COLLEGE OF SCIENCE
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Research Horizons Day

Two-component signaling system BaeSR of *Escherichia coli* in development of antibiotic resistance

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Introduction

- Two-component signaling is one of the stress response mechanisms *Escherichia coli* have for survival adaptability.
- BaeSR two-component system specifically regulates envelope stress response in *E. coli* and is activated by the presence of indole, heavy metal ions, and spheroplasting.
- This system consists of a sensor protein histidine kinase-BaeS and a response regulator BaeR.

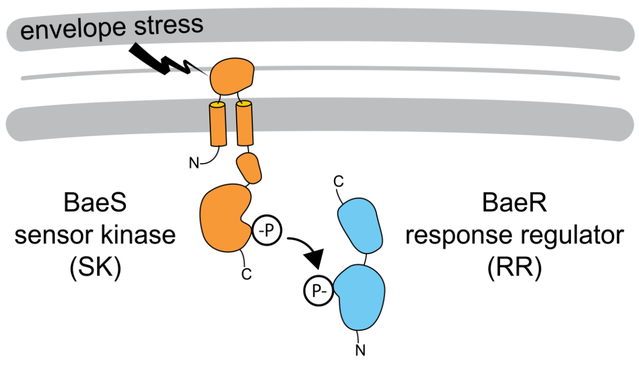

Figure 1-Two-component signaling system BaeSR

- Activated BaeR protein upregulates transcription of about 60 genes including the periplasmic chaperone spy and multidrug efflux pumps MdtABC and AcrD.

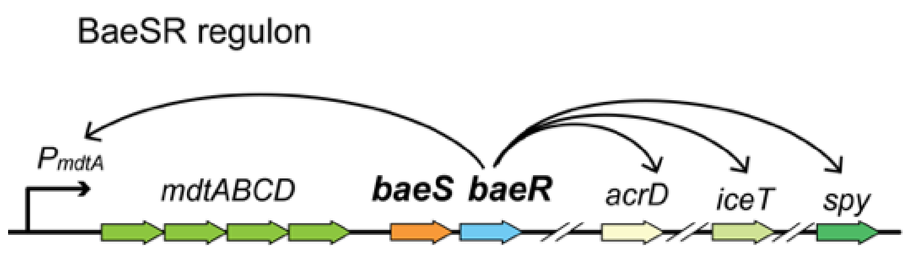

Figure 2-Structure of the *mtd-bae* operon in *E. coli* and major BaeSR regulon effectors

- Treatment of *E. coli* with β-lactam antibiotic carbenicillin resulted in genetic mutations in the *baeSR* locus, leading to increased antibiotic resistance.

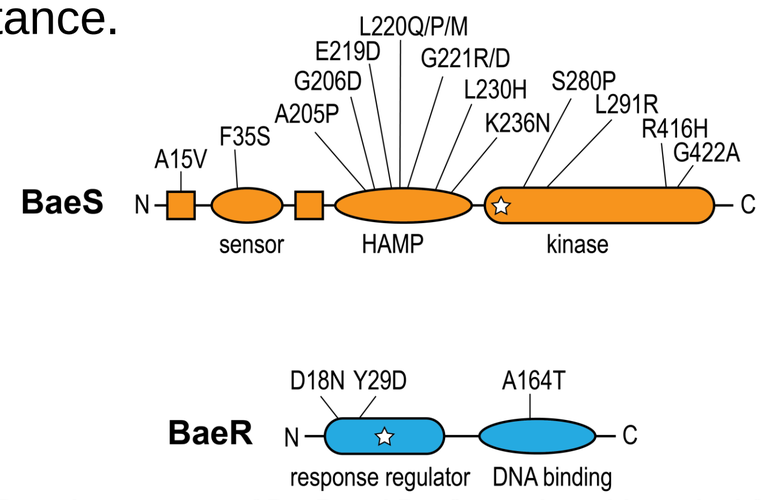

Figure 3- Domain structure of BaeR and BaeS proteins with marked β-lactam selected mutations

Objective

- Characterize functioning of the BaeS and BaeR proteins *in vitro* and analyze effect of the resistance-causing mutations

Results

- BaeS and BaeR proteins were recombinantly expressed in soluble form and were purified using Ni-affinity chromatography.

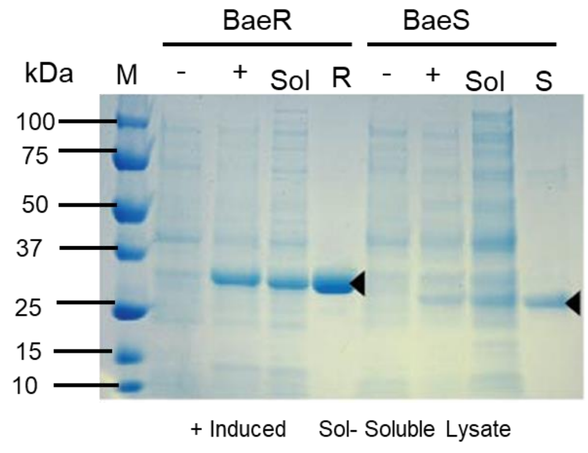

Figure 4-SDS-PAGE analysis of the expression and purification of the BaeR and BaeS (Coomassie stain)

- Designed primers for site-directed mutagenesis of the *baeR* and *baeS* genes.
- Developed primers and optimized PCR conditions for amplification of the promoter (P_{mdtA}) region of *mdt-bae* operon.

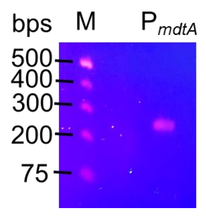

Figure 5-Agarose gel analysis of PCR amplified P_{mtdA} promoter region (ethidium bromide stain)

- Analyzed binding of the BaeR regulator with the cognate P_{mdtA} promoter using electrophoretic mobility shift assay (EMSA).

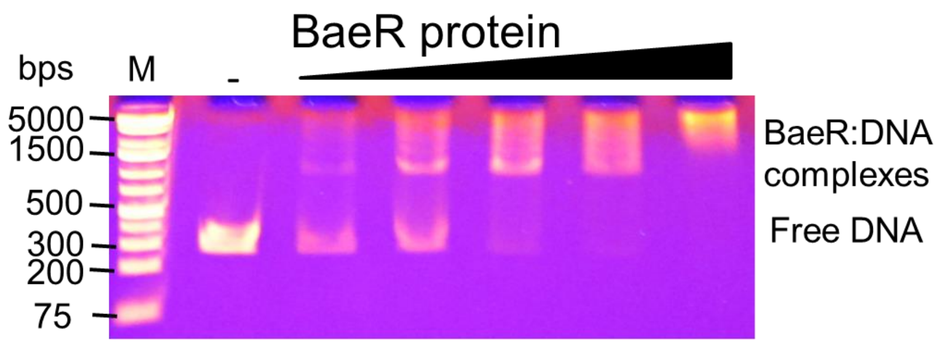

Figure 6-PAGE analysis of P_{mtdA} promoter binding to BaeR protein (ethidium bromide stain)

Summary and Future Work

- We successfully produced BaeSR proteins and promoter DNA and established EMSA protocol for quantification of BaeR:DNA interaction *in vitro.*
- Next we will introduce mutations to BaeS and BaeR proteins using site-directed mutagenesis with the primers we designed and use these altered proteins to analyze the protein:protein and protein:DNA interactions.

Acknowledgements

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