# MAGNETIC RATCHETING OF HYDROGEL DROPS FOR SELECTION OF HIGH MAGNETIC BIOMASS PRODUCTION BACTERIA

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## ABSTRACT

Here we apply magnetic ratcheting to sort clonal cell populations based on the amount of magnetic biomass, magnetosome, accumulated in uniformly sized gelatin microgels, towards the directed evolution of new highly productive strains of magnetic particle-producing bacteria[1]. Magnetic ratcheting system is the versatile sorting tool based on target magnetic contents. We have showed the magnetic ratcheting system can sort out 1.55 times higher magnetosome production population than non-selected population. As the next step, we will do multiple selection cycle with chemical mutagenesis to produce super productive magnetotactic bacteria population.

**KEYWORDS:** Magnetotactic bacteria, Droplet based sorting, Directed evolution, Cell secretion

## INTRODUCTION

Magnetotactic bacteria, like AMB-1, naturally mineralize iron oxide, producing magnetic nanoparticles surrounded by lipid bilayer membranes (magnetosomes). These magnetic nanoparticles are extremely uniform in size and shape, are formed under green chemistry conditions, and can be modified on their surfaces for biomedical applications such as drug delivery, MRI and hyperthermia. However, still magnetosome formation is not sufficiently scaled for industrial use. Thus, directed evolution to isolate clones that rapidly divide while maintaining production of magnetosomes can provide a useful industrial feedstock.

## EXPERIMENTAL

By first encapsulating AMB-1 in gelatin droplets for growth our system can sort out clones which divide rapidly while maintaining high magnetosome production (Fig.1)[1]. A selection process can be divided to five different phases, (1) library generation, (2) encapsulation of AMB-1 in gelatin droplets[2], (3) incubation in droplets for growth and magnetosome production, (4) sorting by quantitative magnetic content using magnetic ratcheting, (5) and further growth and amplification of the selected populations (Fig.2). Single AMB-1 are encapsulated within gelatin gel droplets surrounded by oil using a microfluidic device[2] and then cultivated for 3 days at 30 ℃. Following cultivation microgels are cooled and transferred from the oil to a water phase, where AMB-1 bacteria are maintained in the gelled matrix. Based on the amount of magnetic (number and quantity of magnetosomes), microgels are sorted using a magnetic ratcheting platform[1]. After sorting, magnetic particle formation was quantified using a cellular magnetization ($C_{mag}$) assay.

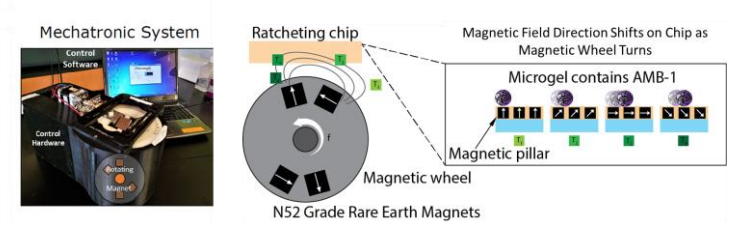


*Figure 1: Magnetic ratcheting system*

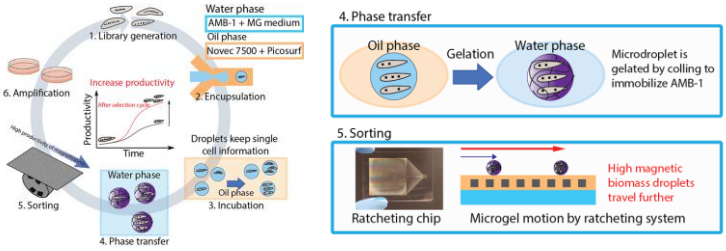


*Figure 2: System flowchart for directed evolution.*

## RESULT ANS DICUSSION

Encapsulation enabled simultaneous selection based on growth and production, which was not previously possible. Importantly, encapsulation did not hinder cell growth to a large extent (7 doubling time in bulk solution vs. 6 doubling time in droplet for 3 days) and allowed for easy recovery after sorting. An optimized droplet size of

70 µm maximized AMB-1 growth, yielding ~ 6 cell divisions creating a large distribution in cell number and increase in the mode of the distribution after 3 days (Fig.3). The magnetic ratcheting system sorted highly productive AMB-1 from the population. After a single selection cycle, preliminary data suggests that $C_{mag}$ and the number of magnetosomes increased compared with control populations in 1.37 times higher. (Fig.4). We also have confirmed that AMB-1 still form magnetosomes even in nanoliter-scale droplets (Fig.4c). Finally, we culture control and sorted population of AMB-1 in 1L bottle and extract magnetosome to compare total productivity. 128.1 mg of magnetosome is produced from sorted population which is 1.55 times more than control population, 82.7 mg. This result show that we successfully sort out not only high magnetosome number population but also high growth rate bacteria.

The magnetic ratcheting system enables quantitative titrated magnetic separations which is not possible with standard magnetic activated cell sorting. As a next step, we plan to introduce random mutagenesis and obtain a larger diversity of clones and operate several sorting cycles to establish increased productivity strains. Extracted magnetic nanoparticles can then be applied to various medical applications.

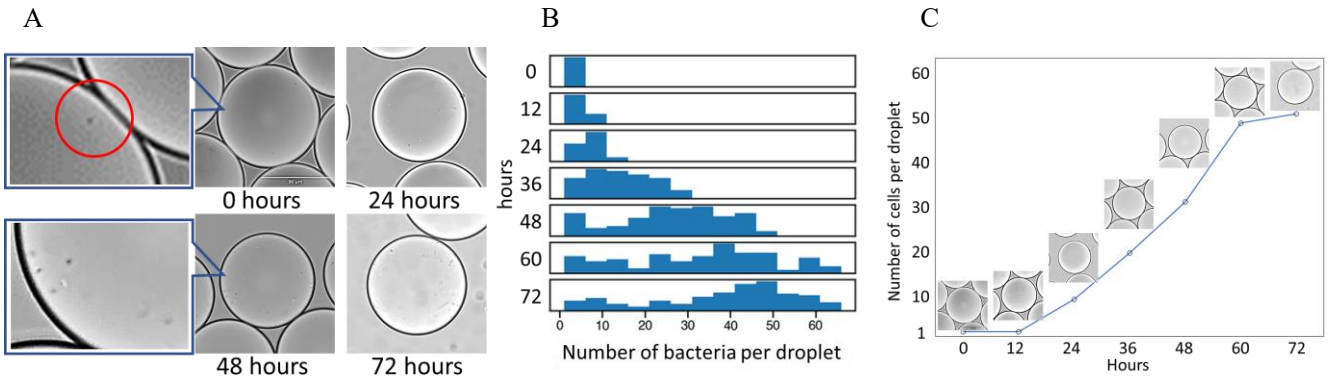


*Figure 3: A) Pictures of encapsulated AMB-1 B) Growth distribution is maximized after 72 hours C) Mode of cells per drop is increased over time.*

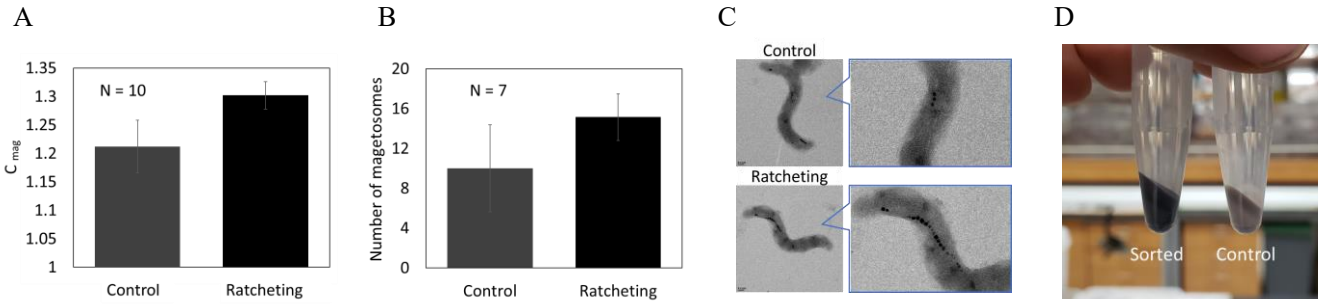


*Figure 4: A-B) $C_{mag}$ , a measure of magnetic content and number of magnetosomes is increased after sorting C) TEM Picture of AMB-1 control and after sorting D)Total production of magnetosome from control and sorted population*


## ACKNOWLEDGEMENTS

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## REFERENCES


[1]Quantitative Magnetic Separation of Particles ; and Cells Using Gradient Magnetic Ratcheting, Murray, C et al., Small, 12, 1891 (2016)

[2]Flow invariant droplet formation for stable parallel microreactors, CT Riche et al., Nat Comm,7,10780 (2016)


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