Phenotypic Analysis of Antibiotic-Producing Soil Bacterium, *Streptomyces coelicolor*

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Abstract

Over the summer, antibiotic production by the soil bacterium *Streptomyces coelicolor* was studied. There were two primary questions to be investigated - what are the physiological benefits of producing the antibiotic actinorhodin (Act), and how does the bacterium tolerate the toxic effects of this redox-active antibiotic? The former question is significant in its application of increasing the efficiency of biosynthesis of medically important drugs. The latter, in a general sense, can provide insight into resistance mechanisms against redox active molecules and reactive oxygen species, the accumulation of which is associated with many degenerative diseases and aging. In order to investigate these questions, the phenotypes and microcolony morphologies of wild type and *S. coelicolor* mutants were studied at the microscopic level.

Introduction to the Mutants

•***ΔsoxR***: In the E. coli model system, SoxR senses oxidative stress in the cellular environment and activating protective measures against reactive oxygen species. In *S. coelicolor* and other antibiotic-producing bacteria, however, evidence suggests that SoxR may be involved in regulating the metabolism of redox-active endogenous antibiotics, including Act.[1]

• ***ΔActAB*:** The act cluster of genes in *S. coelicolor* is responsible for the production and export of the antibiotic Act. The ActAB proteins, specifically, are thought to regulate an efflux pump of Act.

• ***ΔActABΔsoxR* :** Both the actAB operon and soxR are deactivated.

•**M511 (*ΔactII-ORF4*):** This mutant has a deletion of a transcription factor that regulates Act biosynthetic genes. Therefore, this strain does not produce any Act.

Hypothesis

Based on preliminary qualitative observations of previous experiments' micrographs, it was hypothesized that between wild type and mutant populations of *S. coelicolor*, there was a distinct difference in size of microcolonies, or bacterial pellets. Furthermore, it was proposed that two distinct size populations of bacterial pellets co-existed in varying ratios, perhaps indicating differential gene expression or stress responses. It was observed that *ΔActAB* mutant pellets seemed to tend more towards a larger, bloated population with flaky edges, while *ΔsoxR* mutants were typically smaller, more compact, and overproduced the dark pigmented Actinorhodin.

Methods

In order to investigate the proposed hypothesis further, pellet areas over week-long time courses were quantified from daily micrographs of each strain using Fiji ImageJ software. Pellets were traced around their central, dense core, not including protruding hyphae. Area of the selection was converted to micrometers squared using a scalebar-derived ratio of pixels to micrometers. Information was then sorted and analyzed using Excel, adapted from statistical approaches taken in previous experiments.[2]

Results

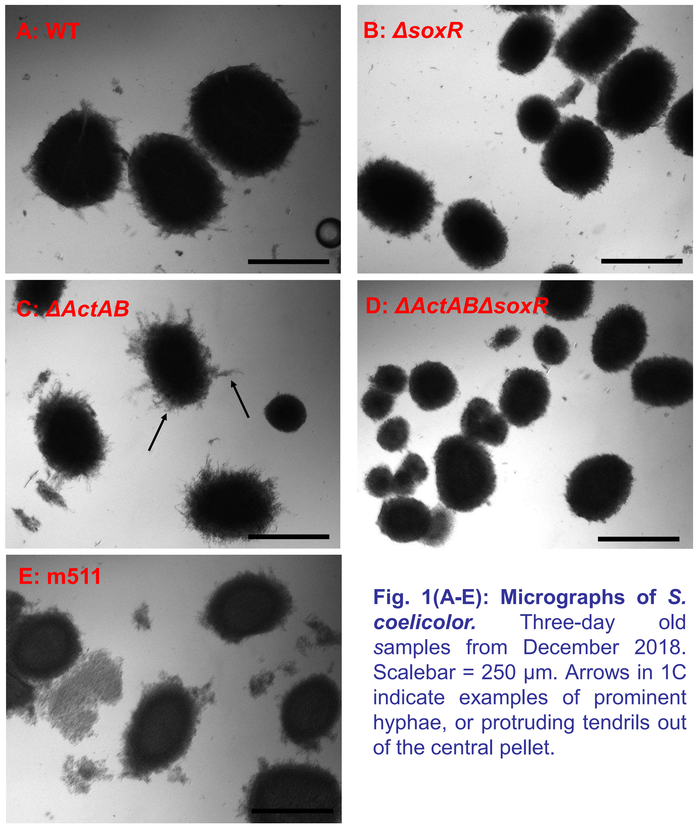

Fig. 1(A-E): Micrographs of *S. coelicolor.* Three-day old samples from December 2018. Scalebar = 250 µm. Arrows in 1C indicate examples of prominent hyphae, or protruding tendrils out of the central pellet.

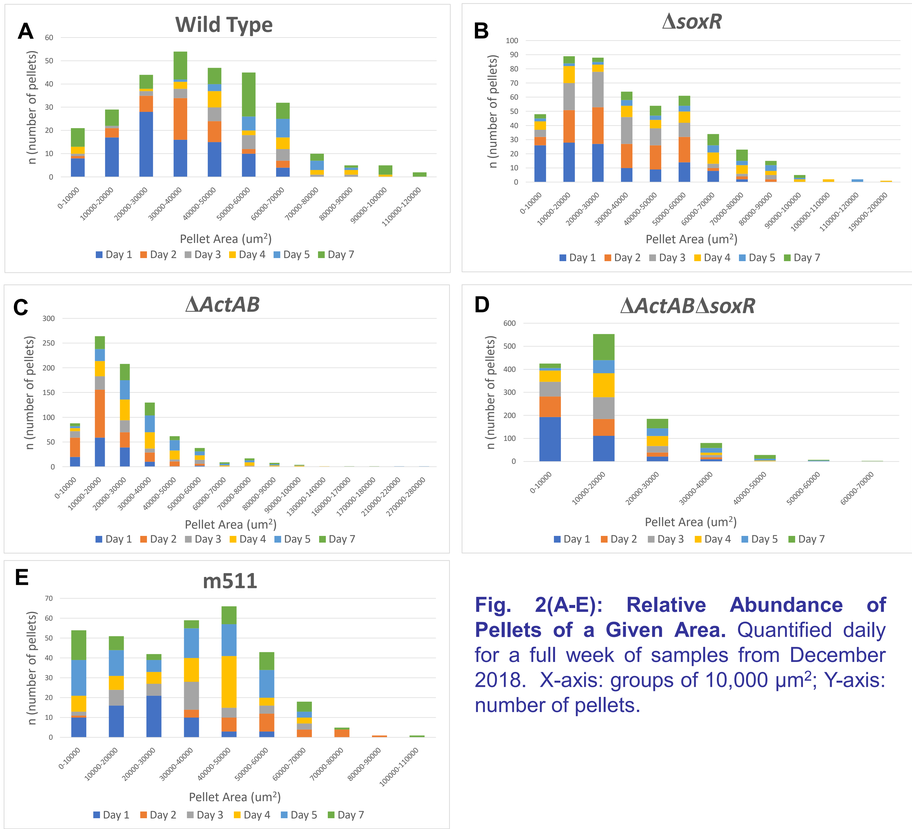

Fig. 2(A-E): Relative Abundance of Pellets of a Given Area. Quantified daily for a full week of samples from December 2018. X-axis: groups of 10,000 µm²; Y-axis: number of pellets.

Discussion

•***ΔsoxR*** is darker than wild type, indicating overproduction of actinorhodin, and pellets are smaller and more compact.

•***ΔActAB*** has two large populations, both of which are flakier and darker than wild type. The larger consists of less pellets, and is extremely bloated, reaching areas of up to nearly 300,0000 µm².

•***ΔActAB ΔsoxR*** has one consistently small population, with relatively short hyphae – leading to a more compact appearance, as seen in *ΔsoxR*.

•**M511** has two distinct populations, one small and compact, and one bloated and flaky - similar to *ΔActAB* pellets. It is paler than all other strains; microcolonies have a pale center surrounded by a darker ring. This could either be a result of lower cell density at the center of pellets, or of an accumulation of another red-pigmented antibiotic near the hyphal regions.

It is still unclear which aspects of the lightness or darkness of pellets in each strain is associated with pigmented antibiotic production versus relative density of pellets, as well as how these factors may indicate overall fitness of the bacteria. Ongoing research is being conducted in order to assess mutant fitness. To do so, quantification data of "live" versus "dead" area of pellets obtained from fluorescence tests – in which live and dead cells are differentially stained with different color fluorescent dyes – has begun to be collected.

Conclusions

While this research is still in progress, quantitative data collected over the summer from a large sample pool supports the original visual analysis of microcolony morphology in *S. coelicolor* mutants. Generally, the following properties were observed - an inability to export Act in *ΔActAB* tends to favor a larger population, while overproduction in *ΔsoxR* leads to the opposite phenotype.

References

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2. Van Veluw, G. J., Petrus, M. L., Gubbens, J., De Graaf, R., De Jong, I. P., Van Wezel, G. P., Wösten, H. A., & Claessen, D. (2012). Analysis of two distinct mycelial populations in liquid-grown Streptomyces cultures using a flow cytometry-based proteomics approach. *Applied Microbiology and Biotechnology*, *96*(5), 1301-1312. .

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