The extraordinary life processes of *Actinomycetales*

Even Spacing

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The team at the Department of Microbiology concentrates on the molecular basis for the processes of chromosome replication and segregation in bacteria

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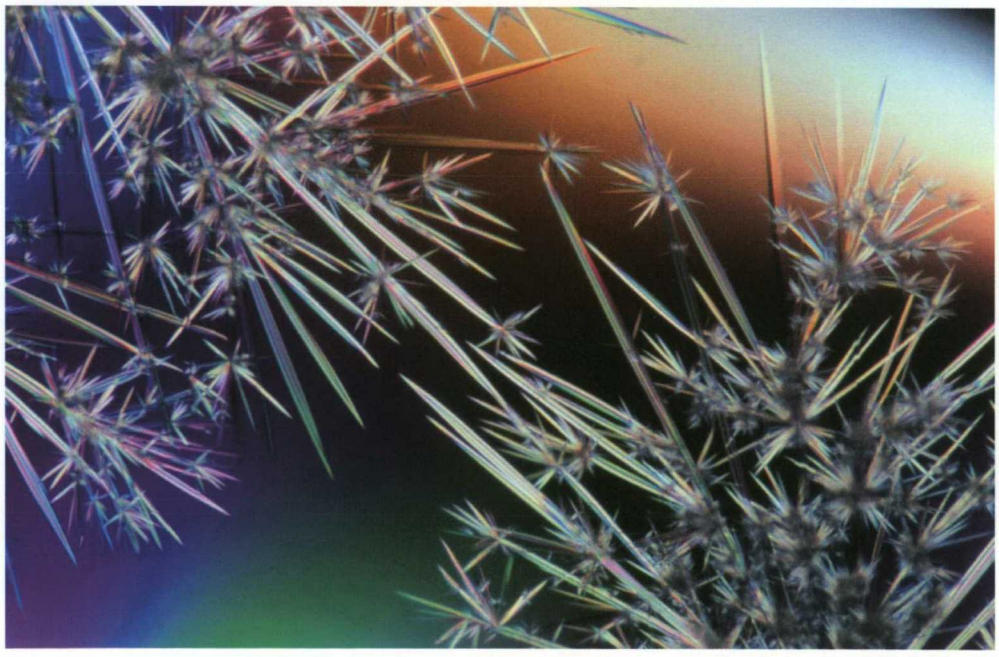
The long hyphae of fungi-like *Streptomyces* bacteria contain many chromosome copies, yet a precisely controlled mechanism ensures only one copy ends up in each newly-formed spore

Bacteria from the genus *Streptomyces* give soil its characteristic odor and play a key role in the carbon cycle in nature. Some of the compounds they produce are dyes which give *Streptomyces* colonies an unusual appearance, while others help them gain an edge over rival organisms: chitinase acts as a weapon against the bacteria's main rival in soil - fungi - while extracellular hydrolytic enzymes enable *Streptomyces* to "feed" on nearly anything. These bacteria are of precious value to mankind because they produce many antibiotics (e.g. streptomycin, neomycin, chloramphenicol, and tetracycline), as well as substances with antiviral and anticancer activity, immunomodulators, herbicides, enzyme inhibitors, and enzymes used in industry.

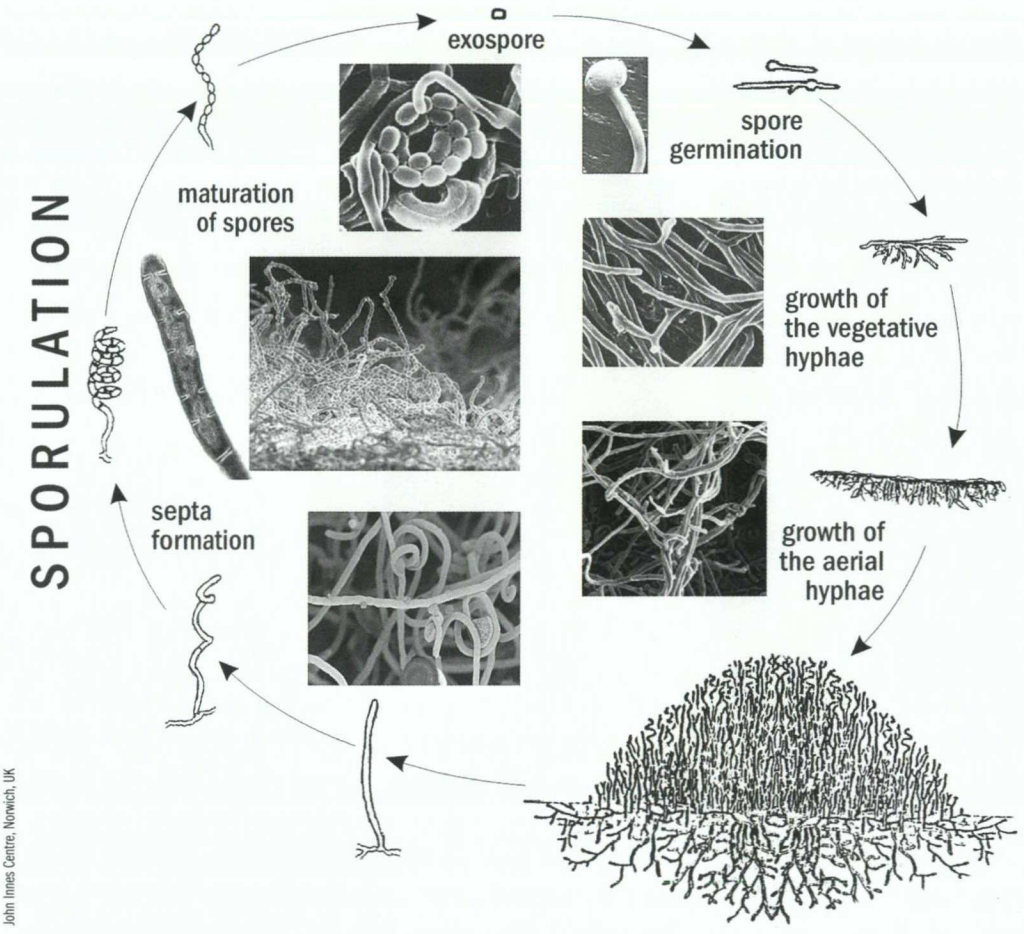
Fungus-like bacteria

The life cycle of *Streptomyces* differs from that of other bacteria, in fact being more similar to that of filamentous fungi. The terminology used to describe *Streptomyces* can therefore sometimes be misleading, since the terms commonly used for fungi are employed. These bacteria grow in the form of a multicellular vegetative mycelium, whose filaments, or hyphae, consist of adjacent elongated cells called compartments. After the nutritive components in the growth medium exhausted, a layer of aerial mycelium forms on the surface of the colony and subsequently differentiates into spore chains. Mycelium growth helps *Streptomyces* colonize soils, and the production of spores resistant to external factors



East News

***Streptomyces* produce many antibiotics, including streptomycin. After being isolated in 1943, it proved effective in fighting tuberculosis bacilli. Pictured here are streptomycin crystals photographed under polarized light**



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Bacteria from the order *Streptomyces* (*S. coelicolor*) have a life cycle that is unusual for bacteria, being more reminiscent of that of fungi

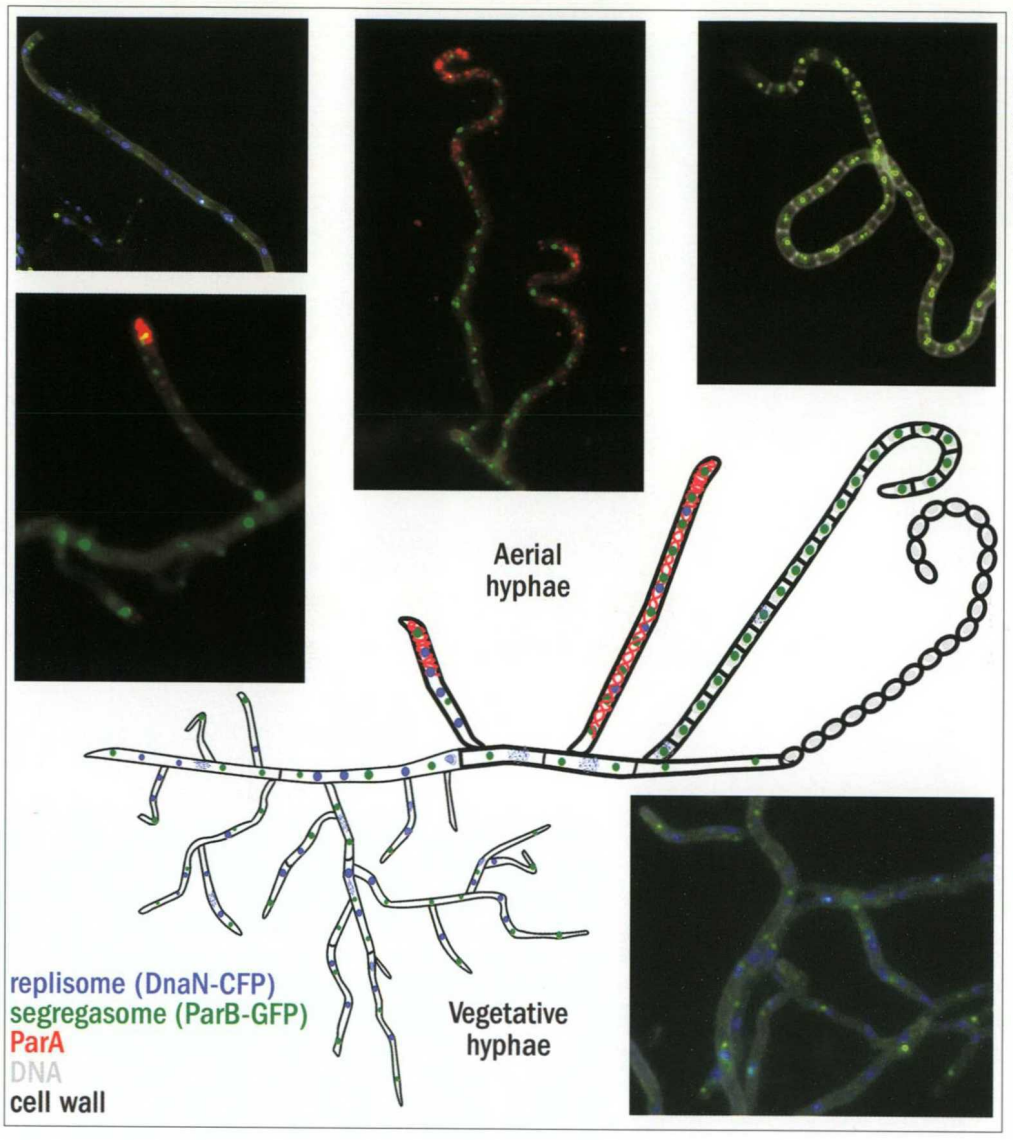
enables them to survive in unfavorable environmental conditions. What is most crucial for the pharmaceutical industry, however, is that differentiation of *Streptomyces* is coupled with the production of secondary metabolites. These traits have led *Streptomyces* to become the target of research that focuses on morphological development - it is intriguing how such processes are successfully regulated, both across the bacteria colony and over time, even in tissue-specific fashion.

An abundance of chromosomes

When considering bacteria, we usually imagine individual cells each containing an individual nucleoid - called the bacterial chromosome. In the case of *Streptomyces*, however, a single mycelial compartment contains several or many chromosomes. Very long aerial hyphae, reaching lengths running tens of μm (while a typical cellular bacteria is only several μm long) may contain even upwards of 50 copies of its chromosome. Moreover, chromosomes within the mycelium are uncondensed and their location seems to be random.

Pondering this unusual trait, we posed ourselves the question of whether all *Streptomyces* chromosomes are copied at the same time, i.e. whether chromosome replication is a synchronic process. To find out, we employed a fluorescent marker - CFP (cyan fluorescent protein) combined with the protein DnaN, a subunit of β polymerase III DNA, which is involved in copying genetic material in cells. The functional polymerase carrying the fluorescent marker was then visible under the microscope - glowing blue in the bacteria cells. This enabled us to trace the replication process in both types of mycelium.

This research indicated that DNA replication is not a random process within a colony, and does not occur with the same intensity everywhere. In the rapidly growing aerial mycelium, where spores are created, chromosomes are in great "demand" (with each of dozens of spores receiving a copy). It was there, too, that we observed the greatest replication activity. In the vegetative mycelium, in turn, the intensity of the replication process was highly varied: we observed



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In *Streptomyces*, the copying of chromosomes (blue dots) occurs in both the aerial and vegetative mycelium. The protein ParA (red color) enables a single copy of the chromosome to be placed into each of the newly forming spores. ParB (green dots) is a component of segregosome, a protein complex helping new copies of the genome to be placed into the daughter cells

both active centers, called replisomes (all the proteins involved in replication gathered in a single place, forming a "DNA replication factory"), visible as distinct fluorescent foci, and scattered fluorescence (resulting from the assembly/disassembly of replisomes). In most of the vegetative mycelial compartments, the number of replisomes was clearly lower than the number of chromosomes present, and in some replication did not occur at all.

Our observations therefore indicate that the replication of *Streptomyces* chromosomes is an asynchronic process, and only selected chromosomes are being copied at any given time. Here we can point out a certain analogy to the replication of chromosomes in higher (eukaryotic) organisms. There, genome replication begins at many locations on the chromosome, but not at all of them at the same time, and so there is a mechanism which determines which replication origin is "turned on" at any given time. Things are similar with *Streptomyces* - only certain chromosomes are chosen for replication, but it is not clear what determines this.

Cells for hire

After genetic material is copied it then undergoes condensation, meaning the close compaction of DNA (making its structure closer and tighter), and segregation, when it is shifted and distributed to the daughter cells. In rod-shaped bacteria such as *Escherichia coli* and *Bacillus subtilis*, the processes of replication, condensation, and

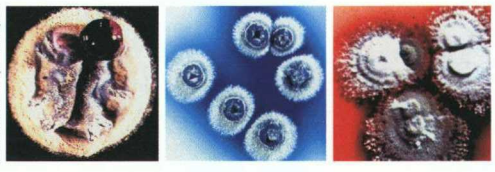
segregation occur simultaneously, with condensation and segregation occurring just after replication is begun. The newly replicated sister regions are then actively shifted towards the pole(s) of the cells, i.e. towards the future centers of the daughter cells. At present we know that the segregation of bacteria chromosomes into the daughter cells is an active and regulated process - requiring a set of necessary proteins and an investment of energy. In *Streptomyces* the process of chromosome condensation and segregation precede sporulation - the spore-forming process. We can even say that condensation and segregation are tissue-specific in *Streptomyces*, because they only occur in the aerial mycelium, right after the end of intensive replication, just as sporulation's characteristic ladder of dividing cross-walls, or septa, are being formed. This process has to be regulated with extraordinary precision: several dozen chromosomes then undergo segregation and condensation and they have to be packed so that each of them is located within the small space of one individual spore.

ParA and ParB

Among the key proteins participating in the segregation of bacteria chromosomes are Par A (an protein with ATPase activity) and Par B (DNA binding protein). In *Streptomyces*, they are responsible for evenly spacing the chromosomes along the aerial mycelium. An absence of these proteins disrupts the segregation of genetic material, giving rise to "empty" spores (without chromosomes). During the course of aerial mycelium development, the protein ParA develops characteristic structures that change as the mycelium grows. In very young and short hyphae, ParA was detected at their tips while in longer aerial hyphae ParA forms long filaments with a double-spiral shape. The function of ParB protein is to bind to a specific site of the bacteria chromosome, called the *parS* sequences. Our research has shown that in the aerial mycelium, protein ParB forms large complexes around the cluster of *parS* sequences in the presence of a ParA filament, consisting of proteins and DNA. They are visible under the microscope as distinct fluorescent clusters (when fused with GFP - green fluorescent protein, ParB glows green). It seems that the ParA protein filament helps to ensure the regular spacing of several dozen ParB complexes between the septa barriers as they are being formed. This spatial organization enables the chromosomes to be spread out with precision - thus ensuring that the dozens of newly formed spores each receive one chromosome.

Dynamic skeleton

Our research has shown that the active and dynamic placement of chromosomes is affected by the enzymatic function of the ParA protein, i.e. the ATPase activity, which involves the breakdown (hydrolysis) of the ATP molecule, an energy carrier. Strains with mutations in the region responsible for ATPase activity and mutants deprived of gene encoding ParA protein demonstrate extensive DNA segregation disturbances, with up to 30% of "empty" spores. In mutant cells, the emerging septa have also turned out to form with irregular spacing. These observations suggest that ParA is not just a sort of scaffolding helping to properly space chromosomes during their segregation, but may also be one of the components of the dynamic cell skeleton (cytoskeleton), which requires energy to function. Like in the cells of higher organisms, the cytoskeleton in *Streptomyces* likely plays a significant role during cell division. It is also thought that the protein ParA could play the role of a check point, coordinating the process of chromosome segregation and cell division. ■



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***Steptomyces* are similar to primitive fungi. Some of the substances they produce give their colonies an unusual appearance**

Further reading:

Hopwood D.A. (2007). *Streptomyces in Nature and Medicine: The Antibiotic Makers*. USA, Oxford: Oxford University Press.

Jakimowicz D., Żydek P., Kois A., Zakrzewska-Czerwińska J., and Chater K.F. (2007). Alignment of multiple chromosomes along helical ParA scaffolding in sporulating *Streptomyces* hyphae. *Mol Microbiol, 65*, 625-41.

Ruban-Ośmialowska B., Jakimowicz D., Smulczyk-Krawczyszyn A., Chater K.F., Zakrzewska-Czerwińska J. (2006). Replisome localization in vegetative and aerial hyphae of *Streptomyces coelicolor*. *J Bacteriol, 188*, 7311-7316.