Application Note

Potential for high-throughput screening of enteric bacteria with gel microdroplets using On-chip Sort
～Operation of On-chip products in anaerobic chamber～

(Collaboration with Dr. Shinji Fukuda, Institute for Advanced Biosciences, Keio University)

Introduction

In recent years, the intestinal microbiota has gained increasing attention due to its importance in modulation of health and disease. However, the research on gut microbiome remains as a challenge since experiment must be conducted under anaerobic condition because most intestinal bacterial are anaerobic, and maintaining an anaerobic environment would often require a anaerobic chamber, which limits the types of tests researchers can carry out. In order to widen the scope of research, we have developed a microfluidic chip-based cell sorter, a microfluidic emulsion droplet generator, and image-recognition single cell dispenser, all of which can fit and be operated inside a standard anaerobic chamber. In this application note, we present the combined use of all three for the proof-of-concept screening of anaerobic enteric bacteria.

Methods

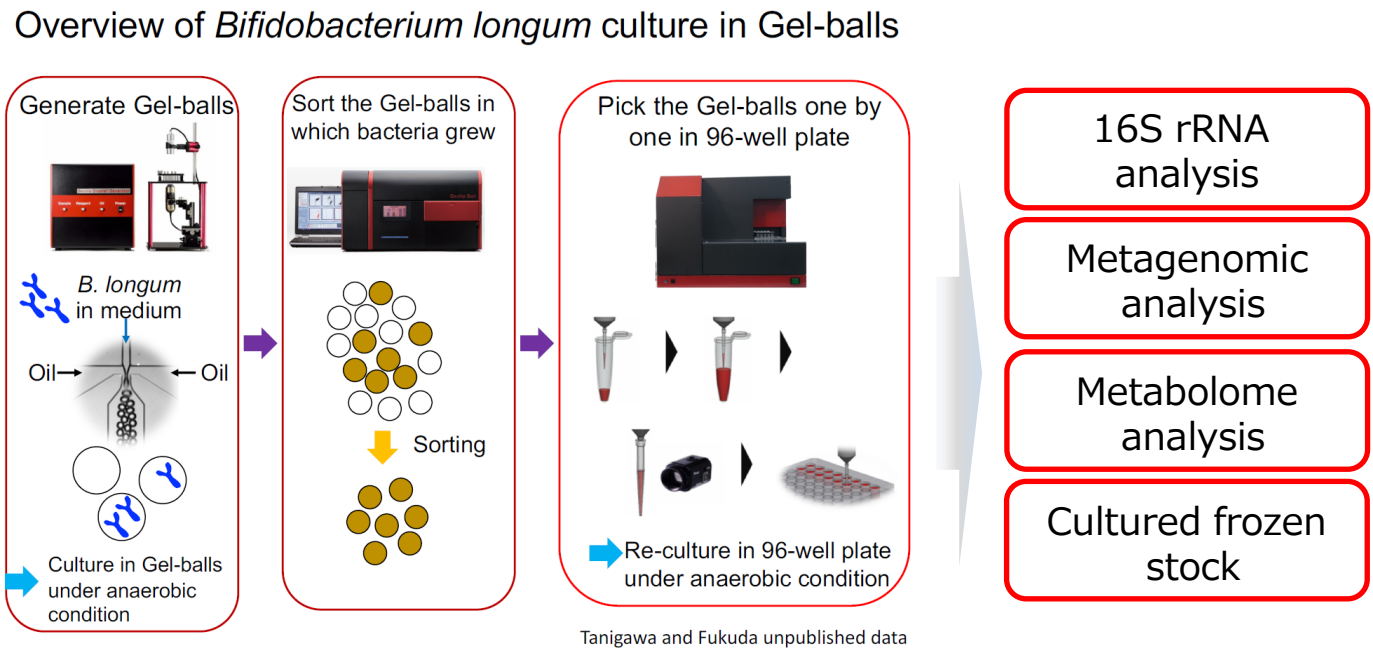

Fig. 1. Workflow of high-throughput screening of *Bifidobacterium longum* using gel microdrops and On-chip Sort. From left to right: Encapsulation of *B. longum* into gel balls using On-chip Droplet Generator and culturing under anaerobic condition; sorting of the gel balls which contain cultured bacteria based on fluorescence using On-chip Sort; dispensing individual collected gel balls into wells of a 96-well plate using On-chip SPiS and culturing them under anaerobic condition; and various downstream applications.

Fig. 1 shows the workflow of high-throughput screening of *Bifidobacterium longum* encapsulated within gel microdroplets (GMDs). Although *Bifidobacterium longum* is an obligate anaerobe, we have used it for a proof-of-concept study within anaerobic chamber. In order to encapsulate *Bifidobacterium longum* within agarose GMDs, On-chip Droplet Generator was used to create Water-in-oil (W/O) emulsion droplets of approximately 30μm. Those droplets were incubated at 37℃ for two days for colonies to form. After exchanging the oil to culture medium, the bacterial membrane was stained with FM4-64 (ThermoFisher Scientific). On-chip Sort was used to sort FM4-64 stained GMDs from the mixture of unstained and empty GMDs. The collected GMDs, which contain *Bifidobacterium longum* colonies formed from single bacteria, were then single-dispensed into 96 multititer plate using On-chip SPiS and re-incubated.

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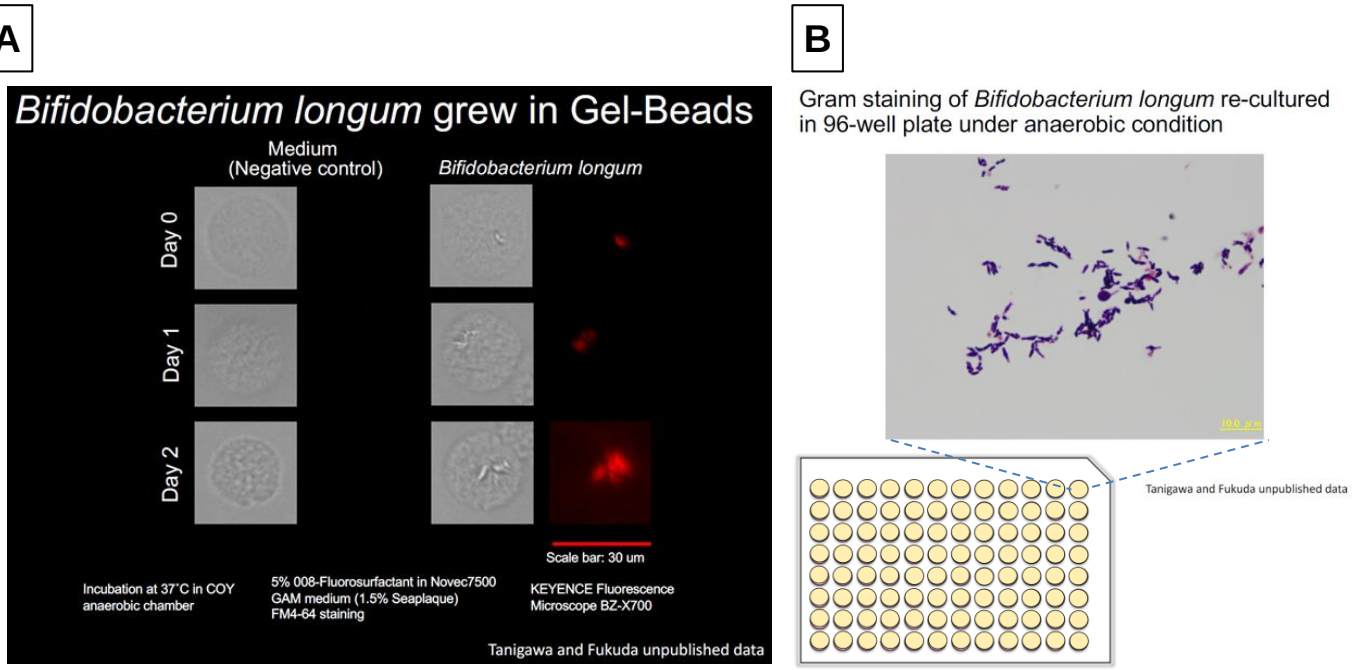


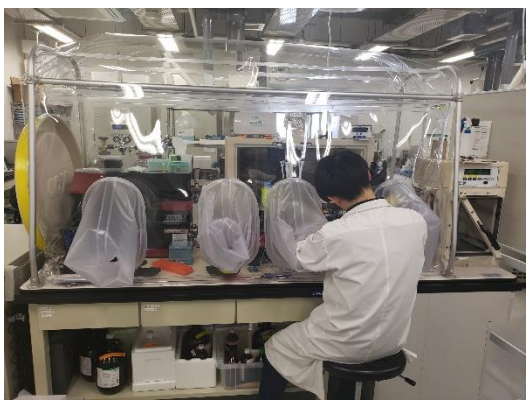
Fig. 2 Encapsulation of *Bifidobacterium longum* within GMD, staining and single-droplet re-incubation. (A) Photomicrographs of GMDs taken 0 days, 1 day and 2 days post-encapsulation. Images on the left show GMDs with only culture medium trapped. Images on the right show GMDs encapsulated with single *Bifidobacterium longum.* (B) Photomicrograph of gram-stained *Bifidobacterium longum* after sorting and single-GMD dispensing into 96 microtiter plate.

Fig. 2 shows the photomicrographs (taken using KEYENCE BZ-X700) of GMDs cultured within anaerobic chamber and taken over 0-2 days. On Day 2, GMDs with high FM4-64 fluorescence signal (i.e. *Bifidobacterium longum* colony) were sorted using On-chip Sort and single-dispensed into 96 wells. The results show that *Bifidobacterium longum* after GMD screening are viable within the well of multititer plate and have shown growth after leaching out from GMD. This outcome proves that the screening combined with sorting inside anaerobic chamber using On-chip products is feasible, and it further implies that screening of anaerobic enteric bacteria is more readily available.

What is an anaerobic chamber

Many intestinal bacteria are completely anaerobic, and when left in aerobic conditions, many of them die over time. This may not be a problem when conducting DRY experiments such as NGS, but when culturing, it is necessary to maintain anaerobic conditions throughout the entire process.

Anaerobic chamber is a device that replaces the air in the chamber with 100% N_2, then fills the chamber with a standard mixture of 96% N_2 and 4% H_2, and uses a palladium catalyst to convert the remaining O_2 in the chamber to H_2O, thereby maintaining an anaerobic (oxygen-free) environment, which is essential for research on anaerobic bacteria. The On-chip systems support the entire process.



(Manufacturer: COY LABORATORY PRODUCTS INC. (USA))
(Photos taken: Mr. Tanikawa from Keio University, Institute for Advanced Biosciences)

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