Overview

Our team project is designing synthetic modules for simultaneous detection of multiple heavy metals such as arsenic, zinc, and cadmium in Escherichia coli. We call this H.M busters! The ultimate goal is to build a micromachine sensing and determining the concentration of heavy metals in a sample solution (e.g. the wastewater). In order to design the system, we will employ two fluorescence proteins (GFP and RFP) and aryl acylamidase as signal reporters. The aryl acylamidase converts a colorless acetaminophen (Tylenol TM) to a brown color substrate. Since H.M busters have three heavy metal promoters, if more than two heavy metals coexist in a solution, the results would be interpreted from the mixed fluorescence and/or color rather than a single signal detection. The successful construction of the synthetic modules in E. coli can be utilized in the form of a lyophilized powder, which can be stored in a drug capsule to make it portable.

Results

We successfully constructed metal detector in E. coli employing natural metal detecting promoters found in E. coli.

1. Zinc detector using RFP as a reporter was constructed and worked at the range of 1–2 mM concentration
2. Arsenic detector using GFP as a reporter was constructed and worked at the range of 0.15–5 mM concentration
3. Cadmium detector using AMD as a reporter was constructed and worked at the range of 0.2–0.4 mM concentration

Summary

We successfully constructed zinc, arsenic and cadmium detector, and confirmed their operation. We registered new part; aryl acylamidase which is used in cadmium detector. It has shown the possibility of a cheaper and more visible detector. Because Tylenol is very cheap, it will be very economical to use aryl acylamidase in any places where fluorescence proteins are used!

Discussion

1. Zinc detector: P\textsubscript{znt}::gfp

The zinc detecting promoter can be induced beyond 1 mM concentration of zinc ion. This range from 1 mM to 2 mM is enough to detect zinc ion in common wastewater. However, when we need to detect higher concentration of zinc ion, we should use dilution methods.

2. Arsenic detector: P\textsubscript{yodA}::gfp

It seems that the cost of the expression of arsenic ion binder is very expensive for microbial cells. In lower levels of concentration, the fluorescence was not enough to be distinguished from the control. A strong inhibitor might exist at the imitation of expression. Compared to zinc ion, the arsenic ion has more harmful effect on the growth of bacteria. This is a drawback of our detecting system considering that the bacteria can be killed while measuring concentrations.

3. Cadmium detector: Synthetic circuit – II: P\textsubscript{ami}::amd

Cadmium detector has a similar problem related to toxicity, as discussed above. Like arsenic ion, cadmium has harmful effects on bacteria. 0.2 mM of cadmium is enough to inhibit the growth of E. coli. The growth of E. coli did not show big difference between 0.2 mM and 0.4 mM concentration of cadmium.

We wanted to detect the proportional change according to concentration with our bare eyes, but the difference was too small to be detected. Because of low fluorescence of GFP and RFP, we considered using a stronger promoter to detect heavy metals. However we did not use it due to standards issues. Some parts registered on the registry did not work, not to mention inaccurate sequencing. The fluorescence GFP and RFP can be seen only with UV irradiation. However, it is possible to see the changes of the color which stimulated in good contrast by metal ion, using aryl acylamidase. The amd gene, which induces brown color by cleaving acetaminophen (Tylenol TM).

References

1. The aro O operon of Escherichia coli Confers Arsenical and Antimicrobial Resistance . A. carlin, W-Shi, Saibil, Dey, and Barry P. Rosen
4. A Propionate-Inducible Expression System for Enteric Bacteria . Sung Kuk Lee and Jay D. Keating