Poster

The role of the transcriptional regulator, Tex, under stress conditions in Lactococcus lactis

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ABSTRACT

Motivation:Tex, originally described in Bordetella pertussis, is a regulator involved in a variety of transcriptional processes and conserved in a wide range of bacteria. However, very little is known about its function in Lactococcus lactis. Preliminary proteomic data have shown that Tex is more abundant in a L. lactis ΔftsH mutant (ftsH knock-out) under cell wall stress. FtsH is a membrane protease involved in regulation assuring cellular protein stability under stress conditions. Moreover, based on previous knowledge, L. lactis ΔftsH presents some distinct phenotypes such as lysozyme resistance and NaCl sensitivity. A link is proposed between those phenotypes and the high level of Tex, which might be due to a higher half-life of this protein, as a consequence of the lack of FtsH protease. In order to get insights on the role of Tex in L. lactis, this work tries to answer two main questions: (i) Is the abundance of Tex regulated by FtsH? and (ii) are the higher levels of Tex causing the resistance and sensitivity

Methods:The gene coding for Tex was amplified by the polymerase chain reaction (PCR), with specific primers designed to clone it under both, a constitutive promoter P32 in pMG36e and the nisin inducible promotor Pnis in pNZ8020. Growth of cells with the empty plasmid (pNZ8020) or overexpressing Tex (plasmid pBL95) in GM17Cm medium was measured by OD at 600 nm in a microplate reader. The L. lactis tex knockout is currently being constructed, by cloning a truncated version of tex in pGhost9, a thermo-sensitive plasmid. Experiments to estimate Tex half-life will be carried out using antigen-antibody tagging in the presence or absence of FtsH.

Results: It has not been possible to clone tex gene under the constitutive P32 promoter. L. lactis cells carrying pBL95 (tex gene under Pnis) growing in GM17Cm medium show a delayed growth related to the nisin concentration. The higher the concentration used, the slower the growth rate indicating that Tex overexpression might have a deleterious effect. Regarding to the lysozyme resistance and NaCl sensitivity, Tex abundance in ΔftsH might be the cause of those characteristics and experiments are in progress to probe it.

Conclusions:Tex functions remain unknown in Lactococcus lactis but constitutive expression and/or too high levels of Tex seem to be deleterious. Further studies are necessary to analyse its role in the generation of new phenotypes and its regulation by FtsH for a better understanding.

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