ERA CoBioTech

BioTech Research & Innovation Hack

2021

ERA CoBioTech Funded Projects at A Glance: SYNTHEROIDS

Synthetic Biology for Industrial Production of Steroids

PART OF EUROPEAN BIOTECH WEEK

INNOVATION IS IN OUR GENES

SYNTHEROIDS

Development of an integrated production process for pharmaceutical steroids using synthetic biology and improved processing technology

Phytosterols are renewable biological resources, which can be converted to precursors of high value clinical steroids by microorganisms

Project coordinator:

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Consortium:

Pharmins Ltd. (Russia)

Sintef Materials and Chemistry - Biotechnology and nanomedicine (Norway)

TU Dortmund -Laboratory of plant and process design (Germany)

Curia Spain (Spain)

Project duration:

15 May 2018 - 30 November 2021

Total budget: 1.7 €M

Bioconversion of phytosterols to new precursors of pharmaceutical steroids

Pharmaceutical steroids are widely used in medicine as anti-inflammatory, anti-allergic agents, sex hormones, etc. as well as in the prevention and treatment of many serious diseases, such as cancer, obesity, diabetes, rheumatoid arthritis, asthma or neurodegenerative diseases, among others. The importance of pharmaceutical steroids has been shown recently, when the steroid dexamethasone was the first drug combating the symptoms of severe cases caused by SARS-CoV-2.
The Syntheroids project aims to develop integrative processes for innovative bioconversion of phytosterols to C22-steroids based on synthetic biology of non-pathogenic Actinobacteria. Phytosterols are renewable biomaterials produced in large amounts as by-product from pulp and paper mills and edible oil producing factories, and some microorganisms are able to convert these phytosterols to other molecules which can be used as precursors for the synthesis of high value clinical steroids.
Although the production of key steroid intermediates (mainly C19-steroids) from phytosterols has been industrially implemented, there is still considerable room for improvement and many challenges remain. The central objective of Syntheroids is to develop an integrated production process for pharmaceutical steroids using synthetic biology and improved processing technology.
A few steroid precursors are today industrially produced from phytosterols, mainly by companies in China, India and the US, although some are located in Europe. Innovative ideas that can expand the list of steroids produced from phytosterols in a single-step biotechnological process are wanted, and this is why two European companies (Curia Spain and Pharmins Ltd.) are active partners in the Consortium. Shorter steroid production pipelines and eco-friendly processes, in compliance with European regulations, will increase the EU-GDP (gross domestic product) as it will increase the companies' competitiveness and reduce the end drug user's medical invoice.

Omics data integration for productive engineering bacterial strains and sustainable integration of Up-and Downstream processes.

The main tools applied in Syntheroids project are the omics techniques, where all the transcribed genes (transcriptomics), the expressed proteins (proteomics) and the produced metabolites (metabolomics) are identified in a determined culture condition.
Complete genome sequencing of steroid-producing bacterial strains and a comprehensive bioinformatics analysis of the assembled genomes has allowed predicting the genes related to steroid metabolism, their operon organization and possible regulation. The transcriptomic, proteomic and metabolomic analyses comparing producing and non-producing conditions has enabled the identification of some genes playing a key role in the proposed branching of the sterol side chain oxidation pathway. The deletion of some of these key genes and the overexpression of other ones has allowed to direct the phytosterol bioconversion toward the target compounds, producing for the first time in a fermentative process some important steroid precursors.
The gene modified not always produced the expected results, and to solve this problem different approach were performed, including the accumulation of different mutations in a same strain. Also, culture conditions were modified with the aim of increase the steroid bioconversion and the reduction of by-products production.
On the other hand, the integration of upstream (fermentation/biocatalysis for product formation) and downstream (separation techniques for product purification) has major potential to design cost efficient and competitive processes. The incentives of process integration are the definition of an overall process optimum (instead optimizing up- and downstream separately without considering the interactions between these steps), overcoming limiting equilibria, avoid (by-)product inhibition, product degradation and convert hydrophobic substances.

This project has received funding from the European Union´s Horizon 2020 research and innovation programme under grant agreement No [722361]

Main results

C22-steroids as phytosterol oxidation by-product of mycobacteria were first described at the beginning of 70thies and its practical interest was rapidly recognized. During decades, C22-steroids were based on microbial strains obtained by random mutagenesis, but the yield, productivity and selectivity were insufficient for industrial use.
Currently, there are sufficient genetic data for rational metabolic engineering. Different bacterial strains have been genetically modified for the production of new C22-steroids, which will be used as starting material for the synthesis of pharmaceutical steroids with application in gastroenterology, endocrinology and other fields of medicine. Some of the initial mutants constructed in the project produced the target compounds and other steroids as by-products. Adding new mutations to the strains and modifying the composition of culture broth the degradation of phytosterols was directed toward the desired compounds. However, the by-products detected can also be used as precursors for the synthesis of other therapeutic steroids, opening new lines of research. The compiled data from omics analyses could be also applied for the engineering of these new strains, as well as the new formulations of the broth cultures aimed to maximize the production. The Syntheroids project has also served for the optimization of downstream process, which can be applied for the purification of steroids with a similar polarity.
The project Syntheroids has practically reached to achieve the proposed objectives, with the production of three target compounds and the identification of fungal strains able to introduce modifications for the production of a fourth steroid. Using one of the new compounds produced, a downstream process has been designed and the scalability to industrial production has been probed.

Future prospect

The goal of Syntheroids, the bioconversion of steroids supported by Synthetic biology, is of major importance for the industrial partners (Curia Spain and Pharmins Ltd.). Both companies will transfer the generated knowledge straight into application, thus ensuring a rapid transfer from invention to practical use.
The Syntheroids project has expanded our knowledge of phytosterol catabolic pathways and their regulation in Actinobacteria, providing innovative solutions for production of steroids for the pharmaceutical industry. Novel engineered strains capable of efficiently forming C22-steroids, have been generated, in addition to new, innovative bioprocesses. These precursors can be used for the synthesis of several therapeutic steroids applied in gastroenterology, endocrinology and other fields of medicine with an enhanced, eco-friendly, and economic process. This process perfectly fits the Bioeconomy concept, as renewable biological resources are converted to economically valuable medical products.
The exploitable output from Syntheroids is the holistic approach where: i) synthetic biology combined with omics analyses have been used to increase the product portfolio and decrease end-product inhibition, ii) a robot based conceptual design methodology together with modelling and simulation will ease product purification and iii) better integrated industrial procedures (up- to downstream) evaluated via costs will decrease production costs.
In addition, the reactions catalyzed by some of the new genes identified are not specific for only one substrate and they can be applied over other steroids not included in the project, which will allow to increase the number of new precursors available in a next future.

A protocol for sorting of Mycobacteria on single cell level using FACS technology (Fluorescent Activated Cell Sorter) has been standardized. The protocol may be used to separate living cells which have been exposed to and are able to tolerate increased concentrations of steroid end-products from dead/ vulnerable cells, to give one example. Also, diverse protocols have been developed for the detection and identification, either by HPLC or by GC, of different types of sterols and steroids. Other standardization protocols are related to downstream processes, mainly concerning to the extraction methods of the various phases of the fermentation broths to increase the value product concentration.

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Methodologies for omics analyses have also been implemented during the Syntheroids project, serving also for future research for the production of other steroid precursors. The response of Nocardioides simplex VKM Ac-2033D to phytosterol and cortisone 21-acetate has been recently published (https://doi.org/10.1186/s12896-021-00668-9), as well as the response to phytosterol of the 9-hydroxyandrostenedione-producing strain of Mycobacterium sp. VKM Ac-1817D (https://doi.org/10.1186/s12896-019-0533-7).
As a result of the tasks of the project, fungal strains providing effective 7β-hydroxylation of ADD have been identified, and the results have also been published https://doi.org/10.1016/j.phytochem.2019.112160). Totally, four peer reviewed paper have been published, and some other more are in elaboration. A second edition of the book Microbial Steroids - Methods and Protocols (https://doi.org/10.1007/978-1-4939-7183-1) will be also published due to the success of the first edition, with more than 25,000 downloads. Two members of the consortium were the editors of the first edition, and all the other members collaborated with different chapters.

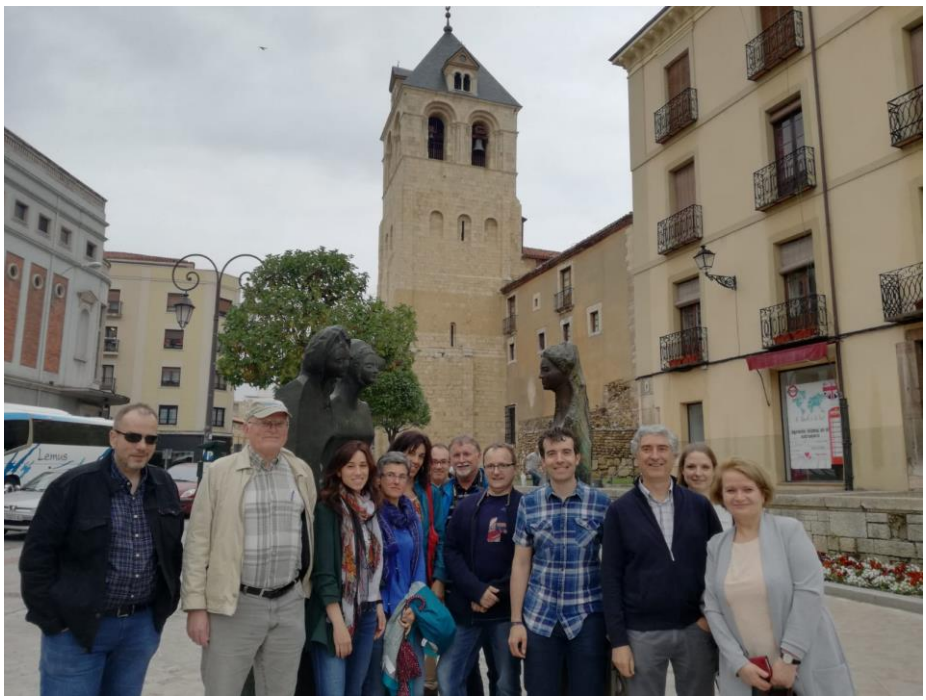

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Figure 1: Syntheroids consortium at the kick-off meeting, hosted by INBIOTEC (Leon, Spain)

Twitter: @syntheroids
Research gate: https://www.researchgate.net/project/Syntheroids-Synthetic-Biology-for-Industrial-Production-of-Steroids

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