JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

pubs.acs.org/JACS

Productive Chemical Interaction between a Bacterial Microcolony Couple Is Enhanced by Periodic Relocation

Chang Kyu Byun,[†] Hyundoo Hwang,[†] Woon Sun Choi,[‡] Toshiyuki Yaguchi,[†] Jiwoon Park,[†] Dasol Kim,[†] Robert J. Mitchell,*[,†] Taesung Kim,*[,†,‡] Yoon-Kyoung Cho,*[,†,‡] and Shuichi Takayama*[,†,§,∥]

[†]School of Nano-Bioscience and Chemical Engineering, Ulsan National Institute of Science and Technology (UNIST), Republic of Korea

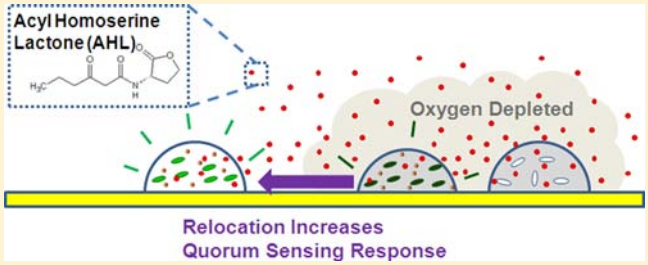
[‡]School of Mechanical and Advanced Materials Engineering, Ulsan National Institute of Science and Technology (UNIST), Republic of Korea

[§]Department of Biomedical Engineering, University of Michigan, Biointerfaces Institute, 2800 Plymouth Rd, Ann Arbor, Michigan 48109-2800, United States

[∥]Macromolecular Science and Engineering Center, University of Michigan, 2300 Hayward Street, Ann Arbor, Michigan 48109, United States

Supporting Information

ABSTRACT: This paper describes a system to study how small physical perturbations can affect bacterial community behavior in unexpected ways through modulation of diffusion and convective transport of chemical communication molecules and resources. A culture environment that mimics the chemically open characteristic of natural bacterial habitats but with user-defined spatiotemporal control of bacteria microcolonies is realized through use of an aqueous two phase system (ATPS). The ATPS is formulated with nontoxic dextran (DEX) and poly(ethylene glycol) (PEG) dissolved in cell culture media. DEX-phase droplets formed within a bulk PEG-phase stably confine the bacteria within it while small molecules diffuse relatively freely. Bacteria-containing DEX droplets can also be magnetically relocated, without loss of its bacterial content, when DEX-conjugated magnetic particles are included. We found that decreasing the distance between quorum-sensing (QS)-coupled microcolonies increased green fluorescent protein (GFP) expression due to increased inter-colony chemical communication but with upper limits. Periodic relocation of the chemical signal receiver colony, however, increased GFP expression beyond these typical bounds predicted by quorum sensing concepts alone by maintaining inter-colony chemical communication while also relieving the colony of short-range resource depletion effects. Computer simulations suggest that such increased productive output in response to periodic nonlethal physical perturbations is a common feature of chemically activated interactive cell systems where there is also a short-range inhibitory effect. In addition to providing insights on the effect of bacteria relocation, the magnetic ATPS droplet manipulation capability should be broadly useful for bioanalyses applications where selective partitioning at the microscale in fully aqueous conditions is needed.



INTRODUCTION

Bacterial relocation caused by physical disturbances, such as contact-mediated bacterial transplant to the body[1] or anthropogenic impacts on soil,[2] and active transport, such as bacterial hitchhiking on zooplankton in lakes,[3] alter the properties of bacterial communities in nature. These changes occur through introduction of new chemicals and nutrients into the community environment and also through disruption or enhancement of bacterial chemical interactions. Such events occur on the micro- to geological scales to alter bacterial community homeostasis and ultimately affect agricultural desertification rates, lake ecology, and human health.[1−3] An understanding of the chemical basis of these and other bacterial community reorganization events would benefit from the ability to construct user-defined, chemically interacting bacterial communities[4−11] where select microcolonies within the community can be physically relocated and the biological effect of the associated chemical environment changes analyzed. Whereas there have been macro-scale research systems to study bacterial relocation effects,[1−3] small-scale experimental systems with length scales on the order of millimeters, as may occur physiologically[12,13] or in nature,[14] are lacking.[15−18] Here, we describe the construction of bacterial communities within an aqueous two phase system (ATPS) environment where select microcolonies can be relocated by magnetic remote control. Furthermore, we demonstrate that microcolony relocation, even in the context of a bacterial microcolony couple in the

Received: October 4, 2012
Published: January 14, 2013

   dx.doi.org/10.1021/ja3094923 | J. Am. Chem. Soc. 2013, 135, 2242−2247

simple aqueous landscape of a culture dish, has significant effects on bacterial protein production due to the interplay between intercolony chemical communication and increased resource availability.[19−22]

EXPERIMENTAL SECTIONS

Preparation of Dextran-Conjugated Magnetic Particle. Amino-dextran (MW: 10 000, Sigma-Aldrich, Co.) was conjugated to Dynabead M-280 Tosylactivated (Invitrogen) by the following procedures. Briefly, 50 μL of a vortexed Dynabead suspension was transferred to a 1 mL plastic tube, centrifuged, and put on a Dynal magnet holder to remove the storage solution. The beads were rinsed additionally with 1 mL buffer A (0.3 g boric acid/50 mL water, then pH was adjusted to 9.5 by 5 M NaOH). To these beads were added 3−6 mg of amino-dextran in 150 μL of buffer A and 100 μL of buffer C (2 g ammonium sulfate dissolved in 50 mL of buffer A). The mixture was incubated on a roller at 37 °C overnight (12−18 h). After flash centrifuging the sample, the dextran-conjugated beads were collected magnetically. After removing the liquid, 1 mL of buffer D (50 mL PBS pH 7.4 with 0.5 g Bovine Serum Albumin) was added and the tube was incubated at 37 °C for 1 h. After removing buffer D, 1 mL of buffer E (10-fold dilution of buffer D in deionized water) was added to rinse the beads. Unreacted, non-DEX conjugated beads (which partition to the PEG phase) were removed by partitioning in a PEG-DEX aqueous two phase system and taking beads only from the DEX-rich phase.

Preparation of ATPS Solutions. Solutions of 10% PEG and 5% DEX ATPS were prepared by mixing 20% polyethylene glycol (PEG, M_w: 8000, Sigma-Aldrich, Co.) and 10% dextran (DEX T20, M_w: 20000, Pharmacosmos) solutions at a 1:1 ratio. The individual PEG and DEX solutions were prepared by dissolving each independently in M9 cell culture medium, which is composed of M9 minimal salts (Difco) with 1% (w/v) tryptone (Difco) and 1% (w/v) glucose (GR grade, Yakuri, Japan) in deionized water (purified by Milli-Q integral 10 system, Millipore).

***Escherichia coli* Transformation and Culture.** Bacteria transformation and culturing was performed as described previously.[23,24] Briefly, each *E. coli* culture was grown on Luria or M9 agar plates with the appropriate antibiotic at 30 °C. A single colony was used to inoculate 5 mL of M9 medium having 1% tryptone and 1% glucose with either 50 μg/mL ampicillin or 5 μg/mL tetracycline added. The cultures were then grown overnight (16 h) with vigorous aeration (250 rpm in a rotary shaker), resulting in an OD_{600} = 1.6−1.9.

Preparation of Bacterial Suspensions in ATPS. Bacterial suspensions were prepared by centrifugation of 800 μL of bacterial cultures at 3000 rpm for 5−10 min followed by removal of the supernatant, and resuspension of the bacterial cells in 50 μL of the ATPS solution.

Bacteria Micropatterning in ATPS. Small (volume range: 0.25−2.5 μL) droplets of bacteria, suspended in the DEX phase with DEX-conjugated magnetic particles, were patterned in PEG phases using a conventional manual pipettor (Eppendorf) to dispense the DEX phase at the desired positions. These experiments were performed using 1−2 mL of ATPS PEG in 60 mm Petri dishes.

Other Conditions. Microscopic conditions and bacteria samples are the following:

For Figure 2, the magnetic particle concentration is 30 mg/mL of Dynabeads M-280 (diameter 2.8 μm) in a DEX rich phase of 5% DEX T20 and 10% PEG 8000 ATPS at room temperature. The images were taken with a Model 250 standard goniometer with DROPimage Advanced v2.4 software (Ramé-hart Instrument Co.).

For Figures 3 and 4, the microscope settings, including the exposure time of 1 s, were kept constant for both bright field images and fluorescent images. Competent cells, strain MG1655, were prepared and transformed with plasmids t9002 (receiver) and k084012 (sender), respectively, transferred from the Registry of Standard Biological Parts (www.partsregistry.org). The "receiver" cell expresses GFP in response to AHL, while the "sender" cell produces AHL. The magnification was 0.7×. The ATPS PEG rich phase is contained in a polystyrene (PS) Petri dish surrounded by rectangular polydimethylsiloxane (PDMS) sidewalls. The magnetic particle concentration was 15 mg/mL of Dynabeads M-280 (diameter 2.8 μm) in a DEX-rich phase of a 5% DEX T20 and 10% PEG 8000 ATPS. M9 medium with 1% tryptone and 1% glucose was used.

RESULTS AND DISCUSSION

We studied the effects of microcolony relocation for a minimal bacterial community comprised of one colony of bacteria that constitutively produces acyl-homoserine lactone (AHL) (these bacteria are referred to as the "sender")[4,5,23] and another colony that responds to AHL by expressing green fluorescent protein (GFP) (these bacteria are referred to as the "receiver")[4,5,23,25] (see Supporting Information 1; Figure S1). Here, "colony" refers to a population of bacteria cultured as a suspension but confined by stable partitioning to the interior of a 0.25 μL ATPS DEX droplet placed in a culture dish with a total of 1−2 mL of an ATPS PEG solution. When these two colonies are micropatterned in the ATPS environment with different distances between them, there are two main responses observed during an 8 h-period: (i) minimal GFP expression at large inter-colony distances (∼20 mm) due to a lack in inter-colony chemical communication and (ii) increased GFP expression due to receipt of the AHL signal that diffuse through the two ATPS phases at small inter-colony distances (∼0.8 mm) (Figure 1A). These distance-dependent results can be explained by the range of influence of the AHL as seen in the computer-simulation of the expected distance AHL molecules diffuse around the sender bacteria microcolony at 0, 2, 4, 6, and 8 h. (Figure 1B).

These AHL concentration-dependent bacterial behaviors observed are consistent with concepts such as quorum sensing,[26,27] diffusion sensing,[28] and efficiency sensing.[29] What is often not considered in these types of sender-receiver bacterial system studies is that quorum sensing responses and associated protein expression are also dependent on the local availability of oxygen and other nutrients and is affected by lack of these resources and other stress factors.[30−36] That is, oxygen depletion due to consumption by the bacteria themselves may limit the rate at which proteins are produced.[19,33,34,37,38] Thus, two conflicting colony positioning requirements for maximizing GFP production exist in our system: (i) the receiver colony needs to be close to the sender to be able to receive sufficient AHL signals, and (ii) the colony needs to be relocated to new areas sufficiently far from any colonies to acquire oxygen more efficiently. Consequently, we analyzed how colony relocation alters the balance between these conflicting needs and can significantly increase the level of GFP expression beyond what is possible without colony movement.

The two different bacterial microcolonies, i.e., sender and receiver, are patterned using modifications of a previously described technology that utilizes aqueous two phase systems (ATPS).[24,39] *E. coli* is stably partitioned in 0.25 μL droplets comprised of the dextran (DEX)-rich phase aqueous solutions within a bath (1−2 mL) of the polyethylene glycol (PEG)-rich phase. To enable relocation of select microcolonies within these aqueous landscapes, we developed dextran-conjugated magnetic particles that stably partition in the DEX phase. Magnetic particle-containing DEX droplets can then be moved selectively (Figure 2) by manipulating a Neodymium (Nd) magnet. For the bacteria relocation experiment, we occasionally observed minimal losses of magnetic microparticles along the trail during the magnetic relocation. However, we could move the whole

dx.doi.org/10.1021/ja3094923 | J. Am. Chem. Soc. 2013, 135, 2242−2247

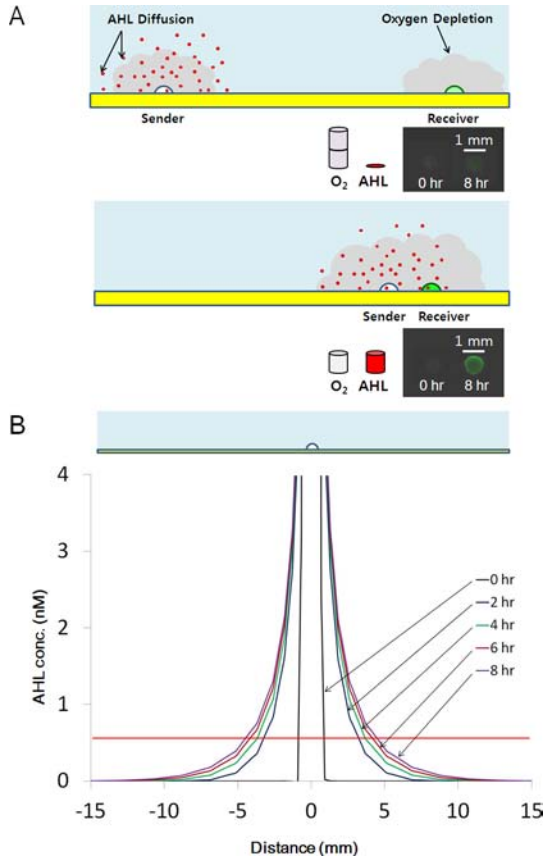


Figure 1. Distance-dependent interaction between signal-producer and -receiver bacteria colonies. (A) When the inter-colony distance is large (~20 mm), AHL signals do not reach the receiver microcolony. Actual images of how fluorescence intensity from the receiver microcolony changes over time under each scenario accompanies each schematic. When the inter-colony distance is small (~0.8 mm), the AHL diffusing from the sender microcolony induces GFP expression in the receiver microcolony. This productive interaction, however, is actually an attenuated response that reflects local oxygen depletion. The bars below each schematic represent relative amounts of AHL and oxygen available to the receiver bacteria colony depending on distance between the sender and receiver colonies. (B) Plots from a computer simulation of how AHL concentration profile around an AHL producing "sender" colony changes. The range of influence of AHL (defined as the region where the concentration of AHL is above half of k_A (0.5 nM, shown as horizontal red line), which is the half maximal rate concentration), extends ~2.9 mm from the edge of the sender colony at 2 h. (details in the Supporting Information, Figure S6A.).

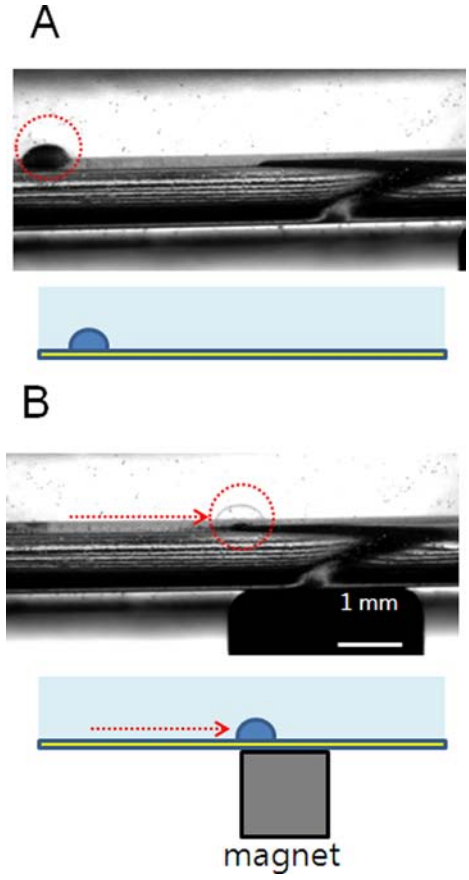


Figure 2. Magnetic remote control of ATPS droplet relocation. (A) A 250 nL DEX droplet containing 30 mg/mL of magnetic particles before relocation. (B) When a cylindrical Nd permanent magnet (3 mm diameter) is brought close to this droplet from beneath the Petri dish, the droplet relocates. The droplet is circled with a dotted line. The droplet is dark in panel A because the magnetic particles are still evenly suspended. The droplet is light in panel B because the magnetic particles have been pulled down toward the magnet.

droplet and bacterial losses were negligible (see Figure S2A in the Supporting Information).

We first studied the effect of relocation on GFP production using just the receiver colony. Even without AHL, the receiver colony expresses low levels of GFP due to leaky expression (Figure 3A). Importantly, the level of GFP produced increases significantly when the colony is periodically relocated (Figure 3A) or otherwise provided with increased amounts of oxygen (see Supporting Information 3; Figures S3A,B). Figure 3B shows a computer-simulation of the expected zone of oxygen depletion around the bacteria microcolony that forms in 120 min. Because the DEX phase of the ATPS confines bacterial cells but allows free exchange of oxygen with the surrounding PEG phase, relocation replenishes the oxygen supply by moving the colony out of this oxygen depleted zone. Simulations of different relocation speeds show that escape from this depletion zone improves with increasing relocation speed up to ~0.1 mm/s, above which there is little additional benefit under our experimental conditions (Figure 3C). As is shown in Figure 3C, to get oxygen replenishment, the magnetic droplet should be moved relatively quickly in order to effectively escape the oxygen-depleted zone. Oxygen likely affects GFP levels through metabolic changes that alter the amounts of proteins expressed and degraded,[19,33,34,37,38] although other oxygen effects such as GFP maturation[40,41] and nonoxygen effects such as nutrient effects[42,43] cannot be completely ruled out as minor contributors (see Supporting Information 4; Figure S4A–C).

To better evaluate the range of possible outcomes from the colony relocation experiments, we used differential equations to model the essence of how GFP production by the receiver colony is modulated by the amount of AHL signal received and availability of oxygen (see Supporting Information 5). Figure 4A shows a model recapitulation of the GFP expression experiments presented in Figure 1 where the AHL signals were maximized but the colony suffers from depleted oxygen levels (see Supporting Information 6; Figure S5). Figure 4B shows a model recapitulation of the experiments shown in Figure 3A where frequent relocation increases oxygen availability, but in

dx.doi.org/10.1021/ja3094923 | J. Am. Chem. Soc. 2013, 135, 2242–2247

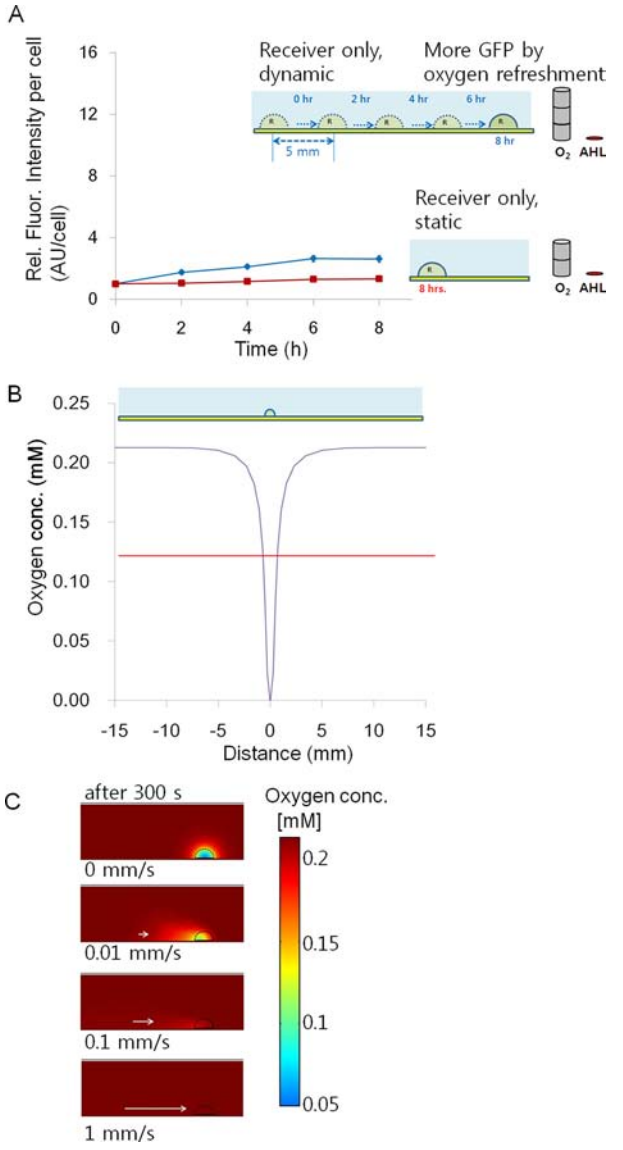


Figure 3. Periodic relocation of the receiver microcolony increases GFP levels through increased oxygen availability in the absence of AHL. (A) Relocation of a receiver microcolony replenishes its oxygen supply and increases GFP fluorescence even without any AHL signal present. (diamonds) Control experiments where the receiver colonies are kept under static conditions. (squares) Receiver microcolony droplets were relocated 5 mm every 2 h. The bars on the right represent relative differences in oxygen availability between the static and relocating scenarios. The error bars show the standard errors (S.E.) from triplicate experiments. The two curves have significantly different area-under-curve (AUC) values as analyzed by *t* test ($p < 0.05$). (B) Plot from a computer simulation showing how a microcolony depletes oxygen in its surrounding media at 2 h. The range of influence of oxygen depletion (defined as the region where the concentration of oxygen is below twice the k_{Ox2} (0.122 mM, shown as horizontal red line), which is the half maximal rate concentration) is relatively shorter than that of AHL diffusion (0.2 mm from the edge of the bacteria microcolony droplets at 2 h, details in the Supporting Information, Figure S6B). (C) Computer simulations show that faster relocation speeds, although with limits, decrease the degree of oxygen depletion around and within a bacterial microcolony.

the absence of AHL. We further modeled what would happen if the receiver colony was relocated periodically in proximity to a sender colony (Figure 4C). Because the range where oxygen

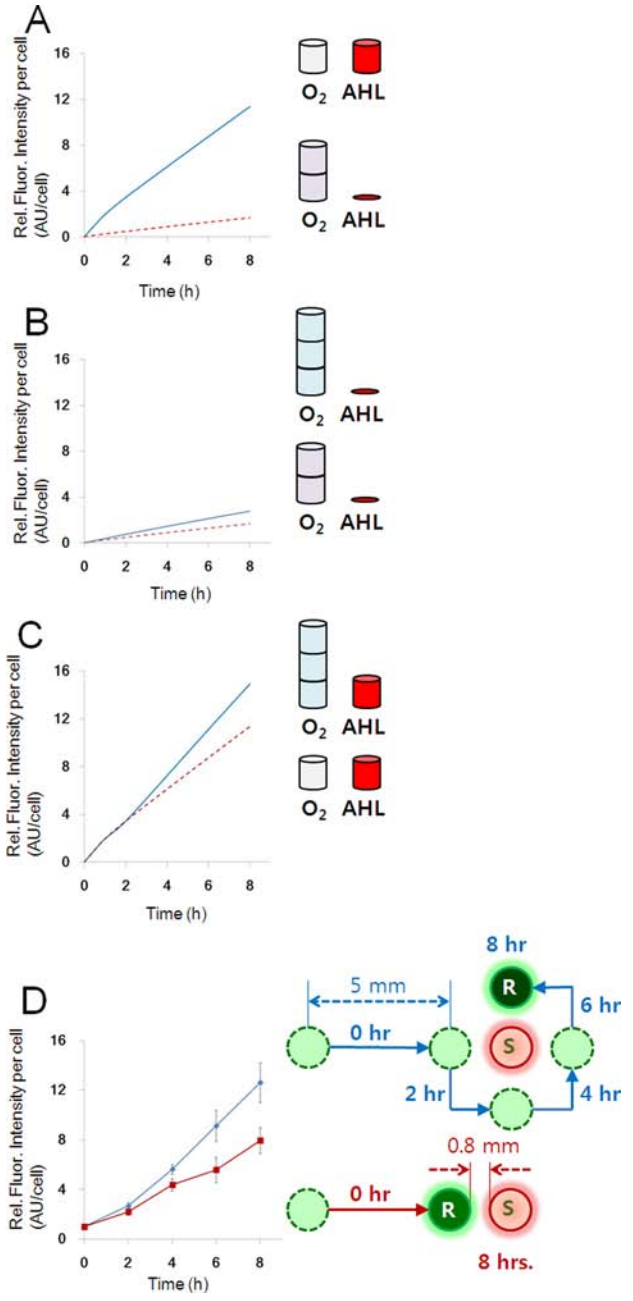


Figure 4. Even in the presence of AHL, periodic relocation of the receiver microcolony increases GFP levels through increased oxygen availability. (A) Mathematical recapitulation of experiments in Figure 1A. GFP production increases with smaller (~0.8 mm between the droplet edges) inter-colony distances (solid curve) compared to larger (~20 mm between the droplet edges) inter-colony distances (dotted curve) due to an increase in AHL signals. (B) Mathematical recapitulation of experiments in Figure 3A. GFP production increases with periodic relocation (solid curve) compared to static conditions (dotted curve) due to alleviation of oxygen depletion. (C) Mathematical prediction of how periodic relocation of a receiver colony around a sender colony (solid curve) may increase GFP production compared to a static colony (dotted curve) due to alleviation of oxygen depletion while maintaining sufficient AHL signals. (D) Experimental results show that periodic receiver colony relocation around a sender colony does indeed increase GFP production (diamonds) compared to static conditions (squares). The error bars show the standard errors (S.E.) from multiple experiments ($n = 4$). The plots have significantly different area-under-curve (AUC) as analyzed by *t* test ($p < 0.05$).

dx.doi.org/10.1021/ja3094923 | J. Am. Chem. Soc. 2013, 135, 2242−2247

depletion negatively influences GFP levels is shorter (~0.2 mm) compared to that where the positive influence of AHL diffusion is observed (~2.9 mm) (Compare Figure S6A with Figure S6B in the Supporting Information 6), we hypothesized and predicted mathematically that there would be relocation scenarios where GFP production would be higher than is possible under static conditions (Figure 4C). This prediction was then tested experimentally (Figure 4D). Indeed, experiments of periodic relocation of the receiver colony around a sender colony at a distance beyond the oxygen depletion zone but within the AHL signaling area (Figure 4D) showed increased GFP expression by the sender colony when compared to static conditions. The term "disturbance" commonly implies negative effects on interactions but we show that for bacterial relationships involving short-range inhibition with longer-range activation, periodic relocation can significantly increase overall productive interactions (Figure 5C).

Unlike in many laboratory cultures that are self-contained and homogeneous, bacterial interactions in nature occur in open but microstructured and dynamic environments. How local concentrations of inducer molecules such as AHL change as a consequence of different numbers, population density, and geometrical distributions of inducer-producing bacteria in such nature-mimicking environments has been actively studied and developed into concepts such as diffusion sensing[28] and efficiency sensing.[29] Bacterial responses, however, are modulated not only by inducer molecule concentrations but also by availability of resources such as oxygen and glucose that alter bacterial metabolism.[30−38] Here, we show that periodic microcolony relocation can significantly alter community behavior by increasing the availability of resources while maintaining high levels of inter-colony chemical communication (Figure 5). Because the ability of a community to produce sufficient quantities of effecter molecules is determined not only by the numbers of co-producers but also by the amount of resources available to them, the sensitivity of the community response to a combination of inducer molecule signals and resource availability is reasonable.[21,22,35] Microcolony relocation can alter either or both parameters so that bacterial communities with similar populations, densities, spatial distribution, and inducer molecule profiles can produce different responses to take advantage of or to cope with such nonlethal physical disturbances.

CONCLUSION

The technology described to allow patterning and relocation of select microcolonies within aqueous landscapes using magnetic remote-control should be broadly useful for a variety of bacterial community interaction studies. Unlike water-in-oil droplet systems, which have been useful for studying the effect of bacterial confinement, the fully aqueous environment of the method described here allows organizing and reorganizing of bacterial microcolonies as physically contained but chemically open interactive systems. A limitation of the technique is that not all bacteria may partition well in the particular ATPS formulation we describe here. There are, however, many biocompatible formulations[44,45] and additives that can optimize the partitioning behavior of different cell types[24,39,46] so that the concept of ATPS microcolony patterning should be applicable to a broad range of bacterial species. The methods should be generally useful for the analysis of dynamic inter-colony interactions that, in nature, are also accompanied by relocation and exchange of chemicals between colonies and with the environment.[2,3]

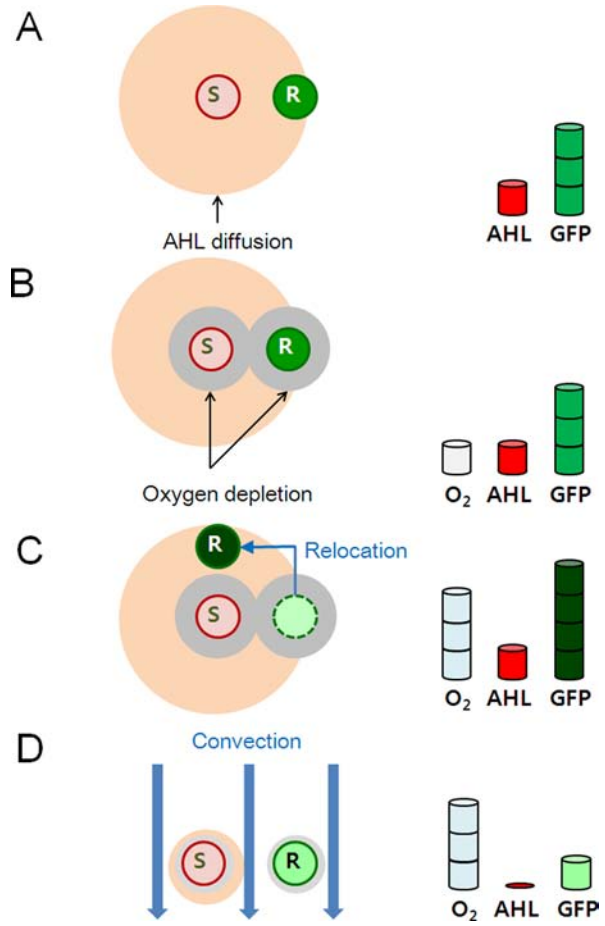


Figure 5. Bacterial microcolonies sense both AHL signals and resource availability to determine if production of proteins may be effective. (A) Conventional concepts of quorum sensing, diffusion sensing, and efficiency sensing only consider inducer molecule concentrations. (B) Bacterial communities, however, are also subject to resource depletion. In our experiments, this range of negative influence has a shorter range that the inducer molecule effects. (C) Select relocation of just the receiver microcolony around a sender colony at an appropriate distance can maintain high inducer signals while escaping from resource depletion. (D) Select colony relocation effects are different from convective effects considered in diffusion and efficiency sensing concepts. For example, when a sender colony is located parallel to a receiver colony in a convective flow stream, the quorum sensing response can be reduced but not increased. The color and height of cylinders at the right of each scheme qualitatively show the availability of resources and signal together with the predicted amounts of protein produced by the signal receiving colony.

Abbreviations. ATPS, aqueous two phase system; DEX, dextran; GFP, green fluorescent protein; PEG, polyethylene glycol.

ASSOCIATED CONTENT

Supporting Information

Additional experimental results; simulation details; equations, figures and graphs. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

takayama@umich.edu; ykcho@unist.ac.kr; esgott@unist.ac.kr; tskim@unist.ac.kr

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

C.K.B. thanks to Prof. Sung Kuk Lee and Prof. Cheol-Min Ghim at UNIST for discussion and advice about bacteria microbiology and simulation. This research was supported in part by WCU (World Class University) program (R322008000200540), SRC (Science Research Center) program (No. 2010-0029438) and by the Basic Science Research Program (No. 2010-0028073, NRF-2011-355-C00054, and 2012R1A1A2044736) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, Korea.

REFERENCES

(1) Costello, E. K.; Lauber, C. L.; Hamady, M.; Fierer, N.; Gordon, J. I.; Knight, R. *Science* **2009**, *326*, 1694−1697.
(2) Requena, N.; Perez-Solis, E.; Azcon-Aguilar, C.; Jeffries, P.; Barea, J. M. *Appl. Environm. Microbiol.* **2001**, *67*, 495−498.
(3) Grossart, H. P.; Dziallas, C.; Leunert, F.; Tang, K. W. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 11959−11964.
(4) Weiss, R.; Knight, T. F. *Comput. Sci.* **2001**, *2054*, 1−16.
(5) Basu, S.; Gerchman, Y.; Collins, C. H.; Arnold, F. H.; Weiss, R. *Nature* **2005**, *434*, 1130−1134.
(6) Tabor, J. J.; Salis, H. M.; Simpson, Z. B.; Chevalier, A. A.; Levskaya, A.; Marcotte, E. M.; Voigt, C. A.; Ellington, A. D. *Cell* **2009**, *137*, 1272−1281.
(7) You, L. C.; Cox, R. S.; Weiss, R.; Arnold, F. H. *Nature* **2004**, *428*, 868−871.
(8) Balagadde, F. K.; Song, H.; Ozaki, J.; Collins, C. H.; Barnet, M.; Arnold, F. H.; Quake, S. R.; You, L. C. *Mol. Syst. Biol.* **2008**, *4*, 1−8.
(9) Danino, T.; Mondragon-Palomino, O.; Tsimring, L.; Hasty, J. *Nature* **2010**, *463*, 326−330.
(10) Tamsir, A.; Tabor, J. J.; Voigt, C. A. *Nature* **2011**, *469*, 212−215.
(11) Ortiz, M. E.; Endy, D. *J. Biol. Eng.* **2012**, *6*, No. 16.
(12) Budrene, E. O.; Berg, H. C. *Nature* **1995**, *376*, 49−53.
(13) Singh, P. K.; Parsek, M. R.; Greenberg, E. P.; Welsh, M. J. *Nature* **2002**, *417*, 552−555.
(14) Grundmann, G. L.; Debouzie, D. *FEMS Microbiol. Ecol.* **2000**, *34*, 57−62.
(15) Carrara, F.; Altermatt, F.; Rodriguez-Iturbe, I.; Rinaldo, A. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 5761−5766.
(16) Connell, J. L.; Whiteley, M.; Shear, J. B. *Nat. Chem. Biol.* **2012**, *8*, 10−13.
(17) Randall, C. L.; Kalinin, Y. V.; Jamal, M.; Manohar, T.; Gracias, D. H. *Lab Chip* **2011**, *11*, 127−131.
(18) Kalinin, Y. V.; Randhawa, J. S.; Gracias, D. H. *Angew. Chem., Int. Ed.* **2011**, *50*, 2549−2553.
(19) Kim, E. J.; Wang, W.; Deckwer, W. D.; Zeng, A. P. *Microbiology* **2005**, *151*, 1127−1138.
(20) Park, S.; Wolanin, P. M.; Yuzbashyan, E. A.; Lin, H.; Darnton, N. C.; Stock, J. B.; Silberzan, P.; Austin, R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 13910−13915.
(21) Keller, L.; Surette, M. G. *Nat. Rev. Microbiol.* **2006**, *4*, 249−258.
(22) Walters, M.; Sperandio, V. *Int. J. Med. Microbiol.* **2006**, *296*, 125−131.
(23) Choi, W. S.; Ha, D.; Park, S.; Kim, T. *Biomaterials* **2011**, *32*, 2500−2507.
(24) Yaguchi, T.; Lee, S.; Choi, W. S.; Kim, D.; Kim, T.; Mitchell, R. J.; Takayama, S. *Analyst* **2010**, *135*, 2848−2852.
(25) Canton, B.; Labno, A.; Endy, D. *Nat. Biotechnol.* **2008**, *26*, 787−793.
(26) Ng, W.-L.; Bassler, B. L. *Annu. Rev. Genet.* **2009**, *43*, 197−222.
(27) Flickinger, S. T.; Copeland, M. F.; Downes, E. M.; Braasch, A. T.; Tuson, H. H.; Eun, Y. J.; Weibel, D. B. *J. Am. Chem. Soc.* **2011**, *133*, 5966−5975.
(28) Redfield, R. J. *Trends Microbiol.* **2002**, *10*, 365−370.
(29) Hense, B. A.; Kuttler, C.; Mueller, J.; Rothballer, M.; Hartmann, A.; Kreft, J. U. *Nat. Rev. Microbiol.* **2007**, *5*, 230−239.
(30) Kim, Y.; Wang, X. X.; Zhang, X. S.; Grigoriu, S.; Page, R.; Peti, W.; Wood, T. K. *Environ. Microbiol.* **2010**, *12*, 1105−1121.
(31) Boyer, M. l.; Wisniewski-Dyé, F. *FEMS Microbiol. Ecol.* **2009**, *70*, 1−19.
(32) Dilanji, G. E.; Langebrake, J. B.; De Leenheer, P.; Hagen, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 5618−5626.
(33) Wagner, V. E.; Bushnell, D.; Passador, L.; Brooks, A. I.; Iglewski, B. H. *J. Bacteriol.* **2003**, *185*, 2080−2095.
(34) Kovacikova, G.; Lin, W.; Skorupski, K. *J. Bacteriol.* **2010**, *192*, 4181−4191.
(35) Dunlap, P. V.; Kuo, A. *J. Bacteriol.* **1992**, *174*, 2440−2448.
(36) Surette, M. G.; Bassler, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7046−7050.
(37) Hansen, M. C.; Palmer, R. J.; Udsen, C.; White, D. C.; Molin, S. *Microbiology* **2001**, *147*, 1383−1391.
(38) Bose, J. L.; Kim, U.; Bartkowski, W.; Gunsalus, R. P.; Overley, A. M.; Lyell, N. L.; Visick, K. L.; Stabb, E. V. *Mol. Microbiol.* **2007**, *65*, 538−553.
(39) Tavana, H.; Jovic, A.; Mosadegh, B.; Lee, Q. Y.; Liu, X.; Luker, K. E.; Luker, G. D.; Weiss, S. J.; Takayama, S. *Nat. Mater.* **2009**, *8*, 736−741.
(40) Krishna, M.; Ingole, B. *Curr. Sci.* **2009**, *97* (7), 1022−1032.
(41) Craggs, T. D. *Chem. Soc. Rev.* **2009**, *38*, 2865−2875.
(42) Chow, D. C.; Dreher, M. R.; Trabbic-Carlson, K.; Chilkoti, A. *Biotechnol. Prog.* **2006**, *22*, 638−646.
(43) Kim, Y. S.; Seo, J. H.; Cha, H. J. *Enzyme Microb. Technol.* **2003**, *33*, 460−465.
(44) Albertsson, P.-A. *Partition of Cell Particles and Macromolecules*, 3rd ed. ; Wiley: New York, 1986.
(45) Mace, C. R.; Akbulut, O.; Kumar, A. A.; Shapiro, N. D.; Derda, R.; Patton, M. R.; Whitesides, G. M. *J. Am. Chem. Soc.* **2012**, *134*, 9094−9097.
(46) Tavana, H.; Mosadegh, B.; Takayama, S. *Adv. Mater.* **2010**, *22*, 2628−2631.