Laboratory Exercise

Using a Microbial Physiologic and Genetic Approach to Investigate How Bacteria Sense Physical Stimuli[S]

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Abstract

A laboratory exercise was designed to illustrate how physical stimuli such as temperature and light are sensed and processed by bacteria to elaborate adaptive responses. In particular, we use the well-characterized Des pathway of *Bacillus subtilis* to show that temperature modulates gene expression, resulting ultimately in modification of the levels of unsaturated fatty acids required to maintain proper membrane fluidity at different temperatures. In addition, we adapt recent findings concerning the modulation by light of traits related to virulence such as motility and biofilm formation in the chemotropic bacterium *Acinetobacter baumannii*. Beyond the theoretical background that this activity provides regarding sensing of environmental stimuli, the experimental setup includes approaches derived from classic genetics, microbiology, and biochemistry. The incorporation of these kind of teaching and training activities in middle-advanced Microbiology or Bacterial Genetics courses promotes acquisition of general and specific techniques and improves student's comprehension of scientific literature and research. © 2014 by The International Union of Biochemistry and Molecular Biology, 42(4):305–322, 2014.

Keywords: *signal transduction; blue light sensing; BLUF-domain containing proteins; motility regulation; biofilm formation; temperature sensing; DesKR*

Introduction

Bacteria can occur almost anywhere on earth under the most varying conditions, developing complex relationships among different microbial groups as well as with the environment. In order to survive in fluctuating environmental conditions, bacteria must be able to rapidly detect changes in their surroundings and react accordingly [1, 2]. For this, bacteria have evolved signal transduction machineries, which sense changes in extracellular and/or intracellular conditions and transmit these signals to various cellular components ultimately eliciting adaptive changes in bacterial physiology, metabolism, and/or behavior. Most of these processes involve ordered sequences of biochemical reactions inside the cell, which are preferentially carried out by enzymes or mediated by second messengers, and result in modulation of gene expression [1].

To illustrate bacterial perception of ubiquitous environmental stimulus such as temperature and light, we combined two sets of exercises extracted from our research experience. One of these exercises is focused on the ability of *Bacillus subtilis* to regulate membrane fluidity in response to changes in temperature through the well-characterized two-component system DesKR [3–6]. This system is composed of a transmembrane sensor, the histidine-kinase (HK) DesK, responsible for the information transfer across the cytoplasmic membrane; and the cytoplasmic response regulator (RR) DesR that represents a transmitter device that couples the phosphorylation signal

[S] *Additional Supporting Information may be found in the online version of this article.*

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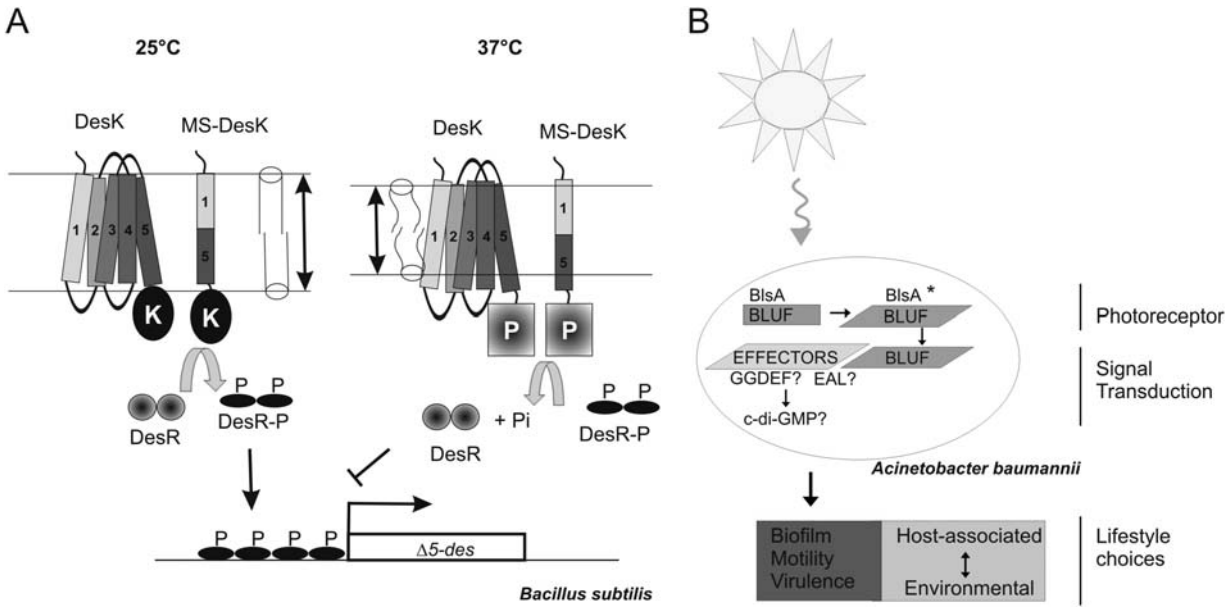

FIG 1 *Schematic representation of signal perception and transduction in the systems selected for this proposed laboratory activity.* a*: Perception of temperature changes and regulation of membrane fluidity in Bacillus subtilis. The soil bacterium B. subtilis adjusts the composition of membrane lipids to cope with temperature variations. The thermosensor DesK is a membrane protein with 5 transmembrane segments that controls the expression of the Δ-5 des gene by regulating the level of phosphorylation of the response regulator DesR. MS-DesK is a chimerical sensor with only one TM, which results from fusing the N-terminal region of the first TM with the C-terminal region of the fifth TM of DesK which mimics the functioning of the full-length sensor.* b*: Sensing and response to changes in light in the opportunistic pathogen Acinetobacter baumannii. Light absorption induces a conformational change in the BLUF photoreceptor BlsA, which is now able to bind unknown effectors that modulate surface motility, biofilm formation and virulence against Candida albicans. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]*

originated in the HK to the transcription regulation of a specific target gene; and an effector enzyme, Δ5 desaturase (Δ5Des) [2, 6; see Fig. 1*A* for details]. DesK senses changes in fluidity through a group of hydrophilic amino acids located at the water/lipid interface of the transmembrane domain (TM) [4; Fig. 1*A*]. At low temperature, the membrane is less fluid (and thicker) and this hydrophilic group gets trapped inside the membrane promoting the kinase state of DesK. However, when the membrane is more fluid (and thinner) this group reaches the aqueous phase triggering events that lead to activation of the DesK phosphatase state. Kinase-on DesK phosphorylates the response regulator DesR, while phosphatase-on DesK removes phosphate from phosphorylated DesR. Phosphorylation of DesR exposes its HTH-DNA-binding motif and enables its binding to the promoter of the desaturase gene [5]. Binding of a tetramer is required to effectively turn on the transcription of the Δ*5-des* desaturase gene [3, Fig. 1*A*]. Once synthesized, the desaturase introduces double bonds into lipids to increase fluidity [2].

As occurs with temperature, light is also a ubiquitous signal that allows organisms to gain information about the external world. For a long time, bacteria were considered insensitive to light, with the exception of phototrophs, which use sunlight as an energy source [1, 7]. Yet, recent studies demonstrated that chemotropic bacteria are also able to perceive this stimulus through photoreceptors, and adjust behaviors accordingly [1, 7–9]. Photoreceptors are chromoproteins consisting of an apoprotein moiety and a chromophore as a prosthetic group, which enables light-induced conformational changes that typically stimulate downstream signaling cascades [1, 7]. We have recently shown that the nosocomial opportunistic pathogen *Acinetobacter baumannii* regulates motility, biofilm formation, and virulence against *C. albicans* in response to light through the photoreceptor BlsA [8, 9]. BlsA contains a BLUF photoreceptor domain at the N-terminal region but lacks an associated effector within the same protein molecule, belonging therefore to the short-type BLUF proteins [8; Fig. 1*B*]. To transduce the signal, these kind of proteins typically associate with partners that contain the effector domains, such as GGDEF and EAL, which are involved in the synthesis and hydrolysis of c-di-GMP, respectively, a ubiquitous second messenger specific to bacteria that ultimately modulates gene expression [1, 7; Fig. 1*B*]. In the case of BlsA from *A. baumannii*, the output domains and proteins acting as partners remain to be identified.

The proposed exercises make use of classical microbiology, genetic, and biochemical tools, which will strengthen students' theoretical background as well as their technical skills in the laboratory. The exercises described here are carried out by advanced Microbiology students, who have received training in basic microbiology techniques

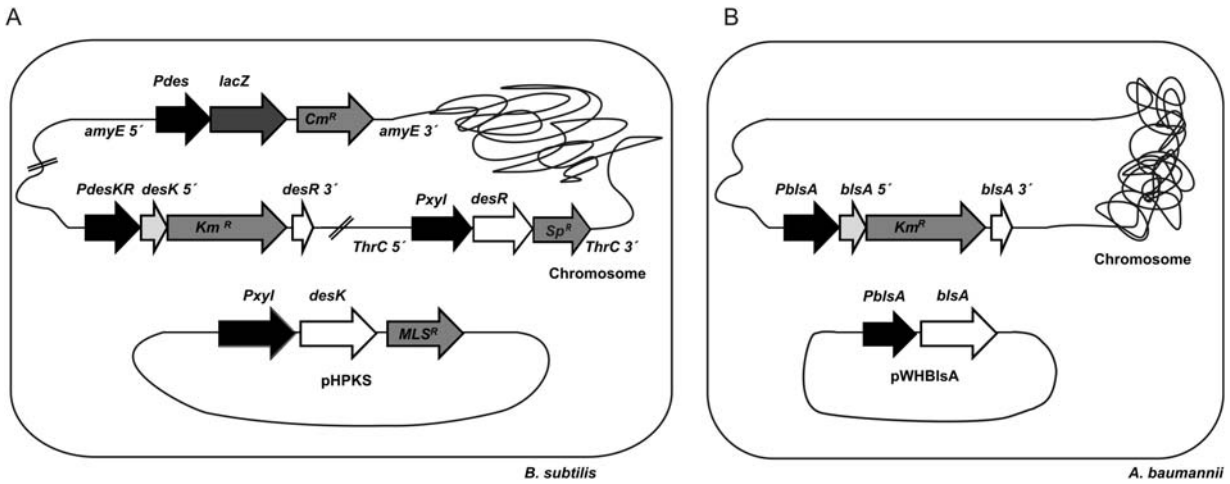

FIG 2

Schematic representation of the bacterial strains and constructions proposed for this classroom activity. a*: Genotype of B. subtilis strain used in this study: the desKR operon is disrupted by insertion of a kanamycin-resistance cassette (Km^R). The strain was further engineered to express desR from the xylose inducible promoter. In addition, it contains a transcriptional fusion of the desaturase (des) promoter to the β-galactosidase reporter gene. Finally, this strain is transformed with plasmid pKPKS carrying each DesK variant (see Materials and Equipment for details).* b*: The blsA gene was disrupted by the insertion of a kanamycin-resistance cassette (Km^R); this strain was complemented with a plasmid expressing the wild type blsA allele from its own promoter.*

previously. The proposed activities are inexpensive and easy to implement in any microbiology laboratory.

Materials and Equipment

The experimental setup requires the use of a 24/37°C incubator, a 25/37°C orbital shaker, an array of nine-LED (light-emitting diode) with an intensity of 6–10 μmol photons/m^2/s (or white light since it includes blue light in its spectrum), a spectrophotometer, and a microcentrifuge as overall equipment. A detailed list of materials, solutions, and equipment required for this activity is provided in Supporting Information Appendix 1.

Procedures

Instructions for Experiment 1: Regulation by Temperature

Rationale

In this activity students will explore signal transduction mechanisms and recognize the transmembrane domains of a signaling protein as relevant for perception of temperature changes. For this purpose, a *desk*$^-$ strain (CM21) is complemented with different DesK recombinant variants that allow the identification of relevant protein regions involved in thermosensing. Functionality of variants is evidenced by the expression and activity of a reporter gene (β-galactosidase) (see Bacterial Strains and Plasmids Section and Fig. 2 for details).

F2

Cultures of *B. subtilis* Strains and Sample Collection

1. Inoculate culture flasks containing 10 mL MM-CASA medium supplemented with appropriate antibiotics (Chloramphenicol 5 μg/mL, Kanamycin 5μg/mL, Erythromycin 1 μg/mL, Lincomycin 15 μg/mL), with a glycerol stock of CM21 strain and its derivatives. Incubate ON at 37°C. This step is performed by teaching assistants.
2. The following day, students inoculate flasks containing 30 mL of MM-CASA plus antibiotics with overnight cultures to a final $OD_{525} = 0.1$, and supplement with xylose 0.1% to induce genes under the control of the *Pxyl* promoter.
3. Grow these cultures to an $OD_{525} = 0.3$ (2 hs) and then divide each of them into two fractions of 15 mL each (time zero in all experiments). Transfer one fraction to 25°C, while keeping the other one at 37°C, to assess the effect of temperature on *des* expression.
4. In all cases, collect three aliquots of 1 mL in microcentrifuge tubes at time 0 (before dividing the cultures) and at 1-hour intervals (1, 2, and 3). One sample is used to follow culture growth by measuring OD_{525} whereas the other two are centrifuged (10,000 g for 1 min at room temperature) to harvest the cells. The supernatant is discarded, and the cell pellet frozen and stored at −20°C for further enzymatic assays.

β-galactosidase Assay

1. Resuspend cellular pellets in 0.65 mL of buffer Z containing 0.25 mg/mL of lysozyme. Incubate the tubes for 10 min at 37°C. A blank is run in parallel replacing the bacterial suspension by 0.65 mL of buffer Z.
2. Add 100 μl of Triton X-100 0.1% and 100 μl of ONPG 4.5 mg/mL. Mix by inversion and incubate the tubes at 28°C for exactly 15 min.
3. Stop the reaction by the addition of 150 μl of Na_2CO_3 1.2 M.

4. Determine OD_{420} in each tube to estimate the amount of o-nitrophenol produced (product generated as a result of β-galactosidase activity). The activity is calculated in Miller units, which takes into account the number of cells in the sample volume (1 mL), estimated by the OD_{525} and the reaction time (15 min). Conversion: Miller units = $OD_{420} \times 1000/ (OD_{525} \times$ sample volume [mL] $\times$ incubation time [min.]). Each point is run in duplicate.

Instructions for Experiment 2: Regulation by Light

Rationale

In this activity, students will observe the effect of light on surface motility and biofilm formation, two traits that are considered as virulence factors in *A. baumannii*, and understand the role of the photoreceptor responsible for perception of the light signal, BlsA, by using isogenic *blsA* mutant and complementing derivatives.

Surface Motility Assays

1. Inoculate motility plates (see Growth Media and Conditions Section for details) with bacteria lifted from overnight LB agar cultures using flat-ended sterile wooden sticks. Motility plates as well as LB agar cultures will be provided by teaching assistants.
2. Incubate plates for 10–12 hours (overnight) at 24°C in the dark or under white light (or emitted by nine-LED, light-emitting diode arrays with an intensity of 6–10 μmol photons/m^2/s, 14).

Biofilm Assays

1. Inoculate haemolysis glass tubes containing one milliliter of fresh LB broth medium with 0.01 mL of an overnight culture grown at 37°C in a shaker, which will be provided by teaching assistants.
2. Incubate cultures of each sample for 4 days stagnantly at 24°C either in dark or under white or blue light. Tubes are run in triplicate for each condition.
3. Biofilms that form on the walls of the glass tubes will be visualized by crystal violet staining [8]. Briefly, each tube is rinsed thoroughly with distilled water and then incubated with crystal violet 0.1% for 10 min. After exhaustive washing, the cells attached to the tube walls are visualized as blue halos.

Safety Considerations

A. baumannii is a biosafety level 2 microorganism. This does not preclude from using it in teaching exercises in middle-advanced microbiology courses, but certain rules should be observed such as laboratory safety guidelines [10] including proper clothing (lab coat), gloves, and footwear. In addition, all biological residues coming from tips, microcentrifuge tubes, and plates are discarded in red bags, which are incinerated afterward. Liquid biological residues, as well as their containers, are first autoclaved before discarding or washing. It is important to mention that the strain used for these exercises, ATCC 17978, is multisensitive. The proposed exercises provide a good opportunity for discussion of biosafety guidelines on organisms belonging to biosafety level 2.

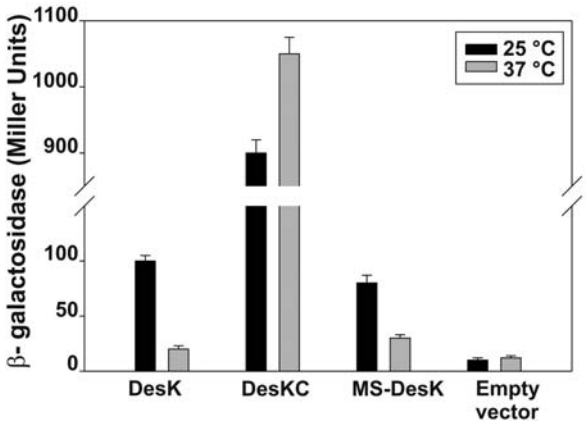

FIG 3

Identification of regions relevant for signaling in DesK using a reporter system. B. subtilis *CM21* desK$^-$ *cells harboring DesK variants were grown at 37°C to an* OD_{525} *of 0.3 and then divided into two samples. One sample was transferred to 25°C and the other kept at 37°C. Aliquots were taken every hour and* β*-galactosidase activities were determined. The values are representative of three independent experiments and correspond to 3 h after the shift.*

Results and Discussion

Experiment 1: Regulation by Temperature

When strain CM21 (desK$^-$) is complemented with pAD4, which codes for wild type DesK, the expression of the reporter gene is regulated by temperature: β-galactosidase is higher at 25°C than at 37°C (Fig. 3). Strain expressing the cytoplasmic domain DesKC (harboring plasmid pCM9) constitutively activates expression of the reporter gene regardless the temperature of incubation (Fig. 3). This experiment clearly shows that the transmembrane domain is essential for temperature modulation of DesK activity. The strain producing a DesK variant that has an unique engineered TM MS-DesK (from pMS-DesK, see Fig. 1 and [4]) behaves similarly to the one producing wild type DesK (from pAD4), that is, it is able to express the reporter gene in a temperature-regulated fashion, indicating that only one transmembrane fragment is enough for signal transduction to the cytoplasmic domain. As a control, CM21 carrying the empty vector (pHPKS) is unable to induce expression of the reporter gene at any temperature (Fig. 3). F3

The exercise is easy and straightforward. Most problems arise if poor lysis of the cells is obtained due to low heat transfer or reduced incubation time in the presence of lysozyme, resulting in a flattened β-galactosidase activity curve (response). These drawbacks are reduced by processing duplicates/triplicates of the samples.

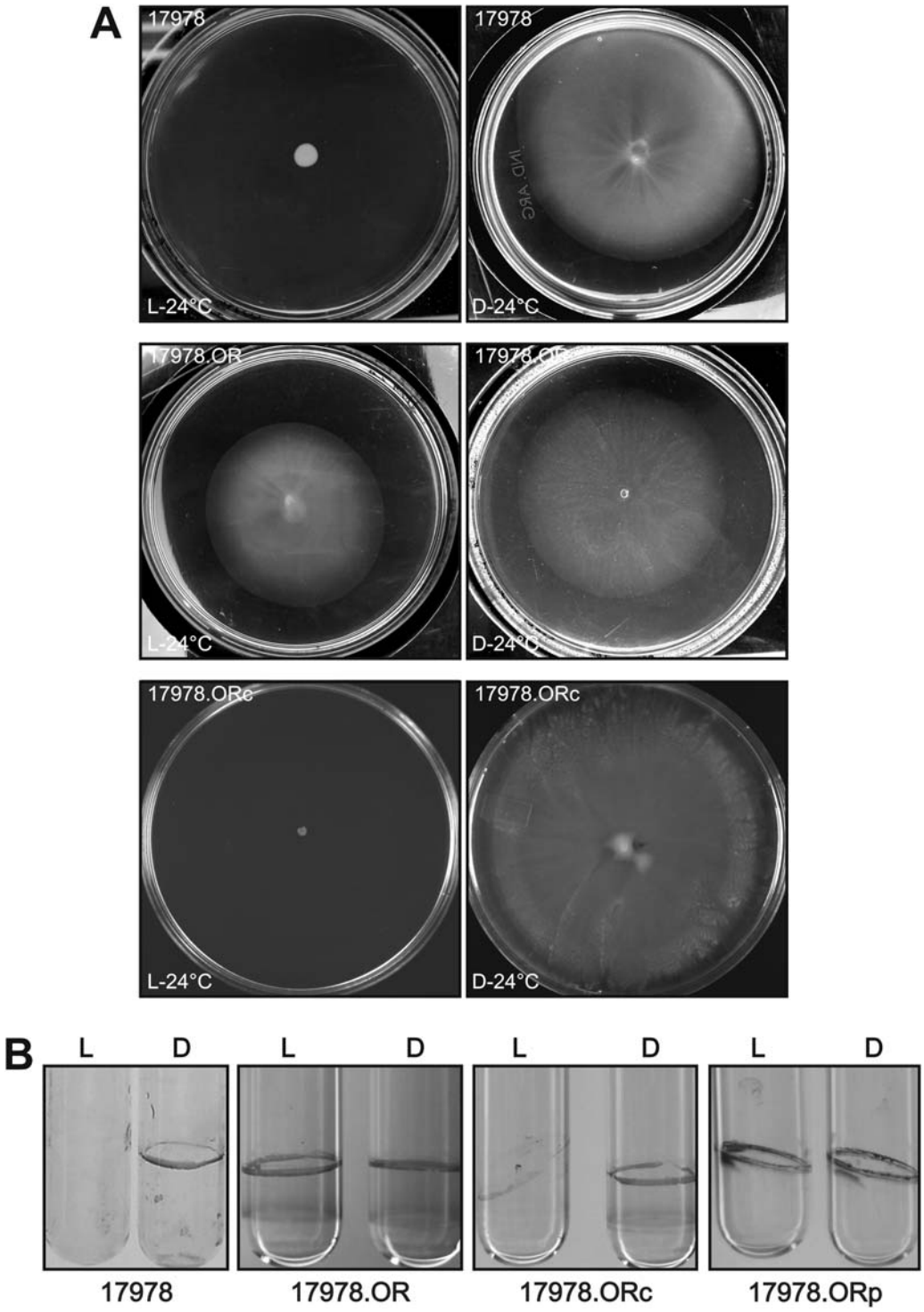

FIG 4

a*: Modulation of motility by blue light through BlsA in A. baumannii. Cells of different strains tested:* A. baumannii *ATCC 17978, the wild type; ATCC 17978.OR, a blsA mutant by insertion of a kanamycin cassette; ATCC 17978.ORc, which expresses the wild type* blsA *allele under its own promoter in a blsA mutant background; and ATCC 17978.ORp, which harbors the pWH1266 empty vector as control; were inoculated on the surface of motility plates. Plates were inspected and photographed after incubated overnight in darkness (D) or in the presence of blue light (BL).* b*. Modulation of biofilm formation by blue light through BlsA in* A. baumannii*. Biofilms corresponding to strains under study were analyzed on glass tubes in the presence of blue light (L) or in darkness, and (D) recorded after static incubation for 96 hours at 24uC by direct visual inspection and staining with crystal violet.*

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Experiment 2: Regulation by Light

Under white or blue light wild type *A. baumannii* strain ATCC 17978 remains at the inoculation point, whereas in the dark it spreads throughout the plate on motility plates (Fig. 4*A*). On the contrary, biofilm formation is scarce under blue light, whereas abundant in the dark (Fig. 4*B*). Therefore, untill now the exercise allows the students to observe that light inhibits both motility and biofilm formation at 24°C (Fig. 4). Further analysis shows absence of photoregulation of these traits when strain ATCC 17978.OR (*blsA* mutant) was used. In this case, the bacteria are able to spread throughout motility plates and form thick biofilms, either in the dark or under blue light (Fig. 4*B*). The wild type phenotype is restituted by complementation of the mutant with the wild-type allele in strain ATCC 17978.ORc, whereas the lack of photoregulation persists in the ATCC 17978.ORp mutant carrying the empty vector, used as a control. The use of these mutant strains shows the involvement of BlsA in these light-regulated bacterial processes.

In these experiments, precautions should be taken to avoid violent movement of motility plates and biofilm tubes, as the movement of plates containing very soft media to incubation chambers could result in bacterial spill resulting in confusing results. Also, biofilms tubes should be treated gently once formed in order to avoid cells to be mixed and resuspended with the liquid. Instructors should make special emphasis regarding this point to avoid spoiling the experiments.

Conclusions

The laboratory activities proposed here include classic microbiology and genetics as well as biochemistry approaches aimed to enrich the students' background of signal transduction beyond the theoretical framework. The inclusion of experiments covering signals as ubiquitous as temperature and light illustrate not only the multiplicity of systems evolved for perception of environmental stimuli in bacteria but also show a variety of techniques appropriate for each system. In the case of the Des system, a wise genetic strategy is adapted directly from the research lab that involves inducible expression in *trans* of variants of the DesK HK in an *desK* mutant background that can be used to analyze the functioning of different regions of this protein. It is worth mentioning the inclusion of a minimal version of the HK, the minimal sensor, which is the smallest fragment of the protein still capable of sensing as well as transducing the signal. The incorporation of this strain in the laboratory activity introduces students to the molecular mechanism of thermo-sensing, allowing discussion and speculations on structural as well as conformational features of the sensor protein. In the case of light sensing, the analysis of a set of strains including *blsA* mutants and the corresponding complementing strains produces straightforward evidence of light modulation of motility and biofilm formation in *A. baumannii*, as well as the involvement of BlsA, the photoreceptor mediating this process.

The design of the practical laboratory session follows that of scientific experiments, in which students collect, analyze, and interpret their own data, and utilize them to support the theoretical part of the course. The discussion session can be further enriched by reading and analyzing papers related to each topic [4, 5, 8, 9], as well as this article. If desirable, an evaluation activity could be included, in which the students summarize the experimental strategies and results in the format of a manuscript, to experience and exercise the process of explaining and writing their own results. The proposed activities have been successfully implemented in the course: "Signal transduction and Regulation of Gene Expression" designed both for graduate students as part of their doctoral program, and for undergraduate microbiology students as an optional course within the curricula. Students found the theoretical concepts and practical activities challenging. They commented that the genetic design triggered the application of similar strategies on their own biological systems. After taking the course, a few students became interested in performing a Ph.D. thesis on this subject, confirming our feeling that the students enjoyed the activities.

Overall, the proposed exercises and procedures are straightforward, cost-effective, do not require expensive equipment, and are reliable and reproducible. For all the aforementioned points, we believe that this is a valuable tool for microbiology students to increase their understanding of signal transduction systems and would provide useful training for future researchers in the microbiology area.

Experimental procedures

Class Outline

The proposed activities were designed to be developed as a module within an advanced Microbiology or Bacterial Genetics course. Students should be acquainted with basic microbiology laboratory skills (aseptic techniques, streaking plates, and pipeting). The laboratory exercises are comprised of two practical sections, temperature regulation of membrane fluidity in *B. subtilis* and light regulation in *A. baumannii*, which can be completed within a 2-week term. A minimum of 3–5 hours per session are necessary for completing the experimental procedures and the discussion of results. A maximum of 16 students should be accepted per class, which should be divided into four groups of four students each. The temperature and light regulation experiments use four bacterial strains each, and therefore, it is advisable that each group concentrate on working with one of these strains for each exercise. Before starting the practical activity, it is recommended to provide a theoretical overview on differential gene expression, signal

TABLE I ***Strains and plasmids required for setting up the proposed activities***

Strain/plasmid	*Relevant characteristics*[a]	*Source/reference*
Strains		
A. baumannii		
17978	Clinical isolate	ATCC
17978.OR	*blsA*::*aph* derivative of 17978; Km^R	(8)
17978.ORp	17978.OR harboring pWH1266; Km^R, Amp^R	(8)
$17978.ORc_{BlsA}$	17978.OR harboring pWHBLSA; Km^R, Amp^R	(8)
B. subtilis strains		
CM21	JH642 *amyE*::P*des-lacZ, desKR*::Km^R, *thrC*::(P*xyl-desR*)	(11)
CM21/pHPKS	CM21 transformed with replicative plasmid pHPKS	(11)
CM21/pAD4	CM21 transformed with replicative plasmid pHPKS carrying a wild type coy of DesK expressed under the xylose promoter	(11)
CM21/pCM9	CM21 transformed with replicative plasmid pHPKS carrying a copy of the N-ter truncated version-cytoplasmic of DesK (DesKC) under the xylose promoter	(12)
CM21/pMS	CM21 transformed with replicative plasmid pHPKS carrying a copy of the chimerical version of DesK (MS-DesK) under the xylose promoter.	(4)
Plasmids		
pWH1266	*E. coli-A. baumannii* shuttle vector; Ap^R, Tc^R	(12)
pWHBLSA	pWH1266 harboring a wild type copy of *blsA* expressed under its own promoter; Amp^R	(8)

All strains and plasmids used in the proposed exercises are available from the authors on request.

Km^R, kanamycin resistance; Tc^R, tetracycline resistance.

[a]*Amp^R, ampicillin resistance.*

transduction in bacteria, and two-component regulatory systems.

Bacterial Strains and Plasmids

T1 Strains and plasmids are listed in Table I (available on request). For temperature regulation experiments, strain CM21 complemented with different plasmids is used. Strain CM21 was obtained by disruption of the *desKR* operon with a kanamycin-resistance gene (Km^r), and was subsequently engineered to express the response regulator DesR from a xylose-inducible promoter. For this, a fusion of the Pxyl promoter to the *desR* gene was integrated in the *ThrC* locus (see Fig. 2*A*). The CM21 strain also carries a transcriptional fusion of the *lacZ* gene to the *des* promoter, which is ectopically integrated at the nonessential *amyE* locus [11], and allows expression of β-galactosidase directed by DesK in its kinase state (low membrane fluidity) (Fig. 2*A*). Strain CM21 was complemented with plasmid pHPKS coding for: (i) wild type DesK [11; pAD4], (ii) truncated DesKC [11; pCM9], which lacks the N-terminal transmembrane sensing region and renders a soluble protein with constitutive kinase activity (unresponsive to temperature variations). This strain is tested to reinforce the concept that the temperature signal is perceived by the TM domain of the sensor. (iii) MS-DesK, the engineered hybrid Minimal Sensor DesK [4; pMS-DesK] with only one transmembrane segment

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that is still able to regulate expression of the desaturase in response to temperature. (iv) Empty vector pHPKS [13]. These different DesK constructions are under the control of the Pxyl promoter as well, so that when xylose is added to the growth media, both components of the operon, DesR and DesK are expressed.

For light regulation experiments, a set of four strains is used: *A. baumannii* ATCC 17978, the wild type; ATCC 17978.OR, a *blsA* mutant generated by insertion of a DNA cassette coding for Km^r; ATCC 17978.ORc, which expresses the wild type *blsA* allele under its own promoter in a *blsA* mutant background; and ATCC 17978.ORp, which harbors the pWH1266 empty vector as control (see Table I for details).

Growth Media, Buffers, and Conditions

Bacillus subtilis cells are routinely grown in LB broth or agar. When indicated, MM-CASA (Minimal Medium-Casa aminoacids) is used. The MM-CASA media contains: SPI Salts 1X, Glycerol 0.5%, Phe 0.01%, Trp 0.01%, Thr 0.01 %, casa aminoacids 0.05 %, FeCl 1 mM, $MgSO_4$ 1 mM. The composition of Spizizen salts (SPI salts) 1X is as follows: $(NH_4)_2SO_4$ 2.0 g/l; KH_2PO_4 14.0 g/l; K_2HPO_4 6.0 g/l; sodium citrate 1.0 g/l; $MgSO_4.7H_2O$ 0.2 g/l. For β-galactosidase assays, buffer Z is required. Buffer Z is composed of: Na_2HPO_4 6 mM, NaH_2PO_4 4 mM, KCl 10 mM, $MgSO_4$ 1 mM, and β-mercaptoethanol 50 mM. Other reagents used for these experiments include 2-nitrophenyl β-D-galacto-pyranoside (ONPG) 4.5 mg/mL, Na_2CO_3 2M and Triton X-100 1%. For lysozyme supplemented buffer Z, 25 mg of lysozyme (from chicken egg white) is added to 100 mL buffer Z.

Acinetobacter baumannii cells are routinely grown in LB broth or agar. When indicated, motility plates (composed of trypone 1%, agarose 0.3%, and NaCl 0.5%) are used. A solution of crystal violet 0.1% in water is necessary for biofilms experiments.

The antibiotics required for these exercises include erythromycin, lincomycin, chloramphenicol, ampicillin, and kanamycin sulfate. Antibiotic stocks are prepared 1000X and stored at $-20^{\circ}C$.

References

[1] Krämer, R. and Jung, K. (2012) Bacterial Signaling, 1st ed., Wiley Blackwell, Federal Republic of Germany.

[2] Cybulski, L. E. and de Mendoza, D. (2011) Effect of membrane thickness on integral membrane proteins activity. Curr. Prot. Pept. Sci. 12, 760–766.

[3] Cybulski, L. E., del Solar, G., Craig, P., Espinosa, M., and de Mendoza, D. (2004) *Bacillus subtilis* DesR functions as a phosphorylation-activated switch to control membrane lipid fluidity. J. Biol. Chem. 279, 39340–39347.

[4] Cybulski, L. E., Martin, M., Mansilla, M. C., and Fernandez, A., de Mendoza, D. (2010) Membrane thickness cue for cold sensing in a bacterium. Curr. Biol. 20, 1539–1544.

[5] Inda, M. E., Vandenbranden, M., de Mendoza, D., Russchaert, J. M., and Cybulski, L. E. (2014) A lipid-mediated conformational switch modulates the thermosensing activity of DesK. Proc. Natl. Acad. Sci. USA 111, 3579–3584.

[6] Najle, S., Inda, M. E., de Mendoza, D., and Cybulski, L. E. (2009) Oligomerization of Bacillus subtilis DesR is required for fine tuning regulation of membrane fluidity. Biochim. Biophys. Acta 1790, 1238–1243.

[7] Gomelsky, M., and Hoff, W. D. (2011) Light helps bacteria make important lifestyle decisions. Trends Microbiol. 19, 441–448.

[8] Mussi, M. A., Gaddy, J. A., Cabruja, M., Arivett, B. A., Viale, A. M., Rasia, R., and Actis, L. A. (2010) The opportunistic human pathogen *Acinetobacter baumannii* senses and responds to light. J. Bacteriol. 192, 6336–6345.

[9] Golic, A., Vaneechoutte, M., Nemec, A., Viale, A., Actis, L., and Mussi, M. A. (2012) Staring at the cold sun: blue light is distributed within the genus *Acinetobacter*. PLoS ONE 8, e55059.

[10] Basic laboratories. in Asamoah-Baah, Ed. (2004) Biosafety Levels 1 and 2, Laboratory Biosafety Manual, 3rd ed., World Health Organization, Ginebra, pp. 9–19.

[11] Albanesi, D., Mansilla, M. C., and de Mendoza, D. (2004) The membrane fluidity sensor DesK of *Bacillus subtilis* controls the signal decay of its cognate response regulator. J. Bacteriol. 186, 2655–2663.

[12] Hunger, M., Schmucker, R., Kishan, V., and Hillen, W. (1990) Analysis and nucleotide sequence of an origin of DNA replication in *Acinetobacter calcoaceticus* and its use for *Escherichia coli* shuttle plasmids. Gene 87, 45–51.

[13] Johansson, P., and Hederstedt, L. (1999) Organization of genes for tetrapyrrole biosynthesis in gram-positive bacteria. Microbiol. 145 (Part 3), 529–538.