Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen

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1. Background & Introduction
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Synthetic biology

Synthetic biology aims to engineer genetically modified biological systems that perform novel functions that do not exist in nature, with reusable, standard interchangeable biological parts. With engineering framework in place, synthetic biology has the potential to make the construction of novel biological systems a predictable, reliable, systematic process.

Pseudomonas aeruginosa

P. aeruginosa, Gram-negative bacteria, widely found in nature, can cause wound infection and suppurative lesions. Pseudomonas aeruginosa , widely distributed in nature and normal skin, intestinal and respiratory tract, is a common clinical pathogen.

The pathogenicity of *P. aeruginosa*

P. aeruginosa colonizes the respiratory and gastrointestinal tract, and causes life-threatening infections to the patients with immunodeficiency such as cystic fibrosis and cancer. P. aeruginosa is still among the leading causes of nosocomial infection primarily because it is intrinsically resistant to many antibiotics and antimicrobials, in part because of its effective efflux systems .

Pyocins S5

Pyocins are narrow-spectrum bacteriocins produced by *P. aeruginosa*. Pyocins are classified into three types: R, F, and S. An earlier study estimated that R and F type pyocins are synthesized by 90% of all P. aeruginosa strains and S type by 70%.

E7 lysis protein

The E7 lysis protein is a key component of the SOS response system in colicinproducing cells and functions to export bacteriocins into the extracellular space under stressful environmental conditions.

The final system

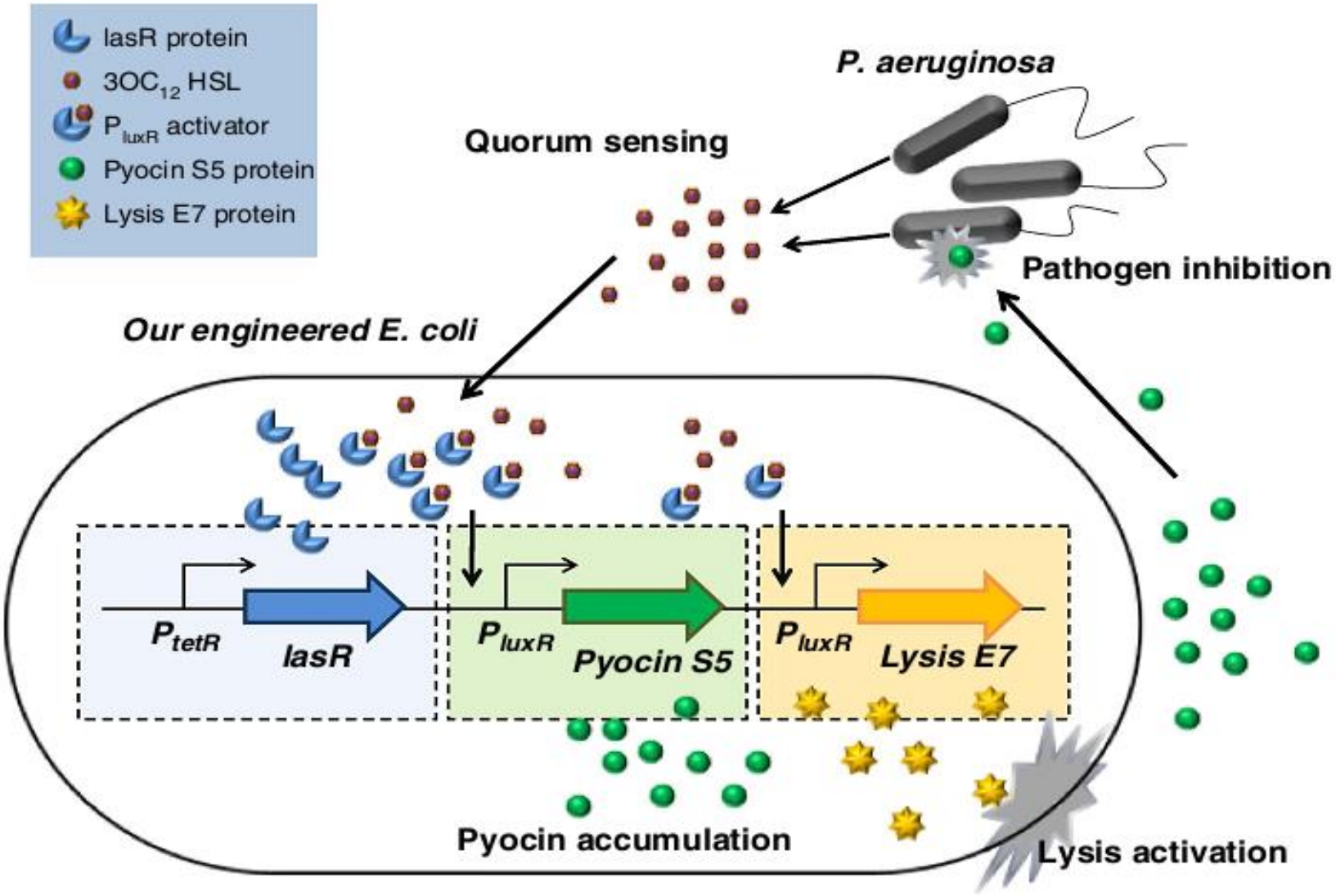
The final system was designed to:

(i) detect AHLs produced by *P. aeruginosa*;

(ii) produce pyocin S5 upon the detection;

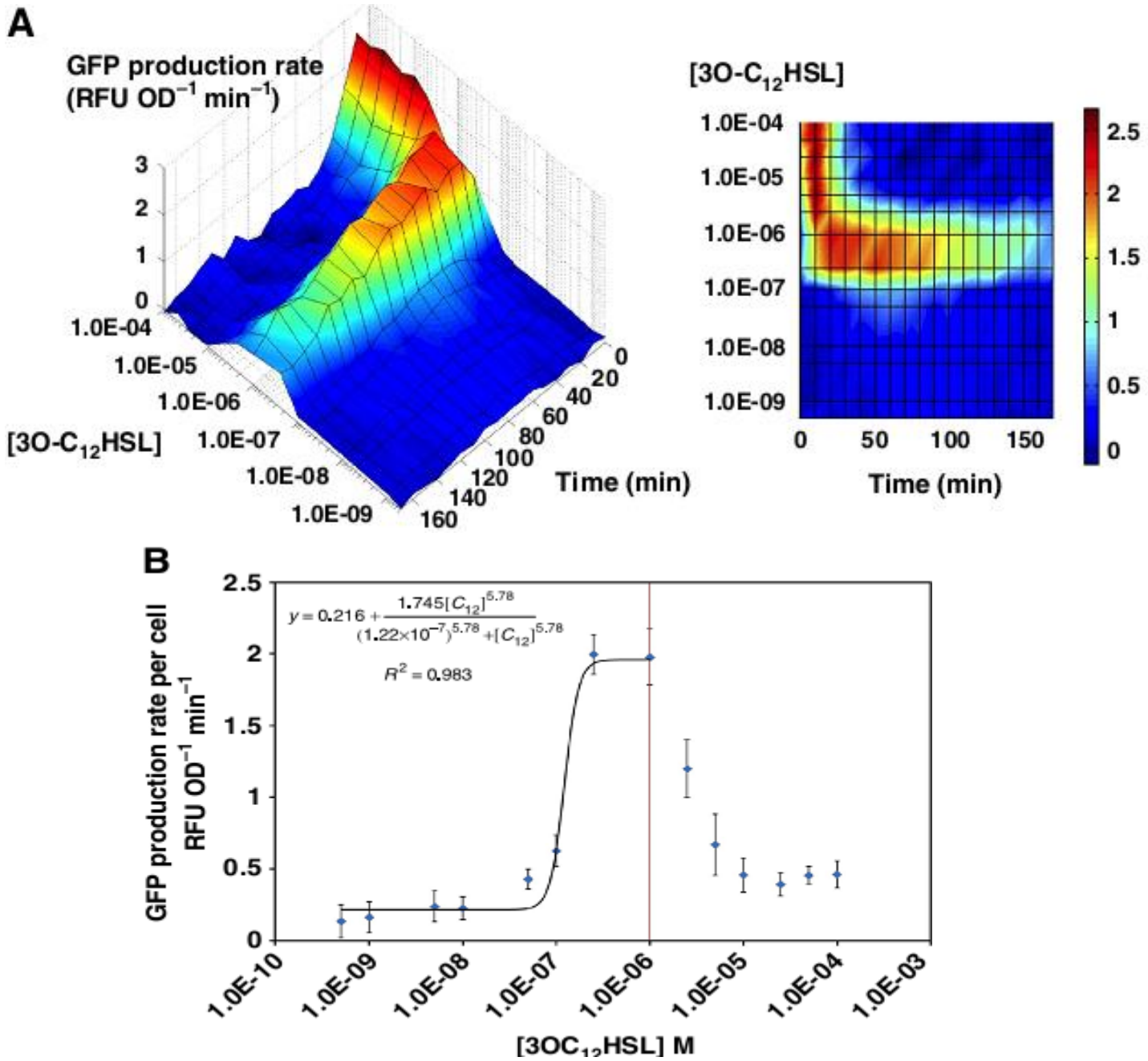
(iii) lyse the E. coli cells by E7 lysis protein so that the produced pyocin S5 is released from the cells , leading to the killing of *P. aeruginosa.*

Schematic of Pathogen Sensing and Killing system



Characterization of the sensing device

To evaluate and characterize the sensing device, the gene encoding GFP was fused to the sensing device (pTetR-LasR-pLuxR-GFP) and the GFP expression was monitored at a range of concentrations of 3OC12HSL.


A
GFP production rate
(RFU OD^{-1} min^{-1})
3
2
1
0
1.0E-04
1.0E-05
1.0E-06
1.0E-07
1.0E-08
1.0E-09
[3O-C$_{12}$HSL]
0
20
40
60
80
100
120
140
160
Time (min)
[3O-C$_{12}$HSL]
1.0E-04
1.0E-05
1.0E-06
1.0E-07
1.0E-08
1.0E-09
0
50
100
150
Time (min)
2.5
2
1.5
1
0.5
0
B
$y = 0.216 + \frac{1.745[C_{12}]^{5.78}}{(1.22\times10^{-7})^{5.78} + [C_{12}]^{5.78}}$
$R^2 = 0.983$
GFP production rate per cell
RFU OD^{-1} min^{-1}
2.5
2
1.5
1
0.5
0
1.0E-10
1.0E-09
1.0E-08
1.0E-07
1.0E-06
1.0E-05
1.0E-04
1.0E-03
[3OC$_{12}$HSL] M

Transfer function of the sensing device

One important characteristic of the sensing device was the transfer function that describes the static relationship between the input (3OC12HSL) and output (GFP production rate) of the sensing device.

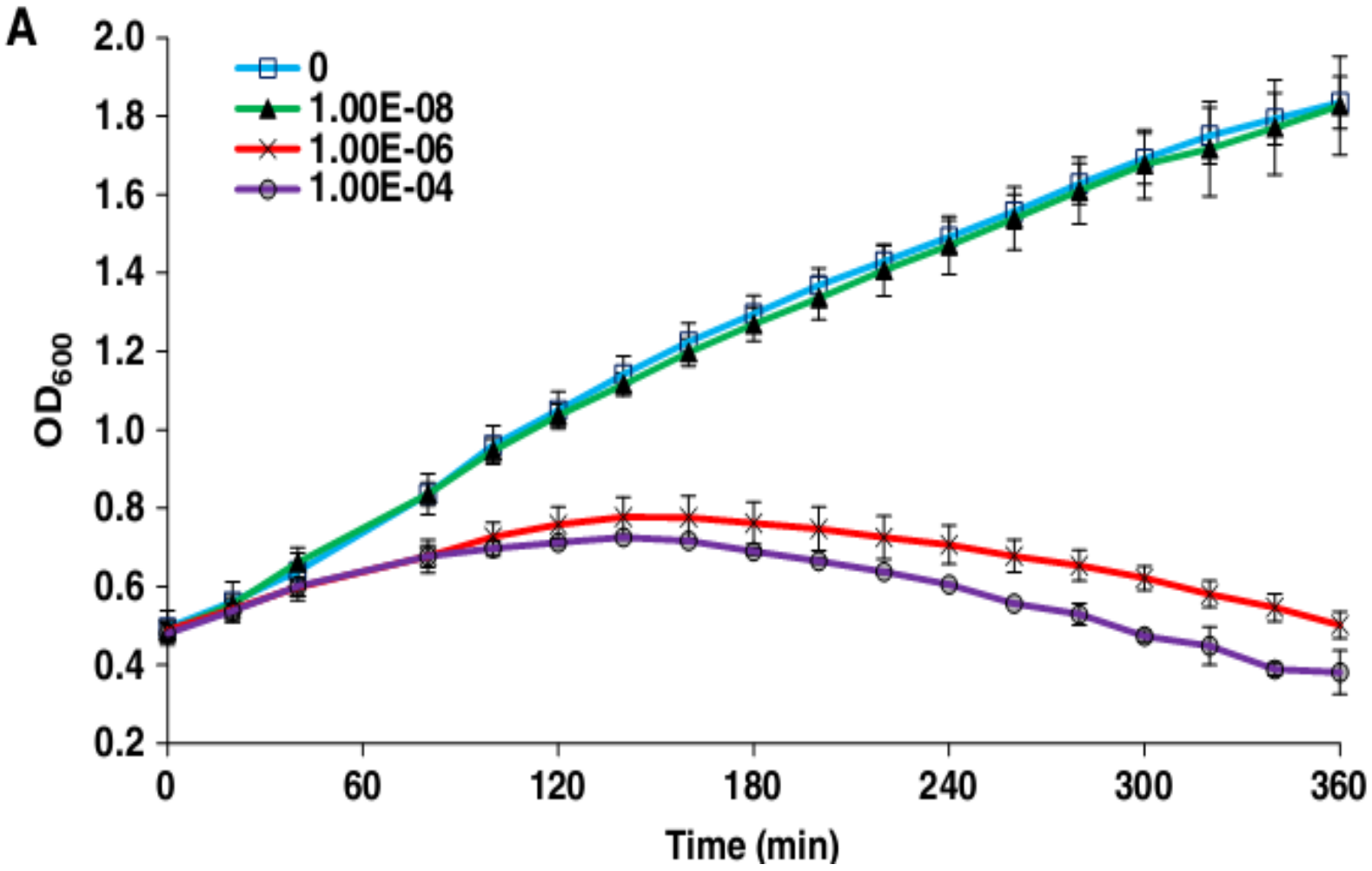
Detection of the native autoinducer produced by *P. aeruginosa*

To verify that the sensing device would be able to sense the amount of 3OC12HSL natively produced by *P.aeruginosa,* the sensing device coupled with a GFP reporter(pTetR-LasR-pLuxR-GFP) was induced using the filtered culture of *P. aeruginosa ln7*, a clinical isolate that is sensitive to pyocin S5. Results show that GFP synthesis rate measured for the isolate ln7 was 1.375 RFU per OD per minute.

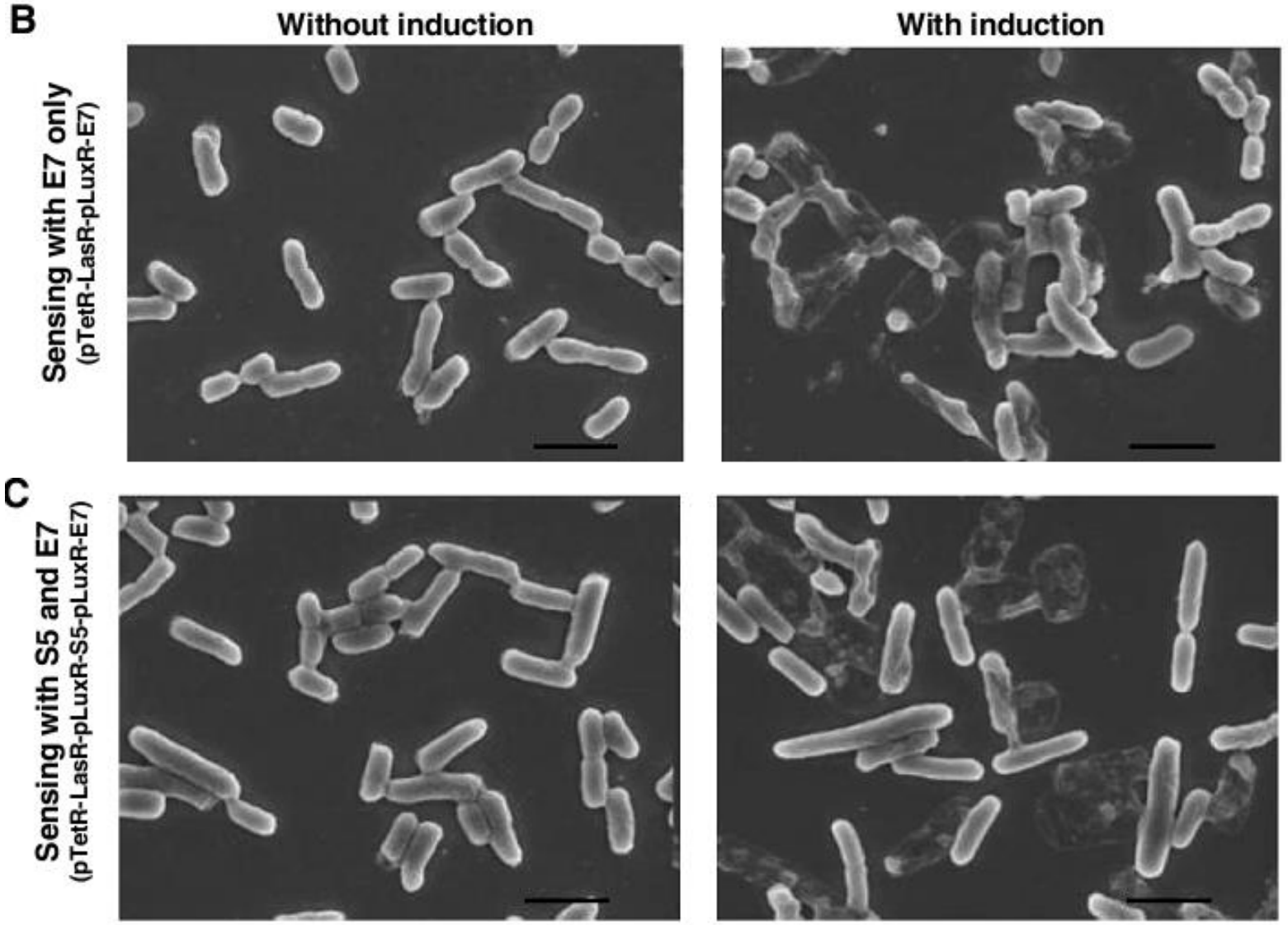
the lysing device

To determine the lysis activity of the system, we characterized the behavior of the E7 lysis protein under the transcriptional control of the sensing device before integrating both the pyocin S5 and E7 genes into the system.

用不同浓度的3OC12HSL诱导表达E7裂解蛋白后的大肠杆菌生长曲线

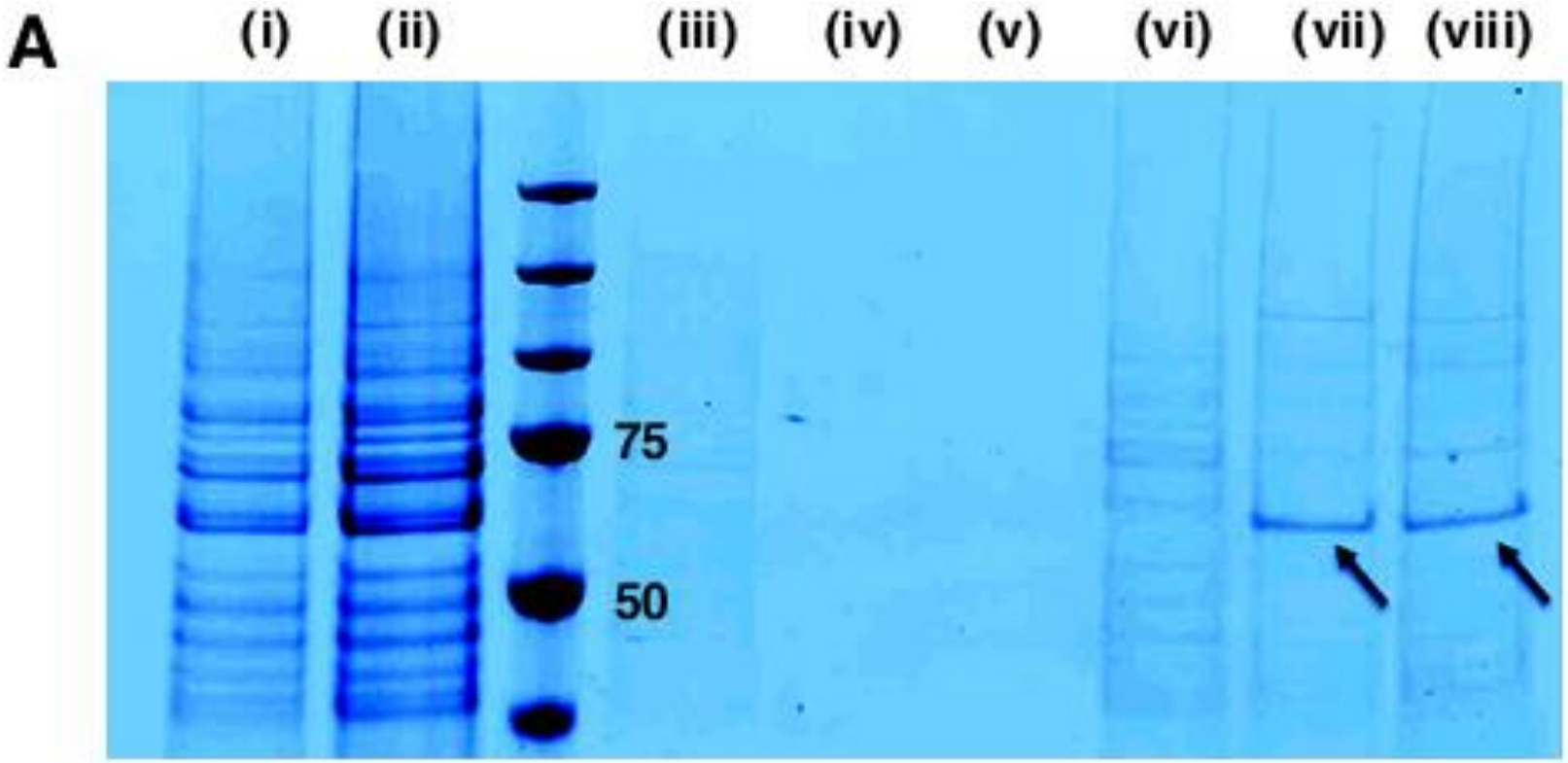


利用FESEM观察裂解蛋白在大肠杆菌表面形态



Verification of the final system with the sensing, killing, and lysing devices

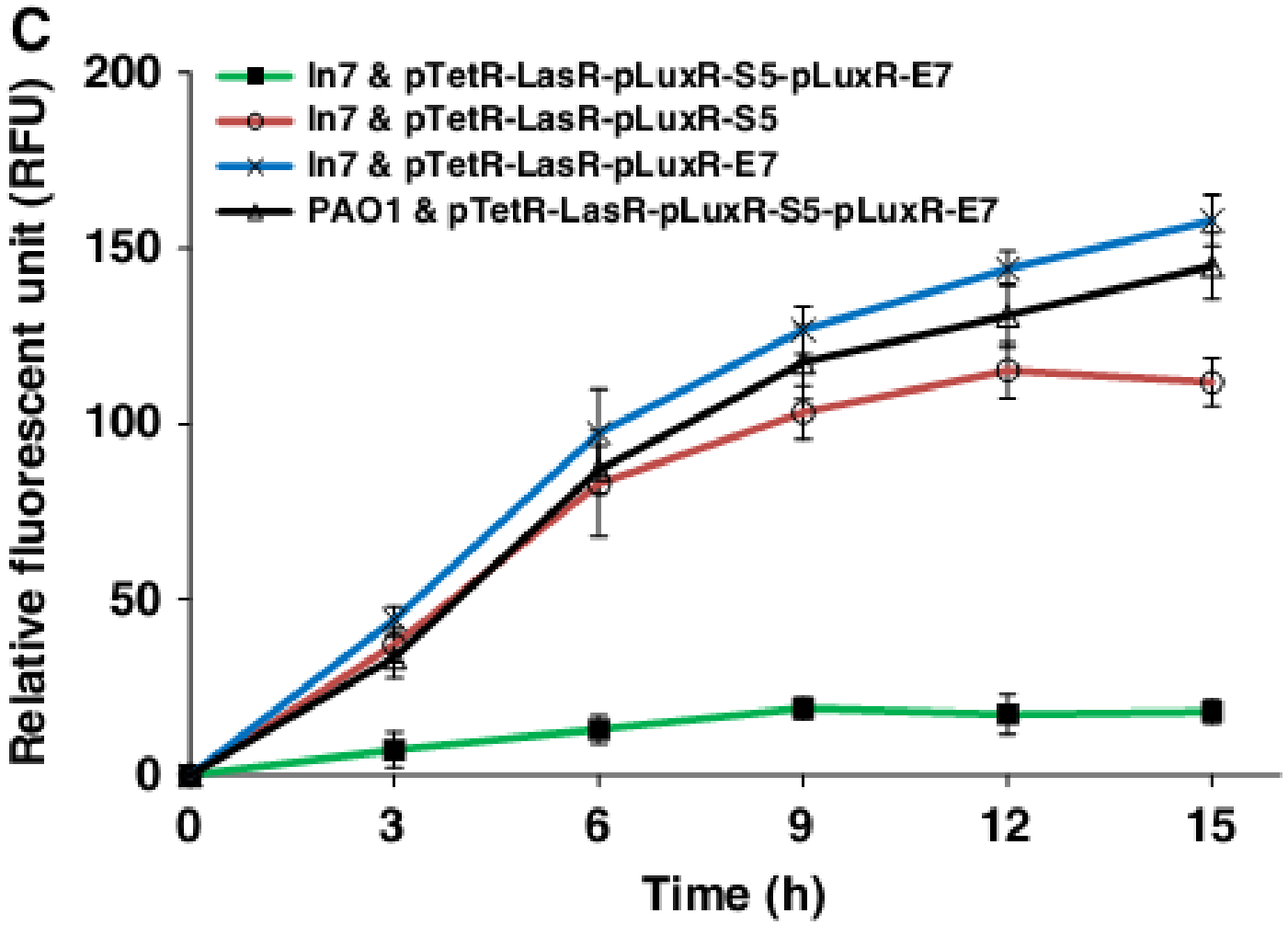
They demonstrated that the engineered *E.coli* are able to sense natively produced AHL 3OC12HSL. To further determine whether the sensing of 3OC12HSL also leads to killing of *P. aeruginosa* as designed, the growth of *P. aeruginosa* was monitored in the presence of the engineered *E. coli* containing the final system.



SDS–PAGE of (i, ii) total extracellular proteins and (iii–viii) IMAC purified His-tagged S5 protein sampled from the extracellular supernatant.

- To verify that our engineered E. coli that contains the final system (pTetR-LasR-pLuxR-S5-pLuxR-E7) exerts a killing activity against *P. aeruginosa* in a mixed culture, we monitored the growth of *P. aeruginosa* co-cultured with the engineered*E. coli* in the ratio 1:4.

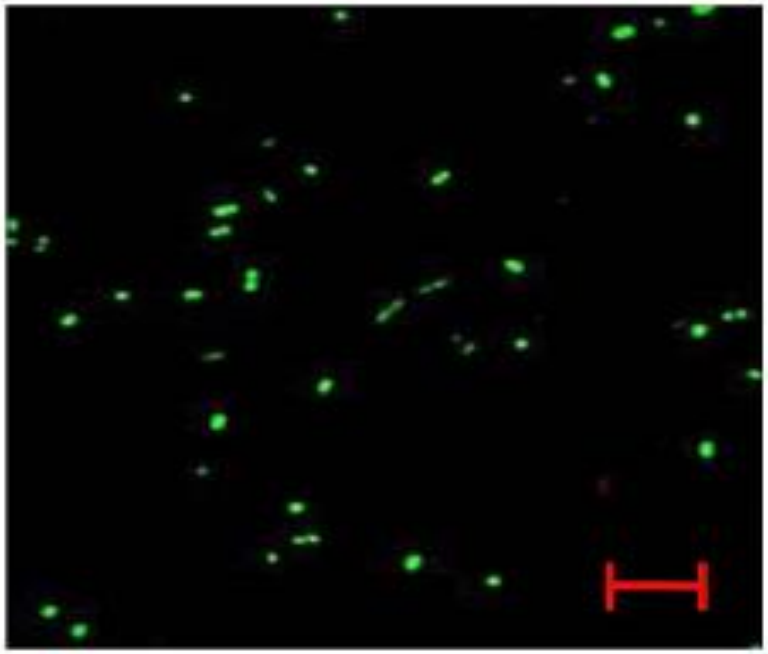
C


Relative fluorescent unit (RFU)
200
150
100
50
0
0
3
6
9
12
15
Time (h)
In7 & pTetR-LasR-pLuxR-S5-pLuxR-E7
In7 & pTetR-LasR-pLuxR-S5
In7 & pTetR-LasR-pLuxR-E7
PAO1 & pTetR-LasR-pLuxR-S5-pLuxR-E7

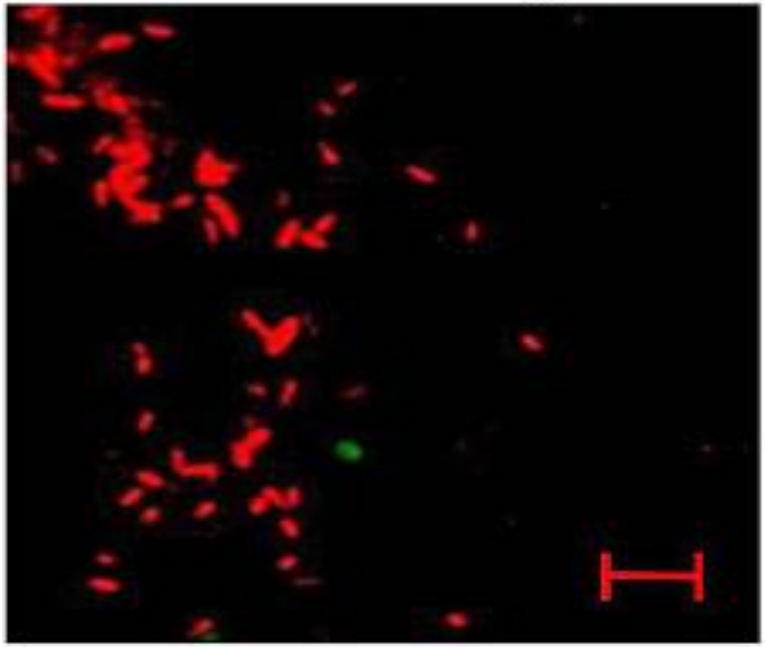
To visualize the extent of biofilm inhibition, biofilm cells with green fluorescence were grown in the presence of engineered *E. coli* on glass slide substrate and examined with confocal laser scanning microscopy (CLSM).

B

Exposed to supernatant of wild-type *E. coli*



Exposed to supernatant of engineered *E. coli* induced with native $3OC_{12}HSL$



Conclusion

This article demonstrated that engineered *E. coli* sensed and killed planktonic *P. aeruginosa*, evidenced by 99% reduction in the viable cells . Moreover, they showed that engineered E. coli inhibited the formation of *P. aeruginosa* biofilm by close to 90%, leading to much sparser and thinner biofilm matrices.

Thank you
for your attention!