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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

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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

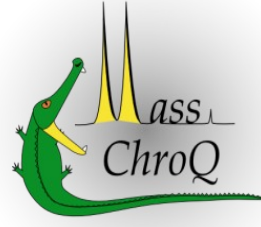
LC-MS/MS

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*.

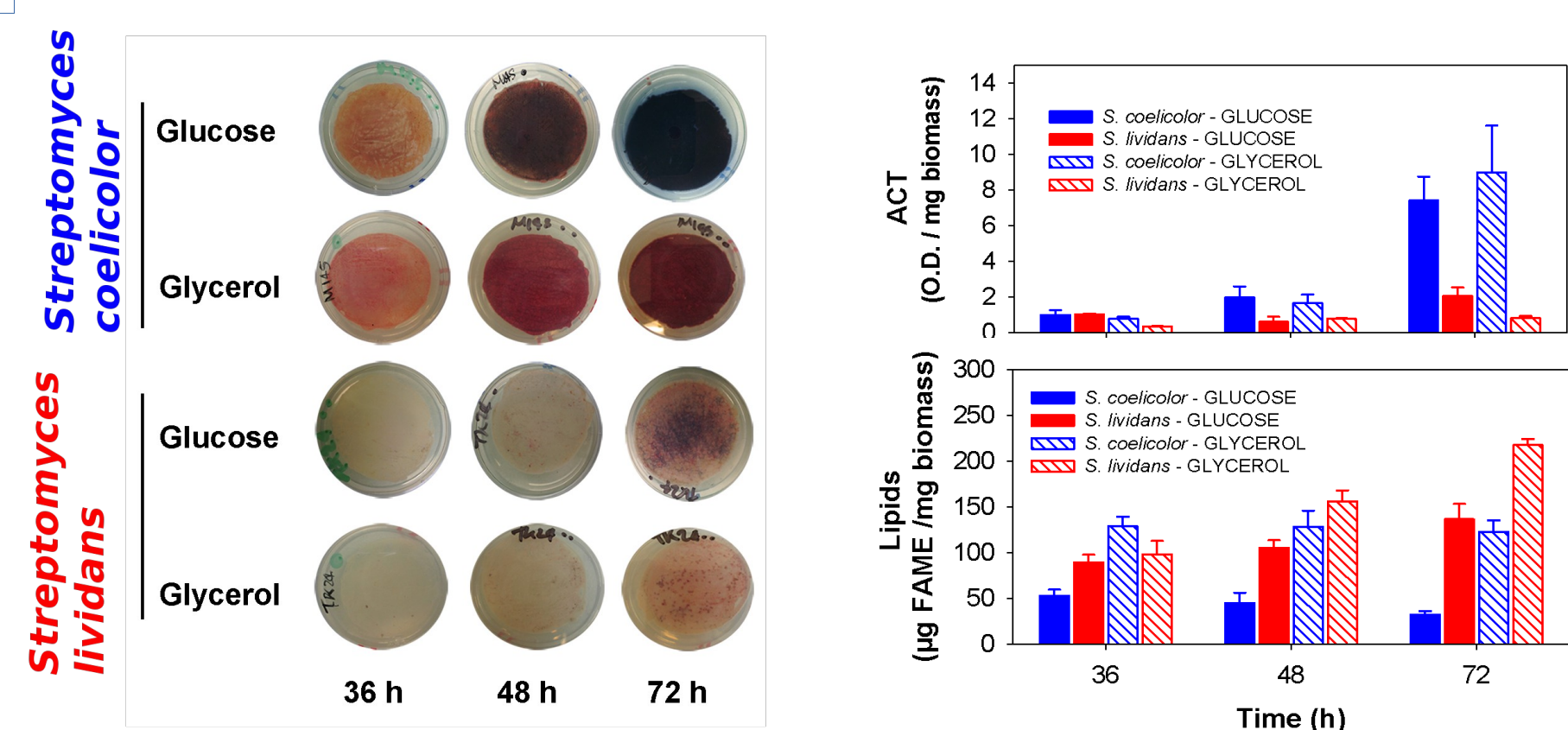


* FREE and OPEN Source software

Spectral counting : suitable for detecting presence/absence of a given protein.
XIC : This method provides high sensitivity to detect small abundance variations.

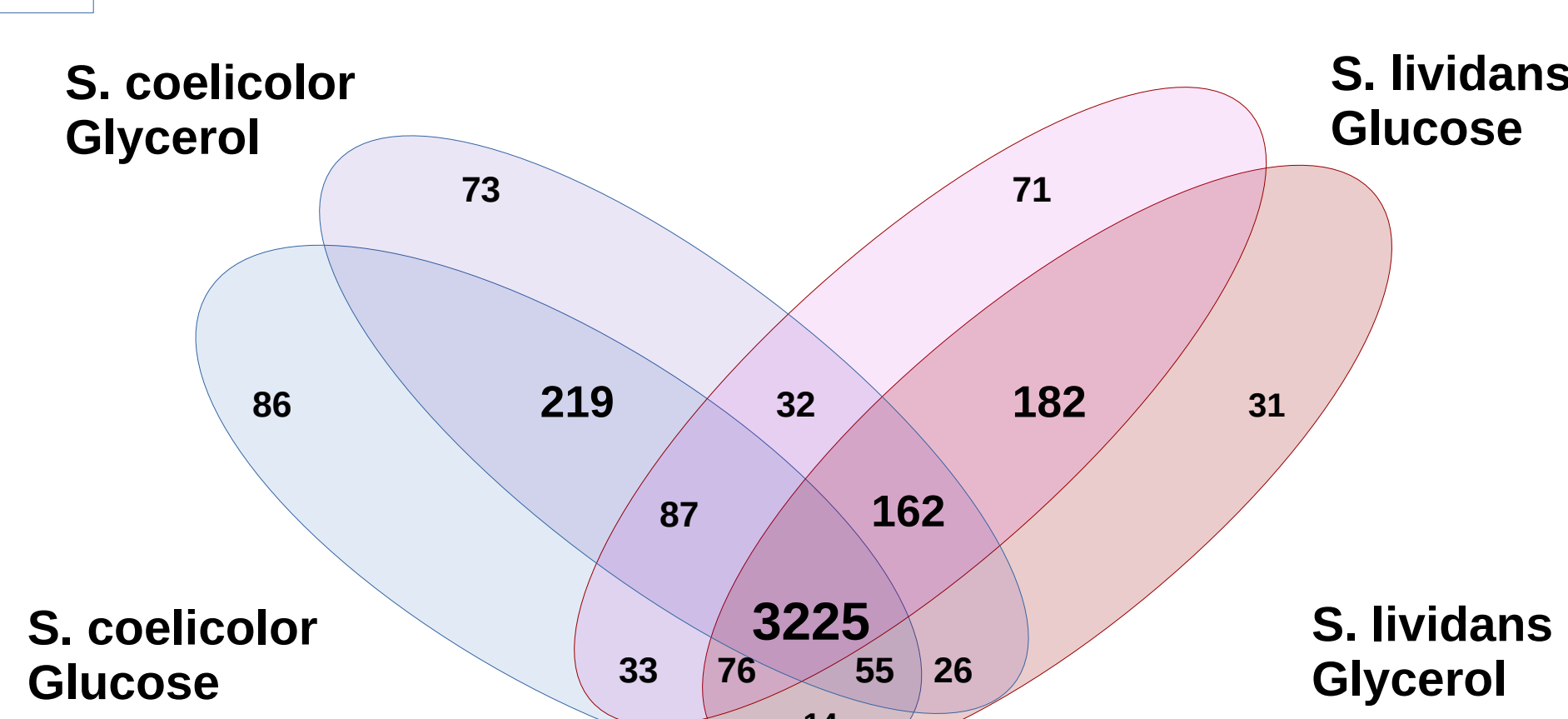
RESULTS

1 PHENOTYPES



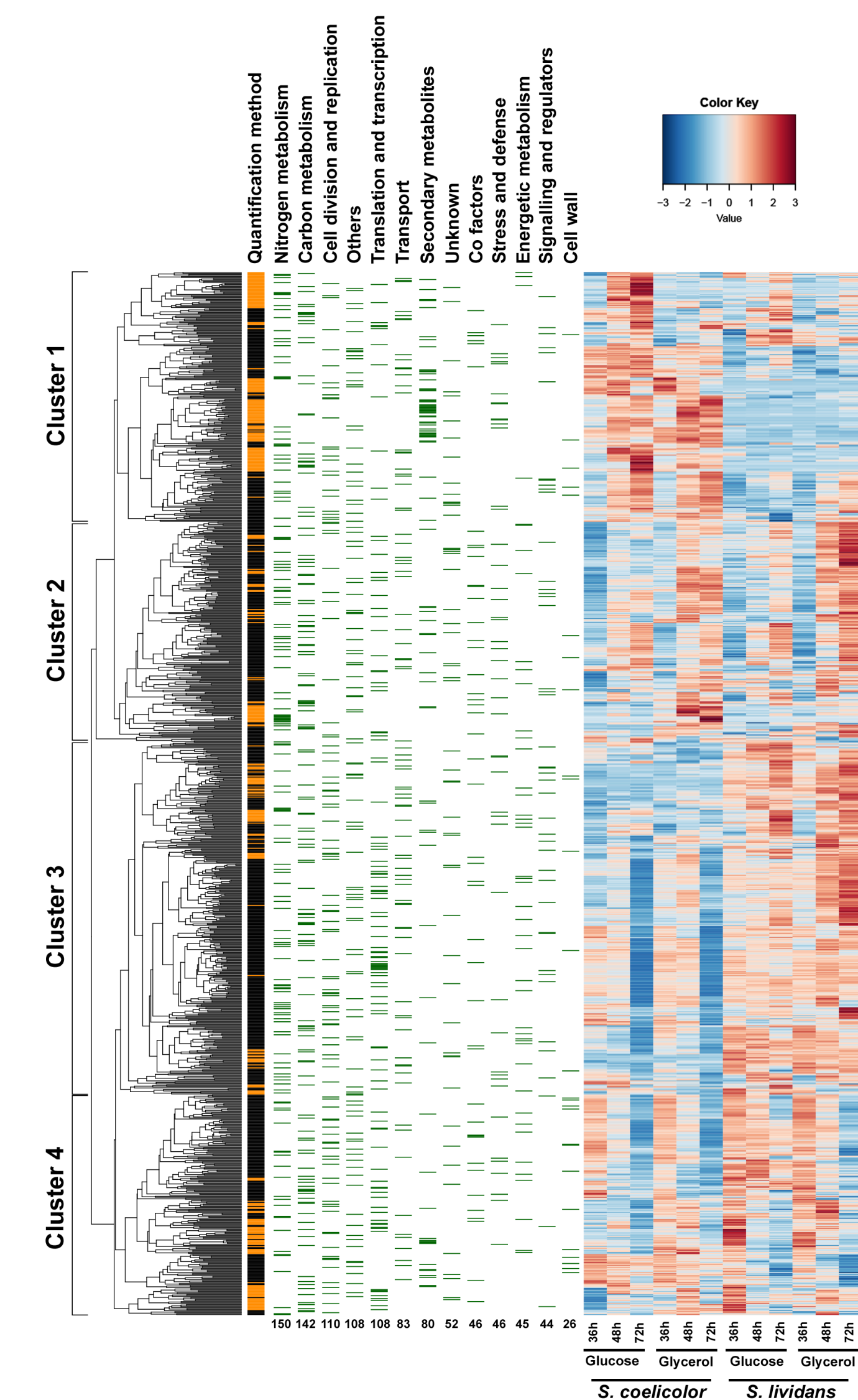
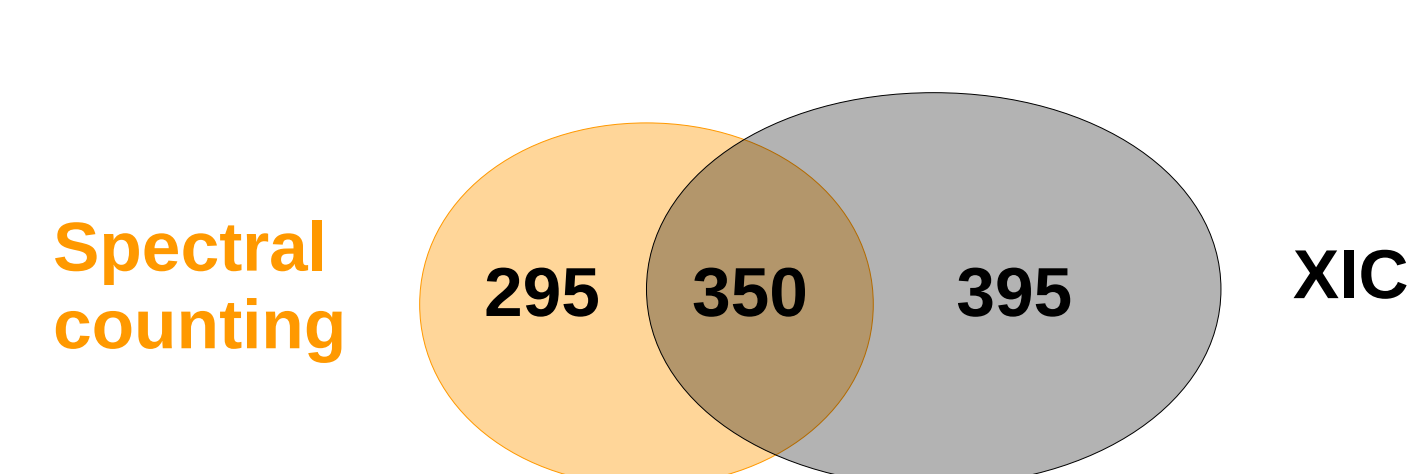
Cultures on solid media showed, in most cases, a reverse correlation between the ability to accumulate lipids (FAMES) and that to synthesize antibiotics (ACT). The sole exception was *S. coelicolor* cultivated on glycerol, that produced both lipids and antibiotics.

2 IDENTIFIED PROTEINS

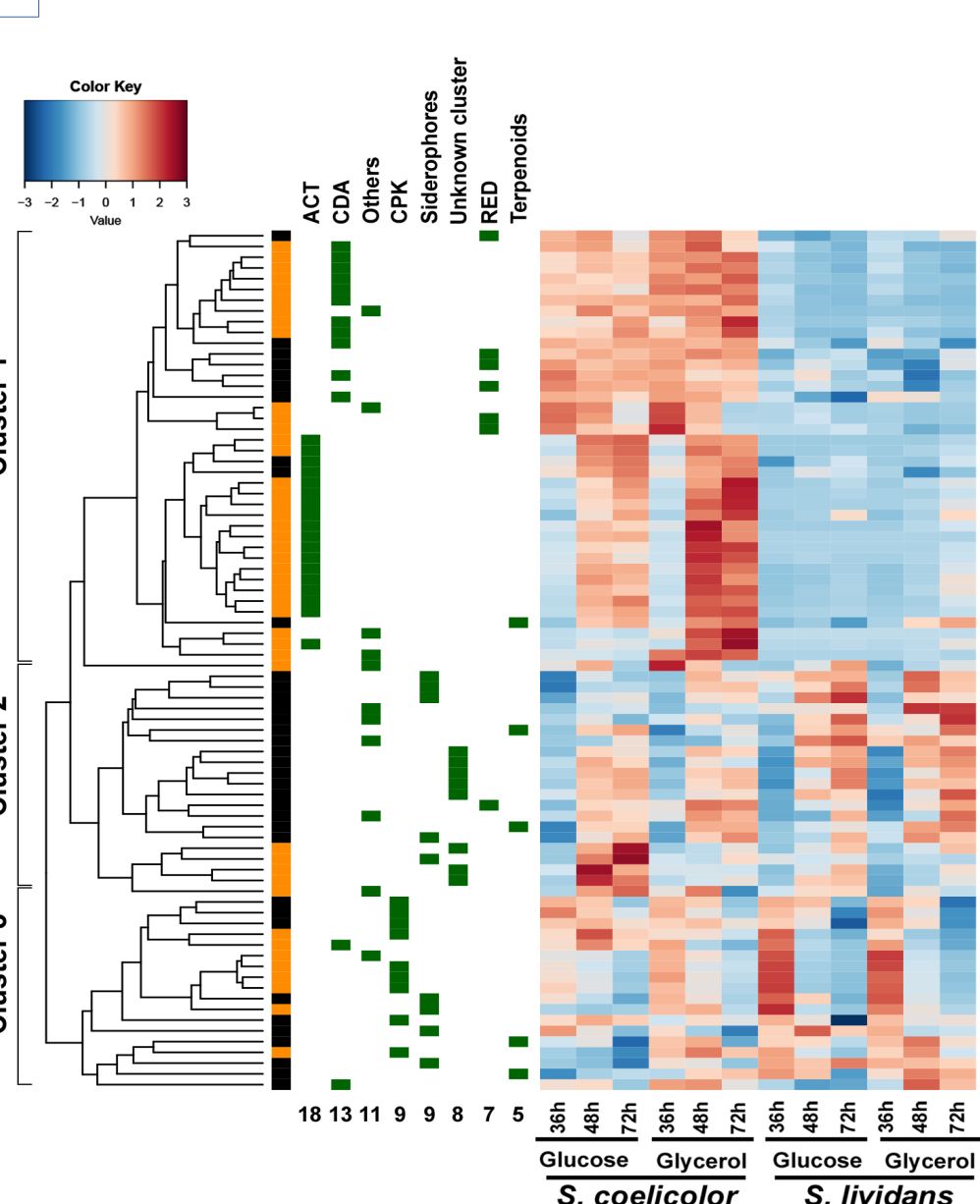


A total of **4372 proteins** were identified. This represents the largest dataset for *S. coelicolor* and *S. lividans* with >52% of their theoretical proteomes.

3 STATISTICALLY SIGNIFICANT PROTEINS



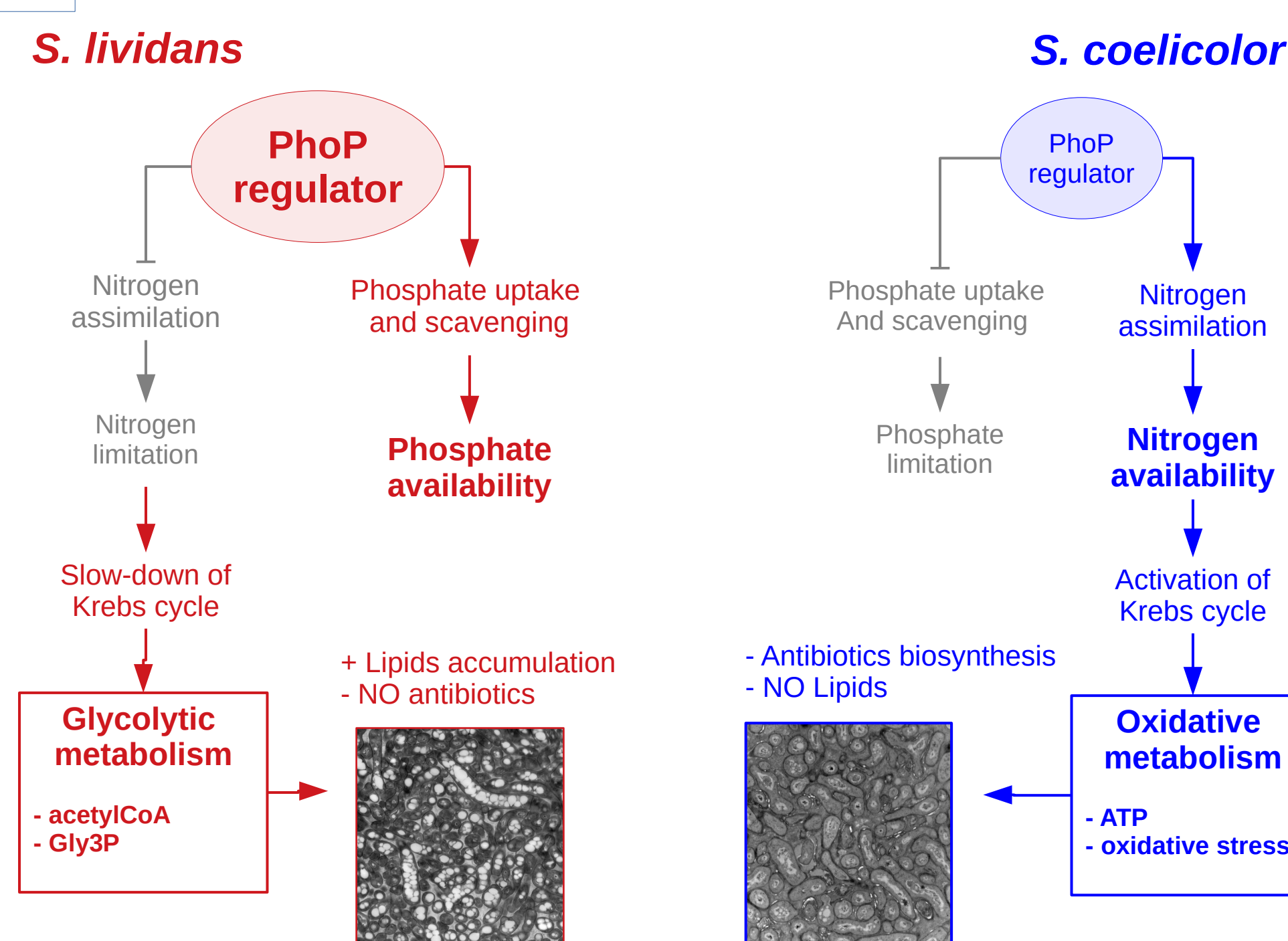
4 ANTIBIOTICS BIOSYNTHESIS



A total of **80 proteins** related to antibiotics biosynthesis showed significant abundance change (ANOVA, adjusted p-value <0.05).

These proteins corresponded to 17 different secondary metabolite biosynthetic clusters.

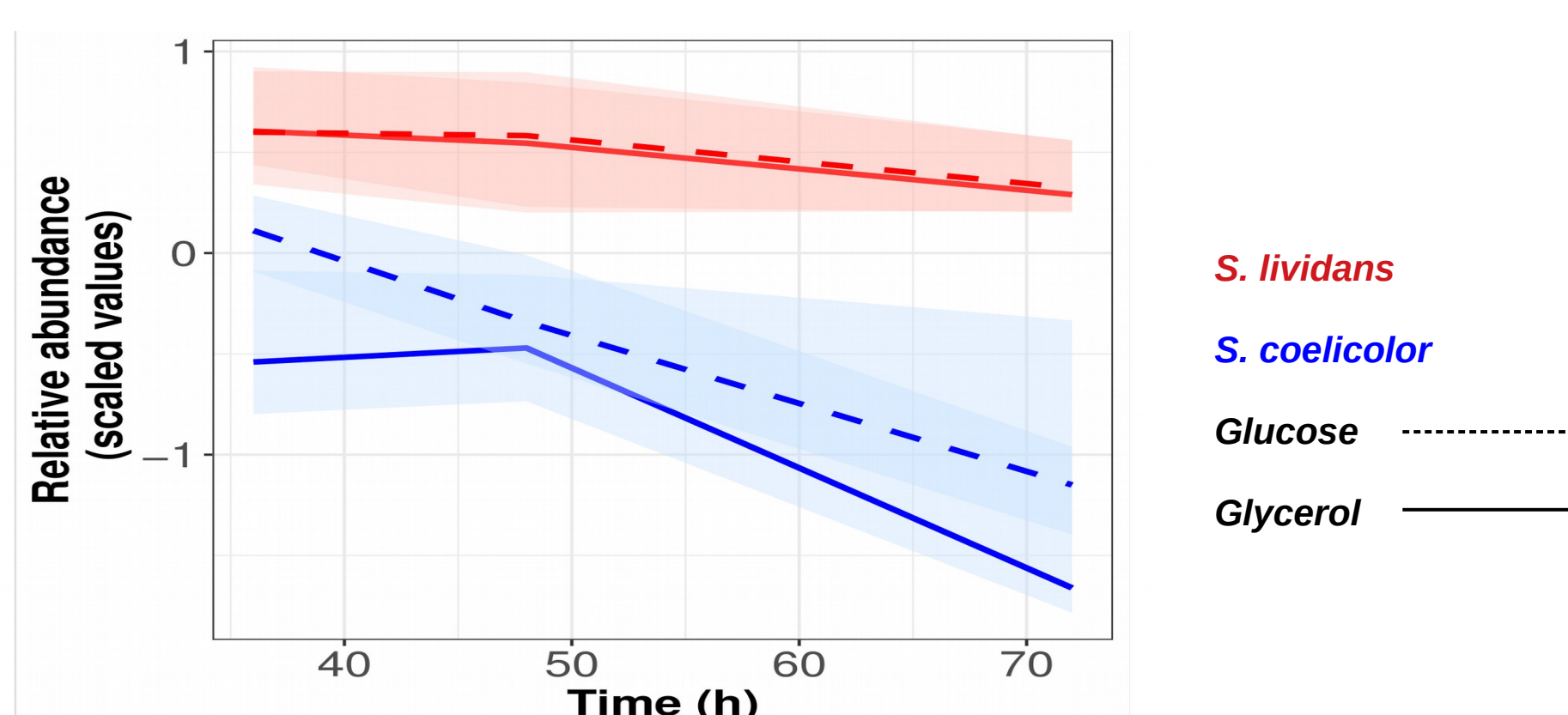
6 PROPOSED MODEL



The low P and high N availability in *S. coelicolor* supported the activation of the Krebs cycle and thus the **oxidative metabolism**, resulting in high ATP. The limitation of P altered respiratory chain resulting into electrons leaking towards alternative acceptors, the induced response is the production of the specialized metabolite ACT ('antibiotic') in order to capture the electrons of the respiratory chain and adjust ATP. This would **reduce oxidative stress**.

Statistical analysis of spectral counts and XICs allowed to detect **1040 proteins** with significant abundance variation according to strain, medium and/or time (ANOVA, adjusted p-value < 0.05). These proteins belonged to 13 functional categories according to their annotation in the databases.

5 PHOSPHATE METABOLISM



The profile of 30 proteins involved in phosphate metabolism showed higher **abundance in *S. lividans*** than in *S. coelicolor*. These proteins included the two component system (TCS) **PhoR/PhoP**, responsible for the positive and negative regulation of phosphate and nitrogen assimilation, respectively.

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