A Study of Bead Capture Efficiency on *Listeria innocua* and *Escherichia coli* Using Non-Specific and Specific Beads

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There have been studies done on the efficiencies of non-specific bead capture[1, 2] and specific bead capture of bacteria within their respective groups [3], however there have not been conclusive studies made on whether specific bead capture of bacteria is more effective than non-specific bead capture. This study focused on whether specific or non-specific bead capture is most efficient. The beads examined were plain polystyrene beads, polystyrene beads functionalized with carboxyl and dimethylamino groups, and a type of immuno-magnetic beads (IMB) known as Dynabeads®. The beads used vary in size, structural properties, and methods of capture. They exhibit different properties that can be crucial in the bead capture process: specific (use of specific antibodies) versus non-specific binding, charge of the material, and hydrophobic versus hydrophilic characteristics. The only specific binding beads are the Dynabeads® (which are coated with Listeria i.-specific antibodies). There are three other beads because if it is determined that non-specific capture is more efficient than the variation of the non-specific beads will help shed some light on why this is the case.

The experimentation used two different bacteria: Listeria innocua and Escherichia coli. Listeria i. is gram-positive and the other, E. coli, is gram-negative. The bacteria were inoculated into LB (Luria-Bertani) media and incubated for one day at 37°C. 210 µl of the bacteria solution was then extracted and diluted 10-fold five times.

Ten µl of beads (five for Dynabeads®) were then mixed into the bacteria solution and incubated for 15 minutes. The unbound bacteria were then isolated through centrifugation (for non-magnetic beads) or magnetic capture (for magnetic beads). They were then rinsed out twice, using PBS (phosphate buffer solution), and between each rinse step diluted 10-fold five times as with the original solution. The remaining pellet solution of bead-bound bacteria was also diluted in the same manner. It should also be noted that the Dynabeads® capture experiment also included Listeria i. grown at room temperature. The dilutions were then plated on Brain Heart Infusion Agar (BHI-Agar) three times each and then were incubated overnight.

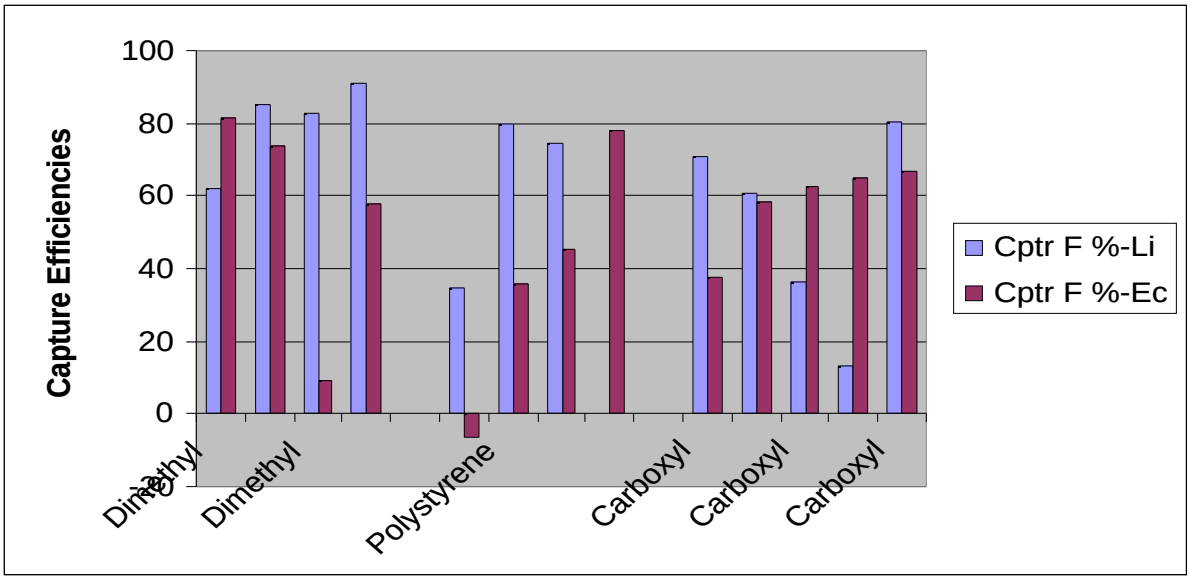

Fig. 1: Preliminary results of data sets for beads with non-specific capture; the number of trials represents the number of times the experiment had to be replicated before the researchers felt that they had achieved reasonably consistent data

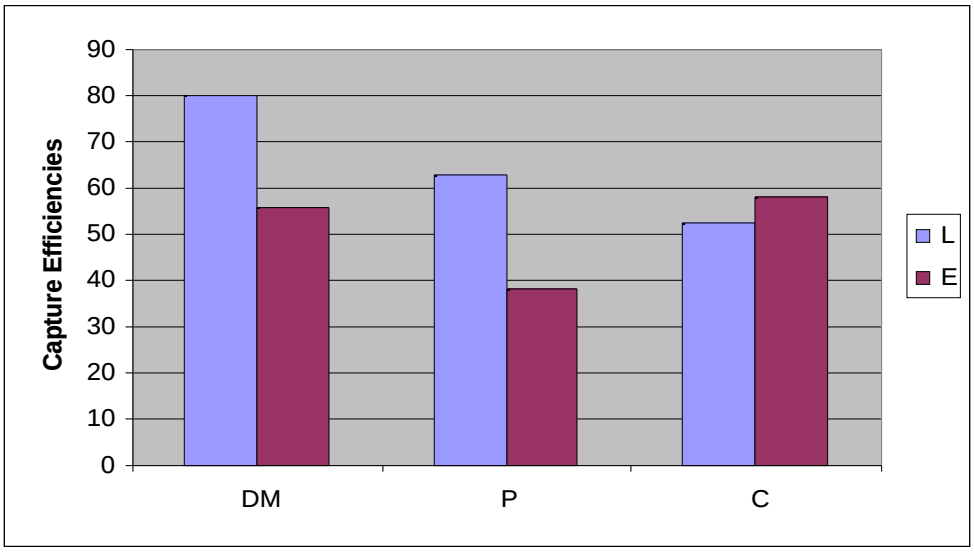

Fig. 2: The averages of the trials in Fig.1

The above results, in Fig. 1, were determined through a series of calculations that stemmed from data extracted from the incubated plates. The plates were counted for number of colonies and then an average was taken of the 3 counts for each step. These averages were then used in a series of calculations to determine capture efficiency. The preliminary results yield data only for non-specific capture as only experiments for carboxyl, dimethylamino, and polystyrene beads have been performed. If non-specific capture were more efficient then there are two possible reasons for this; these reasons could be due to surface charge or to hydrophilic/phobic characteristics. It was expected that the hydrophobic/philic characteristics would not have much of an effect on bacterial adhesion [1]; and that the negatively charged beads would be more effective in binding to both bacteria [2]. Yet our data for non-specific capture shows that the charge data is inconsistent as E. coli binds better to carboxyl beads than Listeria i., and vice versa for dimethylamino beads. E. coli should bind better to dimethylamino as it is gram-negative and should be more attracted to the positively-charged dimethylamino beads; the same

applies for Listeria i. with carboxyl. However these results are not consistent and thus hydrophobicity/philicity could possibly play a role.

This data is raw and has yet to be analyzed, as the experiments for the Dynabead® capture have not been performed yet. Yet when these results are obtained and then they all can be analyzed in full then possibly a clearer solution to the inconsistency visible in the non-specific results can be addressed. There are also plans to perform the bead captures on cantilevers to allow us further understanding on the bead capture process.

References:

1. Lakshmi, S; Kumas, SSP; Jayakrishnan, A. Bacterial Adhesion onto Azidated Poly(vinyl chloride) Surfaces. Journal of Biomedical Materials Research, vol. 61, no. 1, pp. 26-32, July 2002.

2. Ueshima, M; Tanaka, S; Nakamura. S; Yamashita, K. Manipulation of Bacterial Adhesion and Proliferation by Surface Charges of Electrically Polarized Hydroxyapatite. Journal of Biomedical Materials Research, vol. 50, no. 4, pp 578-584, 15 June 2002.

3. Shu-I, Tu; Uknalis, Joseph; Irwin, Peter. The Capture of Escherichia Coli 0157:h7 for Light Addressable Potentiometric Sensor (Laps) Using Two Different Types of Magnetic Beads. Journal Of Rapid Methods And Automation In Microbiology, 17 December 2001.