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# Quantitative proteomics revealed the nature and cause of the different metabolic features underpinning weak and strong antibiotic producing abilities of two model *Streptomyces* species

Aaron Millan-Oropeza<sup>1</sup>, Céline Henry<sup>1</sup>, Clara Lejeune<sup>2</sup>, Michelle David<sup>2</sup>, Marie-Joëlle Virolle<sup>2</sup>

<sup>1</sup> PAPPSO, Micalis Institute, Institut National de la Recherche Agronomique, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France.

<sup>2</sup> Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, F-91198, Gif-sur-Yvette cedex, France.

Contact: aaron.millan-oropeza@inra.fr



## CONTEXT OF STUDY

The *Streptomyces* genus is well known for its ability to produce numerous and diverse bio-active molecules useful to human health. The biosynthesis of these specialized metabolites usually occurs when growth slows down or stops and is triggered by nutritional limitations, especially by phosphate.

Two model species commonly used to understand the biosynthesis of these bio-active metabolites are *S. coelicolor* M145 and *S. lividans* TK24. These species are closely related (95% of orthologous genes) but exert different abilities to produce three well characterized secondary metabolites (CDA, RED, ACT). In presence of glucose as main carbon source, the **high antibiotic** production of *S. coelicolor* was correlated with a **low lipid** content whereas *S. lividans* showed a poor ability to synthesize antibiotics that was correlated with higher lipid content [1].

Despite numerous important scientific contributions over the past 40 years, a systemic understanding of the biosynthesis of these bio-active metabolites and the metabolic feature characterizing the producing bacteria remains incomplete. To progress on the field, a label-free shotgun comparative proteomic analysis was carried out in *S. coelicolor* M145 and *S. lividans* TK24 cultivated under different carbon sources and studied at different times of culture [2].

## METHODOLOGY

### Experimental design (48 samples):

- 2 strains (*S. coelicolor*, *S. lividans*)
- 2 main carbon sources (Glucose, Glycerol)
- 3 time points (36h, 48h, 72h)
- 4 independent replicates

Total proteins  
extract

Double digestion  
(LysC / Trypsin) of  
80µg of proteins

Polymeric solid phase  
extraction (Strata-X)

1 µg peptides

LC-MS/MS

Orbitrap Fusion™ Lumos™ Tribrid™  
(Thermo Fischer Scientific)

- 50 cm column, 216 min analysis
- HCD mode

Proteins  
identification



Proteins  
quantification



X!Tandempipeline\* [3] was used for identification and quantification of number of MS2 spectra (Spectral counting)

MassChroQ\* [4] was used for peptide quantification by area integration on eXtracted Ion Chromatogram (XIC)

Differential  
analysis



Statistical and data analysis were carried out on R\*.

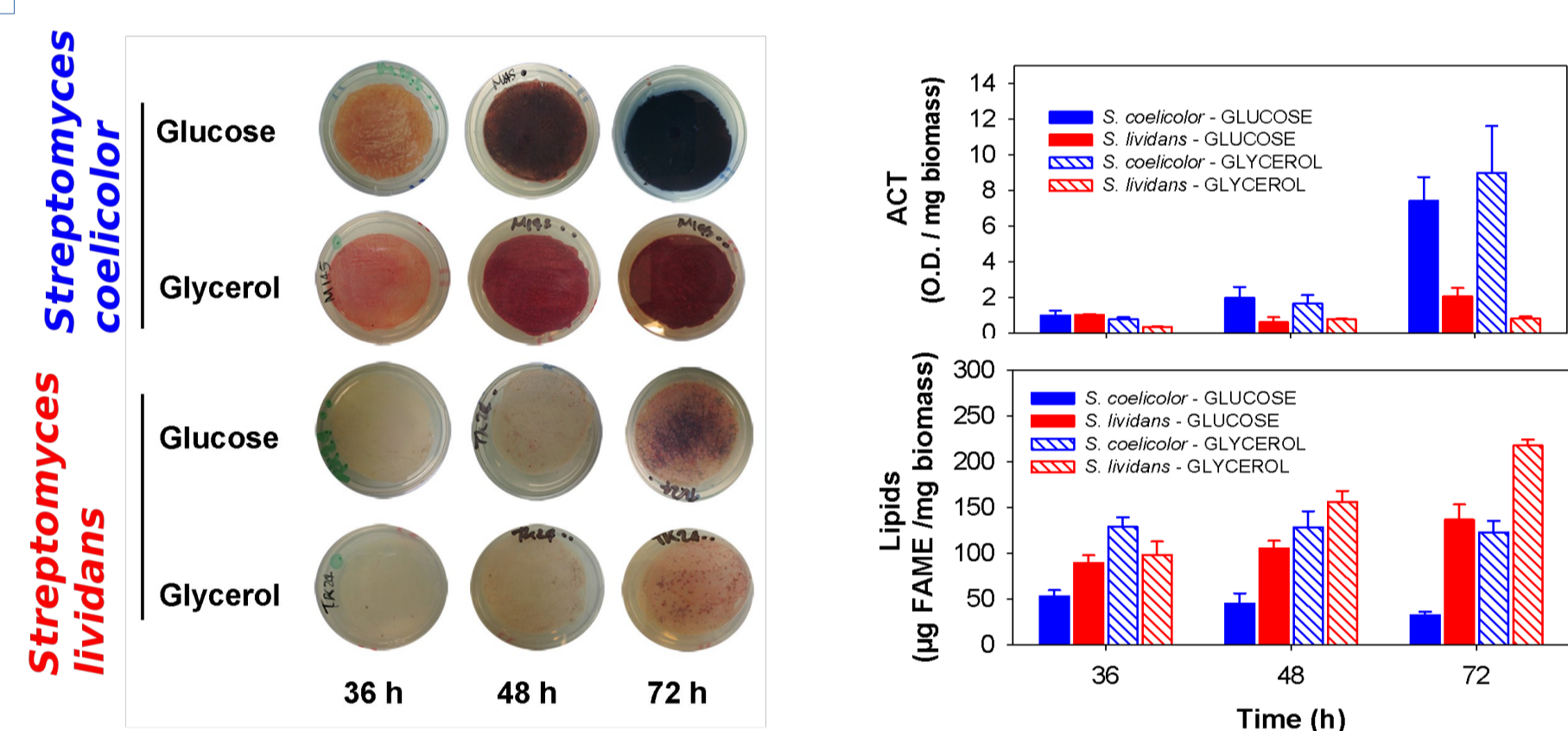
\* FREE and OPEN Source software

Spectral counting : suitable for detecting presence/absence of a given protein.

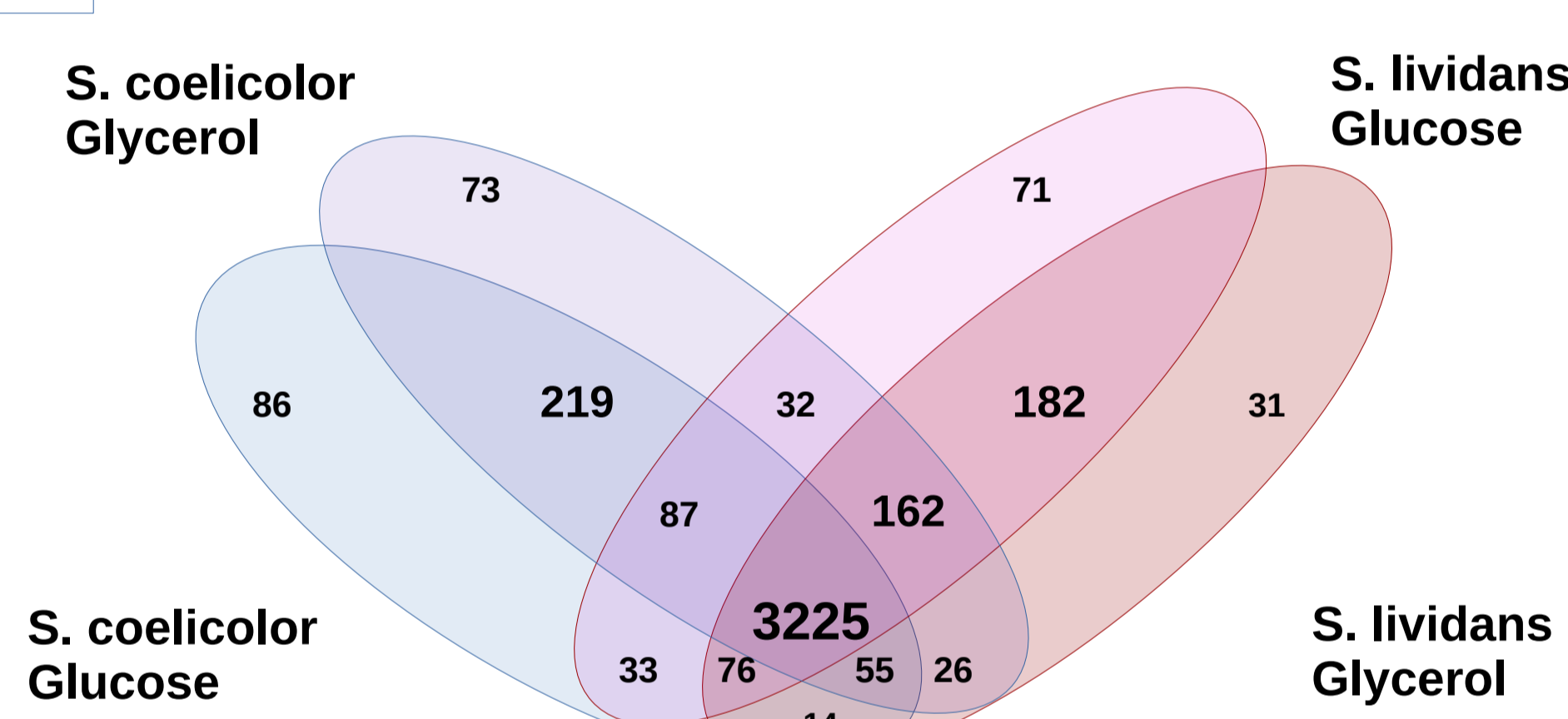
XIC : This method provides high sensitivity to detect small abundance variations.

## RESULTS

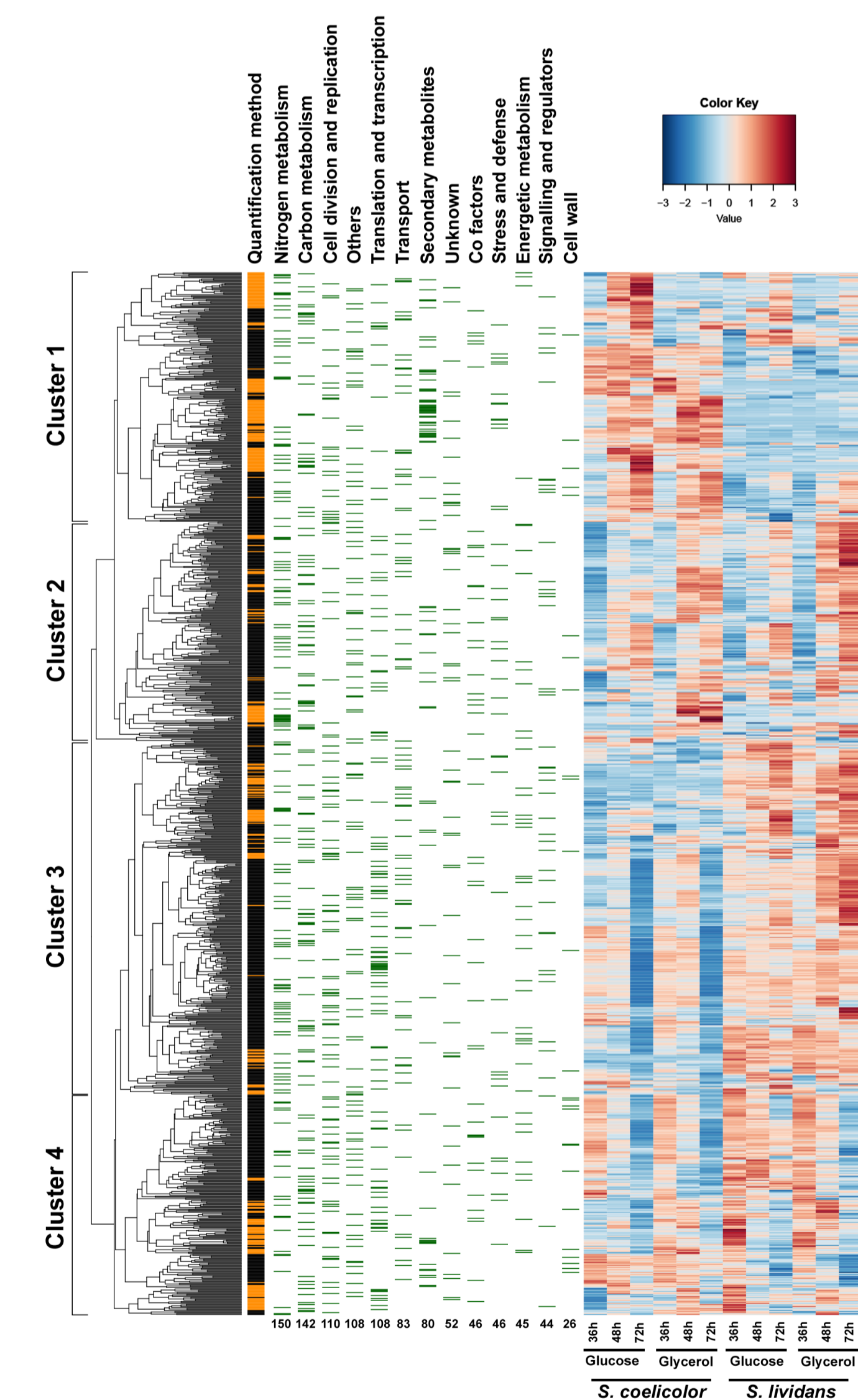
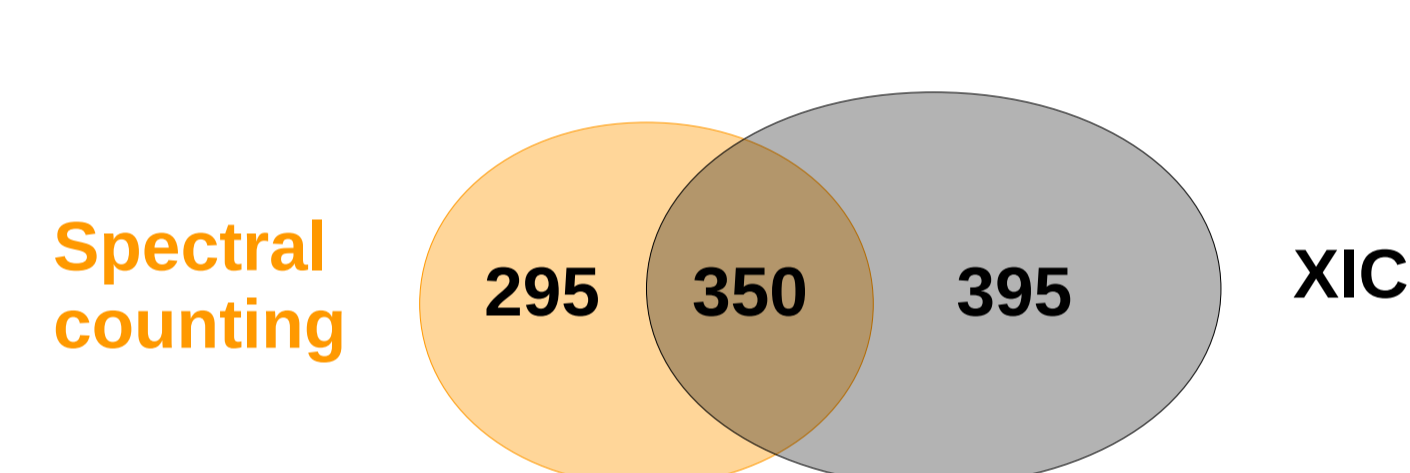
### 1 PHENOTYPES



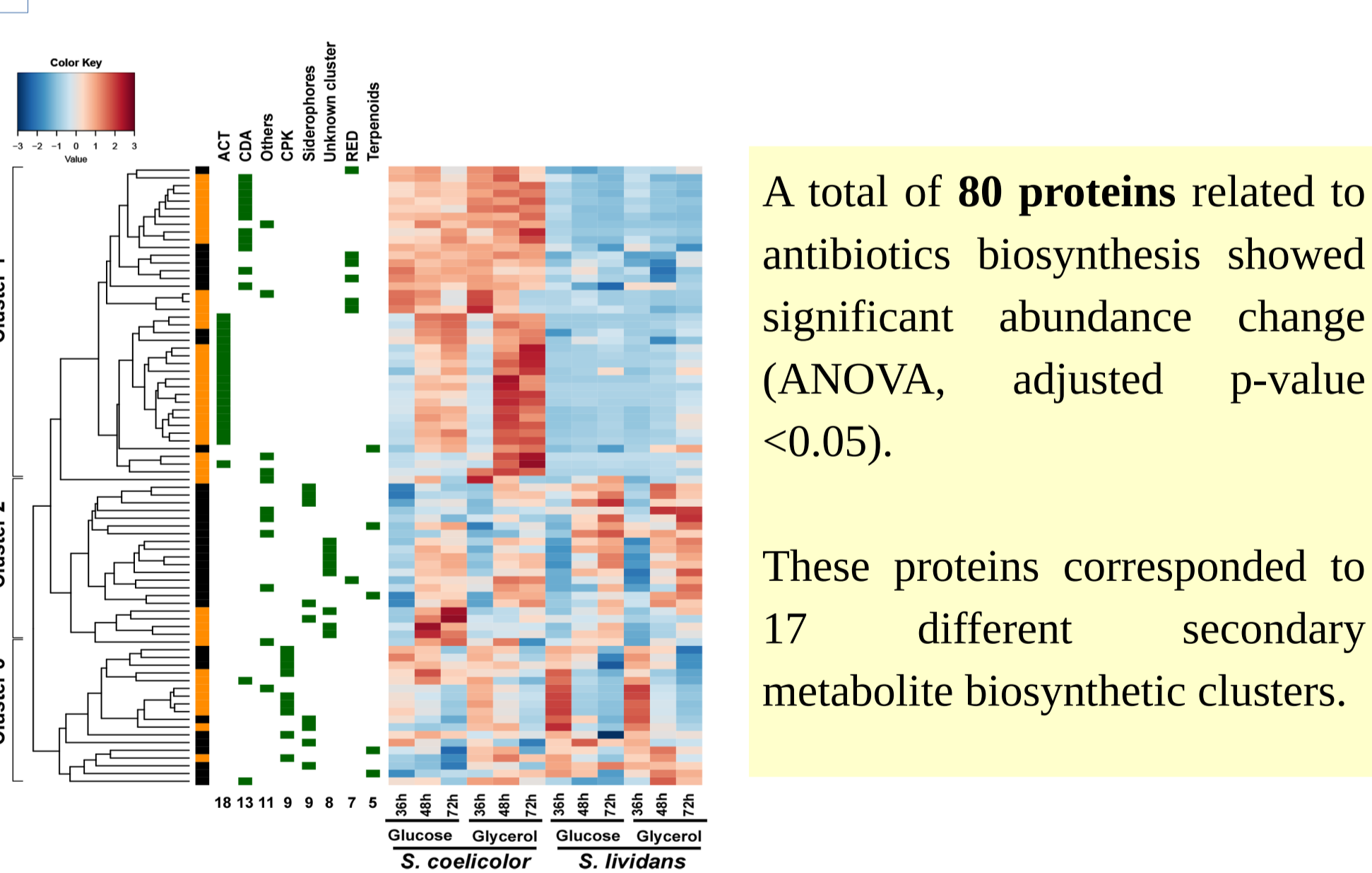
### 2 IDENTIFIED PROTEINS



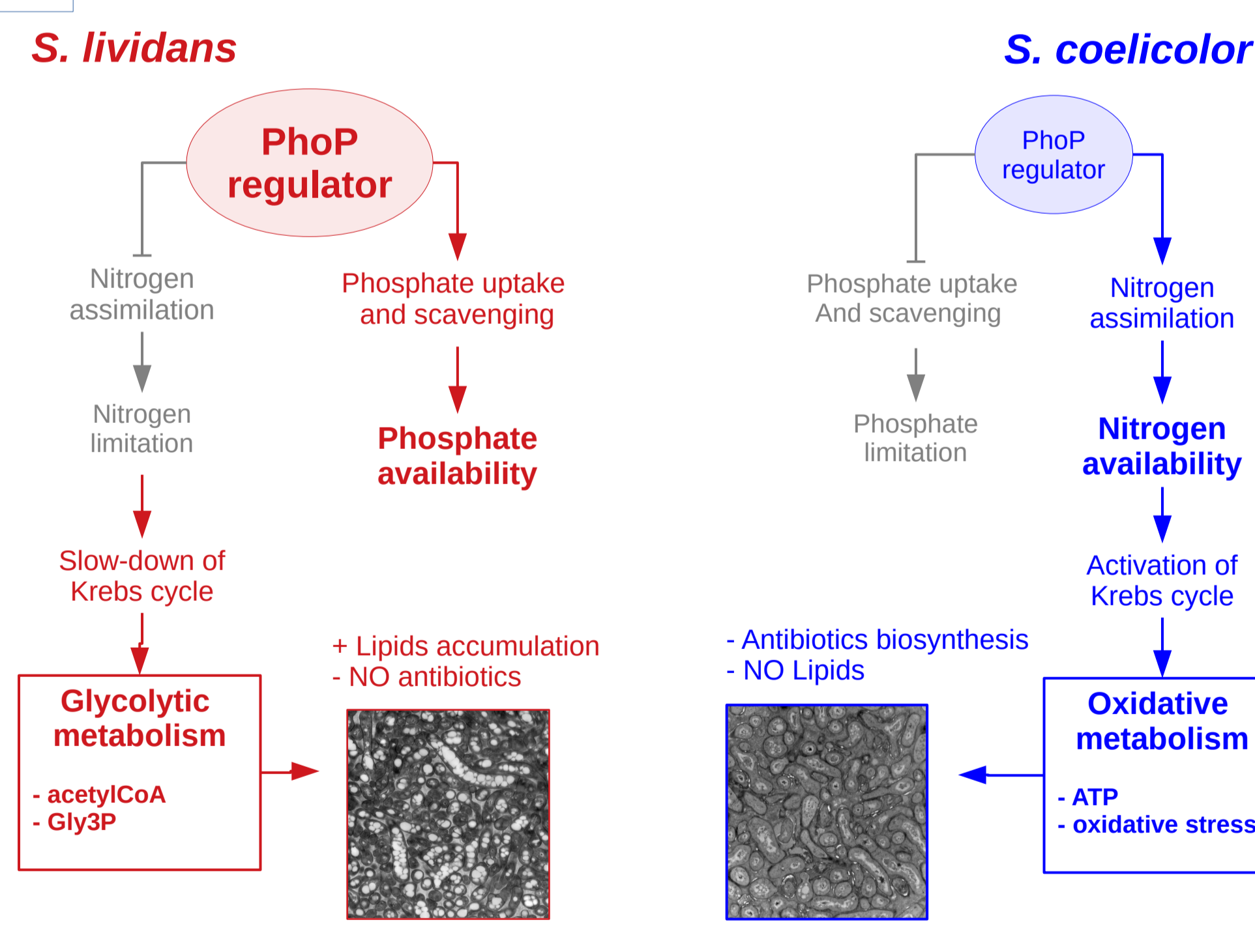
### 3 STATISTICALLY SIGNIFICANT PROTEINS



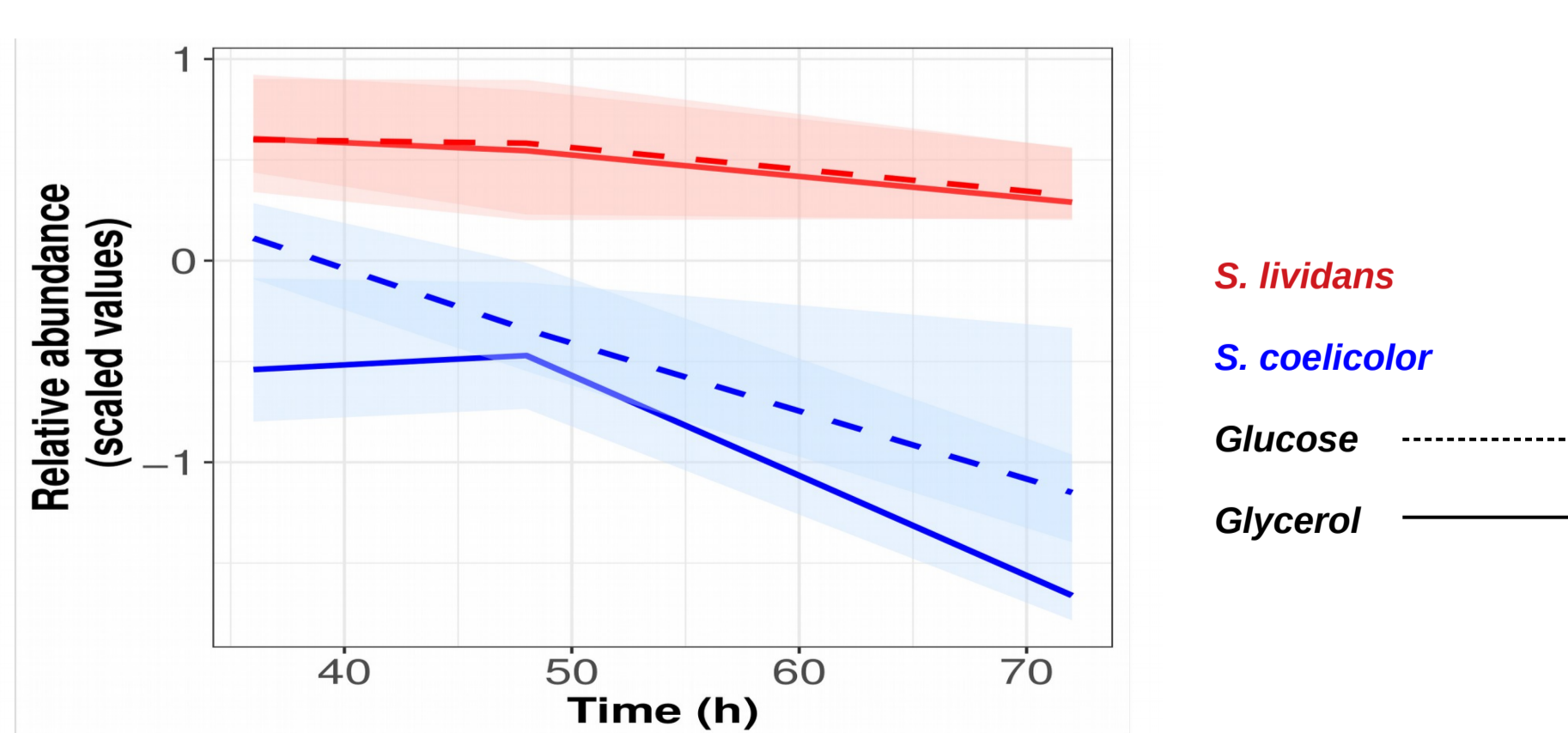
### 4 ANTIBIOTICS BIOSYNTHESIS



### 6 PROPOSED MODEL



### 5 PHOSPHATE METABOLISM



## CONCLUSION

\* This work is pioneering in the elucidation of the basis of the metabolic differences underlying the drastically different abilities of *S. coelicolor* and *S. lividans* to produce antibiotics.

\* The low abundance of the TCS PhoR/PhoP in *S. coelicolor* compared to *S. lividans* led to an alleviation of its regulatory role likely to be responsible of the specific metabolic features of this strain (see model).

\* A novel view of the role of the antibiotics in the physiology of the producing bacteria was proposed. These molecules would play an important role in the regulation of the energetic metabolism of the bacteria in condition of phosphate scarcity.

[1] Le Marechal P, Decottignies P, Marchand CH, Degrouard J, Jaillard D, Dulermo T, Froissard M, Smirnov A, Chapuis V, Virolle M. (2013). Comparative proteomic analysis of *Streptomyces lividans* wild-type and ppk mutant strains reveals the importance of storage lipids for antibiotic biosynthesis. *Appl Environ Microbiol*, 79(19): 5907-17.

[2] Millán-Oropeza A. (2017). Comparative study of the proteome of *S. coelicolor* M145 and *S. lividans* TK24, two phylogenetically closely related strains with very different abilities to accumulate TAG and produce antibiotics. Université Paris-Saclay. NNT : 2017SACL15160.

[3] Langella O, Valot B, Balliau T, Blein-Nicolas M, Bonhomme L, Zivy M. (2017). X!TandemPipeline: a tool to manage sequence redundancy for protein inference and phosphosite identification. *J Proteome Res*. 16(2): 494-503.

[4] Valot B, Langella O, Nano E, Zivy M. (2011). MassChroQ: A versatile tool for mass spectrometry quantification. *Proteomics*. 11(17): 3572-7