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Controlled ultrasound cavitation for DNA delivery into bacteria and yeast

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Sonoporation is an ultrasonic cavitation-dependent method for gene delivery, mostly applied to higher eukaryotic cells and tissues. Cavitation in the vicinity of cells induces pore formation in the cell envelope and mechanical internalization of extracellular DNA. Its application to biotechnology useful organisms is less developed. Here we evaluated the CaviBox® sonoporation device for plasmid delivery into the yeast *Kluyveromyces lactis* and the bacterium *Escherichia coli* BL21, commonly employed organisms for bio-production. CaviBox® tightly controls cavitation through combination of multiple ultrasound beams under strong negative pressures. We evaluated the effect of different cavitation conditions (intensity, duration, duty cycle) and cell suspension media composition on cell viability and functional plasmid uptake. Optimal DNA delivery into *K. lactis* kept in YPD culture medium and incubated with linearized plasmid before sonoporation was achieved at CI 14 for 60 seconds with minimal effects on cell viability. Transformation efficiency was comparable to conventional chemical transformation of this host. Plasmid uptake by *E. coli* occurred in LB culture medium supplemented with 100 mM $CaCl_2$ at CI 12-14 for 20 seconds. Under the same cavitation conditions, transformation was 100 times more efficient when bacteria were suspended in a solution of 25 mM $MgCl_2$, 100 mM $CaCl_2$ and 10% Glycerol. These first results demonstrate the feasibility of DNA delivery into bacteria and yeast species of biotechnological interest by sonoporation, directly in culture medium with minimal manipulation. Improvement of this method holds the potential of simple parallel transformation of large strain collections and or recombinant DNA libraries.

Biography

Gustavo Stadthagen holds PhD in Microbiology from the University of Paris Denis Diderot. He has published more than 10 articles on Molecular Genetics and Biochemistry of Infectious Agents, Cancer Cells and Biotechnology Relevant Microorganisms. He currently works as a Research Scientist at the Unit of Protein and Expression System Engineering of BIOASTER, a new technology research institute on infectiology and microbiology. His research using an integrated approach that includes enhanced recombinant DNA construct design, assembly, delivery and stabilization aims to develop improved protein expression systems for bio-pharma and biotech applications.

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