**Overexpression of anti-*sigE* sRNAs in *Mycobacterium marinum***

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*Mycobacterium* has the ability to adapt to different environmental stresses and σ-factors play an essential role in these adaptations. The expression of σ-factors is regulated via various mechanisms, such as small regulatory RNA (sRNAs). sRNAs play a crucial role in stress responses in bacteria and can fine tune gene expression in response to different environmental conditions. It has become a research hot spot to understand how these changes occur and how they are regulated.

Using total sRNA sequencing (sRNAseq), several sRNAs that target the E mRNA in *Mycobacterium marinum* were identified, with sizes ranging from 20bp to 87bp. They are encoded opposite the *sigE* gene, are completely complementary to *sigE* and show typical characteristics of *cis*-encoded antisense RNA, but the functions and roles of these sRNAs are still unknown.

To study the function of these sRNAs, we overexpressed these sRNAs in *Mycobacterium marinum* by cloning them into a plasmid vector *p*BS401 under the control of a Tet-inducible promoter. The overexpression of one sRNA totally inhibited the formation of biofilm after 7 days’ incubation without shaking and affected cell length in old culture. Then we performed qRT-PCR experiment to analyze the expression level of *sigE* after overexpression of anti-*sigE* sRNAs. The qRT-PCR analysis showed no significant change in the amount of E mRNA in overexpression strains compared with control strain, which indicates the regulation of these sRNAs is not at the transcriptional level. To further study the regulation mechanism of these sRNAs and how the expression level of E affect cell morphology, a *sigE-lacZ* reporter gene construct was successfully made along with a CRISPR interference system against *sigE.*

Our results give insights into the functions of these anti-*sigE* sRNAs as well as in their potential roles in host infection. They also indicate that E, as one of the alternative sigma factors, is involved in biofilm formation in *M.marinum* since the overexpression of its regulators- anti-sigE sRNAs led to the inhibition of biofilm formation. Further studies are needed to find out the relationship between anti-*sigE* sRNAs and *sigE* in *M.marinum*. Hopefully the results will help us better understand gene regulation in *M.marinum* and how *Mycobacterium* responds to environmental changes.