Size-Specific Microfluidic Devices to Capture Microorganisms from the Environment*

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Introduction

It is widely recognized that the overwhelming majority of microorganisms cannot grow in artificial media that is prepared in laboratories. Cultivation-independent approaches, such as rRNA sequencing, have revealed that there remains an immense number of uncultivated species in nature [1,2]. Since then, there have been significant efforts made to overcome these limitations in cultivation. Some endeavors consist of modifications to conventional methods, such as changes in the medium composition (or concentration), gelling agents, and incubation time [3,4]. Other approaches include incubation of microbial cells in their natural environment using membrane bound devices.

Given the large number of unexplored species, high-throughput of microbial discovery requires miniaturization of the growth compartment. We developed a microfluidic device that can be used to capture single bacterial cells from a mixture of species and produce pure cultures from those cells. By having the device located in the cell's natural environment, essential, unidentified growth factors can diffuse into the food chamber through a membrane and allow a single cell type to proliferate and form a large population of clones, which can be later removed for analysis. Initially, cells sense and move toward chemo-attractants diffusing from food chambers. The first cell to fit inside the constriction, blocks the entrance to the food chamber, and prevents other cells from entering. The cell can continue to grow and divide, thus populating the food chamber with a single species (Figure 1) [5].

Materials and Methods

Constriction and large channel patterns were defined on a 3-inch master silicon wafer by using electron beam- and photo-lithography, respectively. The constriction height was created by sputtering chromium metal on the wafer prior to removing the lithographic pattern. Large channels were aligned and created over the constrictions by first applying photoresist onto the wafer and then exposing it to UV light. The completed master wafer was used to make poly dimethylsiloxane (PDMS) polymer chips. The chips were permanently bonded to microscope cover glass using oxygen plasma to create the channels.

The device was then filled with media and a polycarbonate membrane was glued onto the access holes of food chambers to prevent contamination, while allowing exchange of chemicals with the culture. For initial experiments, marine microorganisms *Psychroserpens* sp. and *Roseobacter* sp. were cultured in Lysogeny broth (LB) overnight and mixed to obtain a heterogeneous marine culture. The device was placed into the culture bottle and incubated for few days before imaging with a fluorescence microscope.

Results and Discussion

To demonstrate that our devices can be applied for the separation of environmental microorganisms, we selected two marine species that were recently collected from the environment: *Psychroserpens* sp. and *Roseobacter* sp., which are cocci- and rod-shaped bacteria,

respectively. Both species grew well in the main channel of the devices. Figure 2 (a, b) shows a food chamber connected to the main entrance with a 5 µm wide constriction. Figure 2b, which is a 100x magnified image of the constriction and the food chamber, shows that only *Roseobacter* sp. is present in the constriction and food chamber since only rod-shaped cells can be seen in the food chamber [5]. A sample was removed from the food chamber and cultured on LB-agar plates to confirm that *Roseobacter* sp. was successfully isolated. Interestingly, 5 µm wide constrictions showed multiple lines of cells, but narrower constrictions did not show any bacterial growth. This result suggests that a different or wider range of constriction sizes may be necessary for isolating and separating other species in the environment. This result also raises many questions regarding the behavior of bacteria in confined spaces. Further investigations are being completed to determine the cause of this behavior.

Conclusion

We demonstrated isolation of cells from mixtures containing two cell types, but the concept applies to systems of any complexity. The results presented here validate the ability of our completely passive devices to isolate individual bacterial species from heterogeneous populations, and supports the use of this technique for high-throughput culturing of new microorganisms in virtually any environment. By employing hundreds of such devices in the next step of this project, we expect to isolate previously uncultivated species from the environment.

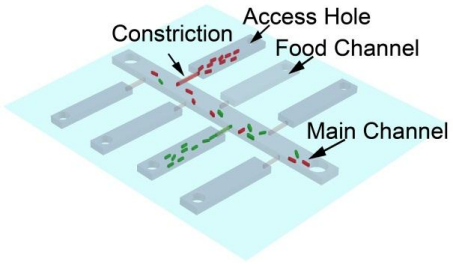

Figure1. Schematics of the microfluidic device.

References

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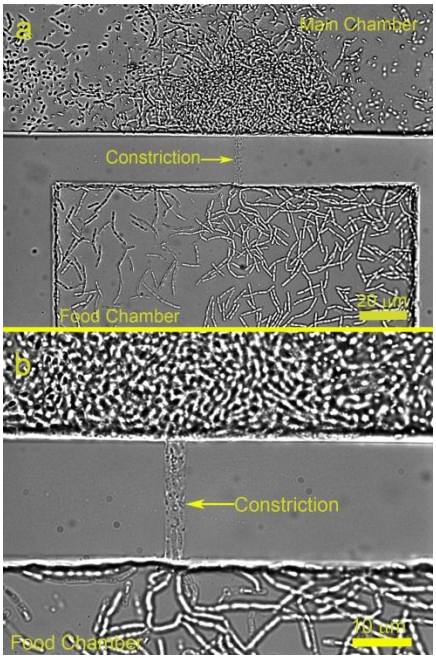

Figure2. Isolation of *Roseobacter* sp. from *Psychroserpens* sp. via a 950 nm tall, 5 µm wide, and 30 µm long constriction. (a) 40x magnification. (b) 100x magnification [5].