Development of the CRISPR/Cas13-based posttranscriptional gene regulation system in Listeria monocytogenes.

P-05.3-08

M. Burmistrz[I], K. Krakowski[I], K. Ścibek[I], A. Krawczyk-Balska[I]

[I]University of Warsaw, Warsaw, Poland

The RNA-targeting Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated (CRISPR-Cas) systems have proven very useful tools both in studying role of bacterial genes as well as modifying their expression for biotechnological purposes. *Listeria monocytogenes* is a Gram-positive, human pathogen. Due to its intracellular life cycle it is considered as a model organism of intracellular infection and a candidate for a vector in gene therapy. Here, we develop the system for post-trascriptional gene regulation based on CRISPR/Cas13 from the *Listeria seeligeri*. The native CRISPR array was extended with the artificial fragment where additional restriction sites were introduced to facilitate incorporation of desired spacer sequences. To validate the system, we decided to target two listerial genes: *hly* and *cadA* responsible for hemolytic activity and heavy metal resistance, respectively. These were chosen due to easy to measure physiological effects. We first analyzed the crRNA processing with northern blot. Then, the effect of anti RNA activity of Cas13 on silenced genes was measured by appropriate *in vitro* assays. In addition to that, their mRNA levels were measured using RT-qPCR method. What is more, the influence of active Cas13 on overall fitness of the bacteria was assessed by growth curve analysis. Taken together our results show that CRISPR/Cas13 system from *L. seeligeri* shows potential for its utilization in other listerial species. However, further studies are required to fully characterize this system. Acknowledgements: This work was supported partially by a grant no. 2016/21/B/NZ6/00963 from the National Science Center, Poland and by the Ministry of Science and Higher Education through the Faculty of Biology, University of Warsaw (intramural grant DSM no. 501-D114-01-1140400).