DEVELOPMENT OF AN EFFICIENT TRANSFORMATION PROTOCOL FOR *ASPERGILLUS SOJAE*, CELL FACTORY FOR ENZYME PRODUCTION

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Introduction. *Aspergillus sojae* is a well-known filamentous fungi, which has been mostly used in the production of the Japanese food "koji", by diverse fermentation processes (1). Furthermore, the potential of this organism as a cell factory for the production of chemicals, pharmaceuticals and enzymes, is promising, and has been evaluated in some groups (2), including ours. However, the lack of an effective transformation protocol for this fungus has been a significant limitation in order to study and understand its biology, and therefore to exploit it more widely for biotechnological purposes. It is our aim to setup a reliable transformation protocol for *A. sojae* based in the *Agrobacterium tumefaciens*-mediated transformation (ATMT) approach. As far as we know, this approach will be the first of its kind for this fungus, a protocol that might be equally applicable to other *Aspergillus* or filamentous fungi species with academic or industrial interest.

Methods. ATMT for filamentous fungi (3) is an approach that has been successfully applied for the introduction of DNA in selected filamentous fungi. ATMT is based in the capacity of *Agrobacterium tumefaciens* to transfer part of its DNA (transferred DNA; T-DNA), contained in the tumor-inducing (Ti) plasmid, to the host cell (Fig. 1). Such T-DNA is typically randomly inserted in the host genome as a single copy (4).

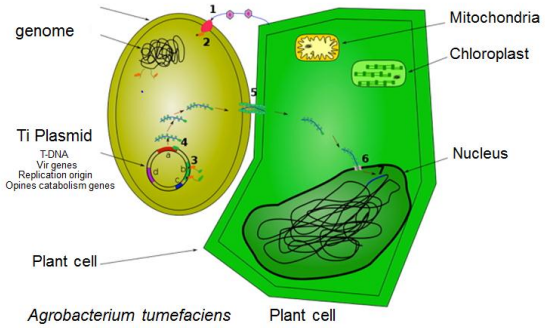

Fig.1 *Agrobacterium tumefaciens* transformation principle. Adapted from Wikipedia.org.

The development of an ATMT protocol for *Aspergillus sojae* strain ATCC 20235, involves the optimization of several sequential steps. Among the key variables are: selection of a dominant antibiotic marker for the fungus; design and construction of a Ti vector; and finally assay diverse co-cultivation conditions between the *Agrobacterium* and *Aspergillus* strain.

Results. So far, we have reached important milestones along this project, like the settings for *A. sojae* selection with Phleomycin antibiotic, and the construction of the Ti vector "pRMegfp" specifically designed to select recombinant *A. sojae* (Fig. 2). pRMegfp vector confers phleomycin resistance (*ble*) and incorporates also the enhanced Green Fluorescent Protein (eGFP) as a reporter gene.

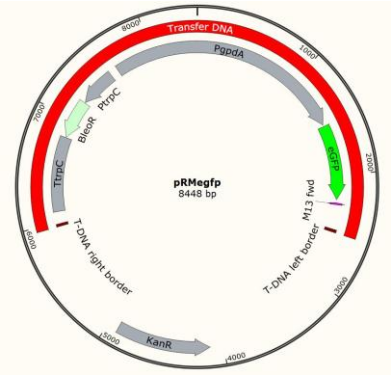

Fig.2 Ti vector pRMegfp.

In ongoing experiments, we are evaluating different co-cultivation parameters to prove the capability of the approach to produce *A. sojae* transformants. These parameters are: *Agrobacterium* strain, *Agrobacterium*:*Aspergillus* conidia ratio, and induction conditions (concentration, time and temperature). Resulting recombinant fungi will be analyzed by PCR, fluorescent microscope and sequencing analyses.

Summary.
-The construction of the pRMegfp vector is complete, and its correct assembly was successful confirmed according to sequencing analyses.
- Current results of this ongoing project will be presented on this poster.

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