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The CRISPR/Cas9 genome editing system to obtain a targeted deletion in the *iucA* gene of *Klebsiella pneumoniae*

Kandina D.*, Sopova J., Velizhanina M.
Center for transgenesis and genome editing, Saint Petersburg State University, Saint Petersburg, Russia
** candyda20@mail.ru*

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Motivation and Aim: *Klebsiella pneumoniae* is one of the most prevalent nosocomial pathogens. Recently, a novel combination of virulence genes (different operons of siderophore synthesis) and antibiotic resistance genes (metallo-*β*-lactamases) has been identified in a single strain, representing a previously unobserved phenomenon [1].
The objective of this study was to create a directed deletion of the plasmid-localised *iucA* gene, which encodes NIS-synthetase and is involved in the synthesis of the siderophore aerobactin. This was done in order to characterize its functional role in the development of hypervirulence in *K. pneumoniae*, which also possesses multiple antibiotic resistance. In pathogenic bacteria, siderophores (trivalent iron chelators) play an important role in virulence and are most often localised on the pLVPK plasmid.
Methods and Algorithms: The pUC19_CRISPR_ΔiucA system, comprising a selective marker for zeocin resistance, is based on the pUC19_CRISPR_ΔpmrA vector [2], which encodes *S. pyogenes* Cas9 nuclease. The Lambda red bacteriophage *λ* recombination system was employed to edit the *K. pneumoniae* genome, as well as genes encoding a guide RNA (sgRNA) and a homologous recombination cassette carrying the desired mutation in the *iucA* gene.
Results: Following the transformation of hypervirulent antibiotic-resistant *K. pneumoniae* strains isolated from patients in St. Petersburg hospitals with the plasmid pUC19_CRISPR_ΔiucA, strains 3911 and 1128 were obtained with a targeted deletion (80 bp) in the *iucA* gene on the pLVPK plasmid, resulting in a shift of the reading frame and formation of a premature stop codon. Two outcomes were observed. In the case of strain 3911, the pLVPK hypervirulence plasmid, which carries the aerobactin synthesis operon, was completely lost by the bacteria. In the case of strain 1128, the desired deletion with a reading frameshift was observed. Qualitative assessment of siderophore production in the resulting mutants on CAS agar revealed a reduction in the area of dye destruction compared to the wild-type strains, indicating a successful knockout of the *iucA* gene.
Conclusion: Consequently, the utilisation of the CRISPR/Cas9 system for the genomic editing of *K. pneumoniae* represents an efficacious methodology, which can be employed in genetic engineering tasks for other *Enterobacteriaceae*.
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