Bioremediation of lead using spore surface displayed proteins

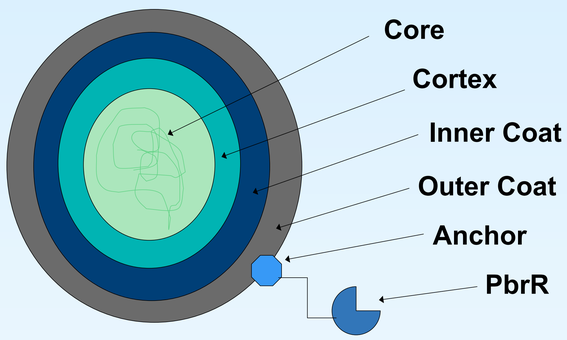
Sofia Diaco
Adviser: Kang Wu
Department of Chemical Engineering, University of New Hampshire

University of New Hampshire

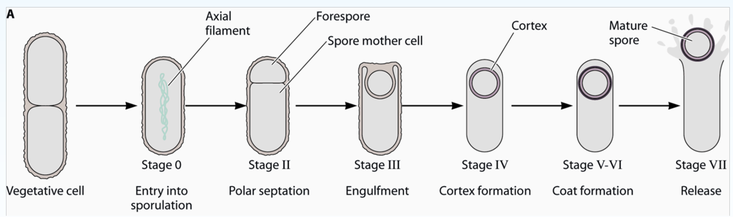
Abstract

Lead is a toxic pollutant very harmful to human health since it accumulates in the body and affects the brain, liver, kidney, and bones. Fetuses can be exposed to lead during pregnancy, which can cause problems with learning later on in the baby's life. The purpose of this project is to display a lead binding protein on bacterial spore surface for the bioremediation of lead from water. Spores from *Bacillus subtilis* are very robust and resistant to various harsh environments. Genetically fused to a spore surface protein, the displayed proteins demonstrate enhanced robustness and can be easily produced through sporulation without the need of further purification. PbrR is a regulatory protein that modulates the lead resistance in bacteria. In this project, we fused seven variants of PbrR to the spore surface protein CotC (CotC-PbrRs). The lead binding affinity and specificity of these PbrR variants on the spore surface will be characterized. The robustness of these spores with PbrR will be also evaluated using wastewater samples.

Introduction

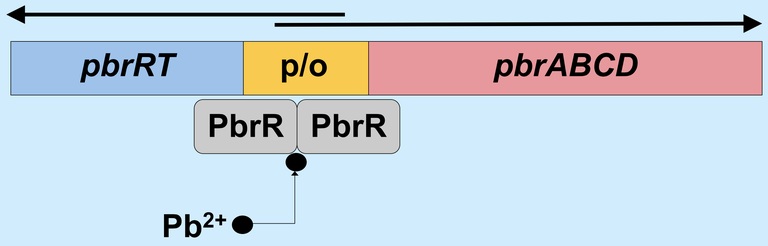


- **Spores, released from vegetative cells through sporulation, have rigid, protective layers around the core which contains the DNA [2].**
- **Spores are able to survive under harsh conditions like extreme temperatures, a wider range of pH levels, radiations, and in the presence of organic solvents**

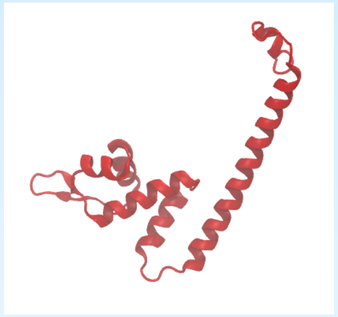


Lead binding protein PbrR

- **PbrR is a protein that regulates the pbr lead resistance operon [3]. The lead ions bind to the PbrR dimer and the resulted complex can bind to the promoter region of the operon and active the expression of genes in both directions.**



- **Structural prediction shows that PbrR is similar to MerR in structure and it has two domains: lead binding domain and DNA binding domain.**

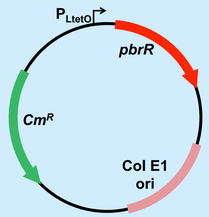


Experimental Design

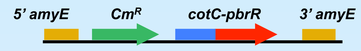
- **3 variants of PbrR (PbrR, PbrR2, and PbrR3), and 4 truncated variants of PbrR (Δ42, Δ60, Δ76, and Δ76Δ126-145) are used in this project to investigate the essential lead binding domain and compare their binding affinity.**
- **Each PbrR variant with a C-terminal His tag is expressed in *E.coli* using the plasmid pPROTet.E for purification of the protein.**
- **Each PbrR variant, with and without His tag, is genetically fused to the anchor protein CotC in *B. subtilis*, an abundant protein in the outer coat [1].**
- **Lead binding affinity of PbrRs displayed on the spore surface and the purified soluble PbrRs will be compared.**

Results

- **Plasmids for the expression and purification of PbrRs in *E. coli* have been successfully constructed.**



- **Linear DNA containing *cotC-pbrRs* (with and without His-tag) for integrating them into the *amyE* site on the chromosome of *B. subtilis* have been assembled.**



- **Transformation of *B. subtilis* was not successful, and it is likely due to the length of the linear DNA being too short. New DNA fragments have been designed and amplified to assemble the integrating DNA to be about 8 kb long.**

Future Work

- **Purify the seven PbrRs using His tag purification column.**
- **Integrate each *cotC-pbrR* into *B. subtilis*.**
- **Collect spores displaying PbrRs and quantify the concentration of PbrR on the spore surface using ELISA.**
- **Measure lead binding affinity of each purified PbrR protein and spores displaying each PbrR variant using a wild type spore as the control at room temperature and neutral pH.**
- **Study the effect of temperature and pH on the lead binding affinity.**
- **Design a process of reversible lead binding using spores displaying PbrRs for lead recovery.**

References

1. R. Isticato and E. Ricca. "Spore surface display," in *Microbiology Spectrum*, 2016.
2. Molecular Biology for Masters. *Regulation of Gene Expression during Sporulation,* [Online]. Available: http://mol-biol4masters.masters.grkraj.org/html/Gene_Expression_I5G-Regulation_of_Gene_Expression_During_Sporulation.htm
3. B. Borremans, et al. "Cloning and functional analysis of the *pbr* Lead Resistance Determinant of *Ralstonia metallidurans* CH34," in *American Society for Microbiology*, vol. 183, no. 19, May 2001.