

Magneto-transformation of *Clostridium pasteurianum* to increase butanol yield

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The adoption of policies favoring the use of renewable biofuels [1] contributed to a steep increase of the availability of crude glycerol (a surplus by-product from the production of biodiesel) which is expected to reach over six times the demand by 2020. *Clostridium pasteurianum* is a gram-positive bacterium able to convert crude glycerol into economically valuable compounds such as butanol that can be used as fuel additive. For this approach to become economically viable, an improvement in the yield of conversion of glycerol to butanol is required which could be achieved through synthetic biology, tuning known metabolic pathways of *C. pasteurianum* by shutting down or overexpressing specific genes. However, this species is known to be hard to transform by conventional methods such as heat shock or electroporation. Magnetofection is a technique invented by Plank and Bergemann in 2001 [2] that has been almost exclusively focused on the transfection of mammalian cells by directing DNA bound to magnetic nanoparticles (MNPs) into the interior of cells through the application of an external magnetic field [3]. The use of MNPs as vectors for transformation of bacteria is still unexplored and has the potential of improving the transformation efficiency of several key microorganisms. This project aims to establish the optimal conditions for transformation of hard-to-transform bacteria by using MNPs with specific sizes and functional groups tailored for bacteria. MNPs are being synthesized and tested using equipment from a spin-off company that produces an external oscillating magnetic field against gradually harder-to-transform bacteria (*E. coli*, *B. subtilis* and *C. pasteurianum*). The magneto-transformation efficiency of our MNPs is being compared with conventional transformation methods and with the commercial MNPs that are normally used for magnetofection of mammalian cells.

[1] Directive 2003/30/EC of the European Parliament and of the Council, 2003

[2] Plank, C., Schillinger, U., Scherer, F., et al. The magnetofection method: Using magnetic force to enhance gene delivery. *Biol. Chem.*, 384 (5), 737-747, 2003

[3] Plank, C., Zelphati, O. and Mykhaylyk, O. Magnetically enhanced nucleic acid delivery. Ten years of magnetofection - progress and prospects. *Adv. Drug Deliv. Rev.* 63, 1300-1331, 2011

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