



SDI Review Form 1.6

Journal Name:	British Microbiology Research Journal
Manuscript Number:	Ms_BMRJ_18922
Title of the Manuscript:	Rare codons optimizer host strain of E. coli improves expression of Clostridium septicum alpha toxin gene
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	This study presents experiments designed to achieve an efficient expression of <i>Clostridium septicum</i> vaccine strain alpha toxin gene in <i>E. coli</i> cells. For this purpose, the pET system was used that is a popular choice. First, I would like to ask author(s) what strains were actually used in this study. I am afraid that it would be difficult to achieve the expression of pET constructs in <i>E. coli</i> BL21 or Rosetta cells. Probably, the author(s) used in this study <i>E. coli</i> BL21(DE3) and Rosetta(DE3) host strains (i.e. λDE3 lysogens). In my opinion some experiments were improperly planned and performed. First of all, to compare the efficiency of <i>csa</i>-toxin gene expression in two <i>E. coli</i> host strains, BL21(DE3) and Rosetta(DE3), the expression of the obtained recombinant plasmid pET22αsep should be examined in the selected bacterial hosts after IPTG induction. Figs 3 and 4 present <i>csa</i> expression only in Rosetta cells. The production level of the CSA recombinant protein (after an addition of IPTG) in the selected hosts was not studied. Thus, why the author(s) consider that rare codon optimizer host stain of <i>E. coli</i> improves the expression of <i>csa</i>-toxin gen. The next stumble, after cloning of genes into pET vectors, non-expression host strain should be used. I can not image to use at this stage <i>E. coli</i> expression strains for transformation and propagation of recombinant pET vectors. Despite these disadvantages, I also found many inaccuracies, mainly in the descriptions of experiments. The low-quality of the technical English in this manuscript and numerous mistakes (not only linguistic), suggest that the manuscript was prepared carelessly.	In this study we used <i>E. coli</i> BL21(DE3) and Rosetta(DE3) host strains The expression of the obtained recombinant plasmid pET22αsep was examined in the selected bacterial hosts after IPTG induction and for this reason a new figure (Fig 4) is inserted and the previous figures 4 and 5 are shifted to figures 5 and 6. I think this stage is optional to use Mistakes were corrected and the author did not prepared this manuscript carelessly Lane 10 was corrected.



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	Examples of mistakes and inaccuracies: Lane 10; an attempt “not attempt”; an active fragment not “active fragment”; - lanes 12,13: in my opinion, first the active fragment of alpha toxin gene was obtained (not extracted) from the vector pJETαsep and then subcloned into the pET plasmid, but not “was cloned and extracted from pJET...”; - lane 14 and all text of the manuscript: Strain names are always written with an initial capital letters; thus Rosetta not rosetta; - lane17: induction with IPTG for 3-6 h not “induction of IPTG ... during 3-6 h after induction”; - lane 21: not “this clone” but the obtained recombinant plasmid; - lane 82: to induce the protein expression not “to induce of protein expression”; - lane 85: a 6-His tag at the C-terminus not “a 6-His tag at C-terminal”; - I suspect that Fig. 2 presents the restriction enzyme analysis of the obtained recombinant vector, but I could not find in the text any information about this restriction enzyme digestion. It was not mentioned the name of any enzyme(s). In addition, the figure quality is very poor. I wonder whether the purification of CSA was necessary to reach the goal of this work. Finally, I would like to note that there is at least one report describing the same cloning procedure of <i>csa</i> into the vector pJET. The same cloning procedure of <i>csa</i> was described in an earlier paper	Lane 12, 13, in the sections 2.1. Cloning & 2.2. Sub cloning, it is completely explained why “was cloned and extracted from pJET. Lane 14 and all text of the manuscript were corrected. Lane 17 was corrected. Lane 21 was corrected. Lane 82 was corrected. Lane 85 was corrected. Fig. 2 in the sections, 2. Materials and Methods & 2.2. Sub cloning, restriction enzyme are mentioned and the procedures are explained. Also a new sentence in this regard is written. Purification has been done as complementary work. It depends on the reviewers’ decision. If it is not necessary, it could be removed from the manuscript. Yes
Minor REVISION comments		
Optional/General comments		