Photochemistry and Photobiology, 2015, 91: 1291–1301

Invited Review

More Than Just Light: Clinical Relevance of Light Perception in the Nosocomial Pathogen *Acinetobacter baumannii* and Other Members of the Genus *Acinetobacter*†

María Soledad Ramírez[1,2], Gabriela Leticia Müller[3,4], Jorgelina Fernanda Pérez[3], Adrián Ezequiel Golic[4] and María Alejandra Mussi*[3,4]

[1]Facultad de Ciencias Médicas, Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM-CONICET), Universidad de Buenos Aires, Buenos Aires, Argentina
[2]Department of Biological Science, Center for Applied Biotechnology Studies, California State University Fullerton, Fullerton, CA
[3]Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina
[4]Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET), Rosario, Argentina

Received 31 March 2015, accepted 21 July 2015, DOI: 10.1111/php.12523

ABSTRACT

A summary of the major findings concerning light modulation in *Acinetobacter baumannii*, which governs aspects related to the success of this microorganism as a nosocomial pathogen, is presented. Particularly, the evidence shows that light modulates the ability of the bacteria to persist in the environment, its virulence against eukaryotic hosts and even susceptibility to certain antibiotics. The light signal is sensed through different mechanisms, in some cases involving specialized photoreceptors of the BLUF-type, whereas in others, directly by a photosensitizer molecule. We also provide new data concerning the genomic context of BLUF-domain containing proteins within the genus *Acinetobacter*, as well as further insights into the mechanism of light-mediated reduction in susceptibility to antibiotics. The overall information points toward light being a crucial stimulus in the lifestyle of members of the genus *Acinetobacter* as well as in other clinically relevant species, such as members of the ESKAPE group, playing therefore an important role in the clinical settings.

INTRODUCTION

Our planet is one of the few places in universe close enough to one star that its whole surface bathes in light, and where life can take place. Each human cell and most forms of life on Earth synchronize their activities taking into account this ubiquitous environmental signal.

In addition to incident solar radiation, humans and other organisms are exposed to artificial and man-made electromagnetic radiation used for indoor and outdoor lighting, medical diagnosis and treatment of diseases (1).

However, the effects light produces on living organisms are far from being completely understood. In the case of bacteria, extensive knowledge has been accumulated for phototrophs, which capture light to convert it into chemical bond energy or electrochemical potential for processes dedicated to satisfy cellular energy requirements (2,3). However, information recalled during the last decades indicates that this signal also plays a role in the physiology of environmental and pathogenic chemotrophic nonphototrophic prokaryotes (3–5). Pertinent examples include physiological responses as diverse and interesting as morphogenesis in *Stigmatella aurantiaca* (6); carotenogenesis in *Streptomyces coelicolor* (7) and *Myxococcus xanthus* (8,9); the general response to stress in *Bacillus subtilis* (10,11); the attachment of *Caulobacter crescentus* to glass surfaces (12); the photophobic response and various biofilm functions in *Escherichia coli* (13,14); biofilm formation in *Idiomarina loihiensis* (15); motility in *Acinetobacter calcoaceticus* and other *Acinetobacter* species (16–20). Light signals perceived by nonphotosynthetic bacteria may even modulate their interactions with hosts (5,21). For instance, light regulates the general stress response and infection of macrophages by *Brucella abortus* (22,23), the virulence of *Agrobacterium tumefaciens* toward cucumber (24); the invasiveness of *Listeria monocytogenes* for enterocytes (25); the nodulation of *Rhizobium leguminosarum* in plant roots (26); the adhesion and virulence of *Xanthomonas axonopodis* v. *citri* strains to the leaves of orange trees (27) and growth and virulence of *Pseudomonas syringae* in Arabidopsis leaves (21,28).

In many cases light is perceived through the involvement of photosensory receptor protein/s. In fact, whole genome sequencing has revealed the wide-spread occurrence of photosensory receptors for UVA/visible light in prokaryotes, being flavin-based blue -light photoreceptors containing BLUF (Blue-Light-Sensing-Using FAD) or LOV (Light Oxygen Voltage) domains among the most frequent (3,29). Environmental genomics and bioinformatic analyses have only recently initiated, and efforts are successfully being conducted to detect further conservative residues in these domains which could contribute to identify novel

*Corresponding author email: mussi@cefobi-conicet.gov.ar (María Alejandra Mussi)
†Part of the data in this study was presented during the 16th International Congress on Photobiology held in Cordoba, Argentina, in September (8th–12th), 2014.

blue-light sensors of these families in whole sequenced genomes (4,5). In this sense, Losi *et al.* have identified sequence logos taking into account conserved residues beyond those functional to the photocycle, as well as considerations derived from secondary structure elements (5). Just in the case of BLUF, these authors were able to identify 1705 bacterial BLUF proteins in 1282 strains, representing 453 species (5). Interestingly, the presence of more than one type of photosensory domain in the same organism has been shown not to be infrequent in bacteria (4,5).

In other cases, light triggers physiological responses through the photosensitation of particular molecules in a photoreceptor-independent pathway (30,31).

Acinetobacter baumannii has been shown to be able to perceive light with the concomitant modulation of important traits related with its success as a pathogen including persistence in the environment, virulence against the eukaryotic host *Candida albicans* (17,19,32; Fig. 1A) and susceptibility to antibiotics (30; Fig. 1B).

In this review, we will summarize major findings regarding light perception in this microorganism as well as other species of the genus *Acinetobacter*.

THE NOSOCOMIAL PATHOGEN *ACINETOBACTER BAUMANNII* SENSES AND RESPONDS TO LIGHT

Some years ago we have found that exposure to light resulted in dramatic differences in motility in *A. baumannii* (17,32). Briefly, cells spread throughout the surface of the plates when incubated in the dark, but formed quiet colonies under blue or white light in motility plates.

We suspected that the phenotype we observed could presuppose the involvement of a photoreceptor, and genome sequence analysis suggested a likely candidate: the only photoreceptor domain-containing protein encoded in the *A. baumannii* genome, which belongs to the BLUF type (A1S_2225 in the case of *A. baumannii* strain ATCC 17978). Disruption of this putative gene eliminated the inhibitory effect of blue light on motility, and spectral analysis of the recombinant protein confirmed its responsiveness to blue light. We therefore designed this active photoreceptor as BlsA for *blue light sensing A* (17).

The responses induced by light through BlsA are not limited to modulation of motility. Blue light also significantly reduced or eliminated the formation of biofilms and pellicles in broth-grown cultures, and enhanced the ability of *A. baumannii* to kill cells of the eukaryotic fungus *C. albicans* (17; Fig. 1A). The ability to interact with and damage cells of *C. albicans* may have relevance to disease conditions caused by *A. baumannii* in humans, as there may be common elements involved in *A. baumannii*-mediated damage to *C. albicans* and to human cells and tissues (32).

The phenomena is distributed within the species, given that *blsA* homologs have been detected in other clinical strains of *A. baumannii*, which are also capable of presenting photoregulated phenotypes (17).

The levels of *blsA* transcripts correlate with temperatures at which the bacteria respond to light—i.e. 24°C but not 37°C (17), as also do BlsA protein levels (M. Cabruja & M. A. Mussi, unpublished). Dependence of light perception on temperature

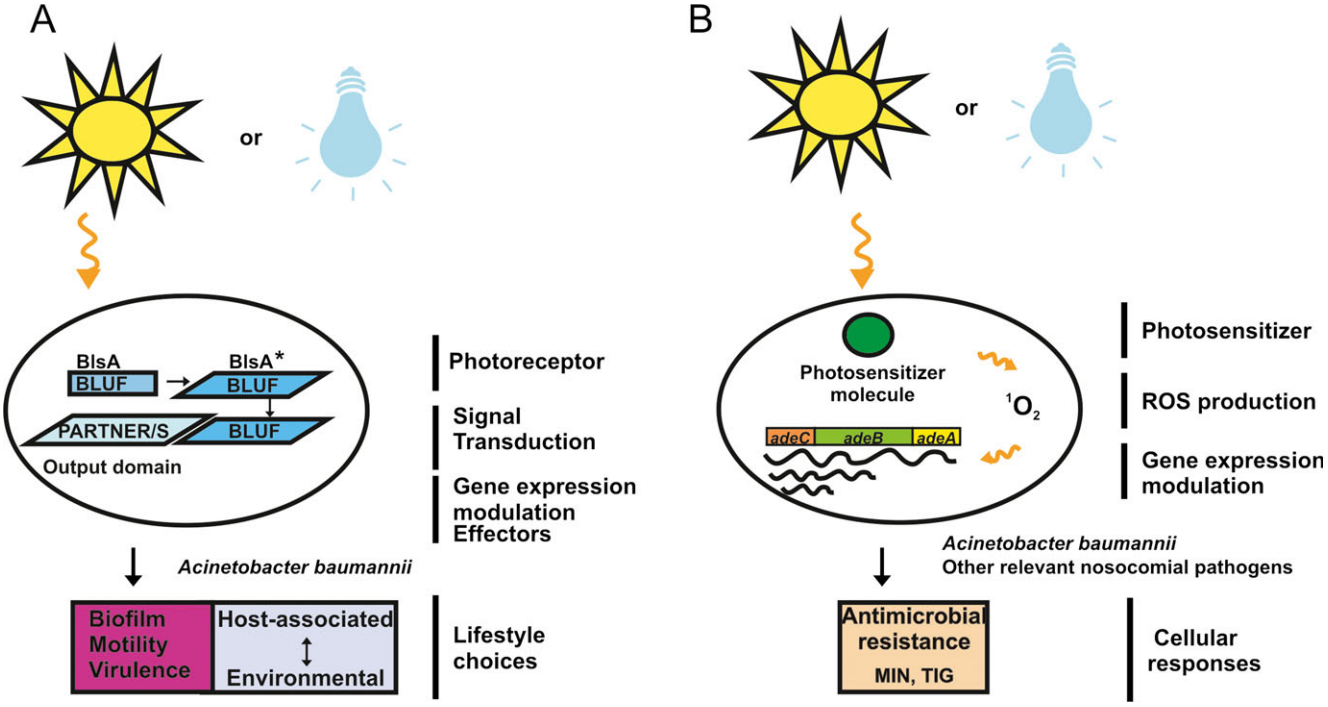

Figure 1. Models for light perception and signal transduction in *Acinetobacter baumannii*. (A) Light absorption induces a conformational change in the *A. baumannii* BLUF photoreceptor BlsA, now able to bind unknown partners that modulate the response, resulting ultimately in regulation of surface motility, biofilm formation and virulence against *Candida albicans* (17). (B) Light can also induce reduction in susceptibility to antibiotics in a photoreceptor-independent manner in *A. baumannii* and other clinically relevant pathogens. In this case, light application could result in the excitation of an unknown photosensitizer molecule with the concomitant production of ROS such as $^1O_2$, leading ultimately to the induction of expression of antibiotic resistance genes such as those coding for the AdeABC pump. This results in reduction in susceptibility to the antibiotics MIN and TIG (30). All these phenotypes occur at 25°C.

prompts speculations on the role of light sensing in the lifestyle of *A. baumannii*. Light and temperature certainly allow bacteria to determine their localization in the media, and play a role in the ability of the microorganism to persist in the environment by modulating motility and biofilm formation (17), a feature particularly relevant in the clinical setting. It is possible to speculate, however, that BlsA would not play a role in systemic infections associated to *A. baumannii* in humans considering the temperature and absence of light in tissues and internal organs. On the other side, the function of this photoreceptor could be important in the pathogenesis of surface-exposed wounds considering the potential exposition of the bacteria to light, and the relative low-moderate temperatures registered in these types of lesions (17).

BLUF proteins are either directly fused within a multidomain protein to an additional domain that regulates the photoresponse or standalone domains that interact noncovalently with an output protein (33). Output domains associated to BLUF identified so far include SCHICH, GGDEF, EAL, cyclases, HTH and PAS (3–5). BlsA contains a BLUF N-terminal domain, but apparently lacks an effector. This necessarily presupposes the requirement of a partner/s protein/s harboring an output domain and acting as a response regulator, whose identity/ies are still unknown (Fig. 1A). Genes coding for BLUF partners are often located in close proximity in the genome, however, in the case of *blsA* no straightforward clue can be clearly deduced, despite some possible candidates are suggested (see below).

The photochemical reaction dynamics of several BLUF proteins have been characterized (34,35). Biradical formation is the first photochemical step in all BLUF domains and is followed by rearrangement of the hydrogen bond network in the flavin-binding pocket (35). The local changes in hydrogen bond orientations are amplified to a global structural alteration involving the β5 strand of the β-sheet which leads to further structural changes in the C-terminal α-helices, ultimately influencing the association of BLUF domains with its partner downstream in the signaling cascade (35). From crystallographic data, the two C-terminal α-helices in BLUF proteins can adopt two different conformations: the α-helices can lie parallel to the β-sheet (e.g. for BlrP1 and the modeled structure of PapB) or can lie perpendicular to β-sheet (e.g. in PixD) (35). The different conformations of the α-helices may reflect light-induced signal transduction to downstream components (35). Recently, Brust *et al.* (33) performed a detailed biophysical characterization of BlsA. Their study showed that residues that H-bond to the C2=O of the flavin in AppA (H44) or PixD (N31) are replaced by F32 (incapable of forming H) bond in BlsA. This is rather surprising since in all sequenced BLUF proteins, there is a residue at this position that is capable of H-bonding to the flavin C2=O carbonyl (33). Mutations to this position in BlsA resulted in faster dark-state recoveries, suggesting that the F32 in BlsA may have been selected during evolution to reduce the rate of dark-state recovery of the *A. baumannii* light-sensing protein (33). Furthermore, data obtained from measurement of the light minus dark steady-state FTIR difference spectrum showed opposite signs between $AppA_{BLUF}$ (1635(+)/1620(−) cm^{-1}) and BlsA (1634(−)/1620(+) cm^{-1}) (33). In $AppA_{BLUF}$, the difference mode has been attributed to structural rearrangement of the BLUF β-sheet. The sign of this protein mode is a unique feature for BlsA because the FTIR difference spectra of other BLUF proteins such as PixD resemble $AppA_{BLUF}$ (33). Consequently, the present results for BlsA point to greater diversity in the structural changes induced by light than previously realized, which are proposed to be directly relevant to the light activated function in *A. baumannii* (33). The weakening of H-bonding in the β-sheet that occurs upon photoactivation in PixD is proposed by the authors to be directly related to dissociation of PixE from PixD (33). Conversely then, the strengthening of H-bonding in the BlsA β-sheet upon photoactivation, revealed by the change in sign of the β-strand marker mode, supports a model for BlsA photoreceptor function in which photoactivation leads to formation of a complex with the downstream target protein rather than dissociation (33).

Overall, BlsA shows idiosyncratic features in comparison to other BLUF proteins, in particular, regarding H-bonding and structural changes induced by light that suggest novel photochemistry and signal transduction mechanisms.

LIGHT SENSING IS DISTRIBUTED WITHIN THE GENUS *ACINETOBACTER*

The potency of new identification technologies including DNA sequencing, Multilocus sequence typing (MLST) and Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) allows nowadays to achieve an accurate identification of microorganisms responsible of nosocomial infections (36–38). This has revealed that many species other than *A. baumannii* within the *Acinetobacter* genus can also cause nosocomial infections, being of particular relevance *A. nosocomialis* and *A. pittii,* other members of the *A. baumannii-calcoaceticus* complex (39), as well as *A. junii, A. lwoffii, A. ursingii* and *A. johnsonii* (40–42).

The phenomenon of light modulation is not restricted to *A. baumannii*, but rather widespread within the genus *Acinetobacter* (18,19), albeit the existence of certain idiosyncratic differences (19). In many of these species, including some strains of *A. nosocomialis* and *A. pittii,* blue light acts as a key factor guiding the decision between motility or sessility at 24°C (19), whereas in *A. baumannii*, light inhibits both motility and biofilm formation (17). Genomic analysis showed the presence of predicted genes coding for BLUF-containing proteins in all *Acinetobacter* sequenced genomes (19). However, the copy number is variable among them. The variable number of genes coding for BLUF-domain containing proteins (from one to six) in the different species analyzed suggests that sensing and responding to light might be of differential importance among them, probably reflecting their different lifestyles and the diversity in niches in which they thrive (19).

Most importantly, light regulation of motility occurs not only at 24°C but also at 37°C in non-*A. baumannii* species (19), in contrast to *A. baumannii* which does not show photoregulation at 37°C (17). This aspect indicates that light modulation could be relevant for infections caused by these bacteria in humans. The differential behavior observed at 37°C compared to *A. baumannii* may result from the extra content of BLUF-domain putative photoreceptors encoded in the genomes of these non-*A. baumannii* species (19).

The four BLUF-domain containing proteins encoded in the *Acinetobacter baylyi* (strain ADP1) genome are active photoreceptors that can replace the *in vivo* functioning of BlsA in *A. baumannii* ATCC 17978 regarding biofilm formation. Similar results were observed for motility, with the exception of ACIAD2110, which only partially complemented the *blsA* mutant (19). Our results therefore show functional redundancy between *A. baylyi* strain

ADP1 photoreceptors and *A. baumannii* BlsA, though partial in the case of ACIAD2110. The participation of the four *A. baylyi* BLUF photoreceptors in photoregulation of motility as well as their possible redundancy in this organism was further explored by Bitrian *et al.* (20). By means of genetic, which included construction of single, double and triple mutants, as well as expression analyses these authors showed that three of the four BLUF-domain-encoding genes, ACIAD1499, ACIAD2125 and ACIAD 2129, but not ACIAD2110 which showed a null phenotype, fully abolished the response to light indicating that they are involved in providing light sensitivity to twitching motility in *A. baylyi* (20). In addition, any of these genes, singly and independently, could restore the blue-light inhibition on motility phenotype in the triple knockout mutant when expressed from a plasmid, and therefore may be considered functionally redundant in *A. bayli* (20). The necessity of the three proteins for inhibition of twitching under blue light in the wild-type strain might be due to a low amount of transcripts and/or protein that each of the BLUF encoded genes individually makes available (20).

The apparent discrepancy respect to the precise role of ACIAD2110 in the overall twitching motility response needs to be further resolved. Yet, it is possible that the difference in behavior observed for ACIAD2110 between the two groups (partial or null participation) originates from the different organisms (*A. baumannii* and *A. baylyi*) in which the studies were performed, implying possibly different signaling cascades as well as processes activated by the photoreceptor in both organisms. This would not be surprising since we have previously shown that the pathways leading to biofilm formation are inversely regulated by light at 24°C between *A. baumannii* and *A. baylyi* (19).

Phylogenetic analysis performed using only the BLUF domains, suggests a common origin for all members of the *Acinetobacter* genus, and could distinguish well-differentiated clusters that group together BLUF homologs from different species, a situation particularly clear for members of the ACB complex (18,19). In similar analyses performed using whole BLUF or LOV-containing proteins sequences from different bacteria, the clustering in distance-trees was dictated also by phylogenetic group proximity as well as by full-protein architecture, i.e. taking into account associated protein domains (4).

Finally, analysis of sequenced genomes in *A. baumannii* as well as in *A. baylyi* strain ADP1 show they only harbor BLUF-type photoreceptors (3). It is worth noting that, with the only exception of *Psychrobacter*, the other genera of the *Moraxellaceae* family, i.e. *Alkanindiges*, *Enhydrobacter*, *Moraxella*, *Oligella*, *Paraperlucidibaca* and *Perlucidibaca*, do not contain genes coding for BLUF-containing proteins. Therefore, BLUF genes must have been acquired by horizontal gene transfer (HGT) in an ancestor of the genus *Acinetobacter*, prior to speciation and possibly after genus differentiation. Then, paralogs in each species must have arisen by gene duplication and/or further HGT. HGT processes involving photosensory proteins have been clearly shown in the case of the LOV photoreceptor modulating virulence in *Pseudomonas syringae,* which is located within an active genomic island acquired by HGT (21). Spread of this genomic island and hence the photoreceptor therein encoded could result in hosts in which sigma factors—controlling global bacterial processes including virulence—are rendered to light control. Further evidence of HGT comes from the LOV photoreceptor present in the α-proteobacterium *Rhizobium leguminosarum* biovar viciae strain 3841, which clusters with those of β-proteobacteria (21).

BLUF GENOMIC CONTEXT ANALYSIS IN DIFFERENT SPECIES OF BACTERIA

It was reported that proteins containing a BLUF-domain function as sensors upstream of phototaxis, nucleotide metabolism and repression of anoxygenic photosynthesis (43).

To retrieve information contained in the genomic context that could possibly provide insights into the partners involved in light signal transduction as well as the diversity of processes directed by light in *Acinetobacter*, we analyzed the genomic context in which BLUF-domain containing genes are immersed in some representative members of the genus whose genomes have been sequenced. As can be observed in Fig. S1 (please see Supporting Information), the close genetic context of *blsA* from *A. baumannii* ATCC 17978 is conserved in most strains of this species included in this study (indicated as same colors along a file, nine out of 15 strains), with the exception of some strains such as *A. baumannii* 6013113 and 6014059, in which variability is detected on the left side genes; or in strains AB056, AB058 and AB059, in which major variability is detected at both sides. In addition, strain *A. baumannii* SDF does not even harbor a *blsA* homolog.

Our *in silico* analysis and resequencing data allowed correction of the original annotation showing that *blsA* is flanked by two predicted coding regions (Fig. S1A). The downstream open reading frame is transcribed in the opposite direction and codes for a hypothetical protein (putative membrane protein), whereas the one located upstream of *blsA* and transcribed in the same direction codes for a predicted protein that belongs to the bacterial oligonucleotide/oligosaccharide-binding fold (BOF) protein family (44). BOF proteins are present in a number of gram-negative pathogenic bacterial strains and have unknown function. All BOF proteins harbor a sec-dependent secretion signal sequence and are predicted to be periplasmatic. In other microorganisms, the BOF protein YgiW, which is preceding members of a two-component system designated *qseBC,* has been characterized. In *Aggregatibacter actinomycetemcomitans*, *ygiW* is coexpressed with *qseBC* genes and regulate biofilm formation (45). In *Haemophilus influenzae*, *ygiW* is cotranscribed with the *qseBC* homolog *firRS*, which senses cold temperatures, ferrous iron and autoregulates its own operon (46). The *ygiW* homolog in *Escherichia coli* has been shown to affect motility and virulence in response to quorum sensing and hormonal signals (47). The homolog VisP present in *Salmonella Typhimurium* has been shown to play a role in stress resistance and virulence through binding to peptidoglycan and LpxO (48). The possibility that the BOF protein present in close proximity to *blsA* in *A. baumannii* is modulating or interacting in some way with BlsA seems plausible, and is currently under study in our laboratory. Other genes and operons in the close surrounding to *blsA* do not provide a clear association with function, at least until what we know so far. It is noticeable the proximity of the *csu* coding system to the *blsA* gene (Fig. S1). However, *in silico* genomic and western blot analyses using anti-CsuA/B serum showed that there is a deletion in the 5′-end of the ATCC 17978 *csuA/*B gene, which results in the abolishment of CsuA/B production (49).

In the case of non-*baumannii Acinetobacter* species, multiple BLUF-domain containing genes (more than two in contrast to only one present in *A. baumannii*) exist in each species (19). These homologs are, in most cases, distributed along the genomes, implying therefore diversity in their genomic contexts. Following is exposed an analysis of some particular cases.

Three BLUF-domain containing proteins are present in *A. nosocomialis* RUH2624. One of the BlsA homologs (ACIRUv1_60150; EEX01065) is located in a similar genomic context as *blsA*, with the difference that the left genes (8 and 9) are missing (Fig. S1). Another homolog present in this strain (ACIRUv1_270092) is in close proximity to a β-lactamase coding gene (ACIRUv1_270091, Fig. 2A); and this poses the question on whether BlsA, transducing a light signal, might be modulating this resistance gene. This possibility, which would not be surprising given our recent results showing modulation by light of antibiotic resistance genes (30), is currently under study in our laboratory. The last homolog (ACIRUv1_920004) is inserted close to an acyltransferase and DNA replication genes (Fig. 2A).

In *A. oleivorans* strain DR1, one of the BlsA homologs (AOLE_NC_014259) is located in a similar genomic context as *blsA*, with the difference that genes in the right (2–5) are missing (Fig. S1). The BLUF-domain containing gene AOLE_05860 is close to a cold shock inducible protein homolog to *cspG* from *E. coli* (Fig. 2B). The homolog AOLE_10045 is in close proximity to a β-lactamase (Fig. 2B), just as is the homolog ACIRUv1_270092 present in *A. nosocomialis* (see above).

In *A. pittii* SH024, one of the three *blsA* homologs (ACISHv1_330004) is in close proximity to a cold shock protein coding gene, such as in the case of *A. oleivorans* (not shown).

In the genome of *A. radioresistens* SH164 there are three (of the five) BLUF-domain containing proteins which are in close proximity to each other: ACIRAv1_110019, ACIRAv1_110021 and ACIRAv1_110028. The homolog ACIRAv1_110019 is in close proximity to a dy-guanylate cyclase. This is one of the few examples where a BLUF-domain containing protein is clearly associated to a putative effector domain of the type previously shown to mediate transduction of the signal in BLUF photoreceptors. Other *A. radioresistens blsA* homologs are in close proximity, again, to a cold shock protein coding gene (ACIRAv1_110028) and an outer membrane protein, OmpW (ACIRAv1_100009) (not shown).

In *A. baylyi* strain ADP1 there are four *blsA* homologs. ACIAD1499 is in close proximity to *mutS* and *fd* in this organism. MutS initiates the process of mismatch repair by recognizing and binding to mismatched bases in double-stranded DNA (50). On the other side, this homolog is close to a ferredoxin coding gene (*fd*), which code for low weight iron sulfur proteins that function as electron transfer agents in some important biological reactions (51). The other three photoreceptor coding genes (ACIAD2110, ACIAD2125 and ACIAD2129) are quite close to each other. In addition, these photoreceptors are in close proximity to iron utilization genes such as siderophore biosynthesis and receptor proteins (Fig. 2C).

Finally, *Acinetobacter* sp. VER3, which was isolated from extreme lakes at the Andean Puna in Argentina (52,53), shows the presence of only one BLUF-domain containing protein located in close proximity to genes coding for a peptidase and alanine racemase, DNA helicase and ribosomal proteins (Fig. 2D).

The overall analysis shows that most BLUF-containing proteins in non-*baumannii Acinetobacter* species are inserted in different genomic contexts than *A. baumannii* BlsA. The presence of genes such as a β-lactamase, *mutS* or iron utilization genes close to these BLUF-domain coding proteins in *A. nosocomialis* or *A. baylyi* suggest that light could be regulating resistance to β-lactams, mismatch repair, or iron metabolism, respectively. Also interesting is the proximity of *blsA* to cold shock like proteins coding genes such as in *A. oleivorans*, *A. pittii* and *A. radioresistens,* which might be possibly related to light sensing at low temperatures.

LIGHT MODULATES ANTIBIOTIC RESISTANCE IN *ACINETOBACTER* AND OTHER SPECIES OF CLINICAL RELEVANCE

We have recently discovered another instance where light becomes important in the lifestyle of *A. baumannii*, a feature shared with other organisms of nosocomial importance. Briefly, under low intensity illumination conditions both with blue, as well as white light, these bacteria show an outstanding reduction in susceptibility both to minocycline (MIN) and tigecycline (TIG) antibiotics when growing stagnantly in plates as well as in liquid media (30). In fact, the application of light resulted in variation in minimal inhibitory concentration (MIC) values to TIG from 2 (in the dark) to 128 μg mL^{-1} (under blue light) in the case of some strains such as ATCC 19606. In a similar way, strain A42 shows fluctuation in the MIC values to MIN from <0.125 in the dark respect to 16 μg mL^{-1} under illuminated conditions (30). These remarkable differences in 64 and 128 folds, respectively, point out the profound influence that light can exert on antibiotic resistance, revealing also the importance of light as a key environmental stimulus governing many processes, which has remained obscure until recently. Beyond susceptibility to antibiotics, this response to light might enhance the ability of the bacterium to persist until conditions are more favorable for growth or until additional resistance determinants can be accumulated.

This behavior was found to be temperature dependent, as it occurs at 24°C but negligible at 37°C. The antibiotics MIN and TIG represent important weapons against multidrug resistant bacteria frequently encountered in the clinical settings (54). In particular, MIN is an old antibiotic that has experienced a rebirth as a result of the emergence of bacteria recalcitrant to common antibiotic treatments (54–56). On its side, TIG, a new glycylcycline antibiotic, has been recently designed and introduced as a MIN derivative (54,55) to treat multidrug (MDR) resistant infections.

The generation of $^1O_2$ results in the same phenotype as the application of blue light, providing strong evidence that light might be modulating resistance through $^1O_2$ production (30). This is not surprising since the production of singlet oxygen by light in the presence of a photosensitizer molecule has been extensively described (31,57). In addition, we show that light induces the expression of genes that have been previously related to resistance to TIG, such as those coding for the efflux pump AdeABC (58–60). In particular, we have determined that in the presence of sub-MIC inhibitory concentrations of TIG, light induces several folds (from 2 to 60) the expression of genes coding for components of the pump respect to dark conditions, both in solid plates as well as in stagnant liquid microplates at 24°C (30). This shows a correlation between reduction in susceptibility to TIG and induction of resistance genes mediated by light. In Fig. 3, we present further evidence by showing that A42 (MDR wild-type strain; 30) cells scraped from LB Difco plates supplemented with methylene blue (MB) and incubated overnight under red light illumination—conditions that account for $^1O_2$

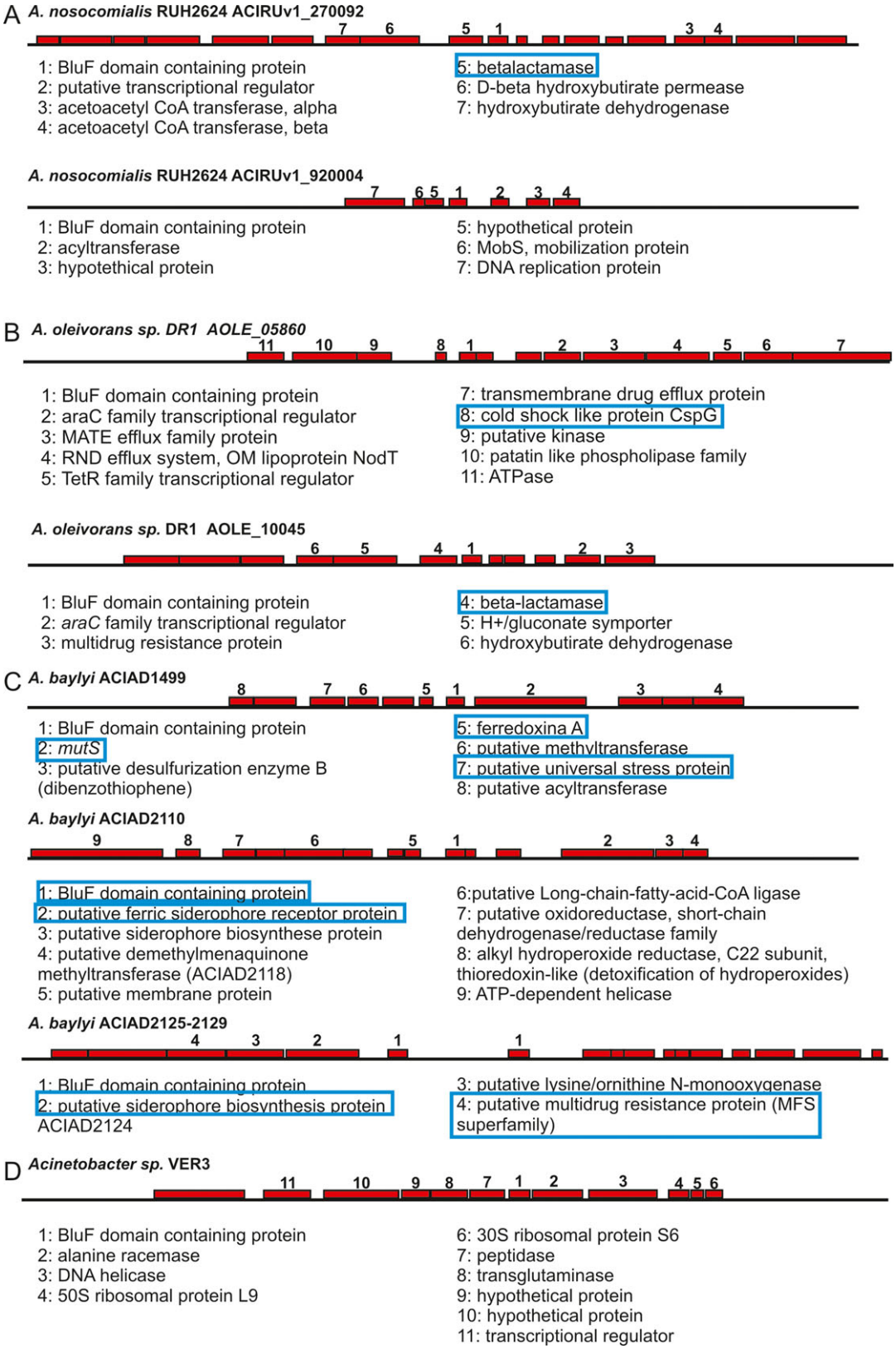

Figure 2. Genomic context of BLUF-domain containing genes in *Acinetobacter*. BLUF-domain containing and neighboring genes present in non-baumannii species of the genus *Acinetobacter:* (A) BLUF-domain containing genes present in *A. nosocomialis* RUH2624, in addition to the BlsA analog. (B) BLUF-domain containing genes present in *A. oleivorans* strain DR1. (C) BLUF-domain containing genes present in *A. baylyi* strain ADP1. (D) BLUF-domain containing genes in *A*. sp. VER3. Only CDS that have an annotated function are indicated. Gene annotations are indicated below the scheme. Within blue boxes are indicated relevant genes, further described in the text. The figure was constructed using information retrieved from the MaGe platform (71; http://www.genoscope.cns.fr/agc/microscope/home/). BLUF coding genes are indicated as 1.

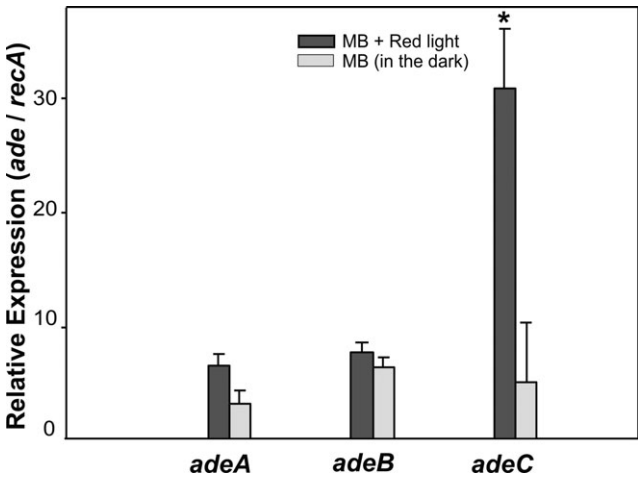

Figure 3. $^1O_2$ triggers induction of expression of the *adeC* transcript levels in *Acinetobacter baumannii*. cDNA from *A. baumannii* A42 cells grown overnight on LB Difco plates supplemented with methylene blue (MB) in the presence of red light (dark gray bars) or in the dark (light gray bars) at 24°C was used as template for qRT-PCR with *adeA*, *B* or *C* specific primers (30). *recA* transcript levels was used as a constitutively expressed internal control. Standard deviations of three independent experiments are shown. Asterisks indicate transcript levels statistically different between light and dark conditions.

generation—result in induction of *adeC* gene respect to darkness. This reinforces the correlation between the application of light, the production of $^1O_2$, induction of components of the AdeABC pump and ultimately, reduction in susceptibility to MIN and TIG (Fig. 1B).

It has been recently shown that the NorM efflux pump, a member of the MATE-family, reduces the level of intracellular ROS in a 8-oxoguanine-(GO) repair-deficient strain, protecting therefore the cell from oxidative stress (61). The mechanism is not yet clearly elucidated but would likely involve the extrusion of specific molecules—by-products of bacterial metabolism—that oxidize the guanines present in both DNA and nucleotide pools (61). This is interesting in the context of the above information, since it could be a precedent to our findings regarding the induction of genes coding for components of the AdeABC efflux pump under blue light, where ROS such as $^1O_2$ are putative intermediates (30).

Whether other pumps or resistance mechanisms are also involved in reduction in susceptibility mediated by light, is a possibility that needs further study. In this sense, it has been shown that the AdeIJK pump acts synergistically to AdeABC in TIG resistance generation (62). Also noticeable is that different strains show differential levels of resistance under blue light for these antibiotics (30). This likely depends on the genetic background, specifically, on the photosensitizer molecules and/or antibiotic resistance determinants present in each strain. In addition, different strains showed induction in different genes of the AdeABC pump, such as *adeAB* in strain ATCC 19606 or *adeC* in strain A42. This is not surprising since plasticity is one of the major features of *A. baumannii*, including genomic context, and regulatory mechanisms. It is therefore possible that regulation of efflux pumps is different depending on the strain. For example analysis by Northern hybridization in strain BB4454 indicated that the three genes were cotranscribed, although mRNAs corresponding to *adeAB* and *adeC* were also present (63).

The conditions of light used in these experiments pose no bactericidal effects in *A. baumannii*. In fact, *A. baumannii* is more resistant to MIN and TIG –and therefore grows to a higher extent- under blue light. In addition, there is no difference in OD between cells incubated under blue light or in the dark in the absence of antibiotics (growth control), when MIC determinations in liquid media in microplates are performed (30).

The difference in resistance to MIN and TIG between light and dark conditions is maximized under low iron levels while, conversely, almost suppressed in the presence of this ion, indicating that its presence constitutes a variable modulating the effect (30).

The possibility that MIN and TIG are being degraded under our experimental conditions does not seem to be the most plausible scenario. Many lines of evidence go in this sense: First, different strains cultured in the same media show different levels of inhibition by MIN and/or TIG under blue light respect to dark conditions at 24°C in LB, whereas some strains that do not even show differences at all (30). For example strains *A. baumannii* strain Ab107 (table 2, 30); *K. pneumoniae* 404 (table 4, 30) and *E. cloacae* strain 9 (table 4, 30) do not show significant differences in MIN and/or TIG in LB between light and dark conditions. Whether the antibiotic was being degraded, differences in inhibition zones in antibiogram plates between light and dark conditions would be observed in all strains. Second, the differences observed in strains A42, A118 and ATCC 19606 in susceptibility to MIN and TIG in LB at 24°C between light and dark conditions are significantly reduced or cancelled at 37°C in the same media. If the antibiotics were being degraded at 24°C, most probably, they would be degrading also at 37°C and the inhibition zone would be similar or greater. Third, the effect is observed in LB media but not in MH or other media such as BM2. If light was exerting a degradation effect on MIN and TIG, it would have probably occurred in the other media too. Fourth, we did not observe differences when TET was tested, an antibiotic belonging to the same family as MIN and TIG. In addition, it is one of the antibiotics shown in other works to be degraded by light (64), meaning that under our experimental conditions, light is not degrading these antibiotics. It should be noted that we are using light sources emitting blue or white light of very low intensity (6–10 μmol m^{-2} seg^{-1}). Furthermore, we do not detect the presence of toxic photoproducts, as the OD (as well as the MIC) of the bacteria increase under blue light.

Finally, our findings show that MIN and TIG antibiotic treatments may be improved by the inclusion of an iron chelator (such as the FDA-approved DSX)—a measure that in addition to keeping the wounds in the dark—would increase the effectiveness in the control of infections involving these microorganisms.

CLINICAL IMPLICATIONS

The manifestation of the pathogenic character of opportunistic pathogens depends on the ecologic triad microorganism–host–environment. Regarding the microorganism, *A. baumannii* is highly versatile, with little nutritional requirements, and a high ability to persist for weeks even in the absence of humidity, e.g. in the form of biofilms. Most importantly, it shows an incredible ability to acquire resistance traits easily and accumulate them in its genome. This, in many cases, leads to the selection of multidrug resistant strains, which ultimately result in the emergence of clonal outbreaks. Conditions of the host considered as risk factors

associated with *A. baumannii* infections include advanced age, immuno-suppression, alteration of the mucous-skin barrier, previous antimicrobial therapy, previous sepsis in the ICU, invasive procedures and long stays in hospitals and ICUs (65,66). Finally, the nosocomial environment constitutes an optimal niche for the successful development of pathogenic behavior since there are a high number of immuno-compromised patients, utilization of antibiotics in high scales that act as selecting agents, and utilization of invasive devices such as catheters, ventilator equipment, etc. The convergence of all these factors determine *A. baumannii* as a successful nosocomial pathogen, a situation that does not take place frequently in other conditions, as for example in the community.

The role and influence of light in the modulation of many, if not all, of these particular aspects has been clearly demonstrated throughout our work in this field (17,19,30). As mentioned before, we have shown that light modulates motility and biofilm formation in *A. baumannii* (17), and other species of the genus *Acinetobacter* (19); factors that directly impact in the microorganism's ability to persist in the environment. In addition, motility along surfaces as well as formation of biofilms in medical devices, hospital rooms, curtains and/or bed clothes, contribute to spread of the microorganisms in the hospital environment and among patients. Modulation of antibiotic susceptibility to certain antibiotics plays an important role in control of nosocomial outbreaks. Recently we have demonstrated that susceptibility to MIN and TIG, two antibiotics used for the treatment of MDR *A. baumannii* infections, is modulated by light (30), enhancing bacterium's ability to persist in adverse conditions such as application of antibiotic treatment. Finally, virulence against *C. albicans* is also modulated by light, allowing speculations that infections in humans might be also modulated by this stimulus. *C. albicans* has been shown to be useful as a model to study interactions between prokaryotes and eukaryotes, either pathogenic or symbiotic (67). Indeed, many of the virulence factors studied in the context of human disease may also have an ecological role within microbial communities (67).

Given that there is no light within internal organs or tissues, it does not seem that light plays a significant role in modulation of systemic infections in humans. Instead, modulation of surface-exposed wound infections by light may be of critical importance, given in addition the relatively lower temperatures recorded in these types of lesions (17). In this sense, *S. aureus* and *A. baumannii*, microorganisms in which we detected modulation of antibiotic susceptibility by light, are known causative agents of skin and soft tissue infections highlighting the importance of our findings.

Temperature of human skin is not equal to that found inside the body. Skin is the largest organ in human body and is in contact with external media. Skin temperature depends on air temperature and time spent in that environment. Even different parts of the body can have different skin temperatures, depending on the conditions. Humans adapt to air temperature changes perceived by the skin by becoming warmer or colder. Measurements inside the body indicate a general temperature of 36.5–37.5°C, whereas skin temperature is below 33°C. Besides, temperature of skin wounds is frequently lower than 33°C (68). In fact, McGuiness and col. reported that wound bed temperatures measured immediately after dressing removal fluctuated between 25 and 37°C (68). These temperature values are quite compatible with our experimental set up.

Regarding light sources, the ones most common to hospital buildings include incandescent/halogen, fluorescent, high intensity discharge and light emitting diodes (LEDs) (69,70). Nowadays, LED technologies is recognized as increasingly convenient even for hospitals (69), and therefore the light sources we are using for the experiments in our work are compatible with those likely present in hospitals, which will definitely bow toward LEDs in the near future.

Globally, the influence of light on phenotypes related to bacterial virulence, persistence and resistance to antibiotics is likely to be easily translated to the clinical setting. Efforts are currently being conducted on generating a convenient animal model to be able to evaluate the effects of light in bacterial–host interactions.

MATERIALS AND METHODS

Transcriptional analysis. A42 cells were scraped from LB Difco plates supplemented with 5 μM methylene blue (MB) and incubated overnight in the dark or under red light illumination, a condition that accounts for $^1O_2$ generation. The cells were immediately mixed with 2 mL lysis buffer (0.1 M sodium acetate, 10 mM EDTA, 1% SDS) in a boiling-water bath. Cell lysates were extracted twice at 60°C with phenol, which was adjusted to pH 4.0 with 50 mM sodium acetate, and then once with chloroform at room temperature. The RNA precipitated overnight at −20°C with 2.5 vols ethanol was collected by centrifugation, washed with 70% ethanol, and dissolved in DEPC-treated deionized water. Total RNA samples were treated with RNase-free DNase I. The integrity of the RNA samples was checked by agarose electrophoresis. RNA samples were collected from three different biological samples prepared in triplicate each time.

First-strand cDNA was synthesized with MoMLV-reverse transcriptase (Promega) following the manufacturer's instructions, using 2 μg RNA and random primers. Relative expression was determined by performing quantitative real-time PCR in an iCycler iQ detection system and Optical System Software version 3.0a (Bio-Rad), using the intercalation dye SYBR Green I (Invitrogen) as a fluorescent reporter, with 2.5 mM.

$MgCl_2$, 0.5 mM each primer and 0.04 U μL^{-1} GoTaq (Promega). PCR primers used in these experiments are described in ref. 19. A 10-fold dilution of cDNA obtained as described above was used as template. Samples containing no reverse transcriptase or template RNA were included as negative controls to ensure RNA samples were free of DNA contamination. Cycling parameters were as follows: initial denaturation at 94°C for 2 min; 40 cycles of 96°C for 10 s, 54°C for 15 s and 72°C for 1 min; and a final extension of 72°C for 10 min. The *adeA*, *adeB* and *adeC* transcript levels of each sample were normalized to the *recA* transcript level for each cDNA sample. Relative gene expression to *recA* was calculated using the comparative $2^{-\Delta C_t}$ method. Each cDNA sample was run in technical triplicate and repeated in at least three independent sets of samples. The ANOVA test was used to determine statistical significance. Further experimental details are described in ref. 19.

Acknowledgements—We acknowledge the LABGeM and the National Infrastructure «France Génomique» for the platform MAGE (Magnifying Genomes), which has been very helpful for the genomic context analyses showed in this review. This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (PICT 2013-0018) to M.A.M. M.S.R, G.L.M. and M.A.M. are career investigators of CONICET. A.E.G. is a fellow at the same institution.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Genomic context of *blsA* in representative members of the genus *Acinetobacter*. A. *blsA* genetic surroundings in *A. baumannii* ATCC 17978 strain. Coding-sequences (CDSs) are located in their corresponding frame. Gene annotations are indi-

cated below the scheme. Within blue boxes are indicated relevant genes, in this case, csu usher chaperone system. *blsA* gene is indicated as 1. Genes resulting from correction of annotations are indicated as big red boxes with the legend MB (putative membrane protein) and BOF (bacterial oligonucleotide/oligosaccharide-binding fold protein), flanking *blsA*. B. Synteny analysis of *A. baumannii* ATCC 17978 *blsA* and neighbor genes, respect to its homologs present in other members of the genus *Acinetobacter*. The figure was constructed using the MaGe platform (71; http://www.genoscope.cns.fr/agc/microscope/home/). Same colors as *blsA* in each file (genes in the same strain) indicate conservation of genomic context respect to *blsA*. CDSs in panel B are aligned with corresponding CDSs in panel A.

REFERENCES

1. Jayanthy, A. and V. Setaluri (2015) Light-regulated microRNAs. *Photochem. Photobiol.* **91**, 163–172.
2. Elías-Arnanz, M., S. Padmanabhan and F. Murillo (2011) Light-dependent gene regulation in nonphototrophic bacteria. *Curr. Opin. Microbiol.* **14**, 128–135.
3. van der Horst, M., J. Key and K. Hellingwerf (2007) Photosensing in chemotrophic, non-phototrophic bacteria: Let there be light sensing too. *Trends Microbiol.* **15**, 554–562.
4. Mandalari, C., A. Losi and W. Gaertner (2013) Distance-tree analysis, distribution and co-presence of bilin- and flavin-binding prokaryotic photoreceptors for visible light. *Photochem. Photobiol. Sci.* **12**, 1144–1157.
5. Losi, A., C. Mandalari and W. Gärtner (2014) From plant infectivity to growth patterns: The role of blue-light sensing in the prokaryotic world. *Plants* **3**, 70–94.
6. Qualls, G. T., K. Stephens and D. White (1978) Light-stimulated morphogenesis in the fruiting myxobacterium *Stigmatella aurantiaca*. *Science* **201**, 444–445.
7. Takano, H., S. Obitsu, T. Beppu and K. Ueda (2005) Light-induced carotenogenesis in *Streptomyces coelicolor* A3(2): Identification of an extracytoplasmic function sigma factor that directs photodependent transcription of the carotenoid biosynthesis gene cluster. *J. Bacteriol.* **187**, 1825–1832.
8. Burchard, R. P. and M. Dworkin (1966) Light-induced lysis and carotenogenesis in *Myxococcus xanthus*. *J. Bacteriol.* **91**, 535–545.
9. Browning, D. F., D. E. Whitworth and D. A. Hodgson (2003) Light-induced carotenogenesis in *Myxococcus xanthus*: Functional characterization of the ECF sigma factor CarQ and antisigma factor CarR. *Mol. Microbiol.* **48**, 237–251.
10. Avila-Pérez, M., K. J. Hellingwerf and R. Kort (2006) Blue light activates the σB-dependent stress response of *Bacillus subtilis* via YtvA. *J. Bacteriol.* **188**, 6411–6414.
11. Gaidenko, T. A., T. J. Kim, A. L. Weigel, M. S. Brody and C. W. Price (2006) The blue-light receptor YtvA acts in the environmental stress signaling pathway of *Bacillus subtilis*. *J. Bacteriol.* **188**, 6387–6395.
12. Purcell, E. B., D. Siegal-Gaskins, D. C. Rawling, A. Fiebig and S. Crosson (2007) A photosensory two-component system regulates bacterial cell attachment. *PNAS* **104**, 18241–18246.
13. Taylor, B. L. and D. E. Jr Koshland (1975) Intrinsic and extrinsic light responses of *Salmonella typhimurium* and *Escherichia coli*. *J. Bacteriol.* **123**, 557–569.
14. Tschowri, N., S. Busse and R. Hengge (2009) The BLUF-EAL protein YcgF acts as a direct anti-repressor in a blue-light response of *Escherichia coli*. *Genes Dev.* **23**, 522–534.
15. van der Horst, M. A., T. P. Stalcup, S. Kaledhonkar, M. Kumauchi, M. Hara, A. Xie, K. J. Hellingwerf and W. D. Hoff (2009) Locked chromophore analogs reveal that photoactive yellow protein regulates biofilm formation in the deep sea bacterium *Idiomarina loihiensis*. *J. Am. Chem. Soc.* **131**, 17443–17451.
16. Hoff, W. D., M. A. van der Horst, C. B. Nudel and K. J. Hellingwerf (2009) Prokaryotic phototaxis. *Methods Mol. Biol.* **571**, 25–49.
17. Mussi, M. A., J. A. Gaddy, M. Cabruja, B. A. Arivett, A. M. Viale, R. Rasia and L. A. Actis (2010) The opportunistic human pathogen *Acinetobacter baumannii* senses and responds to light. *J. Bacteriol.* **192**, 6336–6345.
18. Bitrian, M., C. M. Solari, R. H. González and C. B. Nudel (2012) Identification of virulence markers in clinically relevant strains of *Acinetobacter genospecies*. *Int. Microbiol.* **15**(2), 79–88.
19. Golic, A., M. Vaneechoutte, A. Nemec, A. Viale, L. Actis and M. A. Mussi (2013) Staring at the cold sun: Blue light is distributed within the genus *Acinetobacter*. *PLoS One* **8**, e55059.
20. Bitrian, M., R. H. González, G. Paris, K. J. Hellingwerf and C. B. Nudel (2013) Blue-light-dependent inhibition of twitching motility in *Acinetobacter baylyi* ADP1: Additive involvement of three BLUF-domain-containing proteins. *Microbiology* **159**(Pt 9), 1828–1841.
21. Moriconi, V., R. Sellaro, N. Ayub, G. Soto, M. Rugnone, R. Shah, G. Pathak, W. Gärtner and J. Casal (2013) LOV-domain photoreceptor, encoded in a genomic island, attenuates the virulence of *Pseudomonas syringae* in light-exposed Arabidopsis leaves. *Plant J.* **76**, 322–331.
22. Swartz, T. E., T. S. Tseng, M. A. Frederickson, G. Paris, D. J. Comerci, G. Rajashekara, J. G. Kim, M. B. Mudgett, G. A. Splitter, R. A. Ugalde, F. A. Goldbaum, W. R. Briggs and R. A. Bogomolni (2007) Blue-light-activated histidine kinases: Two-component sensors in bacteria. *Science* **317**, 1090–1093.
23. Sycz, G., M. C. Carrica, T. S. Tseng, R. A. Bogomolni, W. R. Briggs, F. A. Goldbaum and G. Paris (2015) LOV histidine kinase modulates the general stress response system and affects the virB operon expression in *Brucella abortus*. *PLoS One* **10**(5), e0124058.
24. Oberpichler, I., R. Rosen, A. Rasouly, M. Vugman, R. Ron and T. Lamparter (2008) Light affects motility and infectivity of *Agrobacterium tumefaciens*. *Environ. Microbiol.* **10**, 2020–2029.
25. Ondrusch, N. and J. Kreft (2011) Blue and red light modulates SigB-dependent gene transcription, swimming motility and invasiveness in *Listeria monocytogenes*. *PLoS One* **6**, e16151.
26. Bonomi, H., D. Posadas, G. Paris, M. C. Carrica, M. Fredericksonb, L. I. Pietrasanta, R. Bogomolni, A. Zorreguieta and F. Goldbaum (2012) Light regulates attachment, exopolysaccharide production, and nodulation in *Rhizobium leguminosarum* through a LOV-histidine kinase photoreceptor. *PNAS* **109**(30), 12135–12140.
27. Kraiselburd, I., A. I. Alet, M. L. Tondo, S. Petrocelli, L. D. Daurelio, J. Monzón, O. A. Ruiz, A. Losi and E. G. Orellano (2012) A LOV protein modulates the physiological attributes of *Xanthomonas axonopodis* pv. *citri* relevant for host plant colonization. *PLoS One* **7**(6), e38226.
28. Río-Álvarez, I., J. J. Rodríguez-Herva, P. M. Martínez, P. González-Melendi, G. García-Casado, P. Rodríguez-Palenzuela and E. López-Solanilla (2014) Light regulates motility, attachment and virulence in the plant pathogen *Pseudomonas syringae* pv *tomato* DC3000. *Environ. Microbiol.* **16**(7), 2072–2085.
29. Gomelsky, M. and W. D. Hoff (2011) Light helps bacteria make important lifestyle decisions. *Trends Microbiol.* **19**, 441–448.
30. Ramírez, M. S., G. Traglia, J. Pérez, G. Müller, F. Martínez, A. Golic and M. A. Mussi (2015) White and blue light induce reduction in susceptibility to minocycline and tigecycline in *Acinetobacter* sp. and other bacteria of clinical importance. *J. Med. Microbiol.* **64**(5), 525–537.
31. Galbis-Martínez, M., S. Padmanabhan, F. J. Murillo and M. Elías-Arnanza (2012) CarF mediates signaling by singlet oxygen, generated via photoexcited protoporphyrin IX, *Myxococcus xanthus* light-induced carotenogenesis. *J. Bacteriol.* **194**(6), 1427.
32. McBride, M. (2010) Shinning a light on an opportunistic pathogen. *J. Bacteriol.* **192**(24), 6325–6326.
33. Brust, R., A. Haigney, A. Lukacs, A. Gil, S. Hossain, K. Addison, C. Lai, M. Towrie, G. Greetham, I. Clark, B. Illarionov, A. Bacher, R. Kim, M. Fischer, C. Simmerling, S. Meech and P. Tonge (2014) Ultrafast structural dynamics of BlsA, a photoreceptor from the pathogenic bacterium *Acinetobacter baumannii*. *J. Phys. Chem. Lett.* **5**, 220–224.
34. Grinstead, J. S., S. T. Hsu, W. Laan, A. M. Bonvin, K. J. Hellingwerf, R. Boelens and R. Kaptein (2006) The solution structure of the AppA BLUF domain: Insight into the mechanism of light-induced signaling. *ChemBioChem* **7**, 187–193.
35. Masuda, S. (2013) Light detection and signal transduction in the BLUF photoreceptors. *Plant Cell Physiol.* **54**(2), 171–179.
36. Karah, N., B. Haldorsen, K. Hegstad, G. S. Simonsen, A. Sundsfjord, O. Samuelsen and A Norwegian Study Group of Acinetobacter

(2011) Species identification and molecular characterization of *Acinetobacter* spp. blood culture isolates from Norway. *J. Antimicrob. Chemother.* **66**, 738–744.
37. Hsueh, P. R., L. C. Kuo, T. C. Chang, T. F. Lee, S. H. Teng, Y. C. Chuang, L. J. Teng and W. H. Sheng (2014) Evaluation of the Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of blood isolates of *Acinetobacter* species. *J. Clin. Microbiol.* **52**, 3095–3100.
38. Sousa, C., L. Silva, F. Grosso, A. Nemec, L. Lopes and L. Peixe (2014) Discrimination of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex species by Fourier transform infrared spectroscopy. *Eur. J. Clin. Microbiol. Inf. Dis.* **33**, 1345–1353.
39. Nemec, A., L. Krizova, M. Maixnerova, T. J. van der Reijden, P. Deschaght, V. Passet, M. Vaneechoutte, S. Brisse and L. Dijkshoorn (2011) Genotypic and phenotypic characterization of the *Acinetobacter calcoaceticus- Acinetobacter baumannii* complex with the proposal of *Acinetobacter pittii* sp. nov. (formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU). *Res. Microbiol.* **162**, 393–404.
40. Bernards, A. T., A. J. de Beaufort, L. Dijkshoorn and C. P. van Boven (1997) Outbreak of septicaemia in neonates caused by *Acinetobacter junii* investigated by amplified ribosomal DNA restriction analysis (ARDRA) and four typing methods. *J. Hosp. Infect.* **35**, 129–140.
41. van den Broek, P. J., T. J. van der Reijden, E. van Strijen, A. V. Helmig-Schurter, A. T. Bernards and L. Dijkshoorn (2009) Endemic and epidemic *Acinetobacter* species in a university hospital: An 8-year survey. *J. Clin. Microbiol.* **47**, 3593–3599.
42. Traglia, G. M., M. Almuzara, E. Vilacoba, A. Tuduri, G. Neumann, E. Pallone, D. Centron and M. S. Ramirez (2014) Bacteremia caused by an *Acinetobacter junii* strain harboring class 1 integron and diverse DNA mobile elements. *J. Infect. Dev. Count* **8**, 667–669.
43. Singh, A. H., T. Doerks, I. Letunic, J. Raes and P. Bork (2009) Discovering functional novelty in metagenomes: Examples from light-mediated processes. *J. Bacteriol.* **191**, 32–41.
44. Ginalski, K., L. Kinch, L. Rychlewski and N. V. Grishin (2004) BOF: A novel family of bacterial OB-fold proteins. *FEBS Lett.* **567**, 297–301.
45. Juárez-Rodríguez, M. D., A. Torres-Escobar and D. Demuth (2013) ygiW and qseBC are co-expressed in *Aggregatibacter actinomycetemcomitans* and regulate biofilm growth. *Microbiology* **159**, 989–1001.
46. Steele, K., L. O'Connor, N. Burpo, K. Kohler and J. Johnston (2012) Characterization of a ferrous iron-responsive two-component system in nontypeable *Haemophilus influenzae*. *J. Bacteriol.* **194** (22), 6162–6173.
47. Merighi, M., A. Septer, A. Portillo, A. Bhatiya, S. Porwollik, M. McClelland and J. Gunn (2009) Genome-wide analysis of the PreA/PreB (QseB/QseC) regulon of *Salmonella enterica* serovar *Typhimurium*. *BMC Microbiol.* **9**, 42.
48. Moreira, C. G., C. Herrera, B. D. Needham, C. T. Parkera, S. J. Libby, F. Fang, S. Trent and V. Sperandio (2013) Virulence and stress-related periplasmic protein (VisP) in bacterial/host associations. *PNAS* **110**(4), 1470–1475.
49. McQueary, C. and L. A. Actis (2011) *Acinetobacter baumannii* biofilms: Variations among strains and correlations with other cell properties. *J. Microbiol.* **49**, 243–250.
50. Young, D. and N. Ornston (2001) Functions of the mismatch repair gene *mutS* from *Acinetobacter* sp. strain ADP1. *J. Bacteriol.* **183** (23), 6822–6831.
51. Adman, E., L. Sieker and L. Jensen (1972) The structure of a bacterial ferredoxin. *J. Biol. Chem.* **248**, 3987–3996.
52. Albarracín, V., G. Pathak, T. Douki, J. Cadet, C. Borsarelli, W. Gärtner and M. E. Farias (2012) Extremophilic *Acinetobacter* strains from high-altitude lakes in Argentinean Puna: Remarkable UV-b resistance and efficient DNA damage repair. *Orig. Life Evol. Biosph.* **42**, 201–221.
53. Ordoñez, O. F., M. R. Flores, J. R. Dib, A. Paz and M. E. Farias (2009) Extremophile culture collection from Andean lakes: Extreme pristine environments that host a wide diversity of microorganisms with tolerance to UV radiation. *Microb. Ecol.* **58**, 461–473.
54. Bradford, P. A., D. T. Weaver-Sands and P. J. Petersen (2005) In vitro activity of tigecycline against isolates from patients enrolled in phase 3 clinical trials of treatment for complicated skin and skin structure infection and complicated intra-abdominal infections. *Clin. Infect. Dis.* **41**(Suppl 5), s315–s332.
55. Talbot, G. H., J. Bradley and J. E. Edwards (2006) Bad bugs need drugs: An update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clin. Infect. Dis.* **42**, 657–668.
56. Castanheira, M., R. Mendes and R. Jones (2014) Update on *Acinetobacter* species: Mechanisms of antimicrobial resistance and contemporary in vitro activity of minocycline and other treatment options. *Clin. Infect. Dis.* **59**(Suppl 6), S367–S373.
57. Ortiz-Guerrero, J. M., M. C. Polancoa, F. J. Murilloa, S. Padmanabhanb and M. Elías-Arnanza (2011) Light-dependent gene regulation by a coenzyme B12-based photoreceptor. *PNAS* **108**(18), 7565–7570.
58. Rumbo, C., E. Gato, M. López, C. Ruiz de Alegría, F. Fernández-Cuenca, L. Martínez-Martínez, J. Vila, J. Pachón, J. M. Cisneros, J. Rodríguez-Baño, A. Pascual, G. Bou, M. Tomás and Spanish Group of Nosocomial Infections and Mechanisms of Action and 16 Resistance to Antimicrobials (GEIH-GEMARA); Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC) and Spanish Network for Research in Infectious Diseases (REIPI) (2013) Contribution of efflux pumps, porins, and β-lactamases to multidrug resistance in clinical isolates of Acinetobacter baumannii. *Antimicrob. Agents Chemother.* **57**(11), 5247–5257.
59. Higgins, P., T. Schneiders, A. Hamprecht and H. Seifert (2010) In vivo selection of a missense mutation in *adeR* and conversion of the novel $bla_{OXA-164}$ gene into bla_{OXA-58} in carbapenem-resistant *Acinetobacter baumannii* isolates from a hospitalized patient. *Antimicrob. Agents Chemother.* **54**, 5021.
60. Ruzin, A., D. Keeney and P. Bradford (2007) AdeABC multidrug efflux pump is associated with decreased susceptibility to tigecycline in *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex. *J. Antimicrob. Chemother.* **59**, 1001–1004.
61. Guelfo, J., A. Rodríguez-Rojas, I. Matic and J. Blázquez (2010) A MATE-family efflux pump rescues the *Escherichia coli* 8-oxoguanine-repair-deficient mutator phenotype and protects against H_2O_2 killing. *PLoS Gen.* **6**, e1000931.
62. Damier-Piolle, L., S. Magnet, S. Brémont, T. Lambert and P. Courvalin (2008) AdeIJK, a resistance-nodulation-cell division pump effluxing multiple antibiotics in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **52**(2), 557–562.
63. Marchand, I., L. Damier-Piolle, P. Courvalin and T. Lambert (2004) Expression of the RND-type efflux pump AdeABC in *Acinetobacter baumannii* is regulated by the AdeRS two-component system. *Antimicrob. Agents Chemother.* **48**(9), 3298–3304.
64. Chen, Y., C. Hu, J. Qu and M. Yang (2008) Photodegradation of tetracycline and formation of reactive oxygen species in aqueous tetracycline solution under simulated sunlight irradiation. *J. Photochem. Photobiol.* **197**, 81–87.
65. García-Garmendia, J. L., C. Ortiz-Leyba, J. Garnacho-Montero, F. J. Jiménez-Jiménez, C. Pérez-Paredes, A. E. Barrero-Almodóvar and M. Gili-Miner (2001) Risk factors for *Acinetobacter baumannii* nosocomial bacteremia in critically ill patients: A cohort study. *Clin. Infect. Dis.* **33**(7), 939–946.
66. Baran, G., A. Erbay, H. Bodur, P. Ongürü, E. Akinci, N. Balaban and M. A. Cevik (2008) Risk factors for nosocomial imipenem-resistant *Acinetobacter baumannii* infections. *Int. J. Infect. Dis.* **12**, 16–21.
67. Hogan, D. A. and R. Kolter (2002) *Pseudomonas-Candida* interactions: An ecological role for virulence factors. *Science* **296**, 2229–2232.
68. McGuiness, W., E. Vella and D. Harrison (2004) Influence of dressing changes on wound temperature. *J. Wound Care* **13**, 383–385.
69. Ferenc, J. (2011) Lighting the way. *Health Facil. Manag.* **24**(2), 32–36.
70. Natarajan, S. (2013) Hospital services engineering and maintenance. In *Excel Books* (Edited by A. Jain), pp. 157. Excel Books, New Delhi, India.
71. Vallenet, D., E. Belda, A. Calteau, S. Cruveiller, S. Engelen, A. Lajus, F. Le Fevre, C. Longin, D. Mornico, D. Roche, Z. Rouy, G. Salvignol, C. Scarpelli, A. Thil Smith, M. Weiman and C. Medigue (2013) MicroScope: An integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. *Nucleic Acids Res.* **41**(D1), D636–D647.

AUTHOR BIOGRAPHIES

María Soledad Ramírez is a microbiologist Professor in the Department of Biological Sciences at the California State University of Fullerton, CA, USA. She received her Ph.D. in Microbiology from the University of Buenos Aires and her postdoctoral training with a fellowship from the Argentine National Research Council (CONICET). Dr. Ramírez' laboratory studies why certain pathogens are resistant to antibiotics. Her research focuses on the study and identification of mechanisms of antibiotic resistance, their dissemination, evolution, as well as the impact on the morbidity and mortality of bacterial infections.

Gabriela Leticia Müller is a Career Researcher of CONICET. She received her Ph.D. and performed her postdoctoral training at CEFOBI Institute (CONICET and National University of Rosario). She has contributed in the field of NADP-dependent Malic Enzyme in plants and her research interests are currently focused on the analysis of glycine rich-RNA binding proteins during ripening of tomato fruits. Also, she is collaborating in many aspects related to the life style of *A. baumannii*.

Jorgelina Fernanda Pérez is a Clinical Bacteriologist at Hospital Provincial del Centenario de Rosario, Argentina, and a part-time Instructor in Clinical Bacteriology at the Faculty of Biochemistry and Pharmacy at Rosario National University. She has 20 years of experience in clinical laboratory science, particularly in the field of bacteriology.

Adrián Ezequiel Golic is a Fellow at CONICET. He has worked and contributed in the field of light modulation in *A. baumannii* since his Diploma Thesis and is now conducting his doctoral studies under the supervision of Dr. Mussi at CEFOBI with a fellowship from CONICET, Rosario, Argentina.

María Alejandra Mussi is a Career Researcher of CONICET and Instructor of Microbiology at the Faculty of Biochemistry and Pharmacy at Rosario National University. Dr. Mussi's research interests focus on studying the processes modulated by light in *A. baumannii* and other clinically relevant pathogens, as well as the mechanisms leading to the transduction of the light signal.