Speak from "Synthetic Biology: Licensing Bacteria to Kill"

Andrew Jermy:

Pyocin S5-producing *E. coli* inhibited the growth of *P. aeruginosa* by >99%.

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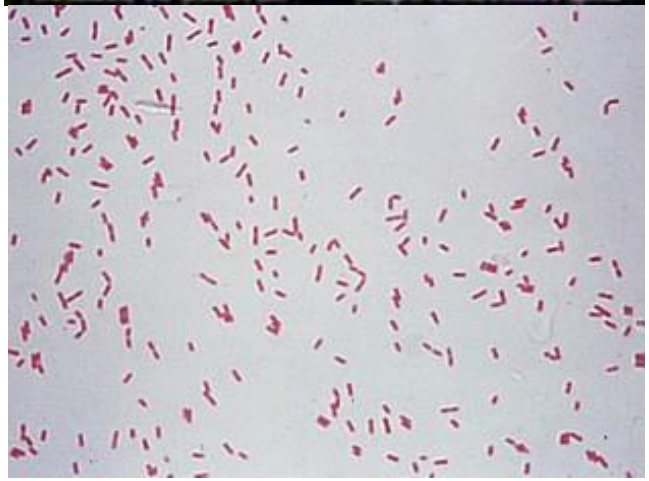
Speaker: Zhi-Ming Xie

Background

Diseases caused by *Pseudomonas aeruginosa* (PA)



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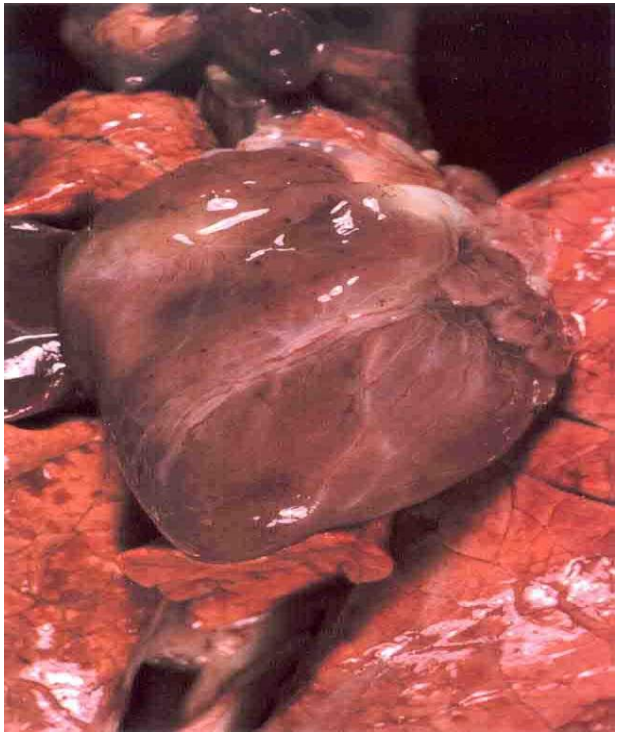

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Several Therapy Strategies

- Antibiotics(one or more species)

  Muti-drug resistance
- Phage therapy: Because of potential employing virus in bacterial infection ,it is limited .
- Development novel ,unconditional antimicrobial strategies is immediately required.

Bacteriocins—Pyocin Type S5

- Ribosomally synthesized antibacterial peptides.
- Specific and effective against closely related species
- New candidate of the next generation antibacterial agent
- Pyocins produced by PA are narrow-spectrum bacteriocins and classfied into 3 types(R,F,S).
- S5 is soluble and consists two parts:the larger can kill while the smaller is prevent the PA from killing (immunity protein).

Quorum sensing

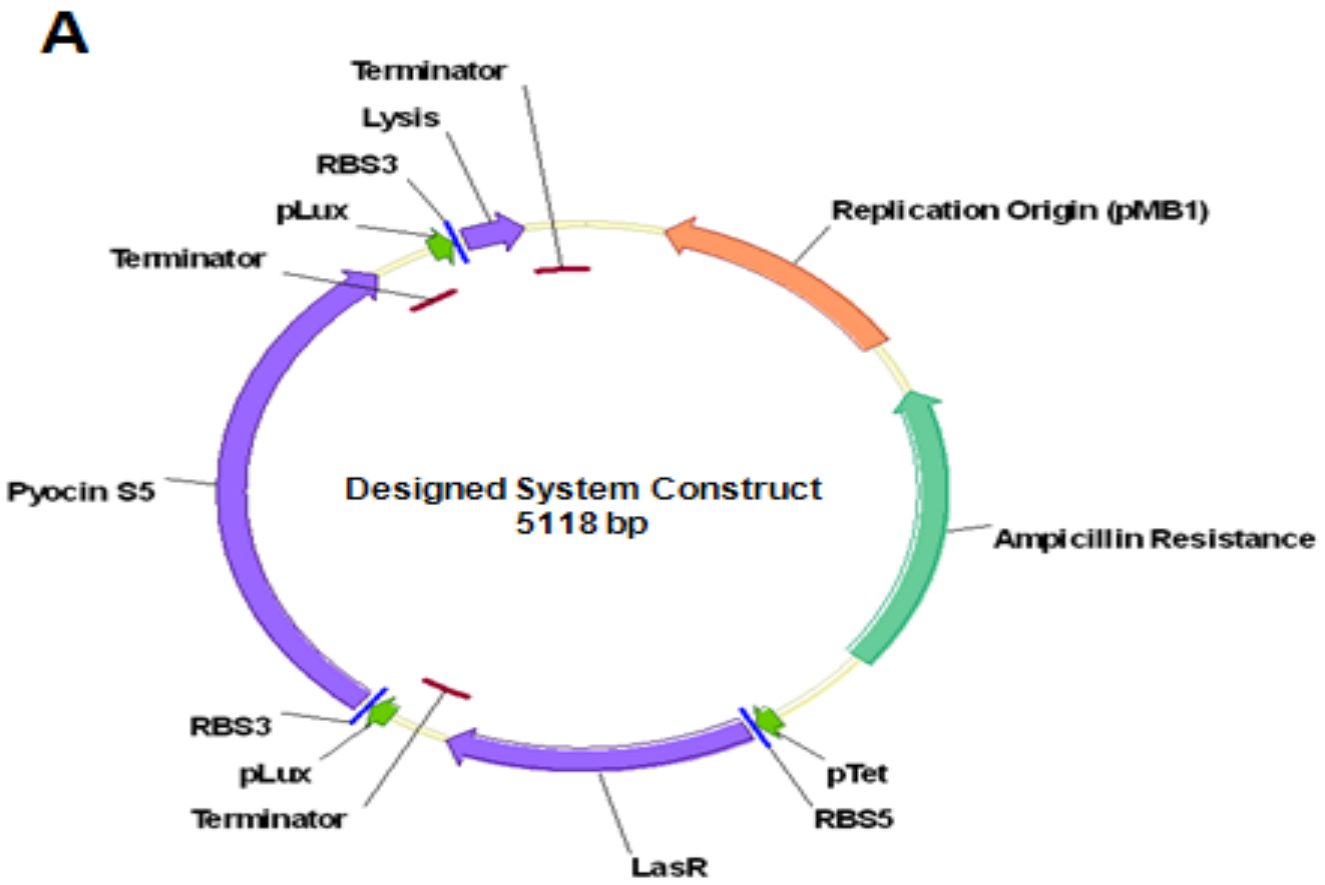
- An organic chemical signaling cascade responsed to different physiological activities(motility)
- PA can produce AHL 3O-C_{12}HSL as the signal.
- Infected Extracellular concentration of AHL 3O-C_{12}HSL is 1.0E-6 to 1.0E-4M.

Goal——Method

Hunter (Detect AHLs)	Detect goal	the Sensing Device
Trigger	Fire	the Lysis Device (E7 Protein)
Bullet	kill PA	the Killing Device (Pycoin S5)

Methods、Results and Discussion

Design of the sensing and killing genetic system



The final engineered system, pTetR-LasR-pLuxR-S5-pLuxR-E7. The system recognizes input chemical signals from *P. aeruginosa* and produces S5 pyocin and E7 lysis proteins.

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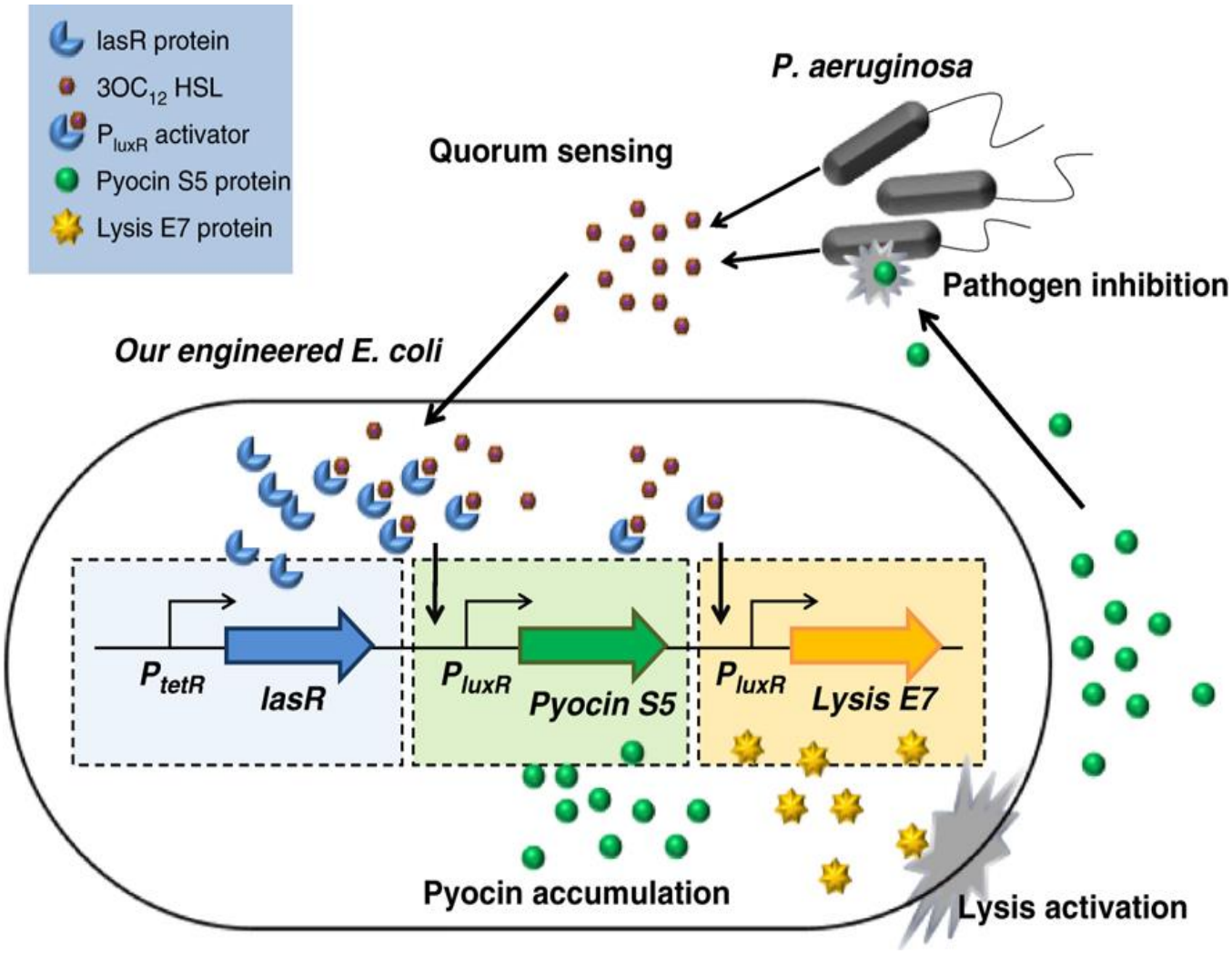


1、$3OC_{12}HSL$ produced by PA caused quorum sensing

2、$3OC_{12}HSL$ can bind tetR expressed constitutively

3、$3OC_{12}HSL$ bind tetR expressed constitutively

Characterization and model of the sensing device (pTetR-LasR-pLuxR-GFP)

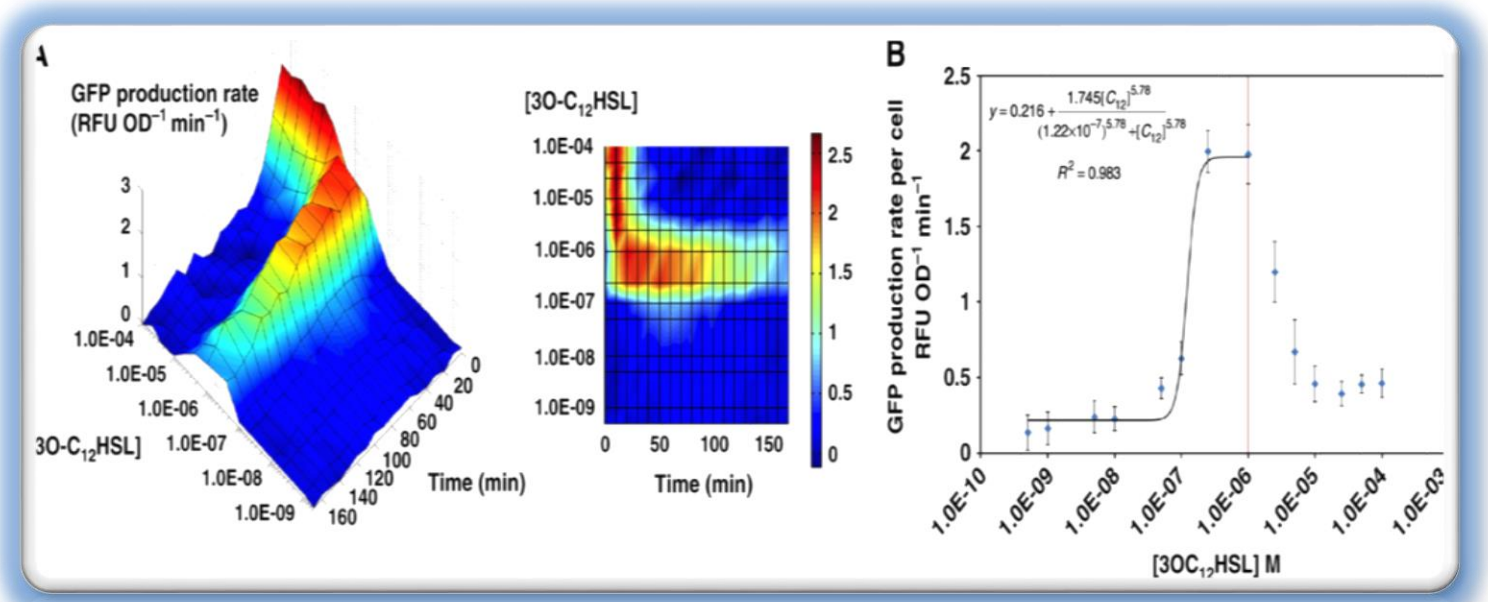


Fig.A：1. 3O-C_{12}HSL ＞1.0E-7M ， GFP sharply increase；
2. 3O-C_{12}HSL＜1.0E-5，GFP sharply decline.

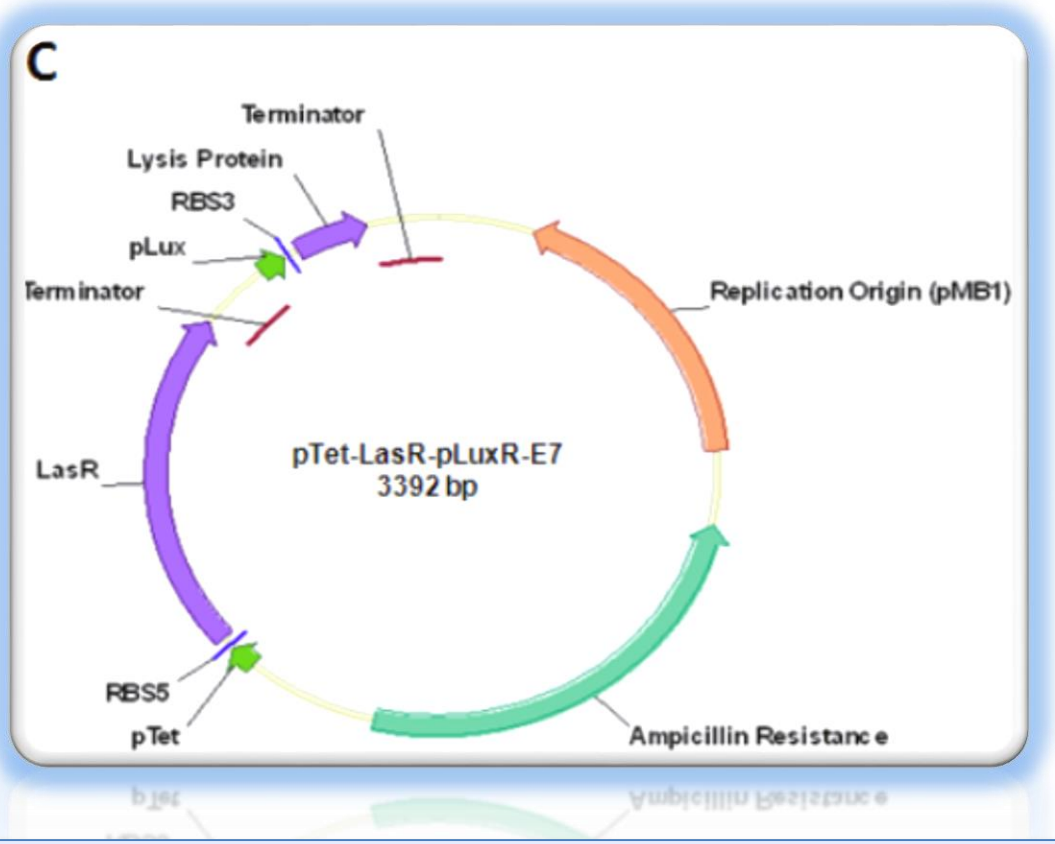
Fig.B：1.The static relationship between input(3O-C_{12}HSL)and output(GFP) is Hill equation(input＜1.0E-6M).
2. 1.0E-6M＜3O-C_{12}HSL ＜3.3E-7M，the output is the maximum(1.96RFU/OD)
3. Half-maximum:1.2E-7M

Summary: The designed sensing device is sensitive enough to detect natively produced by PA(1.0E-6 to 1.0E-4)

Detection of the native autoinducer produced by *P. aeruginosa* (pTetR-LasR-pLuxR-GFP)

- pTetR-LasR-pLuxR-GFP is co-cultured with *P. aeruginosa* ln7(ln7 is sensitive to pyocin S5)
- Result:ln7 was 1.375RFU/OD, between the minimum synthesis rate and the half-maximal of the sensing device.
- Sum: The sensing device can detect the pycoin S5 natively produced by PA.

Characterization of the lysing device (pTetR-LasR-pLuxR-E7)



Methodology: PA and lysis device co-cultured and then induced by 0 to 1.0E-8M $3OC_{12}HSL$. Subsequently , measured the OD_{600} and viewed the cell morphous by FESEM(场发射扫描电子显微镜).

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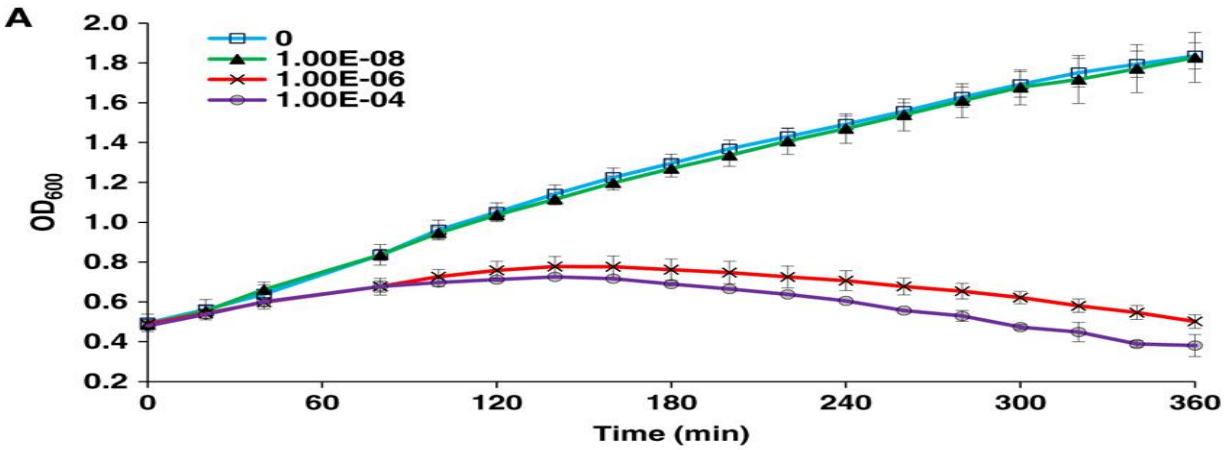

Fig. A: $3OC_{12}HSL$ were 1.0E-6M and 1.0E-4M exhibit the cell growth

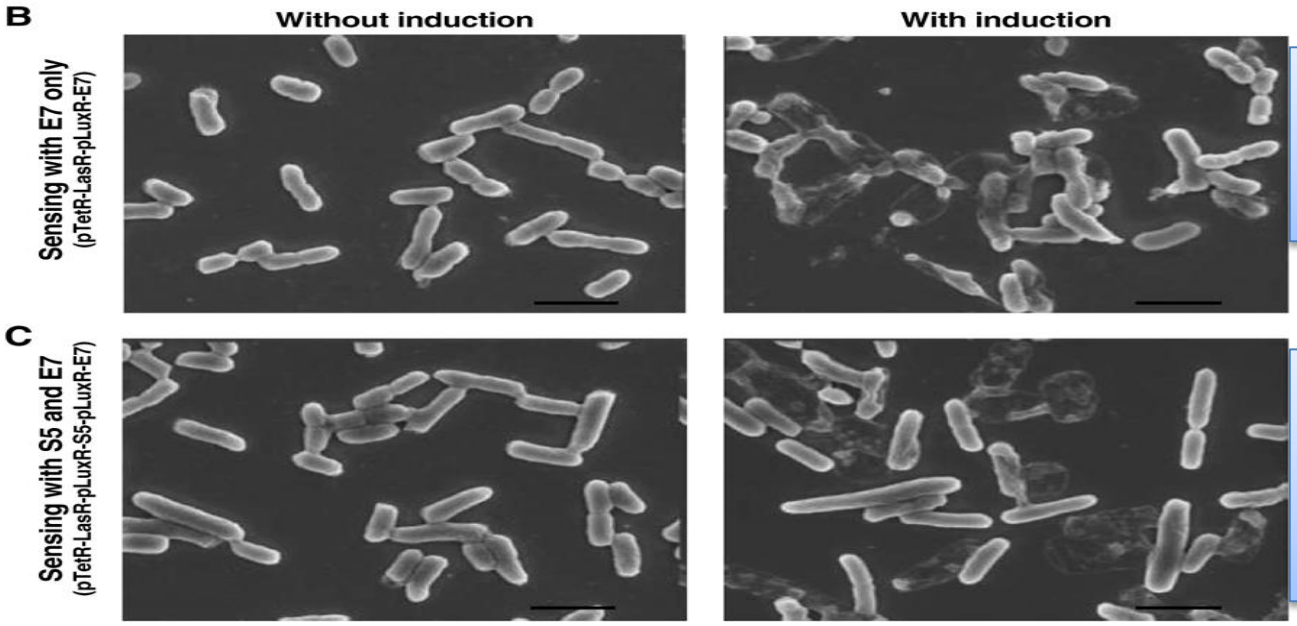

Fig. B: The cell induced by $3OC_{12}HSL$ appeared shriveled with corrugated

Fig. C: PA and the final system co-cultured. The cell induced by $3OC_{12}HSL$ appeared shriveled with corrugated.

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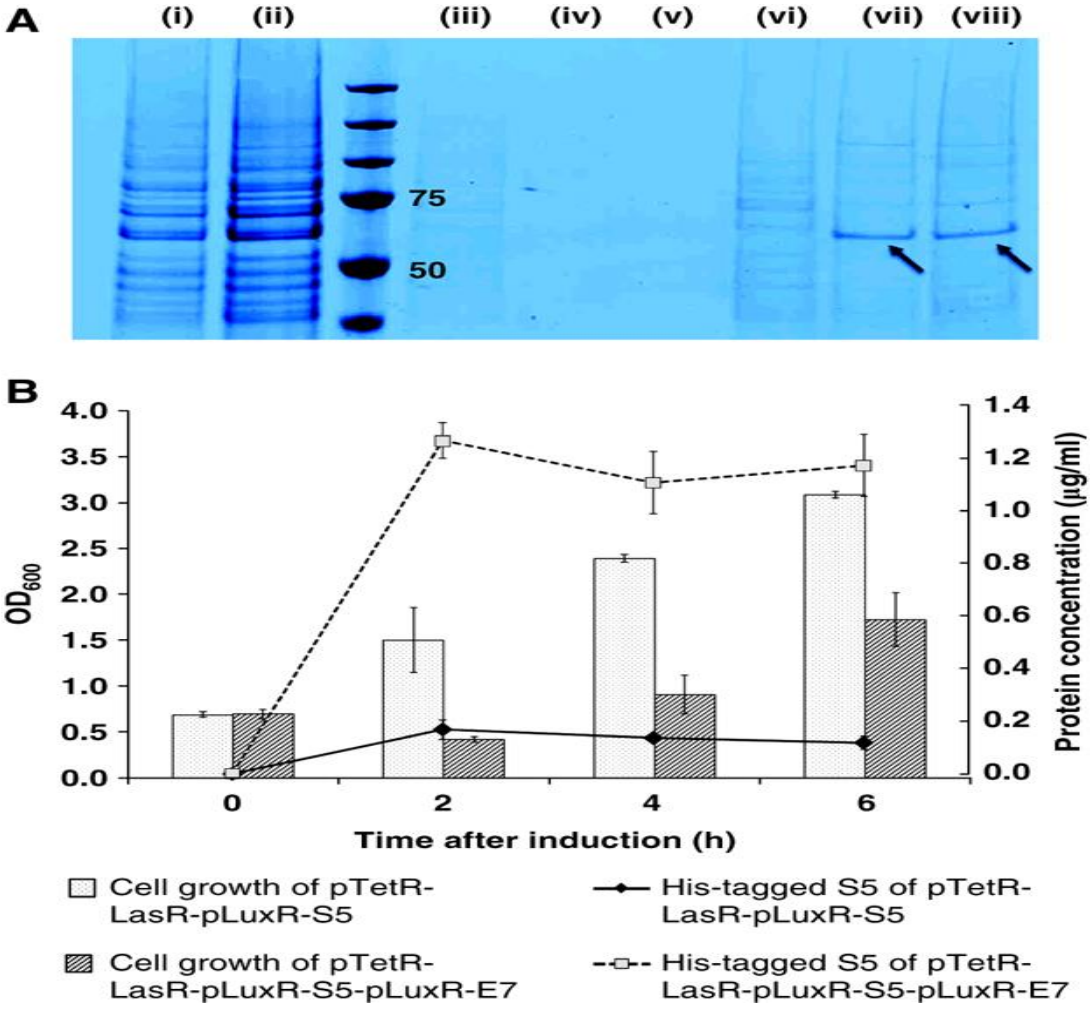


Line：Pycoin S5　　Bar graph：OD_{600}

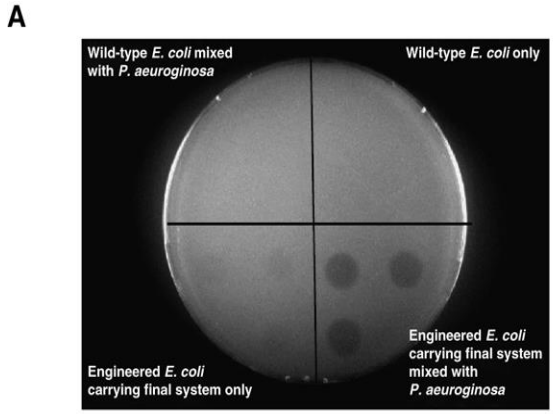
i, iiS5、s5+E7总蛋白；iii–v：S5 诱导0.2.4h后vi-viii：S5+E7诱导0.2.4h后只有S5+E7在诱导2、4h后表达出S5（57kD）

1、impulse release of pyocin S5 at 2 h after induction, followed by a sustained steady-state release in the final system (dotted lines).

2、OD of the final system was characterized by an initial decrease at 2 h after induction, indicative of the onset of lysis, after which the regrowth of engineered E. coli occurs (shaded bar).

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

Fig. A: Only the designed microbe can inhibit PA.



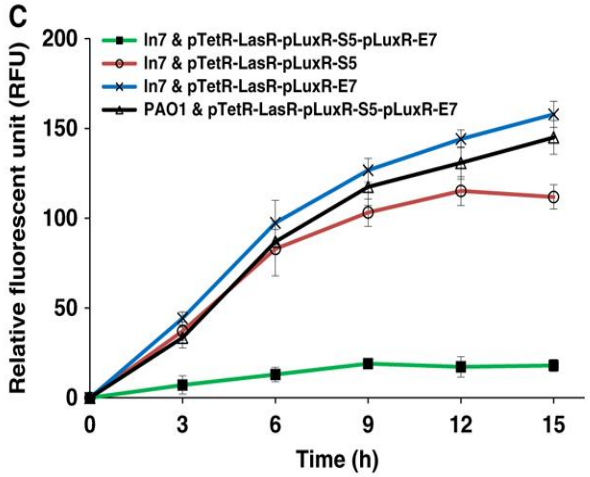
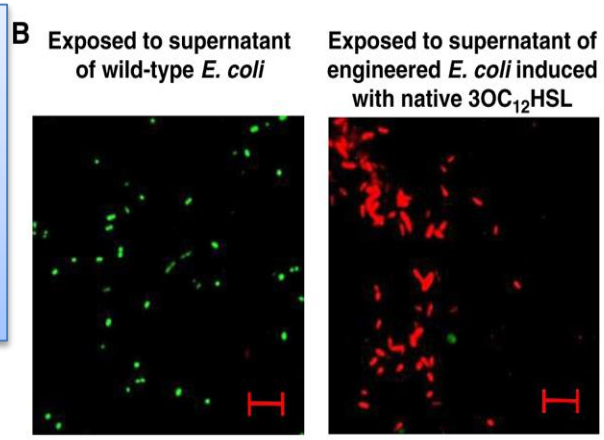


Fig. C: Only the designed microbe can inhibit PA.

Fig. B:LIVE/DEAD Stain.
Dead---PI stained(Red)
Live---SYTO9 stained(Green)



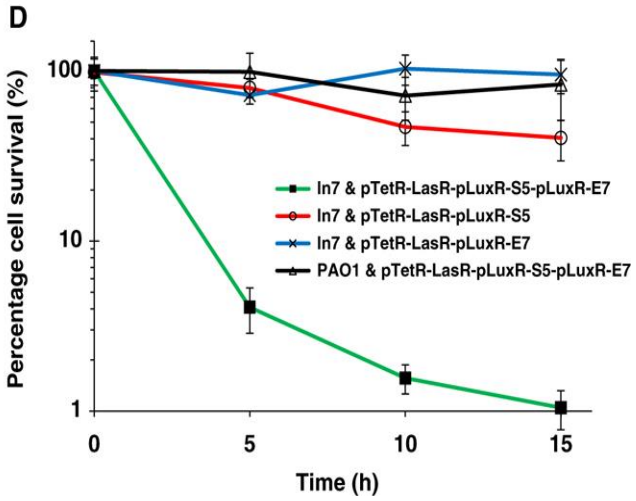


Fig. D: Only the designed microbe can inhibit PA.
（about＞99%）

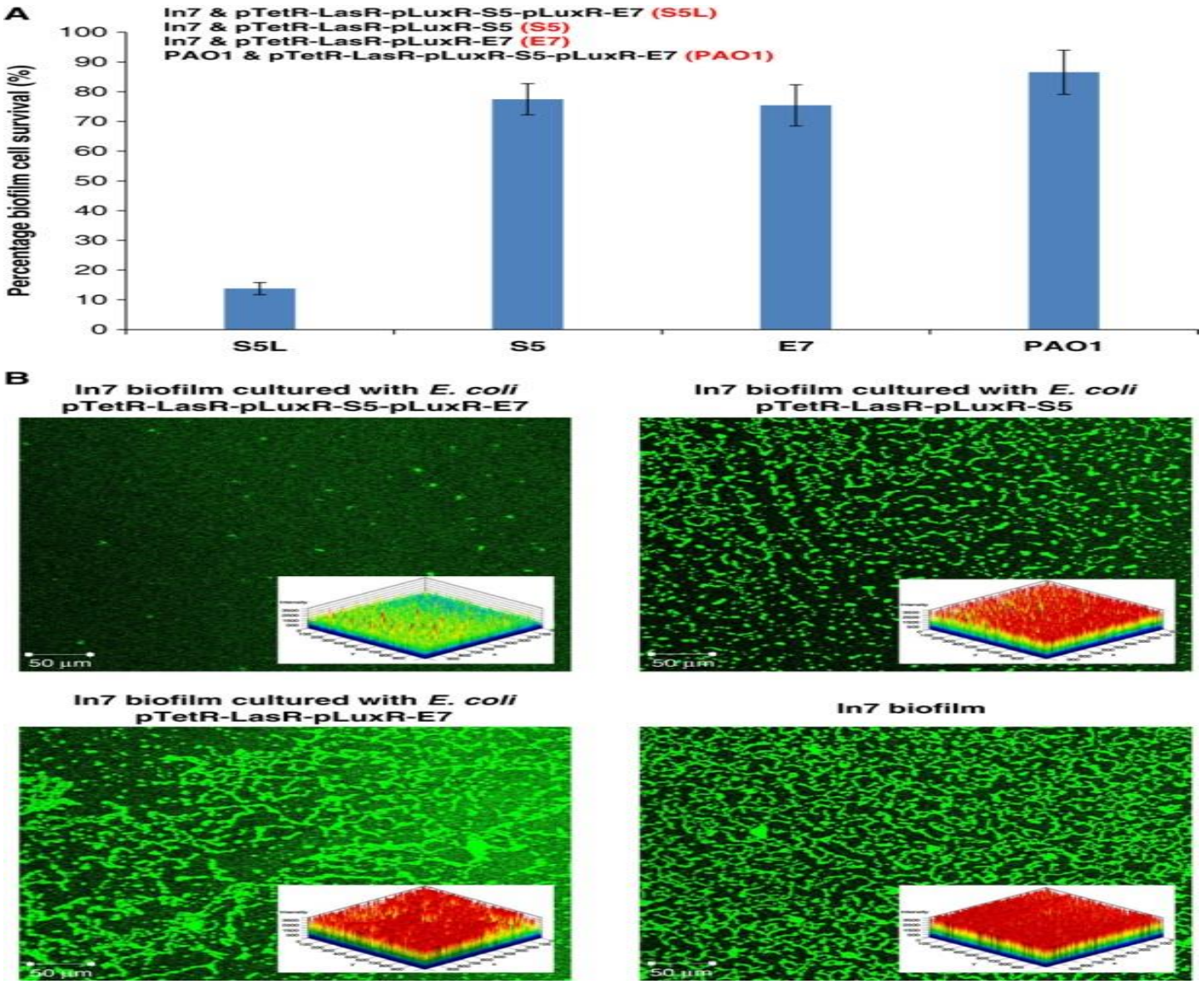


Fig. A: inhibition efficiency is close to 90%.

Conclusions

- 1、The engineered *E.coli* can inhibit biofilm formation during the initial attachment phase and prevent subsequent progression into mature microcolonies（P_{15}）.
- 2、The engineered *E.coli* can effectively inhibitor or kill PA.

Thank you for your patience ！

Now， Let’s Communicate！