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Genetic Circuits and Chemotaxis Induced Bacterial Cloning on Media Plate

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Research Article

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ABSTRACT

Objective: Synthetic biology demonstrates its broad application perspective in the fields of medicine, chemical synthesis, and the production of energy.

Methods: The character that *E. coli* responding to the stimulus is named as chemo taxis which has widely applications such as measurement efficiency of RBS and promoter, suicide mechanism, oscillation timer etc.

Results: A circuit to control the motility of *E. coli* (run or tumble) and form the patterns such as conic curves was constructed. The strength of the promoter and the efficiency of RBS were successfully characterized by using the circuit and chemo taxis that can be used to characterize most of the promoters, the RBS efficiency, terminator efficiency and expression strength of target genes etc. A new suicide mechanism, utilizing the hyperosmotic pressure, was built to induce the growth of *E. coli* Pattern model is the fundamental force in the coordination of multicellular behavior in the bacterial community or a large complex system.

Conclusion: The sources of stress (such as sodium chloride and sucrose) be to generate hypertonic very cheap, convenient and environmentally friendly while antibiotics are expensive and have a bad effect on the environment because of drug-resistant microorganisms.

INTRODUCTION

Synthetic biology is displayed broad application prospect in the field of pharmaceutical, chemical synthesis and energy production. The diversity of their application allowed the exploration of transcriptional regulatory mechanisms and even the development of controllably deliver therapeutics in engineered bacteria [1-4]. The motion of *E. coli* can respond to the stimulation follow the laws. Therefore, precise control can be achieved if we find out the mathematical principles of the motion. Chemo taxis building a deeper understanding of cell differentiation [5]. Cells differentiate and form irregular geometrical organs in their early stage. It remains a secret that how they do this.

The host of the designed network and copy number ratio between the plasmids constructed the network are two main factors that affect the expression of synchronized oscillation. Bacterial chemo taxis [6,7], which is universal in *E. coli*, is defined as migration of bacteria in response to a chemical stimulus. The natural *E. coli* chemo taxis have limited receptor proteins which can

respond to only six kinds of amino acids. Nevertheless, the reprogrammed chemo taxis named pseudo taxis makes the engineered *E. coli* able to respond to molecules, whose receptor proteins do not exist in classical *E. coli*, such as IPTG and L-arabinose, etc.

E. coli have several flagella per cell (4-10 typically), which can rotate in two ways: counterclockwise (CCW) and clockwise (CW) [8]. The former aligns the flagella into a single rotating bundle, causing the bacteria to swim in line, while the later destroy the flagella bundle apart such that each flagellum points in a different direction, reason for the bacterium to tumble. The motility is determined by the phosphorylation state of CheY which is governed by CheZ. In the presence of CheZ, CheY-P is dephosphorylated and produce CheY, thus CheY leads to the flagellar motor rotating CCW resulting in swimming. In the absence of CheZ, CheY is phosphorylated into CheY-P which bind to the flagellar can switch FliM resulting in tumbling [8-10]. Therefore, if no CheZ is expressed (such as *E. coli* CL-1 without cheZ gene), CheY-P couldn't be dephosphorylated so that flagella keep CW, thus *E. coli* keep tumbling and perform non-motile ability on semi-solid culture medium. With enough CheZ expressed, *E. coli* regain chemo taxis ability on semi-solid culture medium. If one kind of molecule (such as IPTG) could stimulate circuit to express CheZ, reprogrammed *E. coli* will have the tendency to migrate to it. We named the reprogrammed chemo taxis pseudo taxis. Therefore, we are able to reprogram bacterial chemo taxis by knocking cheZ gene out of the wild-type genome to control the expression of cheZ by logic gene circuits to manipulate the motion of the cells and let them form patterns such as conic curves.

Conic curves universally exist in nature and are significant for science research, production and living. For example, many planets' orbits are elliptical and parabolic antennas are widely employed in telecommunication [11]. Therefore, we can imitate the orbits of celestial bodies by cell bacterial colony, or form conic curves by precise mathematical laws and apply them in practice. Besides, as aptamers have the potential to respond to almost all kinds of molecules and have already been used to regulate gene expressions such as cheZ to reprogram chemo taxis [12]. In this work, characterizing the circuits we constructed, and mathematical modeling was combined with experiments. It was demonstrated that motile ability is positively associated with the expression strength of cheZ, thus the activity of promoters and efficiency of RBS can be characterized. Meanwhile, a biosafety system was developed which relies on reprogrammed chemo taxis.

Mathematics is the simplest and clearest language, and its value to the development of human civilization is now widely recognized because of its extensive application in science, society and even our daily life. However, the mathematical laws in life sciences are still unclear and even in chaos [13, 14]. Luckily, synthetic biology can overcome these shortcomings on a certain level. Based on this, we design a gene circuit, expecting that mathematical regularities can realize the regulation and control of life activities. We hope our work can inspire people's interests in combining mathematics with synthetic biology.

Methods

Conic curve formation-Parabola and hyperbola

Mathematical definition: In mathematics, hyperbola could be defined as a conic consisting of those points whose distances to a focus, and a directrix, are in a fixed ratio (>1), called the eccentricity (e). Parabola is a conic whose eccentricity is equal to 1.

Hypotheses: Focal points, constant k and the eccentricity ratio e are key factors of conic section. Therefore, we can easily get any eclipse by presetting reasonable focal points with an acceptable constant, as well as parabola or hyperbola by a fixed ratio. Based on these, we need to combine these mathematical concepts with our pattern formation system. If we spot stimulus on semi-solid plate, it will spread from the spotting center out to the periphery. In the spreading process, the concentration is negative correlated to the distance from center. If we draw a line with stimulus, a concentration gradient with square shape will form. And both of the concentration gradients will maintain for a long period. Generally, the stimulus spots are defined as focuses while those lines are defined as directrixes.

There is a threshold ratio of the concentrations of inducer and repressor [15]. This means that more repressor will cause more repression, hence more inducer is needed to relieve the repression, and vice versa. As the concentration of inducer and repressor are directly connected to the concentration of stimulus sources, we can tell the constant k and eccentricity e by combining the relationship between distance and concentration.

Circuit design: The circuit consists of two parts, of which one is named C (constraint), the other is named M (motile) **(Figure 1).** The circuit was transformed into *E. coli* CL-1 which lacks gene lacI and cheZ (ΔlacI, ΔcheZ). In the absence of cheZ, CL-1 adopts non-motile phenotype. Without any exogenous stimulus, *E. coli* will produce background amount of AraC to repress pBAD at limit degree. That means even noL-arabinose involved in, promoter pBAD has leakage expression, so that part C will produce repressor LacI which can bind to the operon of promoter pLac and thus repress its transcription. Because L-arabinose could induce pBAD, within certain concentration range, the more L-arabinose involved in, the more repressor LacI part C could produce resulting the inhibition to chemotaxis. Because of its ability to constrain chemotaxis, this part is named C.

When IPTG involved in, it can relieve the repression from repressor LacI, therefore CheZ is produced to make our engineering bacteria (CL-1) regain motile ability. Within certain L-arabinose concentration range which means certain constraint condition, the more IPTG involved in, the more CheZ is produced leading to stronger motile ability. Because of its ability to make CL-1 motile, this part is named M.

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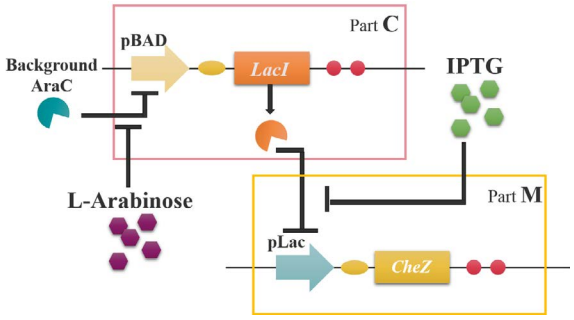

Figure 1. Part C produces LacI to repress the expression of part M. Part M could produce CheZ to make CL-1 regain motile ability.

Materials and methods: Medium: M63 semi-solid medium preparation [16] (Note: Add 0.25 g Agar into an erlenmeyer flask first, then pour into 96 mL H2O, shaking up, continue to add glycerol using pipette. Met, Leu, His, Thr could be premixed for convenience, and the amino acid should be added by injector through 0.22 μm filter membrane. Pay attention that this process must be carried out after high temperature sterilization of the M63 semi-solid medium).

Mix-doting bacteria, inductor and inhibitor on M63 semi-solid medium plate: (i) Pour 20 ml M63 [8] semi-solid medium on a plate, leave the plate air-dried for 90 min in laminar flow clean bench; (ii) Dot 15 μl inductor (IPTG) or inhibitor (L-Arabinose) on the plate through pipette, take care when insert the tip into the medium. Leave the plate air-dried for 20 min in laminar flow clean bench; (iii) Dot 3 μl culture medium at the same position of the inductor (IPTG) or inhibitor (L-Arabinose), leave the plate air-dried for 5 min in laminar flow clean bench; (iv) Seal the plate.

Lineation of inductor and inhibitor on the M63 semi-solid medium plate

(1) Pour 20 ml M63 semi-solid medium on a plate, leave the plate air-dried for 90 min in laminar flow clean bench; (2) Inhale 15 μl inductor (IPTG) or inhibitor (L-Arabinose), doting along the line pre-pianted carefully, bear in mind don't destroy the semi-solid medium when moving alon g the line; (3) Leave the plate air-dried for 90 min in laminar flow clean bench; (4) Repeat the process mix-doting bacteria, inductor and inhibitor on M63 semi-solid medium plate.

Characterization the activity of promoter and efficiency of RBS by chemotaxis

Methods: All three colonies with different promoters were stabbed on the same semi-solid culture medium with 0.02% L-arabione and 50 μg/ml chloramphenicol added in. After 36 hours culturing, difference of chemotactic diameters between each colonies could be distinguished.

Strain culture: (1) Add 50 μl bacterium solution (pLac-RBS (1.0)-CheZ-TT, pLac-RBS (0.3)-CheZ-TT, pLac-RBS (0.01)-CheZ-TT,) into 5 ml fresh LB liquid medium, in which chloromycetin concentration is 50 μg/ml; (2) Culture overnight under the condition of 37 °C, 200 rpm; (3) Add 50 μl bacterium solution from the LB liquid medium above to another 5 ml fresh LB liquid medium, in which chloromycetin concentration is 50 μg/ml; (4) Culture under the condition of 37 °C, 200 rpm for 3 hours. Stock it in 4 °C; (5) Prepare M63 semi-solid medium as above; (6) Draw three dots on a M63 semi-solid medium plate first, and inoculate the three kinds of bacterium solution at the three dots, respectively. The volume of the inoculated bacterium solution is 3 μl; (7) Culture in incubator at 37 °C.

Measurement: (i) Use a ruler to measure the radius of colony from the bottom of the semi-solid medium plate. The initial colony radius is recorded as R1, and the radiuses measured at 12 h, 24 h, 30 h, 36 h, 42 h... as R2, R3, R4, R5, R6, R7 et al.; (ii) Record time and diameter in a table; (iii) Process data with excel.

Black hole

Circuit design: *E. coli* makes use of the EnvZ/OmpR system to reconcile signal transduction in respond to environmental osmolarity changes. EnvZ, a histidine kinase and a response regulator, endured trans-autophosphorylation, the high-energy phosphoryl group is subsequently transferred to OmpR. In our system, the OmpR-controlled promoter (pOmpR) was involved in **(Figure 2).** The expression strength of pOmpR depends upon the medium osmolarity. The EnvZ will phosphorylate more OmpR into phosphorylated OmpR (OmpR-P) while medium osmolarity increasing, resulting in stronger expression intension of pOmpR. In the circuitry design, CheZ is upstream regulated by pOmpR **(Figure 2).**

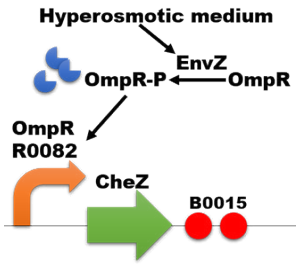

Figure 2. The schematic of osmotic-taxis design.

Construction of *E. coli* strain CL-1: The new genetic circuit, PompC-cheZ on pSB1C3 was introduced into *E. coli* strain CL-1, whose gene of cheZ had been knocked out.

Gradient Plate Experiments (semi-solid medium, Cm 50 μg/ml)

Medium: Medium A [17], with modification (Yeast Extract 0.26%; Peptone 0.27%; K_2HPO_4 0.37%; KH_2PO_4 0.13%; NaCl 0.05%; Glycerol 0.2% g/ml). Sucrose concentration gradient: 0~20%, the concentration gradient was 2%.

Mid-log-phase cell suspensions preparation

(i) Overnight culture of the required strain grow at 37°C in Medium A (Improved) containing appropriate chloramphenicol; (ii) Dilute the overnight culture at the ratio of 1:100 in fresh culture (Medium A), then incubate the culture under the condition of 37°C, 200 rpm, until its OD590 reaches 0.2; (iii) Stock in 4°C. The method of preparing gradient semi-solid medium (0.25% agar) was showed in Support Methods S2-1. Leave the plate incubated at 37°C. During the period of time, measure the diameters of the colony and take them down.

Plates Crossed Experiments (semi-solid medium, Cm 50 μg/ml): Medium: half Medium A (Improved). The concentration of sucrose: 0%, 10%.

The method of preparing liquid medium containing different concentrations of sucrose

(i) Prepare the medium with corresponding ingredients amount in the **Table 1** above; (ii) Add corresponding amount of solid sucrose into the liquid medium; (iii) Wait for sterilization.

Mid-log-phase cell suspensions preparation

(i) Overnight culture of the required strain grow at 37°C in Medium A (Improved) containing appropriate chloramphenicol; (ii) Dilute the overnight culture at the ratio of 1:100 in fresh culture (half Medium A), then incubate the culture under the condition of 37°C, 200 rpm, until its OD590 reaches 0.2; (iii) Stock in 4°C. The method of preparing semi-solid medium with a cross (0.25% agar) was showed in Support Methods S2-2. Leave the plate incubated at 37°C. During the period of time, take pictures of the colony.

RESULTS

Parabola and hyperbola form patterns

Characterization of circuit: The circuit was designed, built and characterized in *E. coli* CL-1. As CL-1 lacks *lacI* gene, promoter pLac won't be repressed by background repressor LacI. We apply gradient test to find out which influence would be made on reprogrammed chemotaxis under the following parameters: the concentration of chloramphenicol, IPTG and L-arabinose.

Characterization of backbone effect: To begin with, the best chloramphenicol concentration was tested. The gradient concentration of chloramphenicol at semi-solid culture medium was shown in **S1 Figure S1.** It was demonstrated that the activity of chemotaxis doesn't have overt linear relationship to chloramphenicol. Interestingly, 50 μg/ml of chloramphenicol gives CL-1 the best chemotaxis. So it was applied to the following characterization.

Characterization of IPTG effect: As promoter pBAD leads to a certain level of expression leakage of LacI, CL-1 has the worst chemotaxis without any stimulus. We added IPTG at gradient concentration and got the results **(Figure 3).** It was found that the activity of chemotaxis keeps increasing when the concentration of IPTG increases from 0 μM to 0.02 μM and gets the best performance with the IPTG range from 0.02 μM to 0.025 μM. We apply 0.025 μM IPTG for our following characterization.

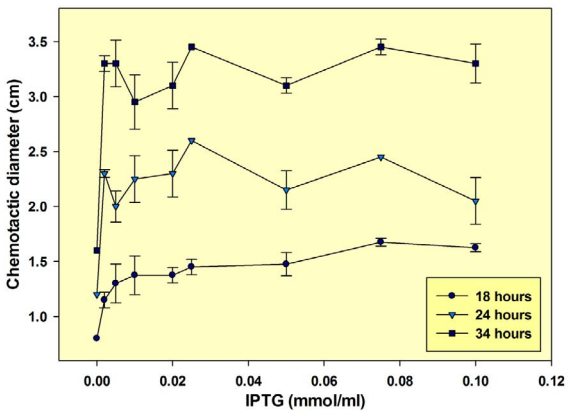

Figure 3. Curve of chemotactic diameter over time under gradient concentration of IPTG.

Characterization of the IPTG tendency: In order to further characterize the IPTG effect, we spotted IPTG and bacteria as the figure show **(S1 Figure S2).** As the concentration of IPTG keeps on decreasing as it spreads out. We spotted IPTG with its

concentration a little higher than the optimum. It was observed that reprogrammed bacteria swim to IPTG source which seemed that the bacteria were attracted by IPTG.

Characterization of L-arabinose effect: As more L-arabinose added in, the expression of promoter pBAD gets stronger, which leads to more LacI produced, resulting in the inhibition to chemotaxis. As our expectation, the activity of chemotaxis keeps going down as the concentration of L-arabinose increases **(Figure 4).** It was proved that 0.2% of L-arabinose has the best inhibitory effect on chemotaxis with 0.025 μM of IPTG added in.

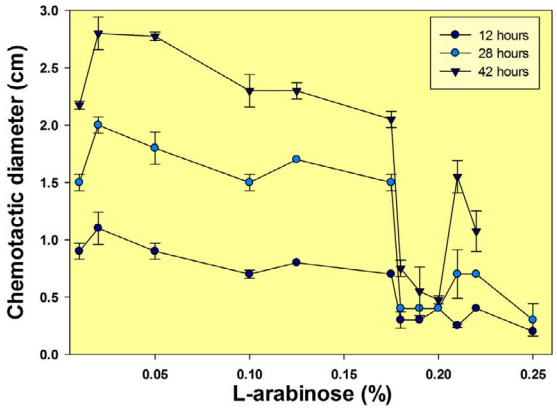

Figure 4. Curve of chemotactic diameter under gradient concentration of L-arabinose.

Parabola and hyperbola: The optimum concentrations of IPTG and L-arabinose, as inducer and repressor, were determined through preliminary experiments. Because the concentration of stimulus will decrease during spreading, so do their effect, we use IPTG and L-arabinose of which concentrations are a little bit higher than the optimum values for our experiments. A straight line with L-arabinose was pictured on the semi-solid culture medium, and a spot with the mixture of IPTG and CL-1 on one side of the line. In the area around the spot, the induction of IPTG is stronger than the repression of L-arabinose, cheZ is expressed and the bacteria adopt motile phenotype. However, when they approach the line where the repressor has a greater effect on the motility, they will lost their motile phenotype and stop.

According to the hypothesis, on the threshold ratio of the concentration of inducer and repressor, their effects are offset and a critical line is formed. Distances of the points on the critical line to the IPTG spot (focus) and the L-arabinose line (directrix) are in a fixed ratio (eccentricity). If the ratio equals to 1, the critical line is a parabola. If the ratio is larger than 1, it is a branch of a hyperbola. Then we conducted experiment to verify the mechanism **(Figure 5B).** Left boundary of the colony is regarded as the critical line. We found that cells have the tendency to swim away from L-arabinose line which is an expected performance of the bacteria.

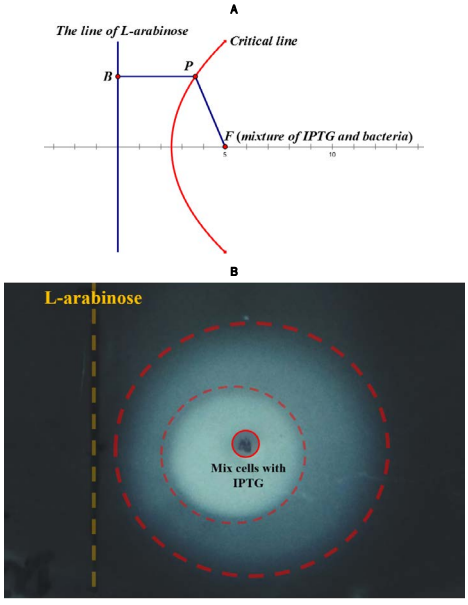

Figure 5A. Schematic of Critical Line model for parabola and hyperbola and B An actual experiment result is shown.

Other function curves-- quasi-hyperbola: After explorations, different ways to arrange bacteria and stimulus on semisolid culture medium were also tried. And some interesting results were obtained. We draw two spots on the semi-solid culture medium, one with L-arabinose **(S1 Figure S3A)** and the other with CL-1 and IPTG. Similarly, on the threshold ratio of the concentration of

inducer and repressor, their effects are offset and a critical line is formed. Distances of the points on the right side of colony boundary to the spot A and the spot B are in a very narrow ratio (**S1 Figure S3B).** Actually, as the critical line is quiet similar to hyperbola, we name it quasi-hyperbola.

Other interesting ways to spot bacteria and stimulus are waiting to be discovered, and the idea can be extended to other function curves and patterns. Two kinds of stimulus to construct a square were took **(Figure 6A).** Two opposite sides were paved by one kind of stimulus to form thin paths. Programmed cells were spotted on the center of the square and we got two oval rings after 24 hours culturing **(Figure 6B).** It was demonstrated that cell rings were stretched by IPTG sides while squeezed by L-arabinose sides. The process and the results were analyzed by mathematical model in MODELLING.

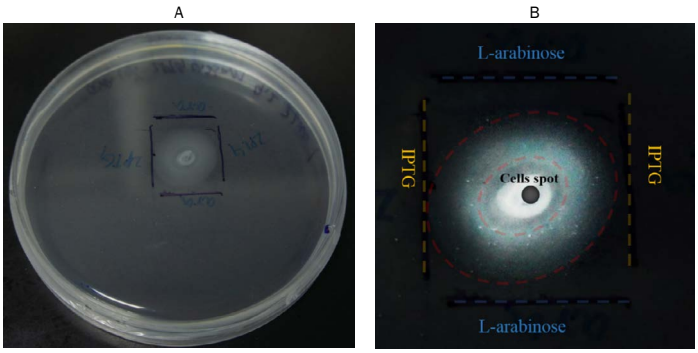

Figure 6A. Spotting cells on semi-solid culture medium with two IPTG lines and two L-arabinose lines and 6B Zoom in to the left picture, two oval rings could be observed on the medium.

In the area around the spot, the induction of IPTG is stronger than the repression of L-arabinose, cheZ is expressed and the bacteria adopt motile phenotype. However, when they approach the line where the repressor has a greater effect on the motility, they will lost their motile phenotype and stop. On the threshold ratio of the concentration of inducer and repressor, their effects are offset and a critical line is formed. Distances of the points on the critical line to the IPTG spot (focus) and the L-arabinose line (directrix) are in a fixed ratio (eccentricity). If the ratio equals to 1, the critical line is a parabola. If the ratio is larger than 1, it is a branch of a hyperbola. (The eccentricity e equals to PF/PB. If e=1, we define the critical line as parabola. If e>1, we define that as one branch of hyperbola.) Left boundary of the colony is expected as the critical line.

CHARACTERIZATION THE ACTIVITY OF PROMOTER AND EFFICIENCY OF RBS BY CHEMOTAXIS

Promoter Yardstick

The limitation of fluorescence measurement: In general, the efficiency of the fluorescence intensity is used to inspect the promoter and RBS, among them, the measured mainly by device [18]. Also, the precision and accuracy of the data is measured by the instrument. Without relative instrument, researchers can't do researches such as interlab study. What's more, if we need to measure fluorescence intensity in continuous time, it calls for continuous sampling resulting in accumulated error.

Chemotaxis can do more: As it was proved that the expression strength of CheZ is positively related with motile ability, a new system with different promoters and RBS efficiency was constructed by chemotaxis. The variable-controlling approach was adopted to compare chemotactic diameter exhibited as the size of the colony between standard components and unknown components by surveying the diameter of colony. The only equipment we need is a ruler.

In order to demonstrate the reliability of the new system, three devices with different promoters which had been characterized by Kelly, J. R. et al. were constructed [19]. All three colonies with different promoters were stab on the same semi-solid culture medium, of which L-arabione concentration is 0.02% and chloramphenicol concentration is 50 μg/ml **(Figure 7A).** After culturing 36 hours, differences of chemotactic diameters between each colony could be distinguished **(Figure 7B).**

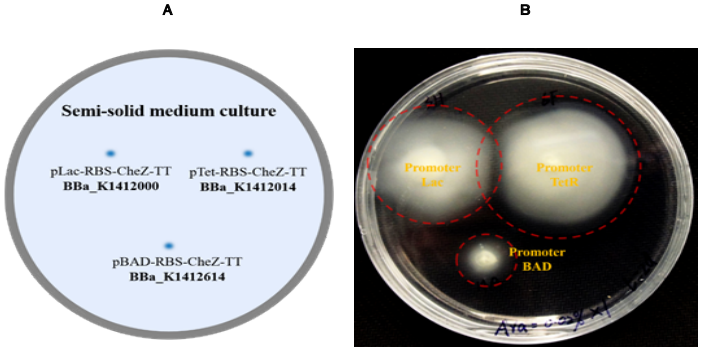

Figure 7A. Schematic of spotting bacteria and B culturing with 0.02 L-arabione for 48 hours, distinguish difference of chemotaxis diameters between each colonies is shown.

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Actually, measuring three colonies hasten diameter in different time and start the shellac set the colony diameter with promoter Lac as 1.0 **(S1 Figure S4).** After 36 hours, the ratio between every colony diameters was determined. If the fixed ratio was we set as the relative promoter activities, according to our characterization, the promoter TetR (BBa_R0040) activity is 1.86 higher than that of the promoter Lac (BBa_R0010). In the previous work [19], the promoter activity between the pTetR and pLac has already been determined, and the ratio (pTetR/pLac) is 1.58. For the system could tell the difference between different promoter activities, it is dependable and available. The characterization of the relative activity of pBAD is carried out with 0.02% inducer L-arabinose in culture. And the ratio (pBAD/pLac) is 0.37. It has important reference value for us to select a different promoter and RBS while data tell us about the comparatively promoter activity of the pBAD (BBa_K206000), while the pBAD (BBa_K206000) can be induced L-arabinose (Promoter activity characterization).

Extensive application: As it was proved that the expression strength of CheZ is positively related with motile ability, a new system with different promoters and RBS efficiency was constructed by chemotaxis. As the results shown, a conclusion, the new system works well with little errors, was drawn. And this method to determine most of the promoters can be standardized. What's more, it would be wonderful as it can be used to determine the RBS efficiency **(Figure 8A),** terminator efficiency **(Figure 8B)** and expression strength of target genes etc **(Figure 8C).**

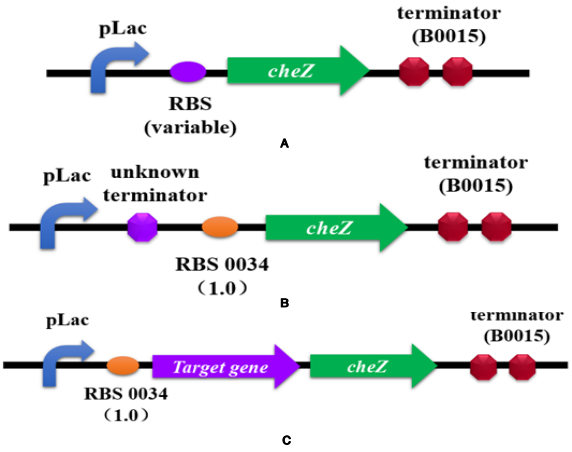

Figure 8. Device to characterize the efficiency different RBS (A) and terminator (B), and device to characterize the expression of target gene (C).

Black hole-suicide by chemotaxis: Thousands of years ago in China, people began to preserve food by curing them, which was recorded in Qimin Yaoshu around 540 AD. Curing is all kinds of food, such as meat, fish and vegetables preservation and flavor process, by adding salt, nitrate, nitrite, or a combination of sugar and it is one of the oldest methods of preserving food [20,21]. Table salt is the primary ingredient used in food curing. Removing water and salt added to the meat to create osmotic solute-rich environment to attract water microorganisms, slowing economic growth. Doing so requires nearly 20 percent of the salt concentration. It has already been proved that 5% concentration of NaCl could inhibit the growth of *E. coli* [22]. However, utilizing hyperosmotic pressure to kill *E. coli* hasn't been fully explored in synthetic biology. In this work, we have put efforts on this topic and developed a system that will contribute to biosafety.

Characterization of circuit

Gradient plate experiments: The semi-solid medium culture with gradient concentration of sucrose was used to characterize the device (BBa_K1412010). And it was assumed that proportional to the radius of dynamic ability and movement. In the plot **(Figure 9)**, when no sucrose was added in, the motile ability was the weakest. The motile ability sustainable growth as the concentration of sucrose increases from 0 to 4%. Then the motile ability goes down mildly as the sucrose concentration rose from 4% to 10%, but the ability is still stronger than that of the ability at concentration 0. A conclusion that our device was working as expectation can be drawn, dynamic capacity decreased (4% to 10%), because the inhibition of hypertonic pressure.

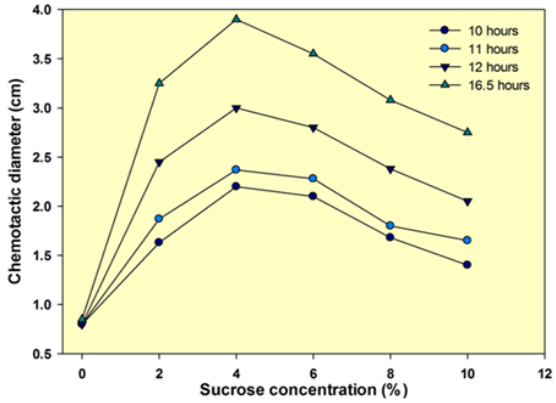

Figure 9. The plot of moving radius versus sucrose concentration.

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Plates crossed experiments: An erect line with water and a horizontal line with 10% sucrose was drawn, and spotted bacteria on the cross **(Figure 10).** Culturing for 48 hours, it was found that reprogrammed *E. coli* has great orientation to high concentration line. It has proved that CL-1 has the trend swimming to high osmotic pressure for high concentration sucrose creates high hyper osmosis.

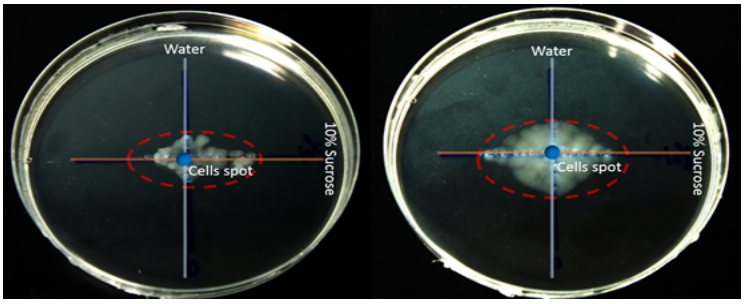

Figure 10. Drawing horizontal line with 10% sucrose and erect line with water.

DISCUSSION

Pattern formation is of fundamental importance in the coordination of multicellular behavior in a community or a large complex system. In physics and engineering, precisely control the size of the parts and verification, validation and predictive capability of engineering system performance lay an important theoretical foundation for the application in actual engineering [23]. In biology, a vast range of intracellular and intercellular coupling mechanisms lead to the formation of patterns that govern fundamental physiological processes, such as embryogenesis, tumorigenesis and angiogenesis [24-26]. Also, engineers can form the space pattern synthesis system while the ability of biomaterials for tissue engineering is a key step, targeted treatment and manufacturing [27,28]. While the number of components as the system grows, more and more difficult to coordinate the input and output components, and their overall impact on production expectations worth, which definitely restrict the application of gene therapy, tissue engineering and fabrication of biomaterials.

Based on this motivation and our experimental results of forming quasi-hyperbola at the present stage, we are going to conduct and adjust experiments according to a more accurate modeling, which expect higher accuracy in the experiments. As to modeling, because nonlinearities and stochasticity arise naturally, tools from the fields of nonlinear dynamics and statistical physics are extremely useful both in the generation of design specifications and for careful comparison between experiment and computational model [12]. Experimental and theoretical analysis revealed that the most important kinetic parameters influence ring development over time. Construction and study the synthesis of multicellular systems, can improve the development of our quantitative understanding of naturally occurring. Then it will lead us to understand and explain nature better. Based on a more accurate experiment and modeling, it will be consider to increasing the communication between the cells, introducing quorum-sensing in order to build more complex mathematical shapes, exerting environmental stimulus. Such systems level bioengineering can synergistically target multiple pathways, symptoms or targets, such as multiple cell populations or organs creating the potential for innovative environmental and therapeutic applications [29].

Develop a new suicide mechanism, utilizing hyperosmotic pressure to kill *E. coli* Based on the characterization, we spotted hyperosmotic pressure spot and reprogrammed CL-1 spot on semi-solid medium culture as figure S5 shows **(S1 Figure S5).** The concentration will decrease with the increase of the distance away from hyperosmotic pressure spot. As osmotic pressure is proportional to the medium concentration, the moving tendency of reprogrammed CL-1 will orient to the hyperosmotic pressure spot. Even to inhibit the osmotic pressure, the motile ability is still more athletic than that of without any inducer. So that reprogrammed CL-1 may even swim towards the high-osmotic site and die. The suicide mechanism is just like the black hole. When the bacteria move into the “event horizon” where the osmotic pressure reaches to the critical value named the killing osmotic pressure, the bacteria can’t go out of the border and be killed finally.

The sources (such as NaCl and sucrose) to generate hyperosmotic pressure are cheap, accessible and environmentally friendly, while antibiotics are expensive and have a bad effect on environmental microbiology because of drug resistance. If our black hole system could be fully developed, it will reduce the barriers to microbiology research especially for the scientists in developing countries.

In conclusion, the circuit to control the motility of *E. coli* (run or tumble) form patterns such as conic curves was constructed. The activity of promoter and efficiency of RBS were characterized by chemotaxis and the circuit. This method can be used to characterize most of the promoters, the RBS efficiency, terminator efficiency and expression strength of target genes etc. A new suicide mechanism, utilizing hyperosmotic pressure to kill *E. coli*, was built. Pattern formation is of fundamental importance in the coordination of multicellular behavior in a community or a large complex system. The cheap sources (such as NaCl and sucrose) to generate hyperosmotic pressure are cheap, accessible and environmentally friendly, while antibiotics are expensive and have a bad effect on environmental microbiology because of drug resistance.

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CONFLICT OF INTEREST

The authors state that they have no competing interests.

AUTHORS' CONTRIBUTIONS

W.J. designed and performed the experiments, and wrote the manuscript; C. T., J. L. C., Y. Y. L., Y. H. C., T. D. Z., R. H. Z., Z. H. C., J.X. H. performed the experiments; B.S.F. supervised the work; and all authors contributed to the discussion of the research.

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REFERENCES

1. Rollié S, et al. Review: Designing biological systems: Systems Engineering meets Synthetic Biology. Chemical Engineering Science 2012;69:1-29.
2. Martinez CA, et al. A synthetic biology approach to the development of transcriptional regulatory models and custom enhancer design. Methods 2013;62:91-98.
3. Vazana Y, et al. A synthetic biology approach for evaluating the functional contribution of designer cellulosome components to deconstruction of cellulosic substrates. Biotechnol Biofuels 2013;6:182-182.
4. Wayne PM, et al. A systems biology approach to studying Tai Chi, physiological complexity and healthy aging: Design and rationale of a pragmatic randomized controlled trial. Contemporary Clinical Trials 2013;34:21-34.
5. Zhuang L, et al. Research paper: Pure populations of murine macrophages from cultured embryonic stem cells. Application to studies of chemotaxis and apoptotic cell clearance. J Immunol Methods 2012;385:1-14.
6. Mandel GN. The Living Regulatory Challenges of Synthetic Biology. Iowa Law Review 2014;100:155.
7. Rousset M, et al. SIMULATING INDIVIDUAL-BASED MODELS OF BACTERIAL CHEMOTAXIS WITH ASYMPTOTIC VARIANCE REDUCTION. Mathematical Models & Methods in Applied Sciences 2013; 23:2155.
8. Topp S, et al. Guiding Bacteria with Small Molecules and RNA. J Am Chem Soc 2007;129:6807-6811.
9. Panisse C. BUILDING AN OPEN INNOVATION ECOSYSTEM. California Management Review 2014;144-171.
10. Fleury M-J, et al. Evaluation of the implementation of the Montreal at home/chez soi project. BMC Health Serv Res 2014;14:557-571.
11. Bunch BH, et al. The History of Science and Technology: A Browser's Guide to the Great Discoveries, Inventions, and the People Who Made Them, From the Dawn of Time to Today. Boston: Houghton Mifflin;2004.
12. Basu SYCHAFHWR A synthetic multicellular system for programmed pattern formation. Nature 434 2005;1130-1134.
13. Luitel BC CULTURE, WORLDVIEW AND TRANSFORMATIVE PHILOSOPHY OF MATHEMATICS EDUCATION IN NEPAL: A Cultural-Philosophical Inquiry. Philosophy of Mathematics Education Journal 2014;28:1.
14. Vitevitch MS, et al. Insights into failed lexical retrieval from network science. Cognit Psychol 2014;68:1-32.
15. O'Gorman RB, et al. Equilibrium binding of inducer to lac repressor.operator DNA complex. J Biol Chem 1980;255:10107-10114.
16. Adler J A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by Escherichia coli. J Gen Microbiol 1973;74:77-91.
17. Kawaji H, et al. Influence of molecular size and osmolarity of sugars and dextrans on the synthesis of outer membrane proteins O-8 and O-9 of Escherichia coli K-12. J Bacteriol 1979;140:843-847.
18. Coussement P, et al. One step DNA assembly for combinatorial metabolic engineering. Metab Eng 2014;23:70-77.
19. Kelly JR, et al. Measuring the activity of BioBrick promoters using an in vivo reference standard. J Biol Eng 2009;3:4-4.
20. http://en.wikipedia.org/wiki/Chemotaxis.
21. Carocho M, et al. Adding Molecules to Food, Pros and Cons: A Review on Synthetic and Natural Food Additives. Comprehensive Reviews in Food Science & Food Safety 2014; 13(4):377.
22. Sun Z WJ, et al. The Inhibitory Function of NaCl to a Few Common Bacteria. Sea-Lake Salt and Chemical Industry 2007;1:104.
23. Oberkampf WLT, et al. Verification, validation, and predictive capability in computational engineering and physics. Applied Mechanics Reviews 2004;57:345-384.

24. Gierer A, et al. A theory of biological pattern formation. Kybernetik 1972;12:30-39.

25. Chaplain MA, et al. Spatio-temporal pattern formation on spherical surfaces: numerical simulation and application to solid tumour growth. J Math Biol 2001;42:387-423.

26. Reinhart-King C: Mechanical and chemical signaling in angiogenesis. Reinhart-King, editor: Heidelberg: Springer 2013.

27. Abramsson A, et al. Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. J Clin Invest 2003;112:1142-1151.

28. Shuguang Z. Fabrication of novel biomaterials through molecular self-assembly. Nat Biotechnol 2003;21:1171.

29. Purnick PEM, et al. The second wave of synthetic biology: from modules to systems. Nat Rev Mol Cell Biol 2009;10:410-422.