Cadmium Catching System Using Cell-Cell Communication

Naoki Saito, Daiki Haraguchi, Takuho Otsubo, Takuto Moriyama, Yoshiharu Otaki
Ryuhei Minei (ADVISER), Assoc. Prof. Shoji Usami (INSTRUCTOR), and Prof. Yoshisuke Nishi (INSTRUCTOR)

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Abstract

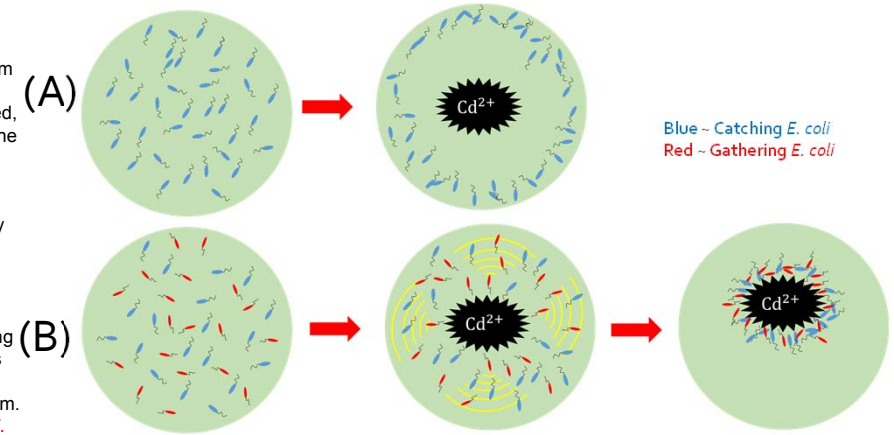
Cadmium is a heavy metal very harmful to many species of organisms. The pollution of cadmium brings a serious problem for human. For example, 'itai-itai' disease in Japan was caused by cadmium poisoning. Nagahama, where our institute is located, is near to Biwa Lake and one of famous rice fields in Japan. If the rice field contains some level of cadmium, harvested rice grain should contain cadmium.

For our safe, the cadmium in rice field must be removed. For this purpose, we have planned to collect the cadmium efficiently with the recombinant *E. coli* (Catching *E. coli*). Catching *E. coli* traps cadmium on the outer membrane. This *E. coli* produces outer membrane protein (Ag43) fused with the heavy metal binding protein (metallothionein).

In doing so, cadmium will be caught and released without killing *E. coli* itself. But *E. coli* was going away from cadmium that was proven by our experiment.

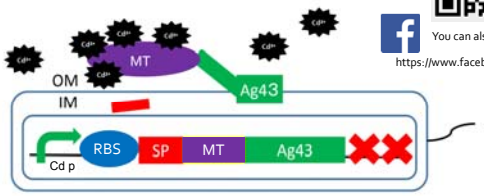
We had an idea to use chemotaxis factor to go toward cadmium. So we created another kind of recombinant *E. coli* (Gathering *E. coli*) that produces aspartic acid, which is a positive chemotaxis factor for *E. coli*. Therefor, both *E. coli* were gathered to the cadmium spot. We characterized the Gathering *E. coli* whether or not this *E. coli* synthesizes aspartic acid and releases in the media in the presence of cadmium ion.

Overview

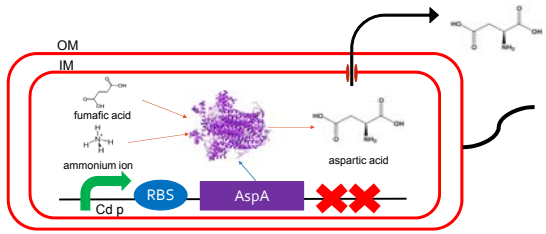


(A) In the case only Catching *E. coli* is put in the system, they go away from cadmium, because they show negative chemotaxis against cadmium. Consequently, they can't catch cadmium effectively. (B) both Gathering *E.coli* and Catching *E. coli*, we can build the collecting system which permits to gather both cells toward the spot of cadmium.

Systems



Catching *E. coli*, in which the construct was illustrated as above, can have the cadmium-trap protein on the outer membrane. The protein consists of a fusion of metallothionine (MT; *SmtA*) with *adventitia* protein (*Ag43*), one of outer membrane proteins, and a signal peptide (SP) of *Ag43*. The genetic origin of *SmtA* is *Synechococcus* PCC. 7942

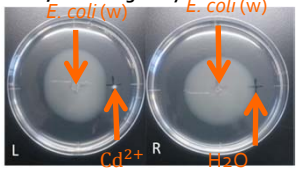


Gathering *E. coli*, in which the construct was illustrated as above (BBa_K1342001), can synthesize aspartic acid that is controlled by the cadmium-dependent promoter. It cosists of zinTp (BBa_K1342005), RBS (BBa_B0034),and *AspA* (BBa_K1342002) with double terminators (BBa_B0015). ZinTp is a promoter inducing gene expression only in presence of cadmium ion. The *AspA* encoding aspartase synthesizes aspartic acid from fumaric acid and ammonium ion.

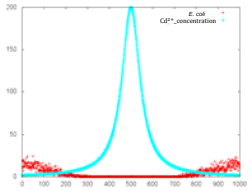
Results

Swarming Assay

***E.coli*, swarming away from cadmium**

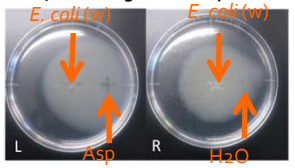


L: cadmium ion (100 mM, 4 µL)
R: H_2O (40 µL)
Incubation: 110 hours
Temperature: 30 ℃

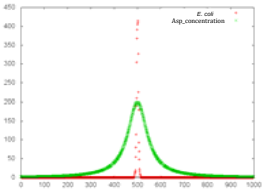


This graph shows the negative chemotaxis profile of *E.coli* by cadmium ion. Assuming that cadmium is spotted at the center of "swarming plate"and *E.coli* shows negative chemotaxis for cadmium, *E.coli* cells were split away from the spotted point.

***E.coli*, swarming toward aspartic acid**

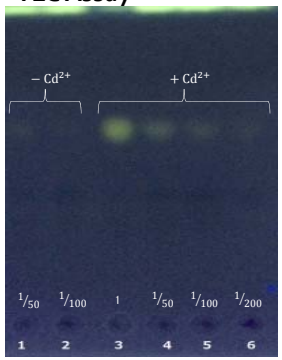


L: Aspartic acid (10 mM, 40 µL)
R: H_2O (40 µL)
Incubation: 108 hours
Temperature: 30 ℃

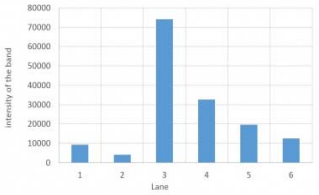


This graph shows the positive chemotaxis profile of *E.coli* by aspartic acid. Assuming that aspartic acid was spotted at the center of "swarming plate" and *E. coli* shows positive chemotaxis for aspartic acid, *E.coli* cells were gathering to the spotted point.

TLC Assay



Analysis of aspartic acid synthesized by *E. coli* (JM109/BBa_K1342001) by TLC
no addition of cadmium culture (diluted at 1:50 by medium (lane1) and 1:100 (lane2) additon of cadmium ion (250µM) no diluted by medium (lane3) diluted 1:50 (lane4), 1:100 (lane5) and 1:200 (lane6).



Estimation of aspartic acid content in spot Each intensity of spots indicating the content of aspartic acid, was estimated by Image J.

Advancement

This graph shows the positive chemotaxis profile of *E.coli* by aspartic acid. Assuming that aspartic acid was spotted at the center of "swarming plate" and *E. coli* shows positive chemotaxis for aspartic acid, *E.coli* cells were gathering to the spotted point.

Policy & Practice

We participated in "The 86th Annual Meeting of The Genetics Society of Japan at Nagahama Institute of Bio-Science and Technology in September 17th-20th, 2014". We proposed the workshop of synthetic biology and invited the general public, iGEM Teams (UT-Tokyo, Kyoto, Osaka, Hokkaido_U, TMU-Tokyo, Tokyo_Tech and Gifu), researchers and instructors of the synthetic biology there, because we wanted to know and learn more about synthetic biology and their societies.

Far future work !
We would like to go further forward to establish the system working in the filed of chemo-attractants like aspartic acid.

Cosmo Bio Co., Ltd.
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