DEVELOPMENT OF MICROFLUIDIC AQUEOUS TWO-PHASE SYSTEM FOR CONTINUOUS PARTITIONING OF E. coli STRAINS
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ABSTRACT
The interaction of bacterial cells with surrounding environment depends on its surface characteristics such as hydrophobicity, hydrophilicity balance and net charge. In this paper, aqueous two-phase system partitioning of Escherichia coli strains based on their difference in surface properties is introduced in a microfluidic system. While aqueous two-phase system is widely use to separate biomolecules on macroscale, the method has not been adapted in microfluidic system. The bacterial cells are partitioned based on their affinity for streams formed by aqueous polymers polyethylene glycol (PEG) and dextran (Dex). Partitioning efficiency of two Escherichia coli strains is currently being optimized.

KEYWORDS: Microfluidics, Aqueous two phase system, Escherichia coli, Continuous partitioning, PEG, Dex

INTRODUCTION
Understanding and analysis of the surface of microbial cells is important in many fields like biotechnology, process engineering, medicine, environmental protection and material technology. For instance, the host immune response is triggered by the cell surface. Additionally, in downstream processing of proteins using bacteria or other cells, non-specific adsorption as well as the existence of multiple bacterial strains can affect the overall operational efficiency. Although bacterial concentration methods such as centrifugation, filtration, and immunomagnetic separation have been reported for food systems, the separation and subsequent concentration of bacterial cells from a food sample during sample preparation continues to be a stumbling block in the advancement of molecular methods for the detection of foodborne pathogens [1].

Although several methods are available for surface characterization, aqueous two-phase system (ATPS) partitioning method has advantage of high selectivity and biocompatibility [2]. In this paper, we use this method to separate two bacteria strains based on their surface property. ATPS is formed by two immiscible aqueous polymer solutions or one aqueous polymer solution and a salt solution. The separation of biomaterials is based on its surface characteristics. Partition of large particles in macro ATPS is severely affected by gravity and it is also difficult to perform continuous partitioning. In microfluidic based system (μATPS), the influence of gravitational force on partitioning is negligible since the direction of particle migration will be perpendicular to gravitational field [3]. In addition, the continuous-flow microfluidic system could potentially enable partitioning of bacterial strains in sample volumes not possible using conventional systems. Moreover in μATPS, the larger surface to volume ratio will decrease the distance a cell has to travel for partitioning to occur [3]. In this paper, we introduce μATPS for partitioning of two E.coli strains (ML308 and BL21).

THEORY
In ATPS, the top phase will be PEG rich and the bottom phase will be Dex rich at equilibrium. PEG and Dex are immiscible non-ionic polymers in nature. Salt is added to give physiological osmolarity to the system and some salts have affinity for particular phase. Uneven migration of ions would leave one of the phases more positively charged than the other and it would introduce electrostatic potential difference between the phases [4]. Each phase has distinctive physiochemical properties like hydrophobicity, hydrophilicity, charge and surface energy and it controls the partitioning of biomaterials when they are introduced in to the system. Fig. 1 shows the electrostatic potential and surface energy forces acting on the cell in the interface. The cells migrate to any of the two phases or stay at the interface depending on its interaction with the system. The distribution of cells between the two phases is described by partition coefficient (P) defined as in equation (1).

\[ P = \frac{C_t}{C_b} \]  

(1)

Where, \(C_t\) and \(C_b\) are concentrations of cells in the top and bottom phase respectively.
**Figure 1:** The forces acting on the cell in the interface of the two phase system. PEG phase is more positive than Dex phase because of uneven partitioning of phosphate ions between the phases

**EXPERIMENTAL**

_E. coli_ strains ML308 and BL21 were cultivated overnight and harvested after reaching an OD$_{600}$ value of 2 to 3 to be used in ATPS experiments. Macro ATPS was prepared with 4.5% (w/w) PEG 6000 and 5.5% (w/w) Dex T500 in 10 mM sodium phosphate buffer, pH 7.4. Bulk PEG and Dex phase was prepared in separating funnel after overnight phase separation. Different concentration of sodium chloride (NaCl) was used to change the ionic strength of the phase system. 50 µl of bacterial sample was added to phase system containing 2.5 ml of both phases in a tube. The contents in the tube was mixed and left for 30 minutes to phase separate. The amount of bacteria in each phase was determined by measuring OD$_{600}$ value of samples from respective phases. The experiment was repeated in phase systems with different salt concentration and the results were compared with other systems.

Microfluidic devices for µATPS and were fabricated by standard soft lithographic techniques using PDMS. Fig. 2 shows an example of a three inlet-three outlet chip used in this work. 4.5% (w/w) PEG 6000, 5.5% (w/w) Dex T500 solution and bacterial cells suspended in 10 mM sodium phosphate buffer solution were individually introduced in to the microfluidic chip using syringe pumps. Fig. 3 shows the inlet and outlet section of the microfluidic chip during the experiment. The flow rates of all three inlets (3; 0.5 and 0.25 µl/min for PEG, Dex and bacteria respective) were manipulated to get stable phase system.

**RESULTS AND DISCUSSION**

Initially, the partitioning efficiency of each _E. coli_ strain was evaluated in a macro ATPS. After partitioning, each phase was separated and analyzed. As can be seen in Figure 4, _E. coli_ ML308 preferred to migrate to hydrophobic PEG phase irrespective of ionic strength of the system. About 99% of the bacterial cell partitioned to the PEG rich top phase. In the case of _E. coli_ BL21 strain, the cells showed affinity for PEG phase only in non-ionic conditions. When salt is added to the system, the bacteria started to migrate towards the interface. Further increase in ionic strength resulted in cell partitioning in the dextran phase (Fig. 5). About 80% of the _E. coli_ BL21 cells migrated to the interface in phase system containing 10 mM NaCl to 100 mM NaCl concentration. 200 mM NaCl phase system resulted in about 50% of the cells partitioned in the Dex rich bottom phase.
Using the optimized PEG and Dex concentrations, µATPS partitioning of E. coli BL21 was examined (Table 1). To keep the interface in the center, the PEG phase was flown at a higher flow rate (3 µl/min) while the Dex was running at 0.5 µl/min (see Fig. 2). The cells are introduced through the center inlet. The collected sample from the three outlets are plated over night and counted. As expected, the cells remained in the interface for the 100 mM salt system (Table 1). However, the cells also stayed in interface even in non-ionic system, indicating the cells are either (i) not migrating away from interface or (ii) the cells are migrating and partitioning in the PEG phase but close enough the middle outlet and extracted from the center.

Table 1: µATPS partitioning of E. coli BL21

<table>
<thead>
<tr>
<th>Phase</th>
<th>% of cells in 0 mM NaCl system</th>
<th>% of cells in 100 mM NaCl system</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG rich phase</td>
<td>2.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Dex rich phase</td>
<td>1.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Interface</td>
<td>96</td>
<td>92.8</td>
</tr>
</tbody>
</table>

Partitioning in µATPS depends on various factors like flow rate of the phases and dimensions of the channel in addition to affinity of the particle to the phases. For improved performance, the flow can be further decreased to increase the time of interaction of the cells with the phase system for increased partitioning efficiency of the bacterial cells. In addition, the ions can be partitioned overnight in bulk system prior the µATPS experiment. Currently, we are optimizing the flow conditions for a stable system using a better syringe pump (low pulsing) to improve the partitioning efficiency.

CONCLUSION

In summary, we introduce microfluidic based aqueous two-phase system to separate bacteria strains. This device once developed can be used as a rapid analytical tool for detecting bacterial contamination in food and process industry. It can also be integrated to lab-on-a-chip (LOC) systems for downstream processing.

REFERENCES


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