One-step transformation of microorganisms

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Background: PgD in Micro Nanotehcnology/ University of Minho/ Portugal
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OBJECTIVES

This project aims to develop products that simplify the laboratory methods used for the genetic improvement of microorganisms with commercial potential that can be applied in biotechnology, in areas such as food industry, environment or energy. These products will constitute a new methodological paradigm through its simplicity and will substitute classical procedures that are more time-consuming, more expensive and technically more demanding. Transformation of microorganisms (introducing DNA into microorganisms for a genetic improvement) is a cumbersome procedure normally needing three main steps, namely: (1) preparation of microorganisms (weakening of natural barriers to receive foreign DNA); (2) a shock step (normally through an electric pulse, jump in temperature or by using ultrasounds); and finally the (3) a recovery step in which the microorganisms are grown in rich medium to recover from the previous step. With our technology we envisage attaining transformation in a single step through spreading microorganisms and foreign DNA on novel transformation petri-dishes.

WORK PLAN

Synthesis of superparamagnetic monodisperse magnetic nanoparticles (with diameters of less than 10 nm) and functionalization with cationic molecules such as dopamine, 4-aminobenzoic acid and 2-pyrrolidinone. Synthesis and functionalization of magnetic nanoneedles. Use external magnetic field or friction forces to induce membrane permeabilization. Synthesized or natural nanomaterials are used to facilitate entrance of DNA in microorganisms such as *Escherichia coli, Bacillus subtilis* and *Saccharomyces cerevisiae*, all of them GRAS microorganisms. The microorganisms mixed with plasmids are spread on culture petri dishes in the presence of nanomaterials and with addition of adequate markers (for instance ampicinin 100µg/mL, IPTG 1mM and X-Gal 40µg/mL for *Escherichia coli.*) transformed colonies are produced (Yoshida & Sato, 2009).

RESULTS

Nanomaterials were synthesized and functionalized with cationic molecules. First attempts to transform non-competent JM109 *Escherichia coli* with pUC19 plasmid and intact BY4741 *Saccharomyces cerevisiae* with pyes2- isc1 plasmid have succeeded by using nanoneedles. Maximum transformation efficiencies obtained for *Escherichia coli* are on the order 1.6×10^5 CFU/µg DNA and 5.8×10^3 CFU/µg DNA for *Saccharomyces cerevisiae*. Essays on *Bacillus subtilis* are in progress.

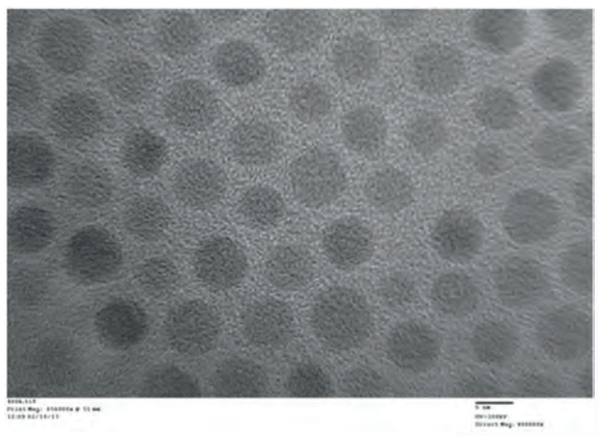

FIGURE 1
Synthesis by thermal decomposition of mono-dispersed magnetic nanoparticles with diameters of about 5nm (S. Sun et al., 2004). Nanoparticles are capped by oleic acid and a ligand exchange reaction is performed with small cationic molecules such as dopamine and 4-aminobenxoic acid to promote dispersion in water and affinity with plasmids. Scale bar is 5 nm.