The orphan regulator Aor1 and its possible histidine kinase in the antibiotic regulation of *Streptomyces coelicolor*

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Introduction

Streptomyces is the largest genus of the phylum *Actinomycetota*. These bacteria have a complex life cycle with a vegetative mycelium of multinucleated hyphae and an aerial mycelium which produces spores for reproduction (**Figure 1**). *Streptomyces* is interesting because is the major producer of antibiotics, which makes this genus important to combat the increasing antibiotic-resistant bacteria [1].

Streptomyces spp. have large genomes with several **biosynthetic gene clusters (BGCs)** to produce bioactive secondary metabolites, but most of them are **cryptic** under laboratory conditions [1]. This secondary metabolism is strictly regulated by multiple factors and regulatory cascades, so it is important to understand this regulatory network to enhance the production of antibiotics and to discover new molecules [2].

Typical **two-component systems** (TCS) are composed by a histidine kinase and a response regulator and they play a crucial role in antibiotic regulation in *Streptomyces*. Several of them have been studied in the model organism *S. coelicolor* [3] and among them the **orphan response regulator Aor1** is a key regulator that controls the expression of several genes of secondary metabolism, including some of cryptic BGCs, and it is related to osmotic stress response (**Figure 2**) [4].

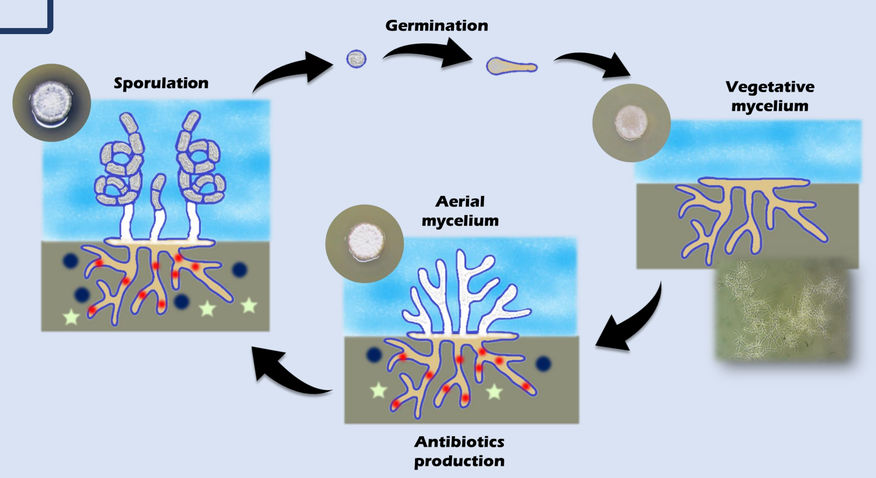

Figure 1. Schematic representation of *Streptomyces* life cycle.

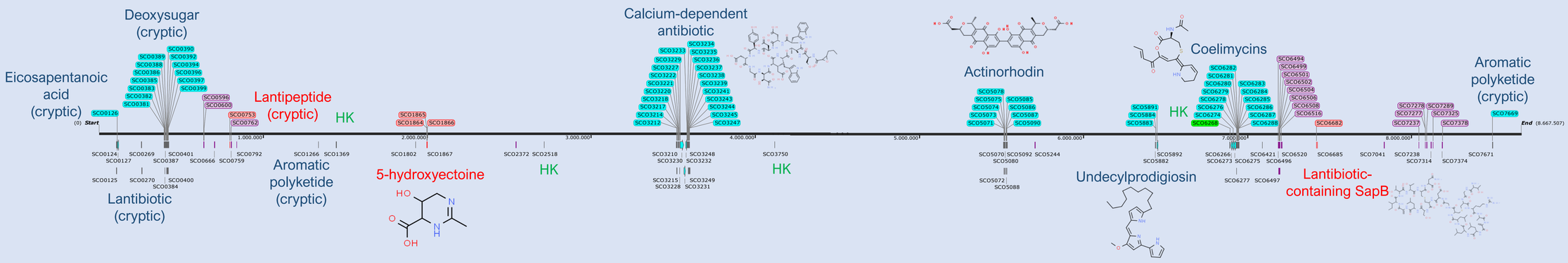

Figure 2. Clusters of **secondary metabolites** up-regulated (red) or down-regulated (blue) in the Δ*aor1* mutant. Genes up-regulated related to osmotic stress are shown in purple. Green genes represent histidine kinases (HK) down-regulated in the mutant. Genomic map has been done with SnapGene software (www.snapgene.com) v7.0.3. Molecules shown were collected from ChemSpider (https://www.chemspider.com/).

Objective

As an orphan regulator, the histidine kinase related to Aor1 remains unknown. The aim of this work is to identify the kinases that could activate Aor1, by bioinformatic prediction and the study of mutants lacking the selected genes.

Materials and methods

Two histidine kinases were selected by their probability to interact with Aor1 according to the bioinformatic tool *Prediction of Interaction Specificity in Two-Component Systems* (https://www.swissregulon.unibas.ch/cgi-bin/TCS.pl) [5].

The mutants lacking these genes were obtained by the CRISPR-Cas9 technology adapted to *Streptomyces* [6]. The deletion of these genes was checked by PCR from genomic DNA of the mutants.

Spores of all used strains were obtained as previously described by Kieser et al. [7]. Colonies with $5 \cdot 10^5$ spores of the parental strain M145, the Δ*aor1* mutant and the new mutants obtained in this work were cultivated on three complex media (LB, R2YE and YEPD) and on a minimum medium (NMMP).

Conclusions

The Δ*3750* mutant has the same phenotype as the Δ*aor1* on YEPD medium, while it has a delay in differentiation on LB medium. Unlike Δ*aor1*, which has a delay in differentiation and secondary metabolism on all media, the Δ*3750* mutant has an increased production of actinorhodin on LB, R2YE and NMMP media (**Figure 4**).

The second kinase, SCO6424, does not seem to be linked to Aor1 because the Δ*6424* mutant phenotype is almost identical to the parental strain. This mutant produces more actinorhodin on LB and YEPD media (**Figure 4**).

The first kinase, SCO3750, whose gene is down-regulated in the Δ*aor1* mutant (**Figure 1**) [4], could be the protein which activates Aor1 and its role in antibiotic production seems medium-dependent. The presence of other kinases and the signals which activate them must be further studied.

Results

Possible interaction with Aor1

The online tool predicted five posible kinases which could actívate Aor1 (**Figure 3**). Since the probabilities were so distant, we selected the first two kinases-encoding genes (*SCO3750* and *SCO6424*), which are orphan (they do not have a response regulator gene associated).

We constructed the mutants in these two genes and checked their phenotype along with Δ*aor1* and M145. We focused on the differentiation of white aerial mycelium and grey spores and the production of blue extracellular actinorhodin and the intracelular red undecylprodigiosin.

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SIB Swiss Institute of Bioinformatics

kinase	regulator	probability
SCO3750	SCO2281	0.66729485
SCO6424	SCO2281	0.192854545
SCO0211	SCO2281	0.0617260435
SCO4598	SCO2281	0.052660324
SCO4597	SCO2281	0.0254642485

Figure 3. Histidine kinases predicted to interact with Aor1 (*SCO2281*).

Visual analysis of the phenotype

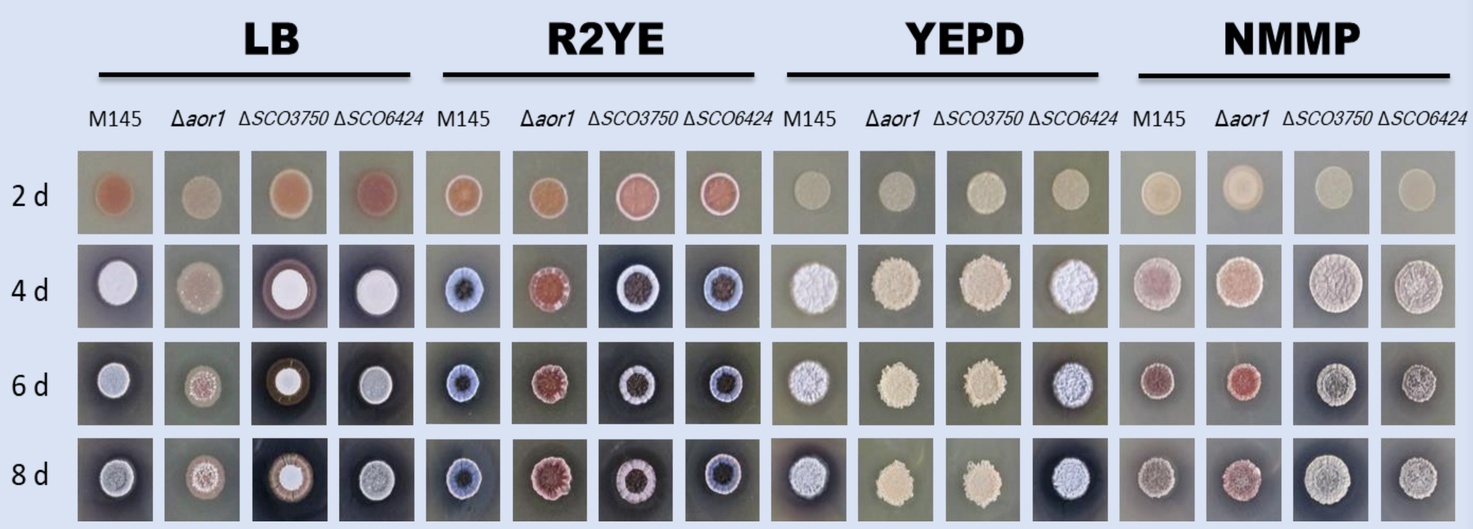

Figure 4. Comparison of the phenotype of the parental strain (M145), the Δ*aor1* mutant and the two mutants in the orphan kinases (Δ*3750* and Δ*6424*) during 8 days on the four studied culture media. Aerial mycelium and spores are indicated by white and grey colour in the colony, respectively. Red undecylprodigiosin is visible inside the colonies before the production of aerial mycelium. Blue actinorhodin appears as a dark halo around the colonies.

References

1. Donald, L. et al. *Microbiol. Res*., 2022, 13, 418–465. https://doi.org/10.3390/microbiolres13030031
2. Xia H. et al. *Front. Microbiol.* 2020, 11:406. https://doi.org/10.3389/fmicb.2020.00406
3. Sánchez de la Nieta, R. et al. *International Journal of Molecular Sciences*, 2022, 23(23), 15085. MDPI AG. http://dx.doi.org/10.3390/ijms232315085
4. Antoraz S, et al. *Front. Microbiol*., 2017, 8:2444. https://doi.org/10.3389/fmicb.2017.02444
5. Burger, L. & van Nimwegen, E. *Molecular Systems Biology*, 2008, 4. 165. https://doi.org/10.1038/msb4100203
6. Tong, Y. et al. *ACS Synthetic Biology,* 2015, 4 (9), 1020-1029. https://doi.org/10.1021/acssynbio.5b00038
7. Kieser, T. et al. *Practical Streptomyces genetics.* The John Innes Foundation, Norwich, 2000.

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