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Referee report on the AUTOREFERAT **K.M. Klimina**

Genetic analysis of Toxin- antitoxin superfamily RelBE in lactobacilli

The topic of the presented dissertation covers a topic of high relevance and importance for our understanding of the interplay between humans and the microbial world. After the analysis of pathogens and their successful control, interestingly by mostly microbial products as antibiotics and the investigation of their producing bacteria like streptomycetes, complex microbial communities are about to become analysed. The knowledge of human microbiome composition, function, and range of variation across multiple body sites has begun to assemble a rich picture of commensal host-microbe and microbe-microbe interactions as well as their roles in human health and disease and their potential as diagnostic and therapeutic tools. Lactobacilli, in particular *L. rhamnosus*, are important and permanent components of the human microbiome. Probiotic actvities have been revealed in numerous *L. rhamnosus* strains. A link between several genes and the probiotic features of *L. rhamnosus* has not yet been established. The properties of *L. rhamnosus* strains are caused not only by the presence/absence of individual genes and gene systems but also by the mechanisms of their regulation. Toxin-antitoxin systems (TASs) play a significant role in the regulation of gene activity. To date, at least five types of TASs occuring in most bacterial and archaeal genera are known. These are involved in apoptosis, antibiotic tolerance, stress

response, and biofilm formation. TASs are likely general elements in bacterial regulatory networks. Type II TASs are usually encoded by two genes that form one operon with a promoter located upstream of the first antitoxin gene. Both, the toxin and antitoxin are proteins. The toxin causes cell death or inhibits bacterial cell growth by targeting RNA, ribosomes, DNA gyrase, or the cell wall. The antitoxin directly interacts with the toxin, suppressing its activity. The toxin protein is more stable than the antitoxin protein. Because the transcription, translation, and/or protease activation are inhibited, the toxin/antitoxin ratio is shifted towards the toxin, causing growth arrest and bacterial cell death. To date there are at least 33 different TAS; one of the well-characterized is YoeB-YefM , belonging to RelBE superfamily. Members of the YefM-YoeB TAS family were found in *E. coli* as well as in Gram-positive microorganisms like enterococci, streptococci, staphylococci, and streptomycetes. The YoeB protein degrades mRNA at the ribosomal A-site that interacts with the 50S ribosomal subunit, thereby inhibiting initiation of translation in *E. coli* . The YefM antitoxin can bind alone or in a complex with the toxin to the operators in the toxin-antitoxin (TA) promoter region thus blocking the operon transcription.

Klimina and her colleagues identified six TASs with toxins belonging to the MazE and RelE superfamilies in the genomes of 11 annotated strains of *L. rhamnosus* . YefM-YoeB TAS was identified in 5 genomes.

PCR analyses revealed that all systems were found in 15 strains of *L. rhamnosus* isolated from humans in central Russia. Cloning of the toxin genes of four TASs on expression vector inhibited *E. coli* growth to various degrees, thus confirming their functionality. TAS YefM-YoeB$_{Lrh}$ was identified in 7 out of 15 laboratory strains. The nucleotide sequences of *yefM$_{Lrh}$ yoeBLrh* genes in diverse strains were identical, only one laboratory strain had one nucleotide substitution in toxin gene. *L. rhamnosus* isolates have been sequenced and deposited.

Cell growth arrest caused by expression of the *yoeB$_{Lrh}$* toxin gene in *E.coli* could be reverted by the expression of a cognate antitoxin gene, *yefM$_{Lrh}$*. The failure to clone the antitoxin and toxin genes together without the TA promoter region is the basis for the hypothesis that the toxin gene *yoe*B$_{Lrh}$ has its own promoter. Klimina describes the structure and transcriptional regulation of genes, encoding TA system YefM-YoeB$_{Lrh}$ in three strains of *L. rhamnosus* comparing stationary and exponential growth phases, the influence of stress factors and mRNA stability. The same TA system is responding to physiological and stress conditions differently in related strains. Using primer extension and RLM-RACE methods three transcription start sites of RNAs in the operon were determined . The promoter region of the operon is preceded by a conserved BOX element occurring at multiple positions in the genomes of *L. rhamnosus* strains. A detailed analysis revealed the presence of BOX elements, occurring as abundant short repeated sequences of about 300 bp with up to 125

copies per genome and modulating gene expression, which were found in Gram-positive bacteria *Streptococcus pneumoniae*. Downstream of and partially overlapping with the 3´end of the *yoeBL*$_{rh}$ toxin gene, a divergently transcribed unexpected RNA was detected.

In this study Klimina and their coauthers investigated the structure and function of a newly identified TA system in *Lactobacillus rhamnosus*. These data are of general interest due to the fact that,sSo far, reports on structure and expression of TA systems in Lactobacilli are rare. In none of the cases reported so far, a combination of detailled *in vitro*- and *in vivo*-analyses has been performed to investigate these genes. For a potential application of Lactobacilli, like probiotica, the conditions of the expression of TA systems are of great importance. The presented data are original an have been published in international Peer-reviewed journals and presented on national and international conferences.

Taken together, these achievements merit

"summa cum laude"

Jena, 0ctober 5. 2015 Dr. H. Krügel