SDI FINAL EVALUATION FORM 1.1

PART 1:

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 Article	**Original Research Article**

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	**Authors' response to final evaluator's comments**
Unfortunately, I am still disappointed in the quality of the manuscript. There are still plenty of mistakes and inaccuracies. Some figures (for instance Fig. 3) are unnecessary. Fig. 4 that shows "comparison of alpha toxin expression in *E. coli*/BL21/pET22β and *E. coli*/rosetta/pET22β" is not convincing. A protein indicated by the arrow is visible in both *E. coli* strains. In such case Western blotting analysis would be much better than SDS-PAGE stained with Coomassie blue dye. In my opinion, the manuscript should be rewritten to improve its scientific language. Therefore, I have to uphold my previous decision. The examples of errors that are still present in the manuscript (not only the abstract text contains mistakes and inaccuracies); my suggestions are marked in red. **Abstract** **Aims:** *Clostridium septicum* is an anaerobic, gram positive bacterium which/that is able to form resistant spores. It produces numerous toxins that cause many diseases such as gas gangrene in humans and braxy in farm animals. In the present study, an attempt was done to express an active fragment of alpha toxin gene of *C. septicum* vaccine strain, in *E. coli* BL21(DE3) and Rosetta(DE3) strains. **Methodology:** In the present study, the active fragment of alpha toxin gene of *C. septicum* vaccine strain was amplified… ?, cloned into… ? and extracted/then obtained from the pJETαsep recombinant cloning vector. After purification and evaluation, it was ligated with the pET22b(+) expression vector and pET22αsep was transformed into *E. coli* BL21(DE3) and Rosetta(DE3) strains. **Results:** Results showed that a recombinant protein was expressed/produced as a soluble protein after IPTG induction in Rosetta(DE3) rare codons/codon optimizer host strain, but not in BL21(DE3). Further optimization on/of expression conditions of the recombinant alpha toxin protein was achieved by incubating the culture of (recombinant) *E. coli*/Rosetta(DE3[pET22αsep] cells in 37°C and induction with 0.5, 1.0 and 1.5 mM IPTG for 3-6 h. Protein expression was evaluated by SDS-PAGE and the recombinant alpha toxin protein was purified using Ni-NTA resin and for its reconfirmation was analysed by Western blot. **Conclusions:** We concluded that, *E. coli* strain Rosetta(DE3) is a suitable expression host for production of *C. septicum* alpha toxin and the obtained recombinant plasmid could be used for further research on production of recombinant vaccine against braxy disease.	

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