

From: PeerJ peer.review@peerj.com

Subject: Decision on your PeerJ submission: "AmrZ is a positive regulator of swimming motility in *Pseudomonas stutzeri*" (#2016:05:10680:0:0:REVIEW)

Date: June 8, 2016 at 8:58 AM

To: David Baltrus baltrus@email.arizona.edu

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PeerJ

Dear David,

Thank you for your submission to PeerJ.

I am writing to inform you that your manuscript - [AmrZ is a positive regulator of swimming motility in *Pseudomonas stutzeri*](#) - has been rejected for publication.

Editor's Comments

REJECT

Both reviewers state that the manuscripts provides little/no new biological or functional insight into the specialised question posed. Reviewer 1 rightly points out that only motility assays are shown and Reviewer 2 rightly raises a number of questions/controls that need to be addressed before the work represents a publishable unit. If these are provided, the work could be reconsidered as a new submission.

Reviewer 1 (Anonymous)

Basic reporting

This manuscript reports that AmrZ of *Pseudomonas stutzeri* is a positive regulator of swimming motility by the analysis of Δ amrZ mutant. As authors

introduced in this manuscript, AmrZ is relatively well studied in many Pseudomonad. Namely AmrZ of *P. fluorescens* and *P. aeruginosa* was reported as a negative regulator of flagellar motility, because Δ amrZ mutant is hypermotile in *P. fluorescens* (Martinez-Granero et al. 2014) and AmrZ represses fleQ gene expression in *P. aeruginosa* (Jones et al. 2014). Contrary AmrZ was also reported as a positive regulator of motility in *P. syringae* pv. tomato DC3000 (Prada-Ramirez et al. 2016).

In this manuscript authors reports one additional example of latter case by the analysis of swimming motility of Δ amrZ mutant in *P. stutzeri*. Authors also showed that complementation of amrZ genes of both *P. stutzeri* and *P. aeruginosa* resulted in the activation of swimming motility. Thus authors showed that although AmrZ of *P. aeruginosa* was reported as negative regulator of flagella motility, it potentially activates swimming motility in *P. stutzeri*. This is an only new finding of this manuscript. However authors did not pay any efforts to clarify different function of AmrZ in *P. aeruginosa* and in *P. stutzeri*.

Experimental design

In this manuscript authors carried out only swimming motility assay. I simply feel insufficiency of data and discussion. There are no experiments to reveal molecular mechanisms for the regulation of flagella motility by AmrZ. I think authors need to identify the target genes of AmrZ of *P. stutzeri* that contributes to the activation of flagellar motility, and investigate their molecular mechanism.

In Figure 1, DBL390 increased motility. Although authors describe “integration of any cassette into the Tn7 site appears to increase motility”, I could not meaning. What is “any cassette” and “Tn7 site”. I think that “DBL390” is not appropriate for analysis of this study.

Validity of the findings

Investigation and finding were quite limited and not enough for paper.

Reviewer 2 (Anonymous)

Basic reporting

see below

Experimental design

see below

Validity of the findings

see below

Comments for the Author

In the present manuscript, Baltrus et al. present evidence indicating that AmrZ is a positive regulator of motility in *Pseudomonas stutzeri*.

While raising interesting mechanistic questions about the regulatory mechanisms of transcriptional regulators at the beginning of the introduction, the manuscript fails to follow-up on with experimental approaches.

As it currently stands, the manuscript simply is a (very short) description of the motility phenotype of a single gene deletion. I don't see how this advances our insight into the mode of action of transcriptional regulators.

At the very least, it would be helpful to see alignments of AmrZ proteins from different strains and corresponding alignments of the putative binding sites upstream of *fleQ*.

It's also not clear if the consensus binding site of AmrZ is known. I presume that this can be at least inferred from the ChIP-Seq data from Jones et al.

or alternatively by experimental approaches (gel shift assays, footprinting etc).

The combination of simple sequence comparisons with the presence and absence of AmrZ binding sites might provide hints about possible regulatory mechanisms, e.g. one would expect to identify a binding site upstream of the transcriptional start site for a positive regulator.

Fig.1:

- Why does the deletion of *amrZ* result in the same motility phenotype in different strain backgrounds, although the parental strains differ significantly in their motility?
- What are the *amrZ* expression levels in DBL332 and DBL390, respectively?
- Is *amrZ* significantly more expressed in DBL390 and does this explain the increased motility?
- Where did the *lacZ* in DBL390 insert? Did the insertion inactivate a negative regulator of motility?

Fig.2:

- A quantification of DBL390 motility is missing as wildtype control. Otherwise one cannot assess the degree of complementation
- What are the expression levels of *P. aeruginosa* and *P. stutzeri* AmrZ? Even though expressed from the same promoter, protein expression levels could be different (e.g. due to different mRNA stability etc).

Minor comments:

- line 38: you talk about the protein AmrZ, not about the gene *amrZ* here.-
- lines 104-105: should read μg (microgram)
- line 138+139: should read 10 mM
- line 142: the duration of incubation is NOT indicated in the figure legends?

- lines 158+160: references are not formatted
 - line 173: 'lines' = 'strains'
 - line 187: missing space
 - line 210: in function of AmrZ for...
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With kind regards,

[Ariel Blocker](#)

Academic Editor, PeerJ

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