



## Novel industrial bioprocesses for production of key valuable steroid precursors from phytosterol



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Project acronym: MySterI

(*Mycobacterial Steroids for Industry*)

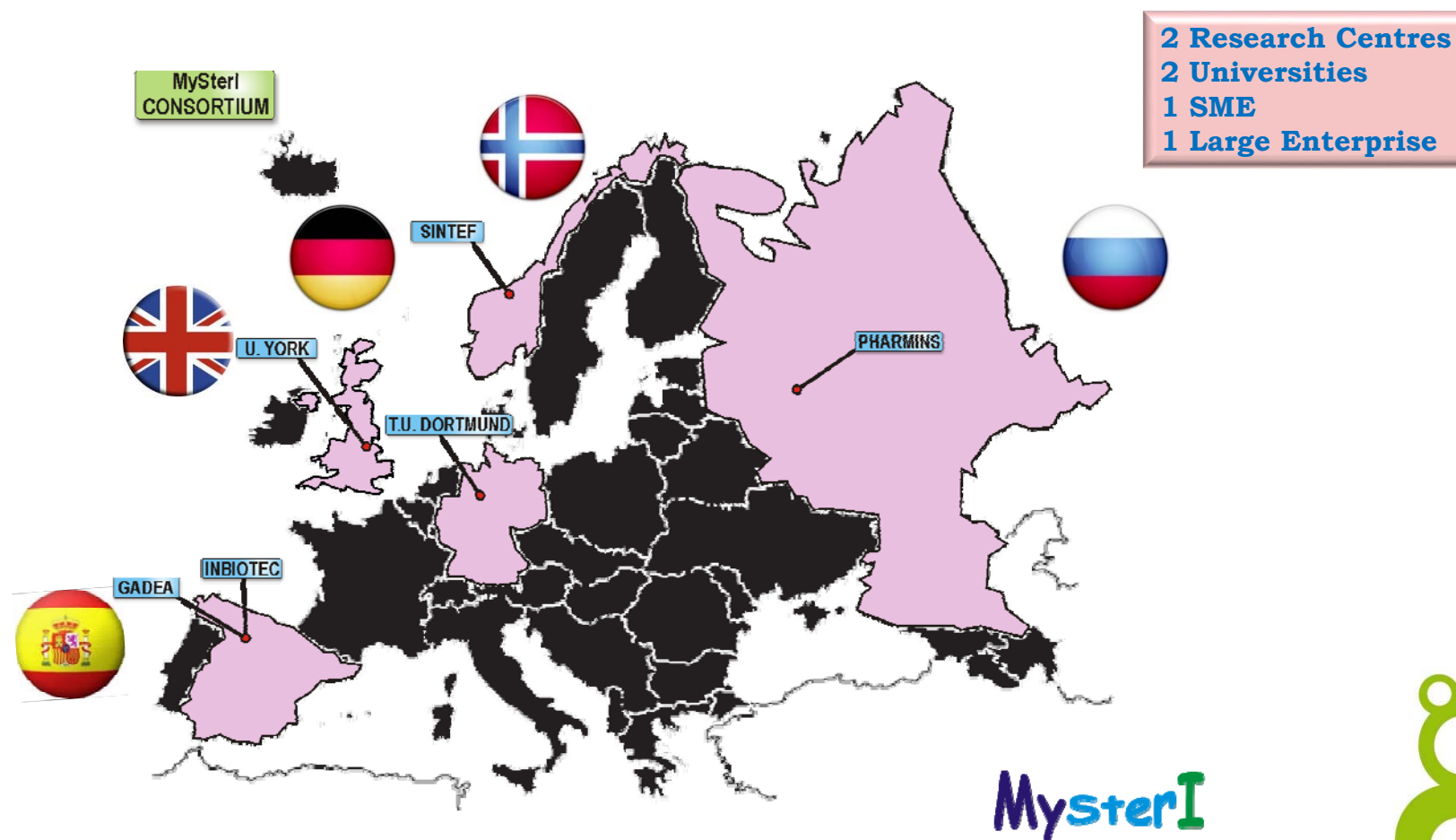
Project no: EIB.12.010

Name: Carlos Barreiro

MySterI

ERA-IB-2 final conference, Berlin, 16./17.02.2016

# Project partners



Project acronym: MySterI  
ERA-IB-2 Final conference, Berlin, 16./17.02.2016





P1: INBIOTEC

# Project partners

- **P1: COORDINATOR:** *Asociación de investigación- INBIOTEC-Instituto de Biotecnología de León (Research Centre). León (Spain).*
- *Dr. Carlos Barreiro, Dr. Antonio Rodríguez-García, Dr. Alberto Sola-Landa*

## MySterI tasks of INBOTEC:

- Genome sequencing *Mycobacterium* sp NRRL B-3805
- Genome mining and annotation
- Transcriptomics (microarrays, RNAseq)
- Proteomics (secretome analysis)

- *Total project budget: 93 000 €*



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P2: Pharmins Ltd.

# Project partners

- **P2: Pharmins Ltd. (SME) Pushchino (Russian Federation)**

- *Dr. Marina Donova*

## MySterI tasks of Pharmins:

- Genome sequencing *Mycobacterium* sp NRRL B-3805
- Biochemical characterization of proteins
- Sterol conversion by modified mycobacterial strains
- Two-steps fermentation to obtain 11- $\alpha$ -OH-AD
- Modification of 11 $\alpha$ -hydroxylase enzymes

- **Total project budget: 123 743 €**



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**P3: University  
of York**

# Project partners

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- ***P3: University of York (University) York (UK)***
  - *Professor Maggie Smith, Dr Jessica Loraine*

## MySterI tasks of U. of York:

- Genome sequencing *Mycobacterium* sp NRRL B-3805
- Genetic tools and strain development
- Development of DNA transformation procedures
- Development of gene knock-out techniques
- Development of promoters to control gene expression

- ***Total project budget: 312 246€***



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P4: Stiftelsen  
SINTEF

# Project partners

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- *P4: Stiftelsen SINTEF (Research centre). Trondheim (Norway)*
- *Mr. Håvard Sletta, Mr. Kjell.D.Josefsen, Dr. Anna Nordborg*

## MySterI tasks of SINTEF:

- Miniaturized cultivations
- Rapid LC/MS analyses for steroids
- Development of fermentation medium for 'omics
- Laboratory scale fermentations and metabolomics

- *Total project budget: 450 000€*



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# Project partners

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- ***P5: Technische Universität Dortmund. Laboratory of plant and process design (University) Dortmund (Germany)***

- *Dr.-Ing. J. Merz, Prof. Dr.-Ing G. Schembecker*

## **MySterI tasks of TU Dortmund University:**

- Key performance indicators capable to handle impurity limits
- Prototyping of a robot based conceptual design methodology
- Design of a prototype of a downstream process

- ***Total project budget: 601 987€***





**P6: Gadea  
Biopharma**

# Project partners

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- ***P6: Gadea Biopharma S.L. (Large Enterprise). León (Spain)***
- *Dr. José L. Barredo, Dr. Marta Rodríguez-Sanz*

## **MySterI tasks of Gadea:**

- Optimization of media and flask cultures**
- Two-steps fermentation to obtain 11- $\alpha$ -OH-AD**
- Improvements for phytosterol bioconversion into testosterone, 11- $\alpha$ -OH AD and DHEA**
- Pilot plant (5-500 L) viability studies and demonstration**

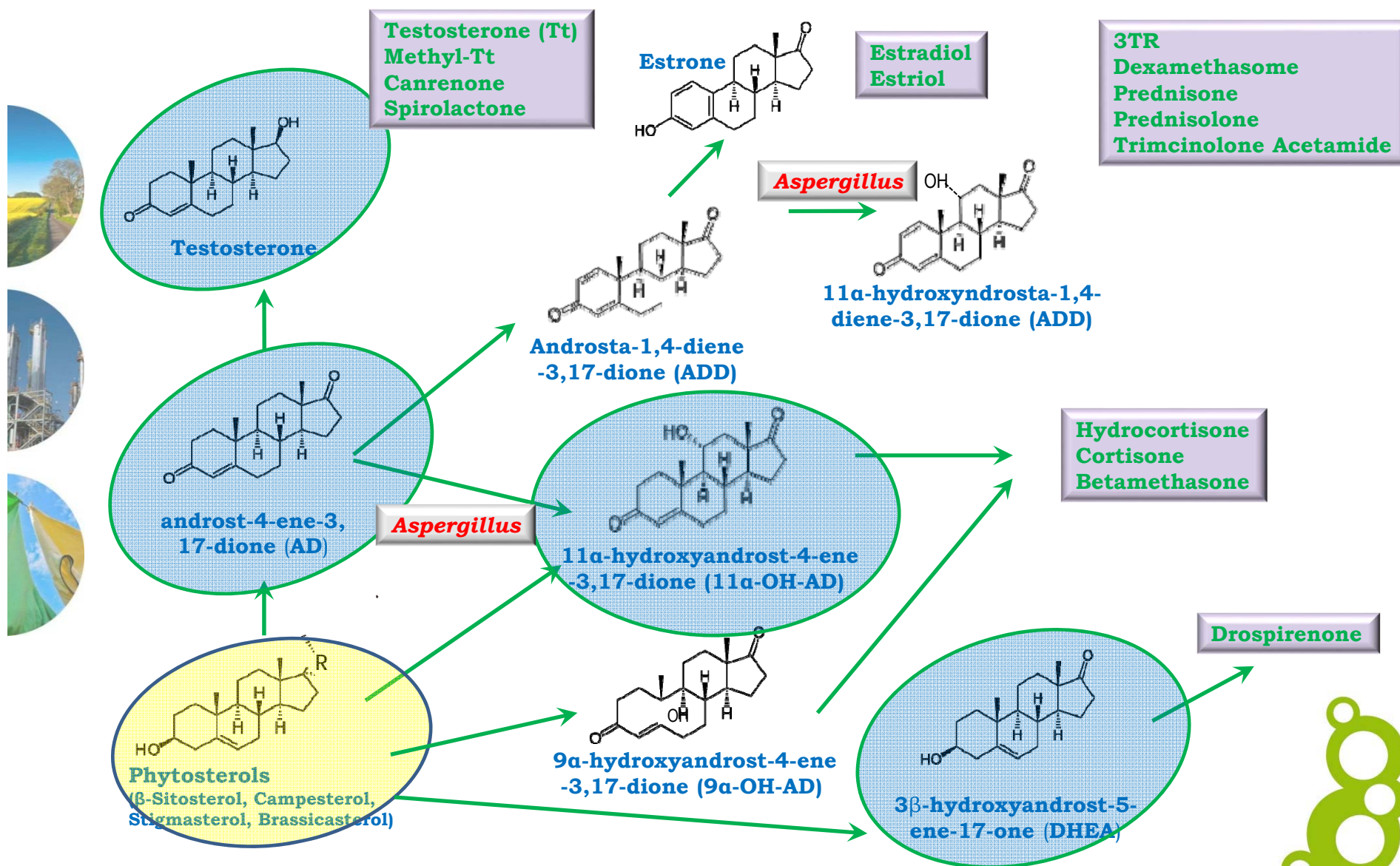
- ***Total project budget: 67 000€***





# Introduction

Why these compounds?





**Phytosterols low water solubility** (1  $\mu\text{M}$ , retards bioavailability)

**Bioaccessibility and bioavailability of phytosterols** (smaller the particle size)

**Cell envelope** (lipophilic cell envelopes containing mycolic acids)

**Affinity uptake of sterols** (direct contact between cells and the substrate)

**Toxic effects of steroid products** (AD and ADD inhibit cell growth)

***In situ* product recovery** (special adsorbents addition)

Wang *et al.*, 2011 (From Soybean Phytosterols to Steroid Hormones). DOI: 10.5772/1007



### *General objective of MySterl:*

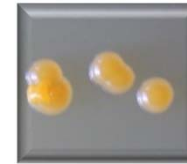
Production of different high value steroid precursors from phytosterols in a single step

### *Detailed objectives of the project:*

- Genome sequencing and annotation of *Mycobacterium* sp. NRRL B-3805 (AD-producer) to identify key bioconversion genes and to enable 'omics tools.
- Understanding of phytosterols bioconversion by means of 'omics technologies.
- Development of the genetic engineering tools for *Mycobacterium* sp. NRRL B-3805.
- Construction of mycobacterial strains capable of producing 11- $\alpha$ -OH-AD, DHEA and testosterone.
- Designing more efficient and eco-friendly methods of production and downstream processing for the three selected compounds.



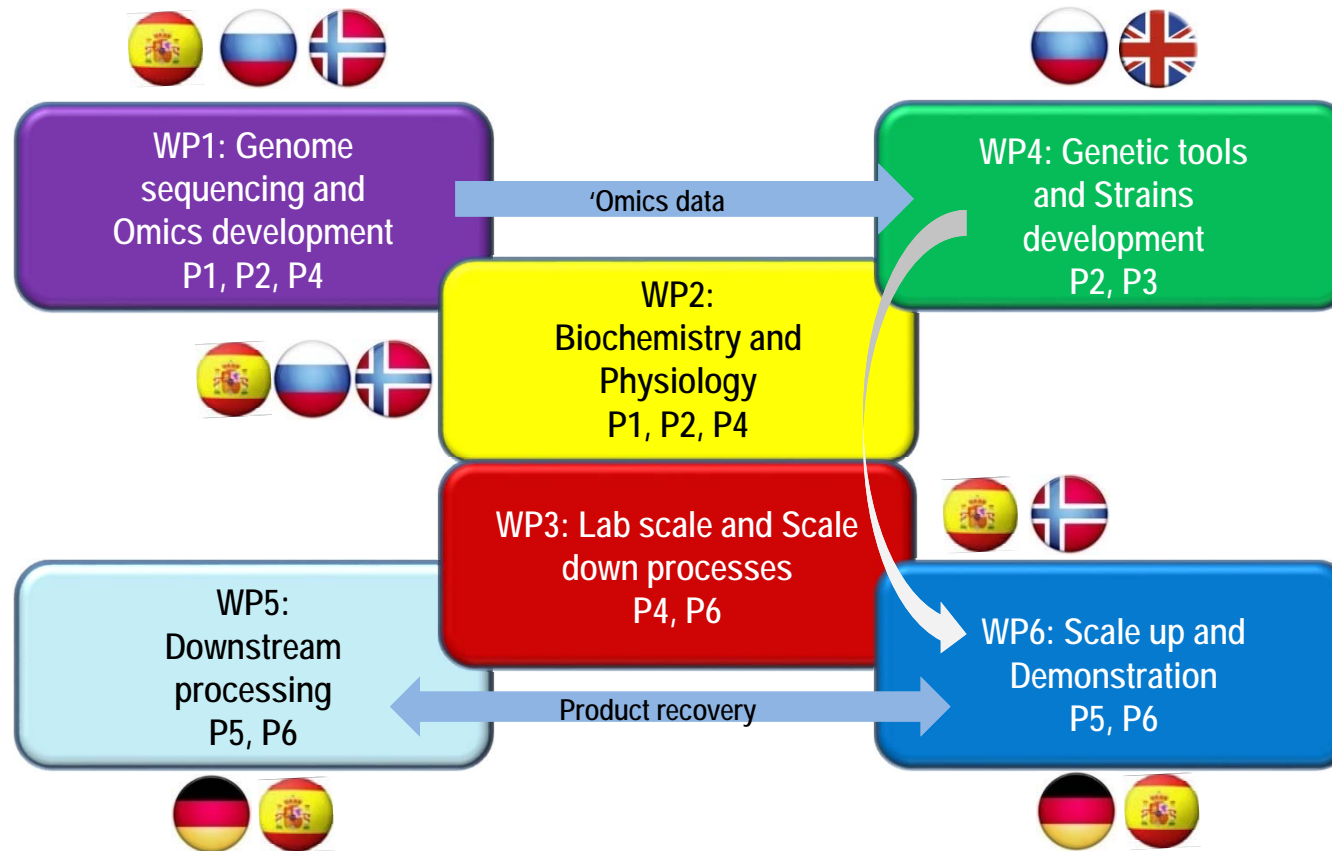
# Introduction



*Mycobacterium* sp NRRL B-3805:  
Fast-growing Mycobacteria  
High AD production  
Low ADD production

- *General project approach:*

## Workpackages Scheme



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# Summary

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- Genome sequencing and annotation of *Mycobacterium* sp. NRRL B-3805 (AD-producer) to identify key bioconversion genes and to enable 'omics tools.



- Understanding of phytosterols bioconversion by means of 'omics technologies.



- Development of the genetic engineering tools for *Mycobacterium* sp. NRRL B-3805.



- Construction of mycobacterial strains capable of producing 11- $\alpha$ -OH-AD, DHEA and testosterone.



- Designing more efficient and eco-friendly methods of production and downstream processing for the three selected compounds.

## *Plans for future:*

- Continue steroids research by Horizon2020
- Two ERA-IB have been applied to 7<sup>th</sup> call



# Project outcome

## Tools

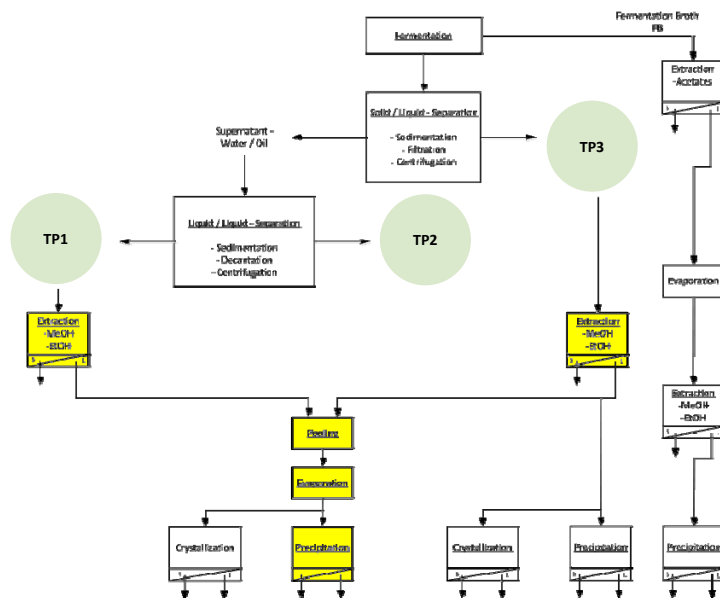


- **Genetics:** DNA transformation procedures, gene knockout technology and a range of promoters for gene expression
- **Physiology:** Established cultivation conditions suitable for 'omics sampling
- **Analytics:** New LC/MS analytical methods for fast measurement of phyosterols and steroids
- **Industrial Processes:** Insight into the transformation process of phytosterols to steroids and impact on cell physiology



# Project outcome

## Greener processes



- Implementation of robot based design methodology
- Development and Automation of process routes for recovering AD:

	$Y_{AD} [\%]$	$X_{AD} [\%]$
Extraction	$96.07 \pm 0.11$	79.0 - 87.6
Pooling	100.00	$86.72 \pm 0.25$
Evaporation	100.00	$86.72 \pm 0.25$
Precipitation	$85.28 \pm 0.05$	$88.41 \pm 0.17$
$\Sigma$	<b>81.93</b>	<b>88.41</b>

- Open topics:
  - Crystallization as alternative to precipitation
  - Evaluation of process route with whole fermentation broth



# Project outcome

# Dissemination



@MySterI\_ERA\_IB



**Congress  
of European  
Microbiologists**



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# General Evaluation

## Publications



Book: "Microbial Steroids". "Methods in Molecular Biology" series of Springer  
Ed.: Dr. José Luis Barredo (Gadea Biopharma)

### • Papers:

- Dovbnya D, Kollerov V, Khomutov S, Malov D, Donova M. A two-step one-pot bioprocess for production of 11a-hydroxyandrost-4-ene-3,17-dione from phytosterol *New Biotechnology*, 2014, v. 31s, s119-120.
- Rodríguez-García A; Fernández-Alegre E; Morales A; Sola-Landa A; Lorraine J; Macdonald S; Dovbnya D; Smith MCM; Donova M; Barreiro C. Complete genome sequence of 'Mycobacterium neoaurum' NRRL B-3805, an androstenedione (AD) producer for industrial biotransformation of sterols. *J. Biotechnol* (Under Review)...

### • Oral presentations:

- C. Barreiro. Production improvement of microbial metabolites by Synthetic Biology. Bangalore India BIO2016, Bangalore, India (2016).
- F. Thygs, J. Merz, G. Schembecker. Automation Strategies in Downstream Process Development. 11th International PhD Seminar on Chromatographic Separation Science, Sundern, Germany (2015).
- F. Thygs, C. Schulze, J. Merz, G. Schembecker. Downstream Process Development: Automation techniques to support experimental investigation. 10th International PhD Seminar on Chromatographic Separation Science, Egmond aan Zee, Netherlands (2014).
- F. Thygs, St. Schuldt, J. Merz, G. Schembecker. Systematic Downstream Process Design: Automation techniques for the purification of natural products. 9th International PhD Seminar on Chromatographic Separation Science, Weggis, Switzerland (2013).

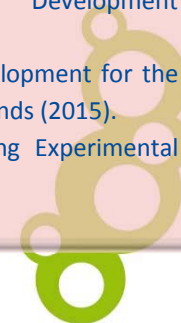
### • Poster presentations:

- C. Barreiro, E. Fernández-Alegre, A. Sola-Landa, A. Morales & A. Rodríguez García; N. Strizhov, D. Dovbnya & M. Donova; J. Lorraine & M. Smith; K. Josefsen *et al* MySterl: a one-step steroid precursors production project from Mycobacterium to industry. FEMS 2015, Maastricht, the Netherlands (2015).
- F. Thygs, J. Merz, G. Schembecker. Miniaturization of Purification Strategies for Systematic Downstream Process Development Scale-up and scale-down of bioprocesses, Dechema Himmelfahrtstagung, Hamburg, Germany (2015).
- A Rodríguez García, E Fernández-Alegre, A Sola-Landa, A Morales, A Ibáñez, RV Ullán, C Barreiro. Genome sequencing and 'omics development for the steroid precursors producer *Mycobacterium* sp. NRRL B-3805. 6th Congress of European Microbiologists (FEMS 2015), Maastricht, the Netherlands (2015).
- F. Thygs, J. Merz, G. Schembecker. Downstream Process Development: Automation Technique to Integrate Operational Steps During Experimental Investigation. ProcessNet-Jahrestagung und 31. DECHEMA-Jahrestagung der Biotechnologen, Aachen, Germany (2014).
- J. Lorraine, MCM Smith. MySterl: Mycobacterial Steroids for Industry. Midlands Molecular Microbiology Meeting. Birmingham (UK).

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# General Evaluation

## International collaboration

- ✓ *Partners interactions (protocols, personnel, strains,...) very beneficial for the achievement of the objectives.*
- ✓ *Supported knowledge very useful in a near future for the **enhancement of the industrial bioconversion process** to obtain steroid precursors.*
- ✓ *Helpful studies focused on **characterization of fermentation broth for a suitable industrial DSP process** for steroid precursors purification.*
- ✓ *“Omic characterization” as **valuable tool to find new target genes** for industrial strain improvement.*



## Contact details

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Prt	Name	Institution	e-mail
P1	Dr. Carlos Barreiro	INBIOTEC	<a href="mailto:c.barreiro@unileon.es">c.barreiro@unileon.es</a>
P2	Dr. Marina Donova	Pharmins Ltd	<a href="mailto:donova@ibpm.pushchino.ru">donova@ibpm.pushchino.ru</a>
P3	Prof. Maggie Smith	University of York	<a href="mailto:Maggie.smith@york.ac.uk">Maggie.smith@york.ac.uk</a>
P4	Mr. Håvard Sletta	Stiftelsen SINTEF	<a href="mailto:Havard.sletta@SINTEF.no">Havard.sletta@SINTEF.no</a>
P5	Prof. Gerhard Schembecker	Technische Universität Dortmund	<a href="mailto:Gerhard.Schembecker@bci.tu-dortmund.de">Gerhard.Schembecker@bci.tu-dortmund.de</a>
	Dr. Juliane Merz		<a href="mailto:juliane.merz@bci.tu-dortmund.de">juliane.merz@bci.tu-dortmund.de</a>
P6	Dr. José L. Barredo	Gadea Biopharma S.L.	<a href="mailto:joseluis.barredo@amriglobal.com">joseluis.barredo@amriglobal.com</a>



# Acknowledgment

MySterI

Novel industrial bioprocesses for production of key valuable steroid precursors from phytosterol

MySterI (Mycobacterial Steroids for Industry)

Project No: EIB.12.010

MySterI wants to thank to:

·ERA-IB

·National Agencies:

-Ministerio de Economía y Competitividad (MINECO), Spain

-Foundation for Assistance to Small Innovative Enterprises (FASIE),  
Russia

-Biotechnology and Biological Sciences Research Council (BBSRC), United  
Kingdom

-Norges forskningsråd (RCN), Norway

-Bundesministerium für Bildung und Forschung (BMBF), Germany

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