BATCH CULTIVATION OF BACTERIAL CELLS IN NANO LITER REACTOR ARRAY

J. Dai¹, S.H. Yoon², H.Y. Sim¹, T.K. Oh²,³, J.F. Kim²,³,⁴,⁵ and J.W. Hong¹

¹Materials Research and Education Center, Department of Mechanical Engineering, Auburn University, USA; ²Industrial Biotechnology and Bioenergy Research Center, Division of Biosystems Research, Korea Research Institute of Bioscience & Biotechnology, KOREA; ³1C Frontier Microbial Genomics and Applications Center, KOREA; ⁴Biosystems and Bioengineering Program, School of Science, University of Science and Technology, KOREA; ⁵Department of Biological Sciences, College of Bioscience and Bioengineering, Korea Advanced Institute of Science and Technology (KAIST), KOREA

ABSTRACT
We have developed a microfluidic reactor array enabling systematic assessment of bacterial cells in a batch culture condition. This system enables creating a series of different growth conditions by preserving different growth factors inside nanoliter reactors and cultivating bacterial cells inside the reactors. From a single on-chip experiment, we determined the effects of carbon sources on bacterial phenotypes, i.e., the pattern of growth curves with Escherichia coli K-12. We demonstrated the systematic assessment of inhibition effect of antibiotics on the growth of Pseudomonas aeruginosa. The present system could be used for systematic phenotypic study of different types of bacterial cells.

KEYWORDS: Batch culture, Nanoliter reactor, Bacterial phenotype

INTRODUCTION
The study of bacterial cells in a batch culture condition is a critical step to understand how bacterial cells adapt to environmental changes, even if they are genetically identical from one genotype. Therefore, tracing the phenotypes associated with the expression of genes under different environmental stimuli can provide useful information in many disciplines [1, 2]. The phenotypic studies are carried out with conventional method which requires sophisticated pipetting procedures. Recently, advanced miniaturized tools have been utilized, such as, high density open-microwell array [3] and microfluidic systems [4]. However, few approaches have been reported to generate a series of culture conditions for bacterial cells in a batch culture condition. We successfully developed a new microfluidic approach allowing different bacterial cell cultivations in nanoliter reactors with different growth conditions. We chart the phenotypes of E. coli and P. aeruginosa by assessing the growth patterns with different carbon sources and various types of antibiotics through both direct cell counting and fluorescence-based measurement.

THEORY
Our microfluidic platform is composed of 24 sets of discrete nanoliter reactors for bacterial cell cultivation in parallel as shown in Figure 1. There are two units; each unit has four different inlets for reagents on one side and one cell inlet on the other side. By introducing bacterial cells to ‘cell in’ channels and providing different media through M1 to M8 channels, eight different culture conditions are tested. Therefore, this array enables flexible hosting of bacterial cell cultures in nanoliter reactors with different culture conditions.

Figure 1. Nanoliter reactor array for bacterial cell growth
EXPERIMENTAL

We have validated the capability of cultivating bacterial cells on our microfluidic system by introducing *E. coli* into nanoliter reactors and monitored the growth of *E. coli*. We charted phenotypic changes of *E. coli* by assessing the growth patterns with different carbon sources through direct cell counting measurement as shown in Figure 2. We have found the different growth patterns due to the different compositions of the carbon sources. We also demonstrated the capability of charting phenotypic changes of bacterial cells in response to growth inhibitors by introducing *P. aeruginosa* with various types of antibiotics which can effectively inhibit growth of *P. aeruginosa* through fluorescence-based growth measurement as shown in Figure 3. We adopted fluorescence measurement as an indirect cell counting method for bacterial cell cultivation. We chose enhanced GFP (EGFP) as a fluorescent marker because GFP is stable inside bacterial cells and more resistant to photobleaching than fluorescent dyes [5]. We observed that different antibiotics have different inhibition effects on the growth of *P. aeruginosa*.

![Figure 2. E. coli growth in a nanoliter reactor. Growth of K12 and Lac\(^{-}\) mutant in M9 minimal medium with different carbon source(s) - no carbon source (I), 0.4 % glucose (II), 0.4 % lactose (III), and 0.4 % lactose + 0.05 % glucose (IV). Relative cell density was calculated by normalizing the initial cell number density to one.](image1)

![Figure 3. Effects of antibiotics on the growth of *P. aeruginosa* harboring EGFP plasmid. (a) The images of fluorescence intensity changes over time (b) Time course of *P. aeruginosa* growth under different types of antibiotics. Relative cell F.I was calculated by normalizing the initial fluorescence intensity of EGFP to one.](image2)
RESULTS AND DISCUSSION

This microfluidic platform is capable of hosting different types of bacterial cells with different growth conditions in a batch culture condition. Moreover, our system can realize simultaneous triplicate experiments to provide reliable data with error bars.

CONCLUSION

Our results show that the present system can generate a series of different growth conditions. The present platform could be used for the screening of useful bacterial genes as well as inhibitors, to help the systematic assessment of environmental factors or genetic modifications on the phenotypes of bacterial cells.

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REFERENCES


CONTACT

*J.W. Hong, tel:+1-334-8447385; jwhong@eng.auburn.edu*