IMMUNOTEC

Robust fermentation production of tacrolimus and related immunosuppressors: Molecular genetics and metabolic engineering to construct a by-product free superproducer

Partners involved in the IMMUNOTEC project

Project coordinator

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Project leaders

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'Thanks to the ERA-Net ERA-IB scheme we have had the opportunity to get funding and collaborate at European Union-wide level in industry-driven research, within a more handleable and focused framework, in terms of scientific and technical scope, compared to the Cooperation programme in FP7.'

Tacrolimus (FK506) is a polyketide compound with potent immunosuppressant activity, widely used to avoid transplant rejection. It has also been used in the treatment of certain autoimmune diseases and dermatoses. Tacrolimus administration, either intravenously or orally, is effective in preventing transplants rejections, being approximately 100 times stronger than the well-known immunosuppressant cyclosporin A. there is a continuous trend of increased use of tacrolimus instead of cyclosporin.

Tacrolimus is produced by Streptomyces tsukubaensis and other Streptomyces species. This compound binds the human FKBP protein, causing a reaction cascade that gives rise to a reduction in the T-cell mediated human immune response, thereby avoiding transplant rejection. This drug is a high-added value pharmaceutical, but its production is technology-intensive, because the levels of tacrolimus produced by the wild type strains are very low.

The project had the following objectives:

1) the genome analysis and cloning of the tacrolimus cluster;
2) the nitrogen regulation of tacrolimus biosynthesis production;
3) the modulation of the oxidative stress response to tacrolimus production; 4) the phosphate control of tacrolimus production;
5) the heterologous expression of the tacrolimus biosynthetic gene cluster; and
6) the improvement of oxygen transfer. The final aim is to obtain overproducer strains of tacrolimus and better fermentation conditions.

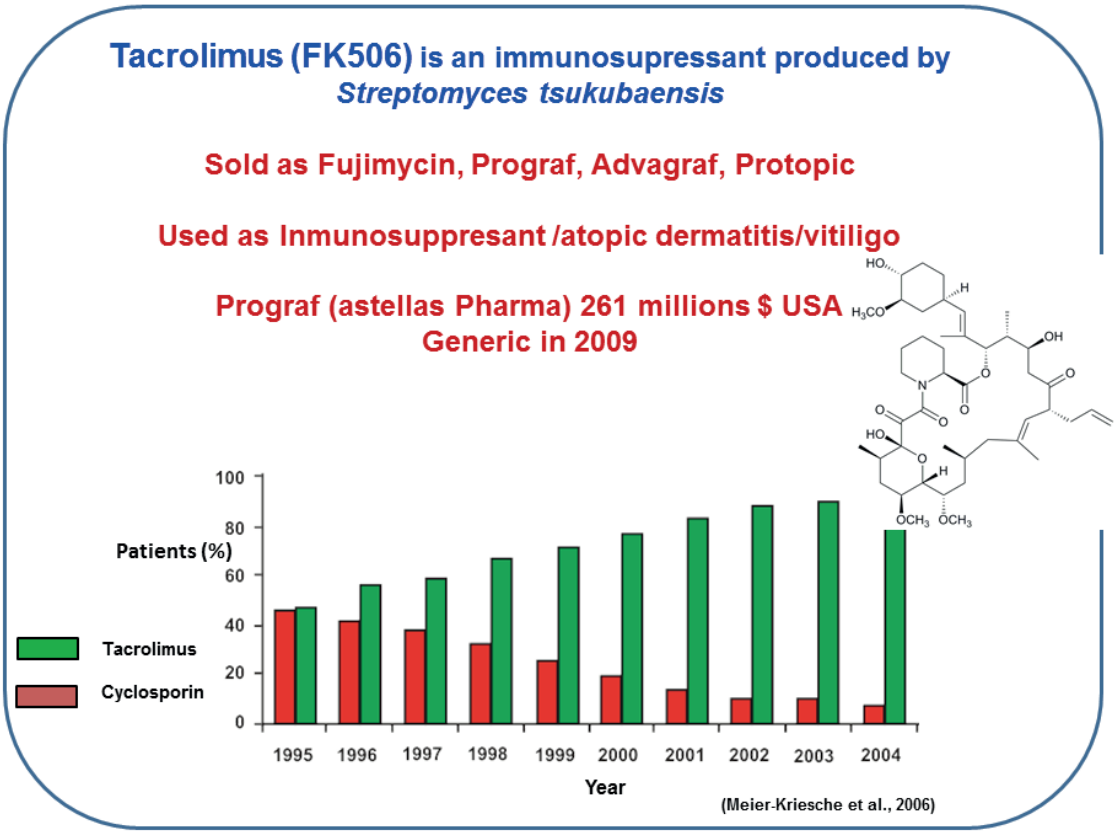
These objectives have been investigated by the groups headed by Professors Paloma Liras and Juan F. Martín (objectives 1 and 4) from Inbiotec (León, Spain); Professors Dr. Wolfgang Wohlleben and Lutz Heide (objectives 2 and 5), from Eberhand-Karls-Universität Tübingen (Tübingen, Germany); Dr. Marta Vaz Mendes (objective 3) from IBMC-Instituto de Biología Molecular e Celular (Porto, Portugal); and Dr. Tania Velasco (objective 6) from Antibióticos SA(León, Spain).

Results

The Streptomyces tsukubaensis genome has been sequenced. It contains a linear chromosome (7.62 Mbp) and two circular plasmids of 24.7 and 31.1 kbp. The genome encodes 6623 proteins of standard size. Thise genome is very rich in sigma factor encoding genes (52) for the regulation of gene expression and contains clus-

ters of genes for 20 secondary metabolites, in addition to the tacrolimus gene cluster. The identification of these clusters will allow the detection of new compounds of still unknown pharmacological interest. The genes for nitrogen assimilation and their regulators have been analyzed. S. tsukubaensis transformants, carrying the S. coelicolor nnaR gene, able to grow on nitrate, have been obtained.

The genes involved in oxidative stress regulation have been studied and related to tacrolimus production, which opens a way to improve the tacrolimus production by modifying the oxygen supply to the cultures.



Tacrolimus overproducer strains have been obtained by transformation with the fkbN regulatory gene, the pipA gene or by knock-out of the aphC gene. These genetic modifications increased the tacrolimus production by 40 to 60% in each case.In summary, a great increase in the knowledge of the molecular biology of tacrolimus production has been achieved and overproducer mutants of the compound have been obtained.

Impact

The knowledge on the molecular biology and the strategies for tacrolimus production will serve to develop more competitive production systems. This, in turn, will result not only in more profitable industrial processes, but also in cheaper and affordable immunosuppressant therapies.