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Manuscript EMBO-2015-41691

Xanthomonas campestris attenuates virulence by sensing light through a bacteriophytochrome photoreceptor

Hernán Bonomi , Laila Toum, Gabriela Sycz, Rodrigo Sieira, Andrés Toscani, Gustavo Gudesblat, Federico Coluccio Leskow, Fernando Goldbaum, Adrian Vojnov, and Florencia Malamud

Corresponding author: Hernán Bonomi, Fundación Instituto Leloir-CONICET

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 23 December 2015

Thank you for the submission of your research manuscript to our journal. I apologize for the delay in getting back to you, but we have only now received the full set of referee reports that is copied below.

As you will see, while referees 1 and 3 acknowledge the potential interest of the findings, both referee 2 and 3 point out that no data is provided concerning the downstream signaling pathway and referee 2 does not support publication of the study in its current form as indicated on the summary evaluation sheet returned with the report. From the referee comments it is clear that data on the signal transduction mechanism have to be provided because two referees feel that without it the manuscript is not suitable for publication.

On the other hand, given the potential interest of your findings, I would like to give you the opportunity to address the concerns and would be willing to consider a revised manuscript with the understanding that all referee concerns must be fully addressed and their suggestions (as detailed above and in their reports) taken on board. Please address all referee concerns in a complete point-by-point response.

Should you decide to embark on such a revision, acceptance of the manuscript will depend on a positive outcome of a second round of review and I should also remind you that it is EMBO reports policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

We now strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure or per figure panel.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

Referee #1:

I enjoyed reading this paper, which I found original and well written. Also, the experiments seem to be of high quality. I wasn't aware of a link between light and virulence in pathogenic bacteria so I learned a few things from reading this work. I think demonstrating relevance of light signaling in a plant pathogen is an important enough advance to warrant publication in this journal pending a response to the comments below.

Comments:

L89. No details are provided about the sequence analysis. I suggest supporting this statement with data (top hits, % similarity etc.) and a phylogenetic tree of BphP.

L94. I don't understand what "Xcc purifications" means. Purifications of what?

Figure EV1B lacks a loading control, which is relevant for the mutant.

L134. Not sure that the word "inhibits" is the most appropriate here. "Negatively regulates" is better.

L169-171. The speculation about PAMPs being hidden is not reall;y appropriate for the results section as it is not supported by data and is just one explanation among many. I would move to the discussion.

L188. Be specific about what these PAMPs are.

Figure EV4. Sliding motility was assessed as a measure of the halo diameter. Where is this data? I only see images of single colonies.

Figure EV5. Same as EV4, where is the quantification?

The model in Figure 5 could be more mechanistic although they did explore the key relevant traits.

They make a good case that this work describes an adaptation to to the phyllosphere environment. How to explain the occurrence of BphP in soil pathogens? How does this relate to previous work on rhizobacteria?

Referee #2:

Manuscript Number: EMBOR-2015-41691V1 by Bonomi et al.

The presented manuscript describes the characterization of a bacterial phytochrome from the plant-pathogen Xanthomonas campestris pv. campestris.

The authors confirmed that the encoding gene is located in a bicistronic operon together with the gene bphO encoding a heme oxygenase. In addition, recombinant protein was shown to possess all photobiological properties of a bona-fide phytochrome. Construction of a chromosomal knock-out

strain revealed that the photoreceptor might be involved in the regulation of virulence. Illumination of the wt strain attenuated virulence in a BphP-dependent manner.

All presented experiments are well-performed and scientifically sound. However, although the authors present some new data pointing towards a possible function of a bacteriophytochrome, the story stops short in providing new mechanistic insights into the signal transduction mechanism. Also, some experiments with the wt and knock-out strain would have benefitted from using different wavelength of light to specifically show the phytochrome effect.

Specific comments:
Introduction:
The authors should state the reason why a phytochrome is able to adopt two distinct light absorbing forms (presence of linear tetrapyrrole chromophore). Does Xcc encode for other putative photoreceptors ? Blue light, UV-receptors? A sentence on this should be included.

Results:
What is the dark isolated form of XccBphP? This should be included in the lower part of the first page of the results (page numbers are missing).
To clearly attribute the effect of light to BphP function it would be nice to include assays using the complementation plasmid that contains the C13S mutation in all described experiments. Furthermore, it would be nice to see if the observed light effect on virulence is dependent on a specific wavelength (red light) and whether this effect can be reverted by illumination with far-red light. This would strongly support a phytochrome-mediated process. The authors only tested light vs. dark. In addition, knock-out of the bphO gene and testing this mutant strain for virulence effects could also strongly support a phytochrome function as such a strain should lose its light sensory function.

One major question that remains unanswered in this manuscript is the downstream signaling. The authors report an influence on EPS and biofilm formation. These processes are known to be controlled by the second messenger c-di-GMP in several bacterial species. Have the authors checked whether the levels of c-di-GMP change upon illumination or in the mutant strain compared to the wild type?
The quantification of EPS via colony diameter seems very vague. Isn't there a more precise way in quantifying EPS?

Minor comments:
Some of the citations do not seem appropriate. For instance the first ones to describe bathy-phytochroms was the group of Richard Vierstra (PNAS 2003).
First bacterial phytochrome citation: add Yeh and Lagarias, Science 1996
To my knowledge the term Soret and Q-band is only used to describe specific absorption regions of porphyrins. The chromophore of a phytochrome is a linear tetrapyrrole.

Referee #3:

The paper submitted by Bonomi and colleagues to EMBO reports and entitled 'Xanthomonas campestris attenuates virulence by sensing light through a bacteriophytochrome photoreceptor' aimed at identifying the role of a bacterial phytochrome in a plant bacterial pathogen, Xanthomonas campestris pv. campestris (Xcc). This bacterium is known to harbor various genes encoding sensors of the environment, among which sensors of light, but their role is generally unknown. The authors analyzed the gene sequences, the protein spectroscopic properties after exposure to various treatments, constructed a null mutant and a complemented mutant to study its aggressiveness on cabbage and determine various phenotypes of the bacterial strains of its in planta effects that could be linked to light perception. The Authors conclude from their study that Xcc perceive light through this phytochrome BphP, and that light perception down regulates various functions involved in virulence. This is a clear cut, well written study allowing to refine environmental parameters influencing bacterial virulence. I have some comments that need to be addressed before publication of this manuscript in this journal and several minor comments.

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Major comments
1. The authors constructed a null mutant by replacement of the target gene with an antibiotic resistance gene cassette. They generated a complemented strain (pXccBphP), which present a behavior that differs significantly from the wild type strain for most of the analyzed phenotypes. Its virulence is lower than that of the wt, its population sizes do not increase in planta during 3 dpi, its production of endoglucanase is far lower than that of the wt, as is the production of xanthan (Figure2, 3, and 4). Results for the pXccBphP are lacking in several tests (Fig 3a and b). These results invalidate part of the demonstration. The authors indicate L. 144 that pXccBph displays XccBphP overexpression; but this is not the expected behavior for a complemented null mutant. To fully validate the hypothesis that the absence of bphP is responsible for the various phenotypes that are observed, the Authors should construct another complemented strain and restore the wild type phenotype, otherwise the demonstration is not complete.

2. No data are provided that allow to decipher the signaling pathway from light perception up to the regulation of one target gene. Some data should be provided to highlight at least some steps of this pathway.

3. This study is focused on one phytochrome from one strain supposedly representing one group of strains. How broad is this light regulation of virulence among plant pathogenic bacteria? This is a far too large question to be answered in this manuscript, however, is it common to all strains from the same pathovar or at least in the species? I suggest that an analysis of the distribution of the bphO and bphp genes in X. campestris and maybe Xanthomonas spp. shall be provided to illustrate the broad biological significance of this study.

Minor comments

L. 60 : add 'it' between 'Interestingly' and 'is'
Title and L.143: the Authors are not referring to the classical sense of virulence in phytopoathology that is the qualitative component of pathogenicity usually evaluated for pathogen faced to a host range, aggressiveness being the quantitative component of pathogenicity. Authors should define virulence.

L. 288-289: It is quite surprising to consider Xcc as a hemibiotroph. I am not sure that this term as a sense for bacterial pathogen. In planta, Xcc live mostly in xylem vessels, which are dead cells. Moreover, this bacterium is able to colonize crop residues and behave as a saprophyte. I suggest that the authors reformulate this sentence.

Fig 2D: stomata seem to remain closed or partially opened in the dark whatever the treatment. The stomatal aperture remains inferior to 2 µm. In light conditions, such an aperture is apparently considered as limited. In this figure axes should have the same scale in order to allow full comparisons of the stomatal aperture during light and dark exposure.

1st Revision - authors' response 20 June 2016

Thank you and the Reviewers for the revision on our manuscript EMBOR-2015-41691V1 and for the opportunity to submit a revised version.

We sincerely appreciate the comments of the three Reviewers and we are convinced that they seek to put our work on firmer ground. Therefore, we have taken the constructive reviewers suggestions to revise the manuscript.

All the reviewers´ comments are addressed below. In the response to the Reviewers, their comments / suggestions / questions are in black and our replies in blue. The insertions made in the revised manuscript (named MS_BONOMI_REVISED_TRACK_CHANGES) are in red type.

Because we understand from the Editor’s and the Referees’ comments that signal transduction mechanism should be provided, we have made a great effort and performed an RNA-Seq to study the transcriptional effects of far-red-light and *Xcc*BphP photoreceptor. This experiment was

originally meant to be performed late this year for a continuing paper but we have decided to do it in advance. We are very pleased to include these results in this EMBO Reports manuscript revision. As it will be appreciated in the revised manuscript presented here, there is a massive transcriptional regulation in the wild-type strain (approximately 25% of its genome, $p < 0.05$) upon illumination with monocromatic far-red light. Moreover, the *Xcc*BphP over-expressing strain, which also shows genome-wide transcriptional changes, indeed exhibits many down-regulated genes that are directly linked to the phenotypes studied throughout our work. This RNA-Seq analysis reveals that *Xcc*BphP acts via transcriptional repression.

We hope that the revised version will be found suitable for publication in EMBO Reports.

Referee #1:
I enjoyed reading this paper, which I found original and well written. Also, the experiments seem to be of high quality. I wasn't aware of a link between light and virulence in pathogenic bacteria so I learned a few things from reading this work. I think demonstrating relevance of light signaling in a plant pathogen is an important enough advance to warrant publication in this journal pending a response to the comments below.
We thank the Reviewer for his/her comment. We appreciate very much that the work resulted of his/her interest.

Comments:
L89. No details are provided about the sequence analysis. I suggest supporting this statement with data (top hits, % similarity etc.) and a phylogenetic tree of BphP.
Following the Reviewer's suggestion, a sequence comparison with relevant members of the phytochrome family was performed, including plant, algae, bacteria and fungi phytochromes. This is now mentioned in the text (**page 5, lines 114-119, Table EV1**). A subsequent thorough sequence analysis within the Xanthomonas genus BphPs was performed to construct a phylogenetic tree, including the nature of the output modules found in this particular genus. This information is included in the new version of the manuscript (**pages 5-6, lines 119-127, Figure EV2**).

L94. I don't understand what "*Xcc* purifications" means. Purifications of what?
We meant "*Xcc* RNA purification". The mistake was fixed (**page 5, lines 99-100**).

Figure EV1B lacks a loading control, which is relevant for the mutant.
The Western blot was repeated and the loading control was included in **Figure EV1B**.

L134. Not sure that the word "inhibits" is the most appropriate here. "Negatively regulates" is better.
We agree with the reviewer, this change was included in the new text (**page 6, line 157**).

L169-171. The speculation about PAMPs being hidden is not really appropriate for the results section as it is not supported by data and is just one explanation among many. I would move to the discussion.
This part was moved to the Discussion section and simplified removing the PAMPs speculation (**page 12-13, lines 359-362**).

L188. Be specific about what these PAMPs are.
This was removed in the new version of the manuscript.

Figure EV4. Sliding motility was assessed as a measure of the halo diameter. Where is this data? I only see images of single colonies.
The sliding motility quantification was included in the new version of **Figure EV5.**

Figure EV5. Same as EV4, where is the quantification?
The quantification of biofilms was performed for **Figure 4C** and exposed in **Table EV2.** Former Figure EV4 confocal microscopy figure could not be quantified by the way data was stored. Hence, we decided to remove this figure as it was redundant with **Figure 4C**.

The model in Figure 5 could be more mechanistic although they did explore the key relevant traits.2
After RNA-Seq analysis, we have included in the **Figure 6A** the candidate genes responsible for

most phenotypes observed throughout this work and that we now know that are transcriptionally down-regulated by *Xcc*BphP.

They make a good case that this work describes an adaptation to the phyllosphere environment. How to explain the occurrence of BphP in soil pathogens? How does this relate to previous work on rhizobacteria?

We appreciate and thank the Reviewer for his/her comment on the work presented. We believe that the light signal is key in some plant infective organisms and it might be acting to "synchronize" with its host rhythms or the day cycle and/or also might be decoding sunlight information (quality and intensity) as a spatial reference. Many different infective soil organisms can benefit from this information sunlight provides. Because light wavelengths are filtered differently in natural environments, for example under a canopy or soil, the microorganisms coding for a particular set of photoreceptor may be a way to integrate complex lightning data to elicit an adaptive response. Soil pathogens might not be an exception here and exploit the same information coded in filtered sunlight and BphPs could have been selected for this reason, along with other photoreceptors. The symbiont *Rhizobium leguminosarum*, similarly to *Xanthomonas campestris*, codes for both a LOV-domain-containing protein and a BphP. It infects its host nascent roots after germination and finally inhabits inside special organelles, termed "nodules", offered by the legume host plant. Hence, its light-induced responses might have evolved to fine tune its infective mechanisms and adaptive responses by the light quality encountered in its niches.3

Referee #2:
Manuscript Number: EMBOR-2015-41691V1 by Bonomi *et al*.
The presented manuscript describes the characterization of a bacterial phytochrome from the plant-pathogen Xanthomonas campestris pv. campestris.

The authors confirmed that the encoding gene is located in a bicistronic operon together with the gene bphO encoding a heme oxygenase. In addition, recombinant protein was shown to possess all photobiological properties of a bona-fide phytochrome. Construction of a chromosomal knock-out strain revealed that the photoreceptor might be involved in the regulation of virulence. Illumination of the wt strain attenuated virulence in a BphP-dependent manner.

All presented experiments are well-performed and scientifically sound. However, although the authors present some new data pointing towards a possible function of a bacteriophytochrome, the story stops short in providing new mechanistic insights into the signal transduction mechanism. Also, some experiments with the wt and knock-out strain would have benefitted from using different wavelength of light to specifically show the phytochrome effect.

We thank the Reviewer for his/her comments and we hope that this new version of the manuscript answers some key relevant questions raised by him/her. Prompted by the need on mechanistic traits and the role of far-red light in this system, we decided to perform an RNA-Seq analysis in order to study the far-red-light and *Xcc*BphP influence in transcription. As it will be appreciated in the revised manuscript presented here, there is a massive transcriptional regulation in the wild-type strain (approximately 25% of its genome, $p < 0.05$) upon illumination with monocromatic far-red light. Moreover, the *Xcc*BphP over-expressing strain, which also shows genome-wide transcriptional changes, indeed exhibits many down-regulated genes that are directly linked to the phenotypes studied throughout our work. This RNA-Seq analysis reveals that *Xcc*BphP acts via transcriptional repression. This information is included in the following new sections: "Far-red light and XccBphP overexpression produce genome-wide transcriptional changes" (**Pages 10-11, lines 269-301, Figure 5**) and "*Xcc*BphP down-regulates transcription of virulence systems" (**Pages 11-12, lines 303-319**), **Table EV3** and in the Discussion section (**Pages 12-14, lines 344-347, 396-400, Figure 6A**).

Specific comments:

Introduction:
The authors should state the reason why a phytochrome is able to adopt two distinct light absorbing forms (presence of linear tetrapyrrole chromophore). Does *Xcc* encode for other putative photoreceptors? Blue light, UV-receptors? A sentence on this should be included.

We agree with the reviewer, the information about the chromophore was added on **page 5, lines 97-98, 110-112** and the only other photoreceptor coded in *Xcc* genome (a LOV-domain-containing protein) was added on **page 4, line 87**, as suggested.

Results:
What is the dark isolated form of *Xcc*BphP? This should be included in the lower part of the first page of the results (page numbers are missing).

When the apoprotein is assembled in the dark, in the presence of BV, the holoprotein *Xcc*BphP state is Pr. After an equilibrium is reached, always keeping the protein in the dark, the Pfr is the enriched state (**page 6, line 143-145**).

To clearly attribute the effect of light to BphP function it would be nice to include assays using the complementation plasmid that contains the C13S mutation in all described experiments. Furthermore, it would be nice to see if the observed light effect on virulence is dependent on a specific wavelength (red light) and whether this effect can be reverted by illumination with far-red light. This would strongly support a phytochrome-mediated process. The authors only tested light vs. dark. In addition, knock-out of the bphO gene and testing this mutant strain for virulence effects could also strongly support a phytochrome function as such a strain should lose its light sensory function.4

To address the issue raised by the Reviewer, we have performed the whole set of experiments including the C13S complemented strain. We have found that this complementation was always insensitive to light stimuli and mostly it recapitulates the *XccbphP* mutant phenotypes. This results corroborates again that the light-sensing capability should be present to complement the genomic *XccbphP* mutation. This new information is included in **Figure 2-4, EV3, EV5 and Tables EV2, EV4**. The Reviewers advice to use monochromatic lights for infections is very well thought, however, due to practical reasons, we have decided to leave it for the future. Regarding bphO mutation, we feel it is not necessary as C13S does indeed abolishes the light sensory functions of *Xcc*BphP. Yet, it is a very nice suggestion that we will keep in mind for the future as the apoprotein might have its own signaling as reported by Fixen *et al* (PNAS, 2014) in other bacteria.

One major question that remains unanswered in this manuscript is the downstream signaling. The authors report an influence on EPS and biofilm formation. These processes are known to be controlled by the second messenger c-di-GMP in several bacterial species. Have the authors checked whether the levels of c-di-GMP change upon illumination or in the mutant strain compared to the wild type?

We indeed agree with the Reviewer: it is likely that c-di-GMP levels might be acting as second messenger in this pathway as is indicated by the EPS and biofilm results. The direct cellular c-di-GMP measurements for the different *Xcc* treatments (light, dark, mutant, complementations) were performed making organic extractions from cells, concentration of the extracts and HPLC-C18 reverse phase column separation and UV-vis detection. However, we were not able to detect c-di-GMP signals from bacterial extractions. We are engaging in a collaborative work with other lab, experts in this matter, to tackle this issue but the timing is not going to allow us to include this future results in this current manuscript.

The quantification of EPS via colony diameter seems very vague. Isn't there a more precise way in quantifying EPS?

We agree with the reviewer and the quantification of EPS on liquid medium was performed by ethanol precipitation as has been previously reported and added this data in the new version of the manuscript (**page 10, lines 246-248, Figure 4B**).

Minor comments:
Some of the citations do not seem appropriate. For instance the first ones to describe bathy-phytochroms was the group of Richard Vierstra (PNAS 2003).
The citation was included (**page 6, line 141**)

First bacterial phytochrome citation: add Yeh and Lagarias, Science 1996.

The citation was included (**page 4, line 74-75**)
To my knowledge the term Soret and Q-band is only used to describe specific absorption regions of porphyrins. The chromophore of a phytochrome is a linear tetrapyrrole.
We have taken this concepts from previously published articles in which Soret and Q bands are used to describe absorption spectra from phytochromes and we have decided to keep this terms in the present manuscript (see: Borucki B *et al*, Biochemistry, 2003; Borucki B *et al*, Biochemistry, 2009; Inomata K *et al*, Biochemistry, 2009; Falklöf O *et al*, J Comput Chem, 2013).

Referee #3:
The paper submitted by Bonomi and colleagues to EMBO reports and entitled 'Xanthomonas campestris attenuates virulence by sensing light through a bacteriophytochrome photoreceptor' aimed at identifying the role of a bacterial phytochrome in a plant bacterial pathogen, Xanthomonas campestris pv. campestris (*Xcc*). This bacterium is known to harbor various genes encoding sensors of the environment, among which sensors of light, but their role is generally unknown. The authors analyzed the gene sequences, the protein spectroscopic properties after exposure to various treatments, constructed a null mutant and a complemented mutant to study its aggressiveness on cabbage5 and determine various phenotypes of the bacterial strains of its in planta effects that could be linked to light perception. The Authors conclude from their study that *Xcc* perceive light through this phytochrome BphP, and that light perception down regulates various functions involved in virulence. This is a clear cut, well written study allowing to refine environmental parameters influencing bacterial virulence. I have some comments that need to be addressed before publication of this manuscript in this journal and several minor comments.
We appreciate the Reviewer's comments and we are glad the work was valued by him/her.

Major comments
1. The authors constructed a null mutant by replacement of the target gene with an antibiotic resistance gene cassette. They generated a complemented strain (p*Xcc*BphP), which present a behavior that differs significantly from the wild type strain for most of the analyzed phenotypes. Its virulence is lower than that of the wt, its population sizes do not increase in planta during 3 dpi, its production of endoglucanase is far lower than that of the wt, as is the production of xanthan (Figure2, 3, and 4). Results for the p*Xcc*BphP are lacking in several tests (Fig 3a and b). These results invalidate part of the demonstration. The authors indicate L. 144 that p*Xcc*Bph displays *Xcc*BphP overexpression; but this is not the expected behavior for a complemented null mutant. To fully validate the hypothesis that the absence of bphP is responsible for the various phenotypes that are observed, the Authors should construct another complemented strain and restore the wild type phenotype, otherwise the demonstration is not complete.
We agree with the Reviewer that may be a more subtle way to complement could recapitulate the wild-type phenotype, such as a knock-in or a using very low copy plasmids. However, we think that a complementation using a plasmidic copy of the gene that overexpresses it does not invalidate our findings. This strain regains its ability to react to light in key experiments performed through this work such as infection and EPS assays. Moreover, overexpression drives some phenotypes of the complemented strain to the opposite direction of the *XccbphP* mutation, due to overexpression. This gave us the information about the gain of function that *Xcc*BphP causes and it is in beautiful accordance with the loss of function the mutant exhibits. For this reasons, and due to the lack of time to construct yet another complemented strain we decided to perform all the experiments for this revision with the complemented strain we already had. We also understand the Reviewer's critic on experiments lacking the p*Xcc*BphP strain, therefore we decided to either complete missing experiments with this strain or remove them.

2. No data are provided that allow to decipher the signaling pathway from light perception up to the regulation of one target gene. Some data should be provided to highlight at least some steps of this pathway.
We agree with the Reviewer and we decided to perform an RNA-Seq analysis in order to study the far-red-light and *Xcc*BphP influence in transcription. As it will be appreciated in the revised manuscript presented here, there is a massive transcriptional regulation in the wild-type strain (approximately 25% of its genome, $p<0.05$) upon illumination with monocromatic far-red light. Moreover, the *Xcc*BphP over-expressing strain, which also shows genome-wide transcriptional changes, indeed exhibits many down-regulated genes that are directly linked to the phenotypes studied throughout our work. This RNA-Seq analysis reveals that *Xcc*BphP acts via transcriptional

repression. This information is included in the following new sections: “Far-red light and XccBphP overexpression produce genome-wide transcriptional changes” (**Pages 10-11, lines 269-301, Figure 5**) and “*Xcc*BphP down-regulates transcription of virulence systems” (**Pages 11-12, lines 303-319**), **Table EV3** and in the Discussion section (**Pages 12-14, lines 344-347, 396-400, Figure 6A**).

3. This study is focused on one phytochrome from one strain supposedly representing one group of strains. How broad is this light regulation of virulence among plant pathogenic bacteria? This is a far too large question to be answered in this manuscript, however, is it common to all strains from the same pathovar or at least in the species? I suggest that an analysis of the distribution of the bphO and bphp genes in X. campestris and maybe Xanthomonas spp. shall be provided to illustrate the broad biological significance of this study.
Following the Reviewer’s suggestion, a thorough sequence analysis within the Xanthomonas genus BphPs was performed to construct a phylogenetic tree, including the nature of the output modules found in this particular genus. This information is included in the new version of the manuscript (**pages 5-6, lines 119-127, Figure EV2**). This6
analysis reveals that *Xcc* is not an isolated case and that BphP light-sensory signaling mechanism is probably conserved at among other *Xanthomonas* pathovars.

Minor comments
L. 60 : add 'it' between 'Interestingly' and 'is'
Modified as suggested (**page 4, line 64**).

Title and L.143: the Authors are not referring to the classical sense of virulence in phytopathology that is the qualitative component of pathogenicity usually evaluated for pathogen faced to a host range, aggressiveness being the quantitative component of pathogenicity. Authors should define virulence.
Due to the Reviewer’s concern, we defined virulence as the “capacity to replicate inside the host”. This was clarified in the text (**page 7, line 166**).

L. 288-289: It is quite surprising to consider *Xcc* as a hemibiotroph. I am not sure that this term as a sense for bacterial pathogen. In planta, *Xcc* live mostly in xylem vessels, which are dead cells. Moreover, this bacterium is able to colonize crop residues and behave as a saprophyte. I suggest that the authors reformulate this sentence.
We understand that it can be a blurred line with this classification. However we think that Xanthomonas is indeed better classified as a hemibiotroph because it is a parasite that initially forms an association with living cells of the host, similar to a biotroph, but at later stages of infection it becomes necrotrophic, actively killing host cells. For the sake of clarity we have reformulated this sentence and included the corresponding citations (**page 12, lines 359-362**).

Fig 2D: stomata seem to remain closed or partially opened in the dark whatever the treatment. The stomatal aperture remains inferior to 2 µm. In light conditions, such an aperture is apparently considered as limited. In this figure axes should have the same scale in order to allow full comparisons of the stomatal aperture during light and dark exposure.
Although the stomata exposed to light typically exhibit a stomatal aperture ranging from 2 to 3.5 µm in *Arabidopsis*, there are variations that depends on many uncontrolled variables. Some differences are due to *Arabidopsis* high stomatal sensitivity to small changes in watering amount and air humidity. These uncontrolled variables are common to all treatments, for that reason we focus in the relative stomatal apertures more than in absolute values. In the experiments presented in **Figure 2C**, although stomatal apertures from negative control under continuous light and no bacteria (untreated) were lower than previously reported values (Gudesblat *et al*, Plant Physiol, 2009; Zeng *et al*, Plant Physiol, 2010), the relative aperture between this control and the wild-type treatment is significantly higher (> 2-fold). In the **Figure 2D** experiment, the stomatal aperture in dark-kept stomata (untreated) presents lower values than those achieved by (i) ABA-induced stomatal closure reported treatments (Gudesblat *et al*, Plant Physiol, 2009, Desclos-Theveniau *et al*, PLoS Pathog, 2012) and (ii) control stomata kept in the light from the same experiment (3-fold). These controls were able to confirm us that the system was able to respond to stimuli properly in each experiment. For these reasons, we are confident in the reproducibility of our experiments because we always ensured that the relative differences between mock and the bacterial treatments were similar. To finish, we agree with the Reviewer´s suggestion and the new version of the figures now share the same scale for easier comparison (**Figure 2C-D**).

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2nd Editorial Decision 11 July 2016

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the full set of referee reports that is copied below.

As you will see, all referees now support publication of your study in EMBO reports. Referee 1 however pointed out that the Coomassie staining of the Western blot shown in Fig. EV1 reveals a different band pattern despite the use of isogenic bacteria. Please explain this difference.

Other points:

- Please include a conflict of interest statement.

- Please complete and provide an author checklist. The template is available for download from our Author Guidelines.

- Moreover, I noticed that you have meanwhile solved and published the crystal structure of XccBphP (Otero et al., April 2016). This related manuscript seems to describe the characterization of XccBphP as photosensor and its spectroscopic properties. Given this earlier paper, the first paragraph in the current manuscript might have to be adapted accordingly to reflect this fact as here XccbphP is still described as "predicted BphP" protein.

- Concerning the schematics in Figure 6: as xanthan production supports biofilm formation and virulence I suggest to replace the inhibitory symbol between those two processes with an arrow.

We look forward to seeing a final version of your manuscript as soon as possible.

REFEREE REPORTS

Referee #1:

They addressed my comments but in the new Figure EV1B. the Western blot band patterns look different which is surprising considering that these are isogenic bacteria. Please explain.

Referee #2:

The authors have presented a nicely revised version of the manuscript and addressed most of my concerns. The only remaining concerin I have is the size of the Figures. If they will be the same size as in the provided PDF, the authors should consider enlarging the text. Especially Figure 1 seems very tiny. I was not able to confirm the structure of the chromophore. The authors should double check the linkage to the Cys residue.

Referee #3:

This paper is a novel version of a manuscript that I previously reviewed. The Authors significantly enriched the manuscript including novel data sets and especially results from RNAseq analysis studying the far-red-light and XccBphP influences on transcription. The Authors took into account most of my comments giving apropriate answers. Nevertheless, I am still of the opinion that another construction of the complemented strain would have been relevant, however there is no doubt that Xcc perceive light through this phytochrome, BphP, and that light perception down regulates various virulence functions. The amount of valuable data that is presented here is high and data are well analyzed. This paper should pave the way for similar analyses in various bacterial plant pathogens. Therefore I recommend acceptation of the present version of the manuscript.

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2nd Revision - authors' response 27 July 2016

Thank you and the Reviewers for the revising again and accepting our manuscript.

Regarding Fig. EV1, we assume that the genetic treatments -lack (mutant strain) or overexpression of *Xcc*BphP (complemented strains)- are the cause of the different band patterns. The RNA-Seq analysis revealed that *Xcc*BphP overexpression produces a differential transcription profile that impacts hundreds of genes. Hence, it is reasonable to propose that the null mutant or complemented strains (absence and overexpression of *Xcc*BphP, respectively) affects their transcriptional profiles and ultimately their protein patterns.

We have followed all the suggestions indicated in your previous email (date July 11th) and included them in the final version EMBOR-2015-41691V3, which we hope is now suitable for publication.

3rd Editorial Decision 02 August 2016

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Malamud Florencia, Bonomi R. Hernán, Vojnov A. Adrián
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2015-41691V2

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures
1. Data
The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions
Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ^2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

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B- Statistics and general methods

	Please fill out these boxes (Do not worry if you cannot see all your text once you press return)
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample sizes were chosen to be as big as possible to maximize detection power.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Yes. In all plant experiments, plants were randomly selected.
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4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
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Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Statistical tests were non-parametrical.
Is there an estimate of variation within each group of data?	Yes. SEM was always calculated and shown in each figure.
Is the variance similar between the groups that are being statistically compared?	Statistical tests were non-parametrical.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	NA
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

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E- Human Subjects

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12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
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14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
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17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	All accesion codes are povided throughout the manuscritp and Extended View files
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	RNA-Seq data are available in the ArrayExpress database (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-4958
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