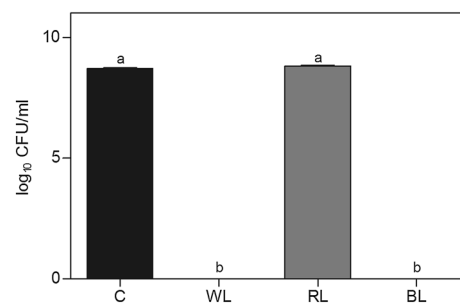


Fig. 1 Evaluation of *A. brasilense* Az39 growth (log₁₀ CFU/ml) in LB-CR medium under dark conditions (Control) or exposed to WL (56 $\mu\text{W}/\text{mm}^2$), RL (13.9 $\mu\text{W}/\text{mm}^2$), and BL (11 $\mu\text{W}/\text{mm}^2$). The plates were incubated for 72 h at 36 °C. The bars correspond to the mean \pm standard deviation of a total of four biological repetitions. The absence of bars indicates growth inhibition. Same letters indicate no significant differences between treatments according to Tukey's test ($P < 0.05$)

exposition to BL, but when exposed to WL, no growth was observed in the same medium (Fig. S1b–d). It is likely that the effect that each type of light has on the bacterium is impacted by the growth medium and the presence or absence of color indicators in the formulation. Exposure to BL only affected growth in *A. brasilense* Az39 when a 75% LB concentration was added to MMAB medium (Fig. S2b). However, exposure to WL, while growing in minimal medium modified by the addition of 50% and 75% LB resulted in bacterial growth inhibition (Fig. S2a). Dimorphic colonies were prevalent, with an approximate 2:1 ratio between smooth colonies (S) and rough colonies (R) when no growth inhibition existed after exposure to WL in MMAB medium enriched with LB. Exposure to BL under the same experimental conditions only brought about smooth colonies (S) (data not shown). These results suggest that the effect of WL on the bacterium depends on the growth medium richness, that is, how much more complex is the medium nutritionally, *Az39* is less tolerant to exposure to WL. Given the behavior of *Az39* when exposed to different wavelengths and grown in media with different compositions, it appears that high nutrient availability makes the bacterium less tolerant to WL or BL exposure. It is likely that the presence of certain types and amounts of nutrients and/or photoactivator molecules affects bacterial growth patterns, in particular at the level

of cell cover, which might determine a non-specific tolerance to light.

Temperature impacts survival in WL and BL

As shown in Fig. 2, growth of *A. brasilense* Az39 was not affected by exposure to RL at 36 °C (Fig. 2a). Furthermore, BL (Fig. 2d) and WL (Fig. 2b) proved lethal when the bacterium was sown in LB media at 36 °C regardless of the presence or absence of Congo Red indicator. Exposure to BL or WL in minimal medium MMAB, by contrast, was not detrimental for growth at the same temperature (Fig. 2b–d). Figure 3 shows that a 28 °C temperature did not inhibit the growth of *Az39* after exposure to dark conditions (Fig. 3a) and RL (Fig. 3c), and a similar observation was made at 36 °C. Exposure to WL (Fig. 3b) and BL (Fig. 3d) at 28 °C inhibited bacterial growth in both rich LB and minimal MMAB media in presence or absence of Congo Red indicator.

Deleterious effect of WL and BL is proportional to the exposure time and light intensity

As shown in Fig. 3, WL decreased bacterial growth from 8 h and it proved to be lethal after 24 h of exposure (Fig. S3a). However, at the last exposure time, BL only caused a

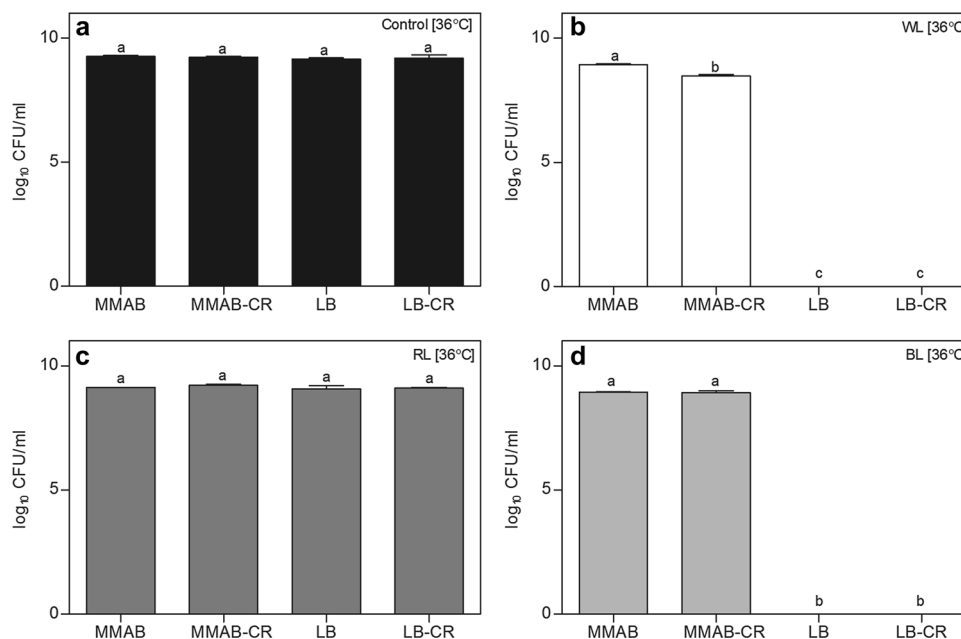


Fig. 2 Evaluation of *A. brasilense* Az39 growth (\log_{10} CFU/ml) in minimal medium (MMAB), minimal medium modified by addition of Congo Red (MMAB-CR), Luria–Bertani medium (LB), and Luria–Bertani medium modified by addition of Congo Red (LB-CR): **a** under dark conditions (Control) or exposed to **b** white light (WL) 56 $\mu\text{W}/\text{mm}^2$, **c** red light (RL) 13.9 $\mu\text{W}/\text{mm}^2$, or **d** blue light (BL)

11 $\mu\text{W}/\text{mm}^2$. The plates were incubated for 72 h at 36 °C. The bars correspond to the mean \pm standard deviation of a total of four biological repetitions. The absence of bars indicates growth inhibition. Same letters indicate no significant differences according to Tukey's test ($P < 0.05$) between the culture medium treatments exposed to the same light conditions

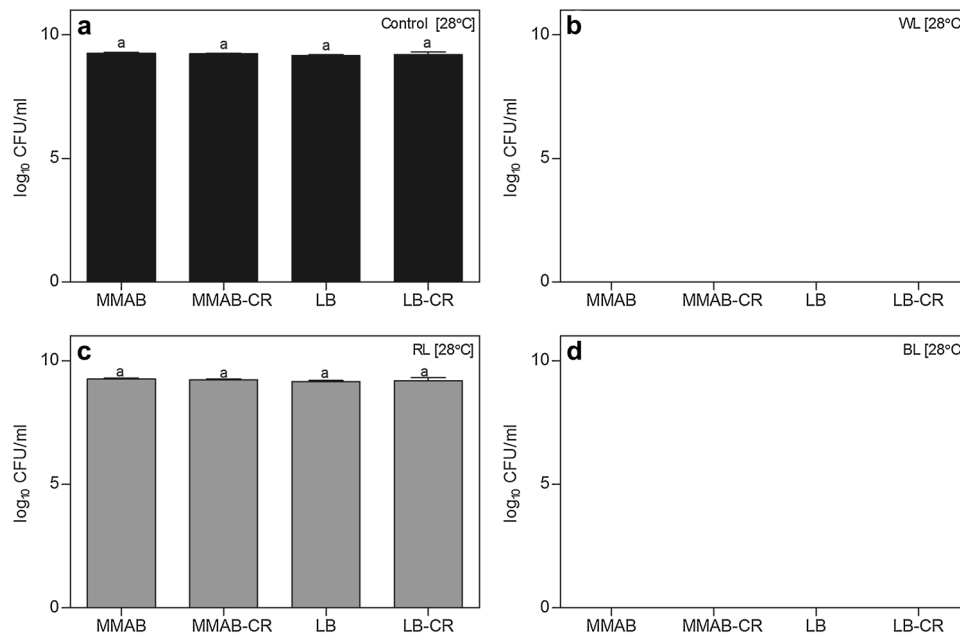


Fig. 3 Evaluation of *A. brasiliense* Az39 growth (\log_{10} CFU/ml) in minimal medium (MMAB), minimal medium modified by addition of Congo Red (MMAB-CR), Luria–Bertani medium (LB), and Luria–Bertani medium modified by addition of Congo Red (LB-CR): **a** under dark conditions (Control) or exposed to **b** white light (WL) $56 \mu\text{W}/\text{mm}^2$, **c** red light (RL) $13.9 \mu\text{W}/\text{mm}^2$, or **d** blue light

(BL) $11 \mu\text{W}/\text{mm}^2$. The plates were incubated for 72 h at 28 °C. The bars correspond to the mean \pm standard deviation of a total of 4 biological repetitions. The absence of bars indicates growth inhibition. Same letters indicate no significant differences according to Tukey's test ($P < 0.05$) between the culture medium treatments exposed to the same light conditions

decrease in bacterial viability, probably due to the beginning of cell damage process (Fig. S3c). In the case of RL, there was no growth inhibition in any of the times analyzed and viability was similar to that of the control under dark conditions. These results suggest that the duration of the exposure to WL can determine the effects on growth, while the impact on viability is observed with a minimum exposure time of 24 h. For BL, partial inhibition was observed at 24 h, which makes it likely that a longer exposure time is required for inhibition to occur compared with WL. There was no growth

inhibition after 24 (Fig. S3b), 48, or 72 h when bacterium was exposed to RL (data not shown).

In nature, sunlight intensity varies according to factors such as temperature and season, but in general terms, 1000 lx intensity usually reaches the ground. Therefore, we evaluated the effect of decreasing light intensities on Az39 growth and development in minimal medium. As can be seen in Fig. 4, the growth in MMAB culture medium of Az39 was inhibited after exposure to intensities of $51 \mu\text{W}/\text{mm}^2$ and $67 \mu\text{W}/\text{mm}^2$ corresponding to BL

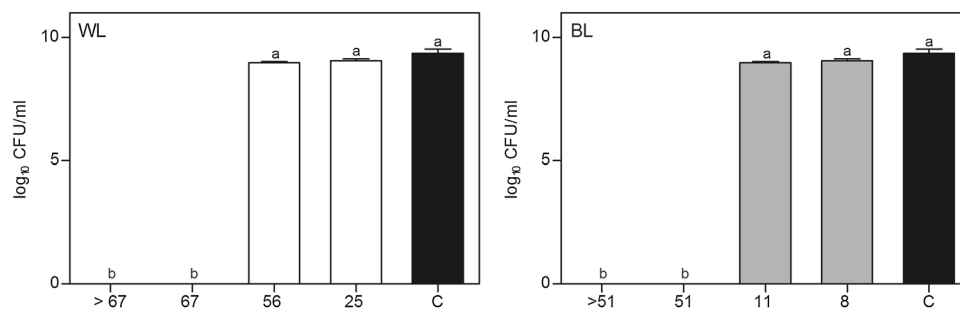


Fig. 4 Evaluation of *A. brasiliense* Az39 growth (\log_{10} CFU/ml) in minimal medium MMAB exposed to different intensities of: **a** with light (WL) [>67 , 67, 56 and $25 \mu\text{W}/\text{mm}^2$] and **b** blue light (BL) [>51 , 51, 11 and $8 \mu\text{W}/\text{mm}^2$]. The plates were incubated for 72 h at 36 °C. The bars correspond to the mean \pm standard deviation of a total

of 4 biological repetitions. The absence of bars indicates growth inhibition. Same letters indicate no significant differences according to Tukey's test ($P < 0.05$) between the light intensity treatments exposed to the same light condition

and WL, respectively. Exposure to either $11 \mu\text{W}/\text{mm}^2$ or $56 \mu\text{W}/\text{mm}^2$, corresponding to BL and WL, did not inhibit growth or development.

Light modifies the swimming motility of Az39 independently of the wavelength

Figure 5 shows how bacterial swimming motility is affected by light exposure. There was a decrease in *A. brasilense* Az39 motility after exposure to each light wavelength, but mainly with WL and BL, which caused a decrease of 87 and 88% in motility (in cm), respectively, compared to the control in darkness, which showed the highest motility under the established experimental conditions. RL also modified the swimming motility, but the reduction percentage was approximately 60% compared to the control. *A. brasilense* Az39 viability was previously evaluated using Swim Medium. In such experiments, the bacterium had the same viability as the control when the plates were exposed to BL and WL at 36°C . This pattern was identical to that observed with MMAB medium (see Fig. 2b–d), and the possibility of the reduction in cell number (CFU/ml) was ruled out due to a lethal effect of WL and BL in Swim Medium plates (data not shown).

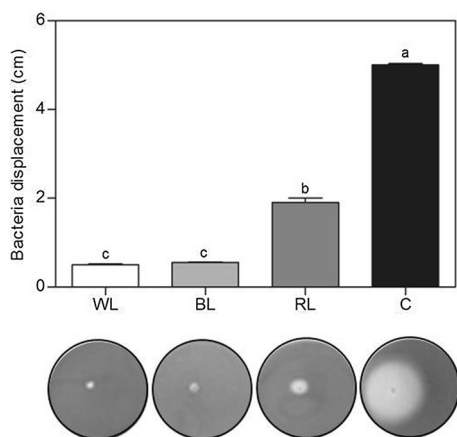


Fig. 5 Evaluation of swimming motility on agar plates inoculated with *A. brasilense* Az39 and incubated under dark conditions (Control) or exposed to white light (WL) $56 \mu\text{W}/\text{mm}^2$, red light (RL) $13.9 \mu\text{W}/\text{mm}^2$, or blue light (BL) $11 \mu\text{W}/\text{mm}^2$. Motility was measured in terms of bacterial displacement under exposure to the light (measured in cm). The plates were incubated for 72 h at 36°C . The bars correspond to the mean \pm standard deviation of a total of three biological repetitions. The absence of bars indicates no displacement. Same letters indicate no significant differences according to Tukey's test ($P < 0.05$) between treatments under the same experimental conditions

Discussion

The effect of light on microorganisms has been studied more than the molecular bases for the effects of light on microbial life (Elías-Arnanz et al. 2011). Most research studies about the effect of light on bacteria began in the 20th century, but it is only in the last 40 years that its molecular pathways have been approached (Ulrich et al. 2005; Wuichet et al. 2010). Several cellular responses have proven to be dependent on light, such as pigment formation, DNA repair, and stress response, as well as multicellular behavior such as the formation of biofilm or sporocarps in the case of myxobacteria (Takano et al. 2005; Sinha and Hader 2002). However, these responses have not been confirmed in *Azospirillum* spp. Kumar and coworkers reported that exposure to red light does not modify the growth or motility of *A. brasilense* Sp7 under controlled environmental conditions. Moreover, they discarded any possible mechanism of tolerance induced by phytochromes in this bacterium (Kumar et al. 2012).

In our experiments, *A. brasilense* Az39 was affected at the growth level by exposure to WL and BL (see Fig. 1). Additionally, BL was lethal in most cases, but there was no difference between RL and the control. Therefore, we assume that the bacterium adapts well to this last type of wavelength, but not to BL or WL. Kumar et al. (2012) support that exposure to red light does not affect bacterial growth in a minimal or rich medium and does not cause significant morphological changes in *A. brasilense* Sp7. Similarly, *A. brasilense* Az39 does not show morphological changes when exposed to RL, regardless of the medium or the culture conditions. However, exposure to BL or WL affected its growth depending on the incubation temperature and the composition of the culture medium.

Kaushik et al. (2002) showed that temperatures below the optimal decreased biomass production and metabolic activity in *A. brasilense* CDJA and *A. brasilense* A40. It is known that a temperature of 28°C decreases growth in *A. brasilense*, increases the formation of protective structures or substances, and decreases metabolic functions in a way that prevents the deterioration of the bacterial cell, but not its growth (Díaz-Saez et al. 2013). In our experiments, the growth and development of Az39 were completely inhibited when bacteria were exposed to WL or BL (see Fig. 3b–d) at 28°C , which allows us to suppose that Az39 was unable to prevent cell death by light exposure at that temperature.

Furthermore, exposure to light at 36°C showed different results. Bacterial growth was inhibited by WL or BL in the rich LB medium (see Fig. 2b–d), while growth in a minimal medium (MMAB) was not affected. In this sense, the composition of culture media affects cellular

physiology, and, therefore, alters bacteria survival capacity (Kram and Finkel 2015). In fact, the enrichment of the culture medium can cause drastic changes in the physiology of the cells and, thus, generates a modification in oxidative states, which in turn leads to an increase in the levels of glycation and the frequency of mutation, and, finally, death (Mironova et al. 2001; Dimitrova et al. 2004). Metabolic stress implies significant morphological changes, including loss of motility, increased accumulation of reserve substances, and increased production of exopolysaccharides, suggesting that cell surface remodeling is a strategy against stress (Bible et al. 2015). For *Caulobacter crescentus*, a non-photosynthetic phototactic and pigment-producing species, it was reported that the biosynthesis of cell surface structures is activated under blue light exposure (Foreman et al. 2012). Konnova et al. (1994) reported that the composition of the outer membrane of *Azospirillum* spp. differs according to the growth phase and culture conditions, which offers certain advantages under unfavorable conditions. In our experiments, the bacterium might have been less tolerant to BL and WL exposure under conditions of high nutrient availability (LB medium) due to the presence of certain nutrients that affected bacterial physiology and cell packing, both of which decreased the non-specific tolerance to light, or due to presence of a higher concentration of photosensitizer molecules (see Figs. 1, 2, S1b, S1d and S2a).

A novel aspect of the effects of visible light on microorganisms has been related to their capacity to biosynthesize photosensitizer molecules that can trigger cell death (Maclean et al. 2008). Several reports indicate that many bacteria have a large number of genes that code for the synthesis of endogenous photosensitizers, which respond to the presence of visible light, but particularly to violet and blue light at 405 and 470 nm, respectively (Ashkenazi et al. 2003; Guffey and Wilborn 2006). The absorption of these wavelengths by photosensitizers generates reactive oxygen species (ROS) that damage cell structures and lead to bacterial cell death (Hessling et al. 2017). In our experiments, bacterial growth was inhibited by exposure to BL or WL but not RL, and this pattern was modified by the composition of the culture medium. Under optimal nutrient availability conditions (LB medium), the bacterium might be less tolerant to BL and WL exposure than under the limited conditions of a minimal medium (MMAB). This is probably due to the presence of a higher concentration of precursors for the biosynthesis of photosensitizers, or nutrients necessary for cell packing, with a consequent decrease in non-specific tolerance to light.

In the last decades, it has been recognized that many bacteria have a high concentration of endogenous photosensitizers (Ashkenazi et al. 2003). Porphyrins, coproporphyrin III, protoporphyrin IX, and uroporphyrin III are the main

endogenous photosensitizers responsible for bacterial photoinactivation (Feuerstein et al. 2004, Maclean et al. 2008). As mentioned, the absorption of visible light by these molecules generates ROS that damage cell structures, leading to cell death. The genomic analysis in *A. brasilense* Az39 showed the presence of at least 14 coding sequences for different photosensitizing molecules. These molecules are synthesized from the metabolism of several amino acids, such as glycine, alanine, aspartate, and glutamate, among others. In our experiments, we used rich medium (LB), with high amino acid concentrations which could increase total porphyrins (data not shown).

A change in motility is common in photosynthetic bacteria, which are capable of detecting alterations in the quantity and quality of the light. Three general types of response to light have been described for bacteria (Häder 1987; Ragatz et al. 1995; Gest 1995): scotophobia or fear of darkness, photokinesis, alteration in motility caused by a light stimulus, and phototaxis, which involves movement towards or away from a source of light. Notably, the direction in which the light radiates is critically determining in phototaxis (Jiang et al. 1998). Bacteria of the genus *Azospirillum* are motile and capable of chemotaxis toward organic acids, sugars, and some aminoacids. Additionally, they are able to follow gradients of oxygen, alternative electron acceptors, and redox active compounds. Most attractant and repellent compounds described so far for this bacterial genus include those that affect intracellular metabolism, leading to the suggestion that most taxis responses correspond to energy taxis in *Azospirillum* spp. (Alexandre 2015). Besides this, no reports have been published until now in relation to the bacterial capacity to respond to light in terms of phototaxis or motility. In this work, we observed a decrease in motility under exposure to WL and BL (see Fig. 5a), in comparison to the displacement reached in darkness (maximum value). However, under RL, motility was lower than in the control, although at the same time greater than under conditions of WL and BL exposure (full-plate irradiation). These results are consistent with the other reports which show that light represses mobility in *A. tumefaciens* and, by contrast, those bacteria grown in the dark are more mobile, adhere to plants better and are more virulent. The reduction in motility under different light wavelengths is a general behavior present in other bacteria of the *Rhizobiaceae* family (*A. tumefaciens*, *A. vitis*, and *Rh. Leguminosarum*) (Oberpichler et al. 2008). Likewise, motility in Az39 was higher in darkness (with half of plate being irradiated) (see Fig. 5b). In both experimental conditions, WL was found to be highly inhibitory for the motility of this bacterium. RL and BL were inhibitory as well, although in a lower proportion.

Some reports showed that light as a stimulus or environmental factor is capable of inducing or repressing the expression of genes related to the synthesis of flagella

(Ramos et al. 2004). The polar flagellum is not only involved in chemotaxis (Grishanin et al. 1991), but also in the first adsorption step when *A. brasilense* colonizes plants (Michiels et al. 1991). Inoculation with *Azospirillum* spp. usually resulted in increased internal colonization of roots, stems, and leaves, indicating high bacterial motility through the plants. Several authors (Baldani et al. 1992; James et al. 1994; Souza et al. 2014) have suggested that the presence of diazotrophic bacteria in xylem vessels may point them out as a route of bacterial migration to different organs. Internal colonization of plant leaves also increased in response to foliar inoculation, suggesting that in this case, the stomata acted as a passive doorway for bacterial migration (Fukami et al. 2016). In summary, in this study, we show that *A. brasilense* Az39 is lethally affected by BL and WL, and that this behavior should be considered when using *A. brasilense* in agricultural practices, including the foliar spray inoculation of soybean, maize, or wheat, among other crops (Puente et al. 2017, 2018; Fukami et al. 2016, 2018). Preininger et al. (2018) have recently compiled the most significant concepts around foliar microbial inoculants and they suggest that efficacy of this products significantly depends on environmental conditions at the time of spraying. Light has a great impact on the viability of microorganisms and their interaction with plant leaf surface. Thereby, formulation should contain protective compounds against the deleterious effect of light to ensure their activity. In our experimental conditions, BL exposure did not inhibit Az39 growth when the bacterium was cultured in a medium modified by the addition of an aliquot of MMAB containing Congo Red indicator (MMAB-CR), in comparison with exposure to WL in LB-CR or BL in rich medium alone (LB). These results show that the Congo Red offers to *A. brasilense* Az39 some protection against exposure to BL when the cell surface composition changes (i.e., production exopolysaccharide) (Budanova et al. 2018) and/or because it absorbs light at 497 nm, in the blue part of the spectrum.

This is the first evidence on the differential behavior by Az39 when exposed to different wavelengths in culture media modified by the addition of the indicator.

Taking into account the consolidation of certain practices in agriculture and the use of *Azospirillum* spp. for foliar spray inoculation in several crops, it is of utmost importance to understand the effect of sunlight or specific wavelengths on such microorganisms, to generate a basic functional model and optimize inoculant functionality in the future.

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Compliance with ethical standards

Conflicts of interest The authors report no conflicts of interest.

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