Reviewer comments

Manuscript: IJMS-3807923

Title: BlihIA - a novel type I restriction-modification system from *Bacillus licheniformis* is sensitive to in vitro inhibition by ArdB antirestriction protein

Decision: Major revision

The functionality of the RMI system from *B. licheniformis* and the inhibitory action of the protein ArdB, as presented by the authors, are depicted with remarkable precision. This precision is expected to significantly contribute to a better understanding and practical application for transformation of the bacterial strain's functional characteristics. Furthermore, the conciseness of the overall explanation and the clear articulation of the findings are highly commendable aspects of the manuscript's organization.

Nonetheless, the terminology used in the manuscript is notably inconsistent or incorrectly presented in several instances, requiring proper revision. Furthermore, a significant omission is observed. It is also considerably regrettable that the experimentally measurement of improved transformation frequency utilizing this system, despite being stated as a research objective, was not performed. Therefore, it is anticipated that supplementing this result would substantially enhance the quality of the manuscript.

In the Discussion section, it is suggested that a comparative analysis with examples of systems possessing similar functionalities would likely offer a more comprehensive understanding of the genetic characteristics observed in the subject strain of this study.

In addition, please refer to the attached minor comments for further revisions. Specifically, it is imperative that the references are consistently formatted according to the journal's guidelines.

Major comments

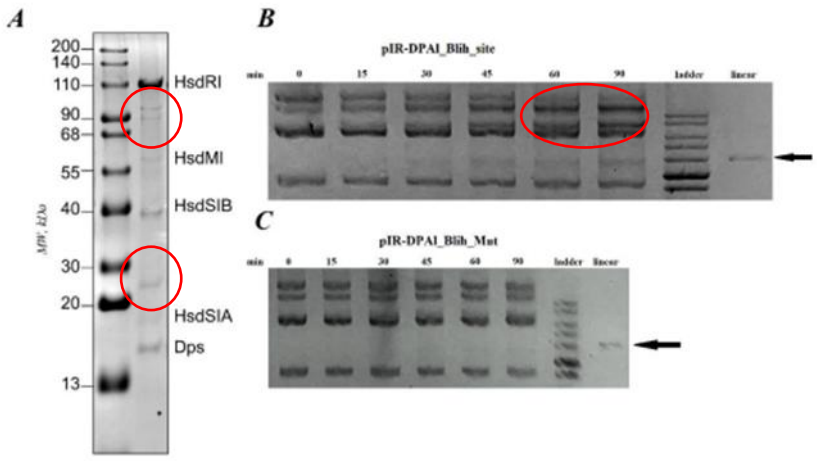
- Could be measure transformation frequency using BlihIA system and its inhibition by ArdB in *B. licheniformis*.
- *E. coli* TG1 genome sequencing data analysis for BlihIA methylation site comparing between TG1 and BlihIA fully expressed. > then found IS1 element and a large 31-kb insert in *fimB* gene. Is this IS1 element translocated by cut-and-paste method?
- In protein purification using BlihIA complex, Dps protein also co-purified. Why? Both proteins interact together? Are there some evidences? Need to discuss.
- In results section, “Endonuclease activity by Lambda phage restriction assay and in vitro BlihIA complex” data was missed. Is it same to EOP counting and linear fragment production? Need to detail explanation the connection between two data.
- References should be followed the journal guideline. Present list was the mixed form.

Minor comments

- Line 23: Please ensure that the keywords are more comprehensively chosen, and a list of all abbreviations used in the manuscript should also be provided.
- Lines 27, 30, 44, 47, 49, 53, 168: reference marking > need to change journal guideline ex. [1],

[2] change to [1,2]

- Line 35: kB should be change to “kilobases (kb)”
- Line 60, 82, 89, 98: “TRD” already include domain, “EOP” assay, *E. coli*, “ONT” – The first instance of an abbreviation in the manuscript must be presented with its full term.
- Line 61-62, 190-191: must be change the names of HsdSIAa and HsdS1Ab subunits (in text) to HsdSIA and HsdSIB (in figure 1), respectively. Or vis versa.
- Line 79: hsdIA change to hsdSIA or hsdSIAb
- Line 84: Please ensure that all cited references are properly indicated within the text, especially for pIRal-2_RM-Ia system
- Line 97, 115: Type 1 R-M system or RMI system. Please ensure that a single, consistent nomenclature is used throughout.
- Lines 142, 143: HsdIA > HsdSIA and HsdIB > HsdSIB
- Line 146: Fig.4A > Fig. 4B
- Line 158: “highly conserved C-terminal D141 residue” Is it point mutation or truncated mutation? If amino acid substitution, it is presented “D141x”. At line 159 and in figure 5B, ΔD141 indicates single amino acid deleted?
- In Table 1, EOP values change to comma to dot. Also, indicate references
- In Fig. 3 legend, BlichIA change to BlihIA in text. RM maybe change to RMI system
- In Fig. 4A. What information is available regarding the unlabeled bands in Fig. 4A? Lane 1 and 2 missed. An explanation is needed concerning what the changing band patterns within the areas indicated by red circles signify in Fig, 4B.



- In Table 2, any redundant content must be removed. In addition, plasmid vector for purification of BlihIA complex include in Table 2, for example, pIR-DPAl-BlihIA-His-TAG.
- It is necessary to explicitly explain the significance of the supplementary files provided. Additionally, the Table descriptions should be clearly organized and presented.