Light controlled heavy metal carrying *E. coli* machines

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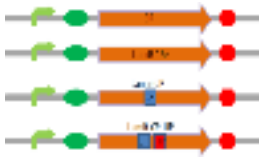
INTRODUCTION

Heavy metals are natural part of environment that we live in. However they have toxic effects when they are above certain concentration. Since they are not degradable and accumulates in living organisms, soil change researches predict that heavy metal contamination may become major problem in close future. Currently bacteria with heavy metal binding abilities have been designed as an efficient and low cost solution for heavy metal contamination. Certain surface proteins have been already reported to tolerate heterologous metal binding peptide insertions to design heavy metal binding bacteria. Given that metals adsorbed to peptides are desorbed under acidic condition, we showed that bacterial metal binding and release can be controlled by light.

MATERIALS & METHODS

1. Plasmids and Strains. *E.coli* DH5α strain was used as a recipient of all plasmids. pLBB9 is derived from PVDL8 plasmid carrying *lamB-153* under *Plac* control. HP(N-Ala-Gly-His-His-Pro-His-Gly-Ala-C) and CP(N-Ala-Gly-Cys-Gly-Cys-Pro-Cys-Gly-Cys-Gly-Ala-C) were inserted between the 153rd and 154th amino acids through BamHI linker. Deltarhodopsin is derived from *H. turkmenica* and expressed under the control of *lac* operator.



2. Metal Adsorption/Desorption measurement. Cells were grown until they reach 0.8 unit. IPTG and Cd^{2+} were added and then were grown 4 more hours and precipitated. Pellet was washed with wash buffer(0.85% NaCl, HEPES) three times and mineralized overnight with nitric acid (65%). Adsorbed metal concentration was measured with atomic absorption spectroscopy. Cells with bacteriorhodopsin were kept under light and precipitated upon 0, 45 and 90 min time points.

MODEL

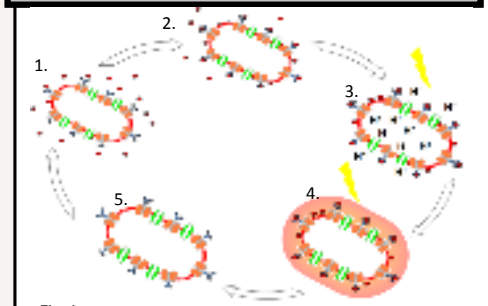


Fig. 1. Bioremediation model describing metal adsorption/desorption under the control of light.

1. Metal Binding Machines(MBMs) are in media to be cleaned
2. MBMs collect metals from the media
3. Cells are removed to another media and kept under light. Rhodopsins are activated.
4. Activated rhodopsins generate local acidic medium around the cell surface
5. MBMs start to release metals

BACKGROUND

Bacteriorhodopsins are retinal-binding integral membrane proteins that function as light-driven proton pumps. Upon binding of retinal molecule its conformation changes due to *cis-trans* isomerization of retinal after light absorption. This conformation change of protein results in efflux of proton.

In our project we used type of rhodopsin called deltarhodopsin(dR) from *Haloterrigena turkmenica* to express in *E.coli.*

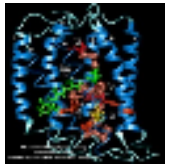


Fig.2.Structure of bacteriorhodopsin· (Theo. Physics Group, Beckman Institute)

The LamB protein is a trimeric outer membrane (OM) protein of *E. coli* sustaining two biological functions. It is used as a surface receptor by a number of coliphages, including phage lambda , and participates in the transport of maltose and maltodextrins across the OM . LamB tolerates insertions of long heterologous peptides at a permissive loop (between structural codons 153 and 154) exposed to the external medium without a loss of function.

RESULTS

To monitor metal binding ability of two different contructs(CP, HPCP) inserted between 153rd and 154th amino acids of LamB we transformed each contruct into *E.coli* DH5α and measured adsorbed metal concentration with AAS. As a control we used LamB-153 without any metal binding insert but BamHI linker. Inserted CP and HPCP peptides resulted in 22 and 13 times more metal adsorption than the control(B9) contruct, respectively. Cd^{2+} was used as a heavy metal.

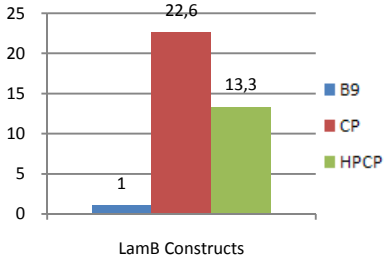


Fig. 3. Metal adsorption efficiency of CP and HPCP inserted within LamB.

To check amount efficiency of metal desorption LamB-CP and LamB-HPCP were cotransformed with BR(bacteriorhodopsin) and HtdR(deltarhodopsin). Upon inoculation cells were subjected to light for specified time intervals and metal(Cd2+) adsorbed on the surface were measured with AAS. Cd2+ on the surface decreased by time.

ColonyI = BR+CP; ColonyII = BR+HPCP;
ColonyIII =HtdR+CP; ColonyIV =HtdR+HPCP;

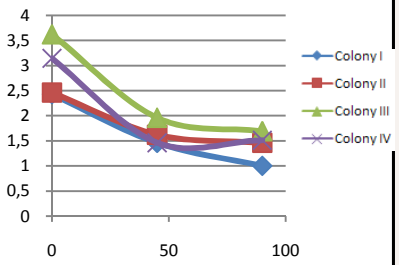


Fig. 4. Rate of metal desorption of different combinations of metal binding peptides and rhodopsins.

CONCLUSIONS

One of the criteria of bioremediation of heavy metals is to avoid further contamination while trying to clean it. So we wanted to design a closed system where minimal external care is needed.

In this work we designed a system with which can we control metal adsorption and desorption using light only. However one problem is still to be solved. The adsorption and desorption should be decoupled. It was already reported that *E.coli* can be engineered to exhibit stable phototactic response. Hence decoupling can also be done by light again, but using specific wavelengths. Moreover, MBMs can also be used to construct ion exchange matrix to uncouple adsorption and desorption.

LITERATURE CITED

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