

Mosquito colonization

Tuesday, October 10, 2017 3:21 PM

Transformation for mosquito colonization

Date: 09/30/17

Name of Task: Transformation of *P. agglomerans* and *E. coli* MG1655 with pSB1C3RFP and interlab plasmid 21H

Who did it: Guilherme and Mireia

Where was it done: Luiziana's Lab, ICB-USP

Methods: (done according to iGEM transformation protocol and Competent cell test *Pantoea agglomerans* (08/18/17))

Quimiocompetent *P. agglomerans*

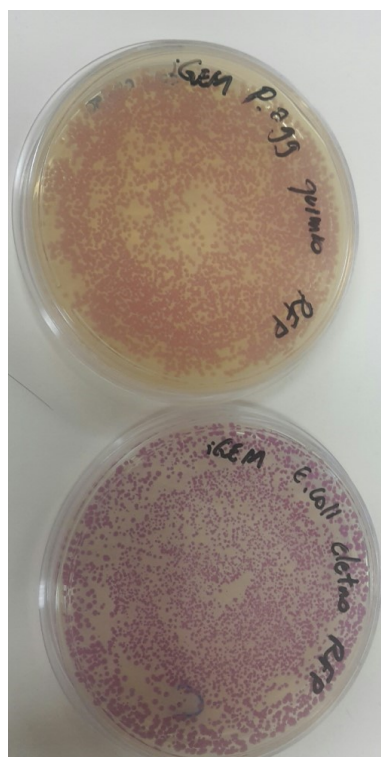
Quimiocompetent *P. agglomerans* from 08/18/17 were pipetted in a 1.5 mL tube together with the respective DNA (pSB1C3rfp/interlab plasmid 21Hgf)

[iGEM heat shock transformation protocol](#) (modified to 5 μ L DNA and 50 μ L electrocompetent cells)

Electrocompetent cells

2 μ L of DNA were pipetted into the electroporation cuvette. 100 μ L of electrocompetent cells (on ice thawed) were pipetted on the DNA drop. The cuvette was flicked to mix, dried with a paper towel and electroporated. (1mm cuvette, 1800V, 5ms)

The electroporated cells were incubated for 1 h at 37°C and 150 rpm in LB medium in a 1.5 mL Eppendorf tube. After the incubation time, the tubes were centrifuged at 13000 rpm for 3 min, resuspended in 100 μ L and plated on LB Chloramphenicol (35,5 g/L) plates.



Result: *E. coli* and *P. agglomerans* transformed with pSB1C3RFP

Date: 10/02/17

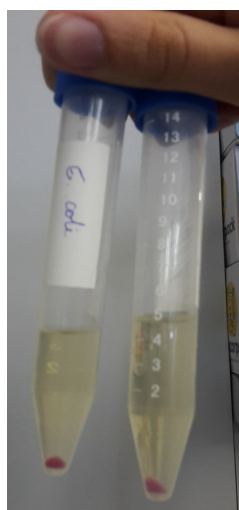
Name of Task: Inocule preparation of *P. agglomerans* and *E. coli* MG1655 with pSB1C3RFP

Who did it: Karent and Mireia

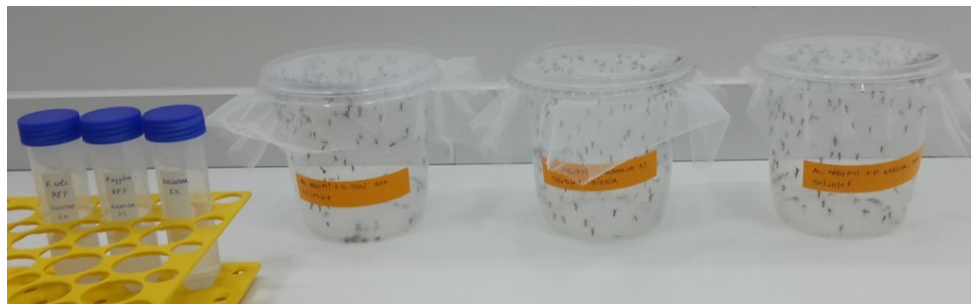
Where was it done: Margareth and Luiziana's Lab, ICB-USP

Methods: (done according to Wang *et al.*, 2017 protocol)

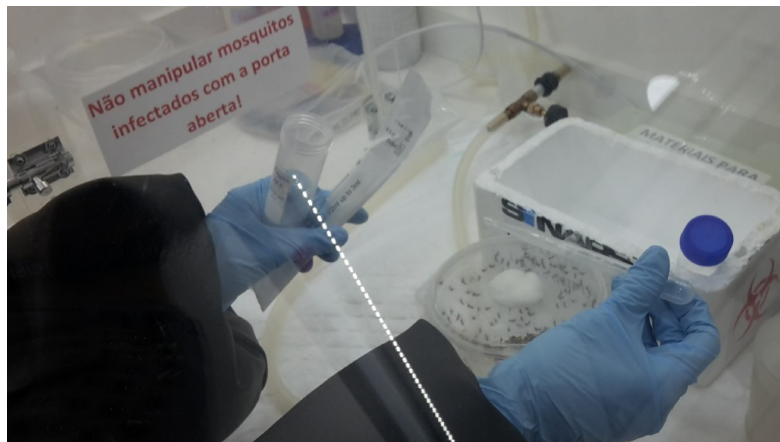
The *E. coli* MG1655 and *P. agglomerans* transformed were cultured in LB broth and on agar plates at 28°C. Bacteria cells were harvested by centrifugation (3,000 g, 10 min), washed twice in **sterile phosphate buffered saline (PBS)**, and resuspended in 5% (wt/vol) sterile sucrose solution to obtain 10⁷ cells/ml. The bacterial suspension was added to cotton pads and provided to adult mosquitoes for 8 days, and then bacterial cotton pads were replaced with new cotton pads containing 10% sucrose solution later of 48 hours.

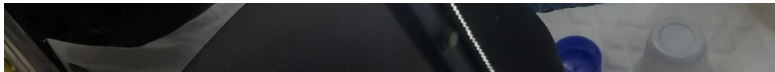


Preparation of bacterial suspension



Bacterial suspension with the adult mosquitoes for the colonization test





Inoculation of bacterial suspension in the adult mosquitoes

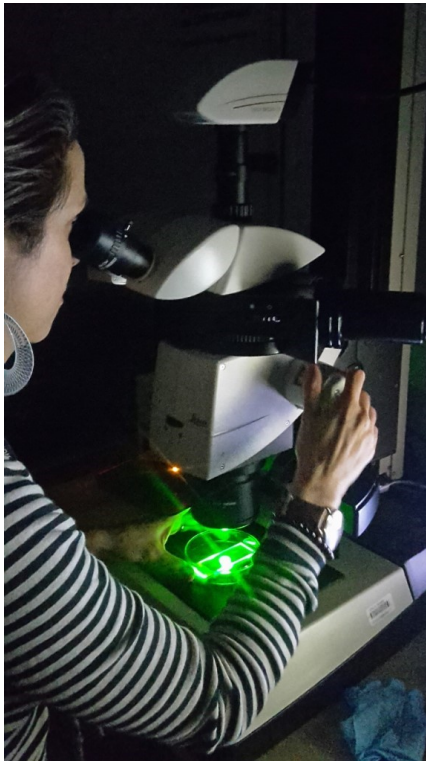
Name of Task: Colonization and quantification of *P. agglomerans* and *E. coli* MG1655 with pSB1C3RFP in mosquitoes

Who did it: Karent, Mireia, Ga and Natalia

Where was it done: Margareth's Lab, ICB-USP

Methods: (done according to Wang *et al.*, 2017 protocol)

To investigate colonization of *P. agglomerans* and *E. coli* MG1655 in different tissues of mosquitoes, adult mosquitoes were fed for **48 h** on a cotton pad with 5% sterile sucrose solution and containing 10^7 cells/ml of *P. agglomerans* and *E. coli* MG1655 with pSB1C3RFP. The bacteria-fed mosquitoes later were feed on a 10% sucrose solution. The midguts and ovaries were dissected and homogenized in sterile PBS. The fluorescent of RFP protein was confirmed by fluorescent microscopy.

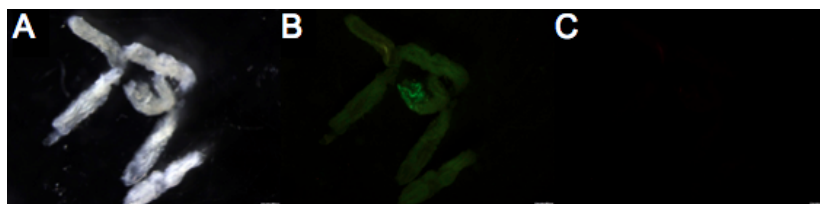




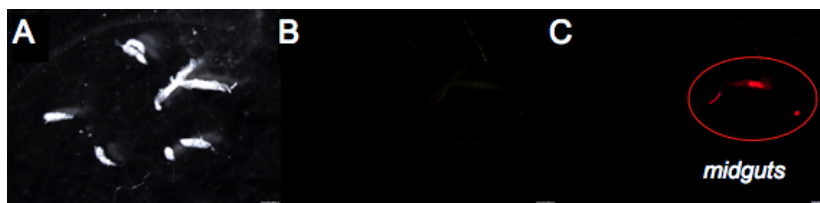
Results



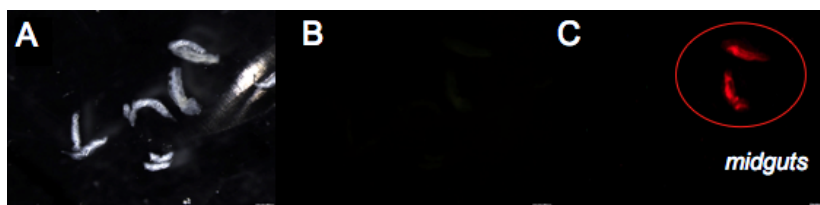
Five ovaries of mosquitoes fed with sacarose to 24h (Negative control). A five ovaries microscope, B five ovaries with green filter, C five ovaries with red filter.



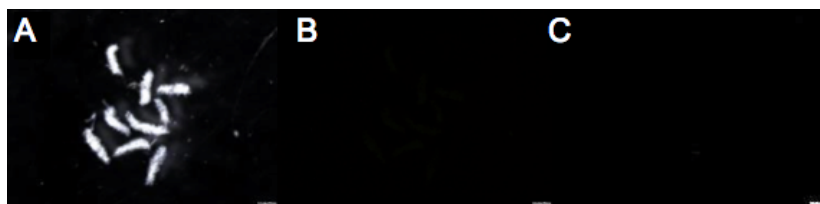
Six midgut of mosquitoes fed with sacarose to 24h (Negative control). A Six midgut microscope, B Six midgut with green filter, C Six midgut with red filter.



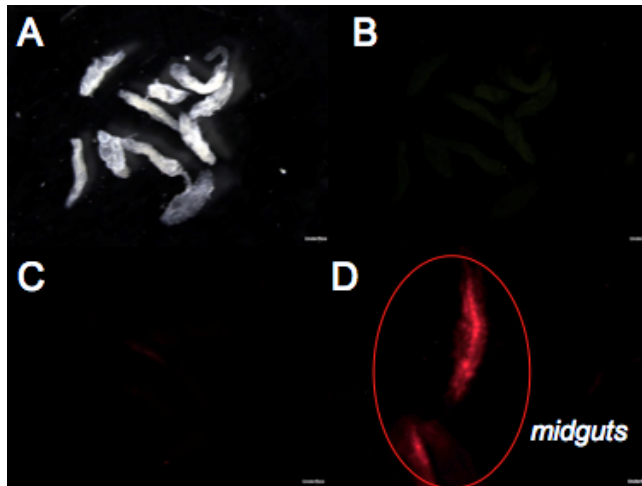
Six ovaries and two midgut of mosquitoes fed with *P. agglomerans* to 24h. A Six ovaries and two midgut microscope, B Six ovaries and two midgut with green filter, C Six ovaries and two midgut with red filter.



Seven midgut and two ovaries of mosquitoes fed with *P. agglomerans* to 24h. A Seven midgut and two ovaries microscope, B Seven midgut and two ovaries with green filter, C Seven midgut and two ovaries with red filter.



Nine ovaries of mosquitoes fed with *E. coli* to 24h. A Nine ovaries microscope, B Nine ovaries with green filter, C Nine ovaries with red filter.



Ten midgut of mosquitoes fed with *E. coli* to 24h. A Ten midgut microscope, B Ten midgut with green filter, C Ten midgut with red filter, D two midgut with red filter from another spot.

We confirmed the colonization of *P. agglomerans* and *E. coli* strains in the midgut of female mosquitoes fed by 24h with the cotton pads containing the bacterials. The others samplings did not show the colonization of the mosquitoes.