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DISSERTATION TITLE

Analysis of the dynamics of the *Streptomyces venezuelae* growth, differentiation and chromosome segregation

ABSTRACT

Streptomyces are appreciated for their ability to produce a vast number of secondary metabolites applied in industry, medicine and pharmacy including most of commonly used antibiotics of natural origin. *Streptomyces* exhibit complex lifecycle, which involves formation of multigenomic vegetative mycelium and later, in response to nutrient depletion, aerial hyphae which differentiate into chains of unigenomic spores. This process is called sporulation. Sporulation starts after cessation of aerial hyphae growth and requires synchronized cell division. The ladder-like structure of regularly spaced septa is formed in the aerial hyphae. This process is initiated by the polymerization of FtsZ protein. FtsZ forms Z rings in the place of future sporulation septa. Shortly before sporulation, chromosome replication in multigenomic aerial hyphae slows down and, tens of chromosomes are condensed and segregated simultaneously. Similar to most prokaryotes ParA and ParB proteins of *Streptomyces* are responsible for chromosome segregation. ParB specifically binds *parS* sites clustered in the central region of the chromosome. This leads to formation of large nucleoprotein complex called segrosome. ParA is a weak ATPase and forms filaments spreading along hyphae responsible for regular distribution of the ParB-*parS* complexes. Regardless of intensive research on *Streptomyces* differentiation for the last 60 years, the details of the sporulation process still remain unclear.

Here, I used the time lapse experiments to analyze the influence of ParAB proteins on the sporogenic hyphae extension rate, differentiation and formation of sporulation septa. All the experiments have been performed for a novel model species – *S. venezuelae*. It was shown that *parA* deletion leads to acceleration of hyphae extension rate, whereas *parB* deletion results in decreased hyphae extension rate. Analysis of ParA-EGFP, ParB-EGFP and FtsZ-YPET localization proved that the cessation of sporogenic hyphae growth coincides with the distribution of regularly spaced segrosomes and Z rings. Additionally, ChIP-seq

technique showed that by enhancing ParB complexes formation, ParA is involved in the segrosome organization. My studies revealed that chromosome organization maintained by ParA and/or ParB proteins as well as topoisomerase I (TopA) affects the Z rings formation. *parB* and *parAB* deletion resulted in accelerated Z-rings formation. Lowering of the TopA level triggered assembly of the Z rings in the extending aerial hyphae.

These analyses proved that ParAB proteins are necessary not only for the proper chromosome segregation but also by controlling the chromosome organization they affect the sporulation septa formation. They regulate the hyphae length, growth rate and differentiation.