Streptomyces coelicolor in an oxygen-limited liquid environment: adapt and escape

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Streptomyces coelicolor in an oxygen-limited liquid environment: adapt and escape

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Introduction

Streptomycetes are mycelial soil bacteria that undergo a complex developmental cycle on solid media. Spores germinate and form a branched, vegetative mycelium.

Several signals trigger the formation of hydrophobic aerial hyphae that differentiate further into reproductive chains of spores. Differentiation is accompanied by the production of secondary metabolites, e.g. antibiotics.

We have studied the development of *Streptomyces coelicolor* in standing liquid cultures. The media in these cultures show steep oxygen gradients [1] which are similar to those found in flooded soils. For example, nutrient rich media were already anoxic 1-2mm below the surface. How does *S. coelicolor* cope with this? Recent research indicates that this bacterium adapts and escapes.

Despite the oxygen limitation in the standing liquid cultures, *S. coelicolor* readily colonised the medium (Figure 1), implying the presence of an active anaerobic metabolism [1]. Growth of *S. coelicolor* under anaerobic conditions had not been reported before. Yet, various genes within the genome are predicted to be involved in low oxygen stress, nitrate and nitrite respiration, and fermentation (http://www.sanger.ac.uk/Projects/S_coelicolor/). This indicates that this bacterium is fully equipped to grow under these conditions.

Hyphae in the aqueous anaerobic environment not only grow freely in the medium but also attach to and grow over the hydrophobic surface of the well (Figure 1) [1, 2]. Attachment of hyphae was reduced in strains in which the *rdlA* and *rdlB* genes had been deleted. These genes encode for homologous secreted proteins called rodlins. Rodlins are only produced by hyphae in contact with a hydrophobic environment such as a hydrophobic solid or the air and form (or are part of) a rodlet-decorated outer cell wall layer (Figure 2).

The finding that the *rdl* genes are not only expressed under aerobic conditions, but also in oxygen-limited conditions, suggests that regulation of these genes and possibly other developmentally regulated genes as well, is not signalled through oxygen levels.

After a period of submerged growth, hyphae in the liquid standing culture migrated to the air interface (Figure 1) [1]. How hyphae move to this interface is currently being studied. Possibly, buoyancy of *S. coelicolor* is provided by the formation of gas vesicles encoded for by two gas vesicle gene clusters that are contained in the genome. Hyphae in shaken liquid cultures do not float when these cultures are no longer shaken which indicates that shear forces may have a negative effect on flotation.

The observed decrease in oxygen tension and nutrient limitation in standing liquid cultures could form additional triggers for becoming buoyant. At the air interface floating colonies were formed that produced sporulating aerial hyphae (Figure 1) similar to those on solid agar media. Interestingly, the floating colonies were fixed at the air interface by a rigid light reflecting film. However, this film does not seem to be involved in enabling hyphae to escape the water to grow into the air. This was concluded from a recent study showing that chaplins (hydrophobic cell surface proteins involved in aerial mycelium formation) fulfil this function.

A strain in which six out of eight chaplins genes were deleted was strongly affected in its formation of aerial hyphae [3, 4]. However, the light reflecting film was still formed [3]. Aerial growth on solid medium could be restored in the mutant by applying purified chaplins to the colony surface [3], indicating that chaplins might act as surfactants. Indeed, mixtures of chaplins were shown to lower the water surface tension from 72-28mJ m^{-2}.

This surface activity is accompanied by major conformational changes in the proteins. At the water-air interface, chaplins assemble into small amyloid-like fibrils that are rich in ß-sheet [3]. Chaplins are the first reported example of functional amyloid-like proteins in Gram-positive bacteria and only the second in the prokaryotic domains.

Future research will focus on metabolism under oxygen limited conditions and on the role of the light reflecting film formed at the water-air interface. Identification of the molecules that make up this film will be the first step.

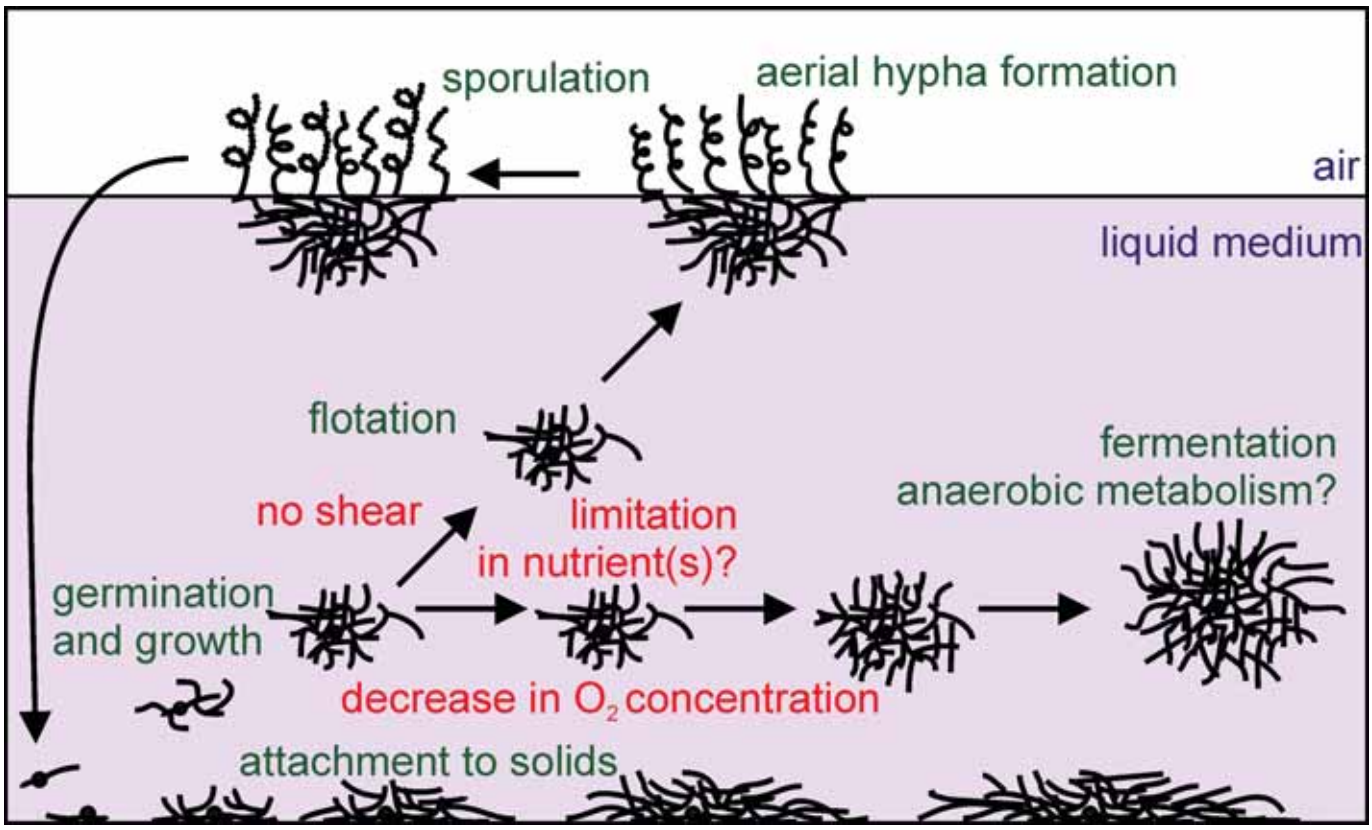

Figure 1. Standing liquid cultures of *S. coelicolor* demonstrate an extended life cycle, attachment to hydrophobic solids and novel metabolic pathways.

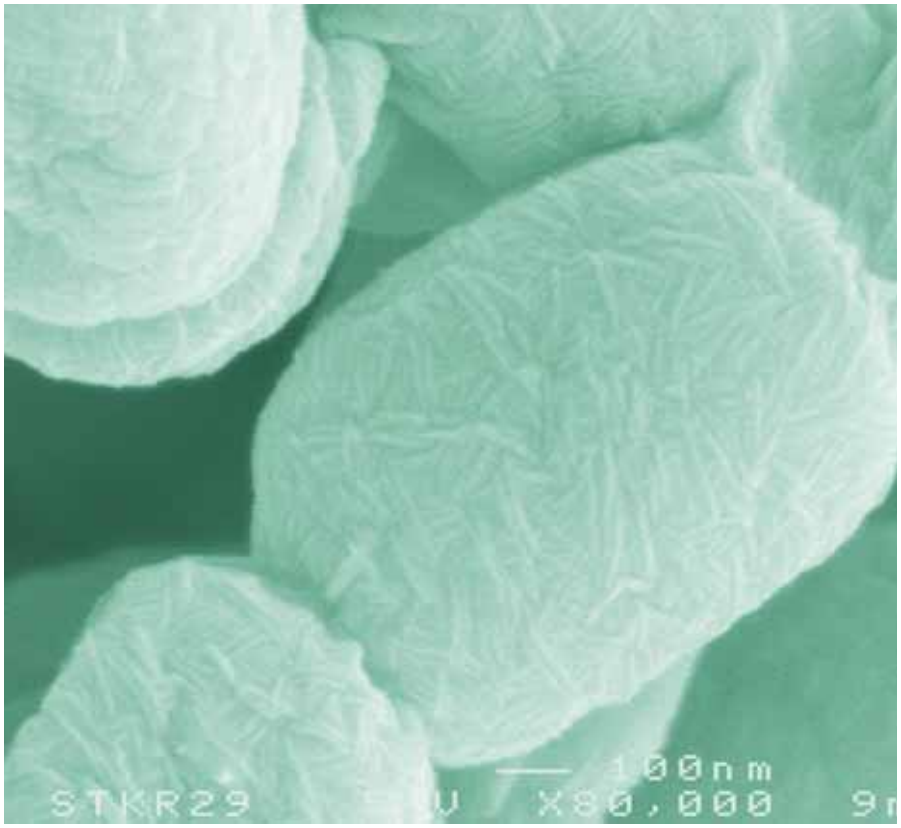

Figure 2. The outer surface of aerial structures of *S. coelicolor* is characterised by a typical ultrastructure called the rodlet layer.

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