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Pre-sporulation stages of *Streptomyces* differentiation: state-of-the-art and future perspectives

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Abstract

Streptomycetes comprise very important industrial bacteria, producing two-thirds of all clinically relevant secondary metabolites. They are mycelial microorganisms with complex developmental cycles that include programmed cell death (PCD) and sporulation. Industrial fermentations are usually performed in liquid cultures (large bioreactors), conditions in which *Streptomyces* strains generally do not sporulate, and it was traditionally assumed that there was no differentiation. In this work, we review the current knowledge on *Streptomyces* pre-sporulation stages of *Streptomyces* differentiation.

Keywords

antibiotic; differentiation; programmed cell death; secondary metabolism; sporulation; *Streptomyces*

Introduction

Streptomycetes are gram-positive, mycelium-forming, soil microorganisms that play important roles in mineralization processes in nature. They have great socio-economic relevance, as they produce several clinically relevant secondary metabolites (antibiotics, antitumorals, immunosuppressants, etc.) (Hopwood, 2007). Streptomycetes have complex developmental cycles that resemble filamentous fungi, forming hyphae and mycelia. They also have sporulation and programmed cell death (PCD) processes and are considered multicellular prokaryotic models.

Differentiation and development of *Streptomyces* in solid cultures

The traditional *Streptomyces* developmental cycle mainly focused on the sporulation phases occurring in solid cultures. After spore germination, a completely viable vegetative mycelium (substrate) grows on the surface and inside agar until it differentiates to a reproductive (aerial) mycelium that grows into the air, producing spores at the end of the cycle (reviewed in Flärdh & Buttner, 2009). This developmental model has been refined with respect to the stages preceding aerial mycelium formation and sporulation (Fig. 1). A young, compartmentalized mycelium (MI) was reported to die early on, following a highly ordered sequence (Manteca *et al.*, 2005, 2006a). Subsequently, the viable segments of this mycelium differentiate into a multinucleated second mycelium (MII). MII grows inside the

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culture medium (substrate mycelium) until it starts to express hydrophobic covers and grows into the air (aerial mycelium) and ends by forming spores (Manteca *et al.*, 2007). Prior to sporulation, there is a second round of PCD affecting substrate and aerial mycelium (Wildermuth, 1970; Mendez *et al.*, 1985; Miguelez *et al.*, 1999). MI, MII, and PCD were mainly described in *Streptomyces antibioticus* ATCC11891 (Miguelez *et al.*, 1999; Manteca *et al.*, 2005) or *Streptomyces coelicolor* M145 (Manteca *et al.*, 2007). However, the existence of these developmental stages can be considered general to the *Streptomyces* genus, as they were observed in all the streptomycetes analysed: *Streptomyces griseus* IFO 13350, *Streptomyces avermitillis* MA-4680, *Streptomyces cinereoruber* ATCC19740, as well as hundreds of unclassified streptomycetes, examined during the screening experiments aimed at discovering novel secondary metabolites (Yagüe P, Genilloud O, Manteca A, unpublished results).

Spore germination

Spore germination constitutes the first step of *Streptomyces* development (Fig. 2). However, the mechanisms activating germination remain somewhat vague. Spore germination comprises a sucession of distinctive steps, which were organized nicely by Hardisson *et al.* (1978) into three stages: darkening, swelling, and germ tube emergence. Darkening only required exogenous divalent cations (Ca^{2+}, Mg^{2+} or Fe^{2+}) and spore energy reserves. Calcium was reported to accumulate in the spore covers and be released during germination (Eaton & Ensign, 1980; Salas *et al.*, 1983). Wang *et al.* (2008) demonstrated that calcium regulation could be mediated, at least in part, by *cabC*, a gene encoding an EF-hand calcium-binding protein. Trehalose was demonstrated to be consumed during the early stages of germination (Hey-Ferguson *et al.*, 1973; McBride & Ensign, 1987). The second stage, swelling, needed an exogenous carbon source, and the last stage, germ tube emergence, required additional carbon and nitrogen sources.

Spore germination is highly regulated and can be externally modified. Hirsch & Ensign (1976) reported that the latency preceding germination of *S. viridochromogenes* spores was eliminated by gentle heat shock, a method that is routinely used to synchronize spore germination in *Streptomyces* (Kieser *et al.*, 2000). Guijarro *et al.* (1983) revealed the existence of a protein fraction that rapidly degrades during germination and that might be regulating this process. Mikulík *et al.* (1984) demonstrated that RNA and protein synthesis began in the first 5 min following spore inoculation, a fact that was later confirmed by Strakova *et al.* (2013). Ribosomes were described as being complex, with melanine-type pigments forming insoluble aggregates, rendering them inactive in the dormant spores (Mikulík *et al.*, 1984). Haiser *et al.* (2009) demonstrated the importance of cell wall hydrolases in both spore formation and spore germination. The existence of germination inhibitors excreted by germinating spores was discovered in *Streptomyces viridochromogenes* by Grund & Ensign (1985) and its chemical nature was subsequently characterized by Petersen *et al.* (1993). These inhibitors were also identified in *S. coelicolor* (Song *et al.*, 2006). Cyclic AMP is involved in the regulation of germination (Süsstrunk *et al.*, 1998); this regulation is mediated, at least partially, by the cyclic AMP receptor protein (Crp) (Derouaux *et al.*, 2004; Piette *et al.*, 2005). NepA has been described as a structural cell wall protein involved in maintaining spore dormancy in *S. coelicolor* (de Jong *et al.*, 2009). Noens *et al.* (2007) identified SsgA as a protein marking cell wall sites where germination takes place.

Overall, important information has already been obtained concerning *Streptomyces* germination. However, there is still much to discover to fully understand the biochemical pathways regulating this important process.

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Primary compartmentalized mycelium (MI)

MI is completely compartmentalized and is different from substrate and aerial mycelia, which are multinucleated (Manteca *et al.*, 2005). Compartmentalization of this mycelium was studied by fluorescence microscopy, using membrane (FM 4-64, Cell Mask) and cell wall (WGA, vancomycin) fluorescent stains, as well as electron microscopy (Manteca *et al.*, 2005; Manteca & Sánchez, 2009) (Fig. 1). MI septa membranes did not generally display thick cell walls; moreover, they were curved, probably due to the osmotic cellular pressure which could not be supported by their thin cell walls (Manteca *et al.*, 2005; Manteca & Sánchez, 2009) (Fig. 1b). The function of MI thin septa remains unknown. They may facilitate intercellular communication inside *Streptomyces* hyphae.

Jakimowicz & van Wezel (2012) described the existence of two different septa in *Streptomyces*: substrate and aerial mycelia septa and spore septa. The formation of the two types of septa is regulated by different mechanisms (Willemse *et al.*, 2011). MI septa would constitute a third type of thin septa that is structurally different from substrate/aerial and sporulation septa. This would make *Streptomyces* a very unusual organism, with three distinct septa associated with different developmental stages. Mechanisms regulating MI septa formation have yet to be discovered. FtsZ is one of the key proteins involved in cell division in bacteria. FtsZ was proven to participate in the formation of substrate/aerial/sporulation septa and its mutation gives rise to a non-sporulating syncytial mycelium having no septa (McCormick *et al.*, 1994). Surprisingly, FtsZ mutant tolerated strong mechanical breakage (McCormick *et al.*, 1994); the reason for this resistance remains unknown. One possibility could be the existence of some kind of septa in the FtsZ mutant similar to those with thin cell walls present in MI.

Secondary multinucleated mycelium (MII)

MI mycelial segments which remained viable after the first round of PCD started to grow as multinucleated hyphae (MII), whereas dead segments were progressively dismantled (Manteca *et al.*, 2006b) (Fig. 1). Cellular debris generated by MI dead cells was present in the extracellular medium (cytosolic proteins; diaminopimelic acid, D-alanine, and other amino acids originating from cell wall degradation; nucleolytic activities; DNA or RNA fragments, etc.) (Manteca *et al.*, 2006a). They were also observed under the electron microscope: García (1995) reported that substrate mycelium in *S. antibioticus* was ‘embedded among an intercellular material’ and Manteca *et al.* (2005) described the complete disorganization of MI dying cells. MII growth is completely viable on the surface and inside agar (substrate mycelium) until it undergoes a new round of PCD (Wildermuth, 1970; Mendez *et al.*, 1985; Miguelez *et al.*, 1999; Manteca *et al.*, 2006a). The remaining viable MII hyphae start to form hydrophobic covers (chaplin-rodlin layer) (reviewed in Claessen *et al.*, 2006) and grow into the air (aerial mycelium). Substrate mycelium was considered the vegetative mycelium, whereas aerial mycelium hyphae were considered specialized hyphae destined to sporulate (Chater, 1984). Aerial mycelium would re-use nutrients released by the substrate mycelium during the second round of PCD (a kind of cannibalism) (Mendez *et al.*, 1985) and antibiotics would be produced by the substrate and/or aerial mycelium to prevent competition with other microorganisms during sporulation.

Substrate and aerial mycelia (MII) are multinucleated (reviewed in Jakimowicz & van Wezel, 2012). The existence of these multinucleated hyphae is very unusual and its biological relevance has yet to be revealed. Other well characterized filamentous bacteria, such as *Cyanobacteria*, are not multinucleated (reviewed in Singh & Montgomery, 2011). The obvious advantage to being multinucleated would be to facilitate the distribution of nutrients and biochemical signals, but with a very important risk in nature, as any damage

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would spread to the whole colony. Other mycelial microorganisms, such as fungi, also have multinucleated mycelia at temporary specific stages which are usually related with reproduction (reviewed in Glass & Kaneko, 2003). As discussed below, when *Streptomyces* development was analysed in soils, MI was the predominant mycelium and MII was a transitory phase preceding sporulation, which suggests that MI may be the true vegetative mycelium in nature.

The transition from substrate to aerial mycelium was extensively studied (Fig. 2). *Streptomyces coelicolor* mutant strains defective in different stages of hydrophobic cover formation (aerial mycelium) were used for the genetic and biochemical analysis of *Streptomyces* differentiation. Bald mutants (defective in aerial growth) regulate the 'sky-pathway', which activates the expression of genes related with hydrophobic cover formation (Rdls Chps, SapB) (Fig. 2). Elegant revisions of the state of the art of these developmental pathways have already been published (Claessen *et al.*, 2006; McCormick & Flärdh, 2012).

The mechanisms regulating the absence of septa in MII or their presence in MI remain unknown (Fig. 2). Some authors have described the potential of substrate hyphae to septate and form spores prior to aerial mycelium differentiation, a feature known as 'ectopic sporulation' or 'de-programmed sporulation'. Kelemen *et al.* (1995) described ectopic sporulation in a mutant strain lacking a DNA fragment near glkA in *S. coelicolor*. Kwak & Kendrick (1996) and Ohnishi *et al.* (2002) have described the same process in class III bald and NP4 mutants of *S. griseus*. Sporulation was also reported in substrate mycelium of wild *Streptomyces carpinensis* strain (Miguelez *et al.*, 1997). Ohnishi *et al.* (2002) postulated the existence of unknown, specific mechanisms inhibiting septa formation in substrate hyphae. Manteca *et al.* (2010a, b) analysed differences between MI and MII proteomes, identifying several putative regulatory proteins differentially expressed in both types of mycelia. These experiments were recently extended to MI and MII transcriptomes (Yagüe *et al.*, 2013). Further work will be necessary to characterize the biochemical pathways controlling the transition from MI to MII (Fig. 2).

Compartmentalization of tip ends of aerial mycelium and sporulation

The last stage of *Streptomyces* development in solid cultures corresponds to hypha septation and spore formation (Fig. 1). *Streptomyces whi* mutants defective in different stages of sporulation were used for the genetic and biochemical analyses of these developmental stages (Fig. 2). Sporulation is beyond the scope of this review. Revisions of the state of the art of genes and proteins regulating sporulation already exist (Claessen *et al.*, 2006; Flärdh & Buttner, 2009; Jakimowicz & van Wezel, 2012; McCormick & Flärdh, 2012).

Differentiation and development of *Streptomyces* in liquid cultures

Most *Streptomyces* species do not sporulate in liquid cultures and it was widely accepted that no morphological differentiation took place in these conditions. Secondary metabolites would be produced by the substrate mycelium at the stationary phase after a transient growth arrest (Granozzi *et al.*, 1990; Neumann *et al.*, 1996; Novotna *et al.*, 2003; Zhou *et al.*, 2005; Chouayekh *et al.*, 2007). Despite that, sporulation was reported in liquid cultures for several streptomycetes, such as *Streptomyces venezuelae* (Glazebrook *et al.*, 1990), *S. griseus* (Kendrick & Ensign, 1983), *Streptomyces chrysomallus* (Kuimova & Soina, 1981), *S. antibioticus* ETHZ7451 (Novella *et al.*, 1992), *Streptomyces albidoflavus* SMF301 (Rho & Lee, 1994), or *Streptomyces brasiliensis* (Rueda *et al.*, 2001). Sporulation was also seen to be activated in several *Streptomyces* species under nutritional downshifts, including the model strain *S. coelicolor* (Koepsel & Ensign, 1984; Daza *et al.*, 1989), and was also observed in several streptomycetes liquid cultures during the screening experiments aimed at

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discovering novel secondary metabolites (Yagüe P, Genilloud O, Manteca A, unpublished results).

New aspects regarding *Streptomyces* development (MI, MII, PCD) in solid cultures were extended to liquid cultivation (Manteca *et al.*, 2008) (Fig. 1a). Similar to solid cultures, there was a young, compartmentalized mycelium (MI) that differentiated to a multinucleated mycelium (MII). The MII emergence was preceded by a transient growth arrest, which was the consequence of MI PCD. The only mycelial phases present in liquid were MI and MII without hydrophobic layers (Fig. 1). It was demonstrated that MII is the antibiotic-producing mycelium. This was the first time that antibiotic production was associated with differentiation in liquid cultures (Manteca *et al.*, 2008). The lifespan of MI in liquid cultures was longer than in solid media (around 17 h in solid vs. 48 h in liquid) (Manteca *et al.*, 2007, 2008). MI compartmentalization correlated well with the traditionally accepted existence of a specific phase at the beginning of the development – 'the middle of the exponential phase' in which protoplasts could be formed in *Streptomyces* liquid cultures (Okanishi *et al.*, 1974). Protoplast formation by MII multinucleated mycelium was almost non-existent, a feature that can, in fact, be used to fractionate MI and MII mycelia (Manteca *et al.*, 2010a).

Proteomic (Manteca *et al.*, 2010b) and transcriptomic (Yagüe *et al.*, 2013) analyses demonstrated that differentiation in liquid was much more similar to solid cultures than might be expected within the context of the classical *Streptomyces* developmental model. Proteins and transcripts involved in primary metabolism were up-regulated in MI, whereas proteins and genes involved in secondary metabolite biosynthesis were up-regulated in MII. The most remarkable differences between MII from solid and liquid cultures involved proteins regulating the hydrophobic cover formation and sporulation (Manteca *et al.*, 2008, 2010b). Differentiation of MII after mycelia growth arrest is not enough to guarantee secondary metabolite production, as it can also be regulated by environmental signals, including components of the culture medium, such as nitrogen (Aharonowitz, 1980), carbon (Sánchez *et al.*, 2010) and phosphate (Chouayekh & Virolle, 2002; Martín, 2004).

Streptomyces development in conditions resembling nature (soils)

The significance of the first compartmentalized mycelium was obscured by its short lifespan in usual laboratory culture conditions (Manteca *et al.*, 2005, 2008). This might be attributable to the relatively high cell densities attained in laboratory culture conditions, which provoked massive cell death, differentiation, and sporulation. Natural growth conditions imply discontinuous growth and limited colony development (Williams, 1985). When *Streptomyces* development was analysed in conditions resembling nature (soils inoculated with poor spore inocula), a new developmental cycle emerged in which MI was the predominant mycelium (Manteca & Sánchez, 2009) (Fig. 3). Spore germination was a very slow, non-synchronous process that commenced at about 7 days and lasted for at least 21 days. The mycelium did not clump into dense pellets and remained in the MI compartmentalized mycelium phase for a long time. Even after 1 month of incubation, PCD, MII or sporulation were not detected. It is clear that in nature, cell death and sporulation must take place at the end of the long vegetative phase (Wellington *et al.*, 1990; Anukool *et al.*, 2004) when the nutrient imbalance gives rise to bacterial differentiation. As already commented above, the absence of compartmentalization in the vegetative *Streptomyces* mycelium (substrate) was unique in filamentous bacteria and difficult to understand due to the fragility of a multinucleated mycelium in nature. If we consider development in conditions resembling nature, compartmentalized MI would in fact be the dominant stage and multinucleated MII would be a transient antibiotic-producing structure, facilitating nucleic acid division and preceding sporulation (Fig. 3).

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Streptomyces programmed cell death

Bacterial PCD can be defined as any type of genetically controlled cell dismantling involving the activation of specific cell death transducers, regulators, and effectors (Engelberg-Kulka *et al.*, 2006). PCD was described in bacteria from different taxa, such as *Bacillus* and *Escherichia coli* (Engelberg-Kulka *et al.*, 2006), *Anabaena* (Ning *et al.*, 2002), *Caulobacter* (Hochman, 1997; Bos *et al.*, 2012), *Streptococcus* (Guiral *et al.*, 2005), *Staphylococcus* (Chatterjee *et al.*, 2010), and *Myxobacteria* (Søgaard-Andersen & Yang, 2008). With few exceptions, such as the toxin-antitoxin modules from *E. coli*, the competence-sporulation processes from *Bacillus subtilis* (both reviewed in Engelberg-Kulka *et al.*, 2006) and the competence processes of *Streptococcus pneumonia* (Guiral *et al.*, 2005), the biochemical pathways controlling bacterial PCD, as well as the biological role of this process, are poorly understood (reviewed in Engelberg-Kulka *et al.*, 2006). These three, well characterized bacterial PCDs are regulated in distinct ways, and there is no general biochemical model applicable to all bacterial PCD.

Miguelez *et al.* (1999) and Manteca *et al.* (2006a) demonstrated that *Streptomyces* death phenomena associated with development present the characteristics of programmed cell death. Biochemical parameters, such as the degradation of the cell wall and membrane, DNA/RNA degradation, corroborated the existence of a highly regulated, active cellular suicide that entails the activation of specific degradative enzymes (Manteca *et al.*, 2006a). Among these enzymes there was a precursor of sequence non-specific nucleases involved in massive chromosomal degradation (Nicieza *et al.*, 1999) and the sequence-specific nuclease (endoG) that produced chromosomal bands analogous to those that appear in the programmed cell death of eukaryotic cells (apoptosis) (Cal *et al.*, 1996; Samejima & Earnshaw, 2005). A proteomic analysis revealed that PCD in *S. coelicolor* was accompanied by the appearance of enzymes involved in the degradation of cellular macromolecules, regulatory proteins, and stress-induced proteins (Manteca *et al.*, 2006b). Sevillano *et al.* (2012), identified the first functional toxin-antitoxin system in *Streptomyces* that could be related to PCD. Bacteria having complex life cycles (streptomycetes, cyanobacteria, etc.) harbour several eukaryotic signalling domains and are considered to be the evolutive origin of these domains (Zhang, 1996; Aravind *et al.*, 1999; Koonin & Aravind, 2002; Petrickova & Petricek, 2003). In all, 244 genes (3% of all *Streptomyces* ORFs) harbour these kinds of domains (Table 1). Further work will be necessary to characterize the biochemical regulation of *Streptomyces* PCD and to determine whether the genes described above, including those encoding for proteins harbouring eukaryotic type signalling domains, are involved in its regulation.

The biological function of *Streptomyces* PCD remains somewhat unclear. It was reported to be involved in the generation of nutrients to be consumed by the aerial/sporulating mycelium, a kind of cannibalism (Mendez *et al.*, 1985; Miguelez *et al.*, 1999). If we consider that the best known bacterial PCDs, those occurring in *Streptococcus* and *Bacillus*, are involved in competence (taking fragmented DNA by transformation) (Guiral *et al.*, 2005; Engelberg-Kulka *et al.*, 2006), and that in the case of *Bacillus*, this process precedes sporulation and antibiotic production, an analogous process might also be happening in *Streptomyces*: appropriate DNA fragments would be produced by specific nuclease activities (Cal *et al.*, 1996) and the lysis of MI mycelium (Manteca *et al.*, 2006a) and incorporated by the multinucleated MII followed by recombination and the formation of a huge battery of variable spores. Several authors have hypothesized about the existence of horizontal gene transmission (HGT) phenomena in *Streptomyces* and other actinomycetes (Wiener *et al.*, 1998; Ueda *et al.*, 1999; Egan *et al.*, 2001; Metsä-Ketelä *et al.*, 2002; García-Vallve *et al.*, 2003; Kawase *et al.*, 2004; Nishio *et al.*, 2004; Doroghazi & Buckley, 2010) but the mechanisms generating this HGT remains poorly understood. Conjugative plasmids

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(reviewed in Thoma & Muth, 2012) and transduction (Burke *et al.*, 2001) may contribute in some way to this HGT, but competence/transformation may also occur. *Streptomyces* PCD precedes MII differentiation and sporulation and it was postulated that components released during the degradation of these dying cells could be producing diffusible signals in the form of amino acids/peptides (Sánchez & Braña, 1996) or N-acetylglucosamine (Rigali *et al.*, 2006), thereby inducing differentiation. Further work will need to delve into the biological significance of *Streptomyces* PCD.

Conclusions and future perspectives

Streptomyces growth in nature differs substantially from that observed in ordinary laboratory cultures, a fact that must be borne in mind when development is analysed. MI is the vegetative mycelium and predominates in nature. Under stress conditions (nutrient/oxygen limitation etc.) it suffers a PCD and differentiates to a multinucleated mycelium (MII) that forms spore chains at the end of the cycle. Multinucleated MII would facilitate rapid growth and nucleoid division prior to sporulation. MII produces antibiotics that are decisive in helping the bacterium compete with other microorganisms.

Streptomyces research has classically focused on the aerial mycelium formation and sporulation phases taking place in solid cultures. By contrast, pre-sporulation stages, including differentiation in liquid cultures, have been largely ignored. The new insights regarding presporulation stages of *Streptomyces* in combination with future work aimed at understanding the biochemical regulation of these processes will be key to comprehending and optimizing hyphae differentiation in industrial fermentations, as well as improving the screening for new secondary metabolites from natural *Streptomyces* strains.

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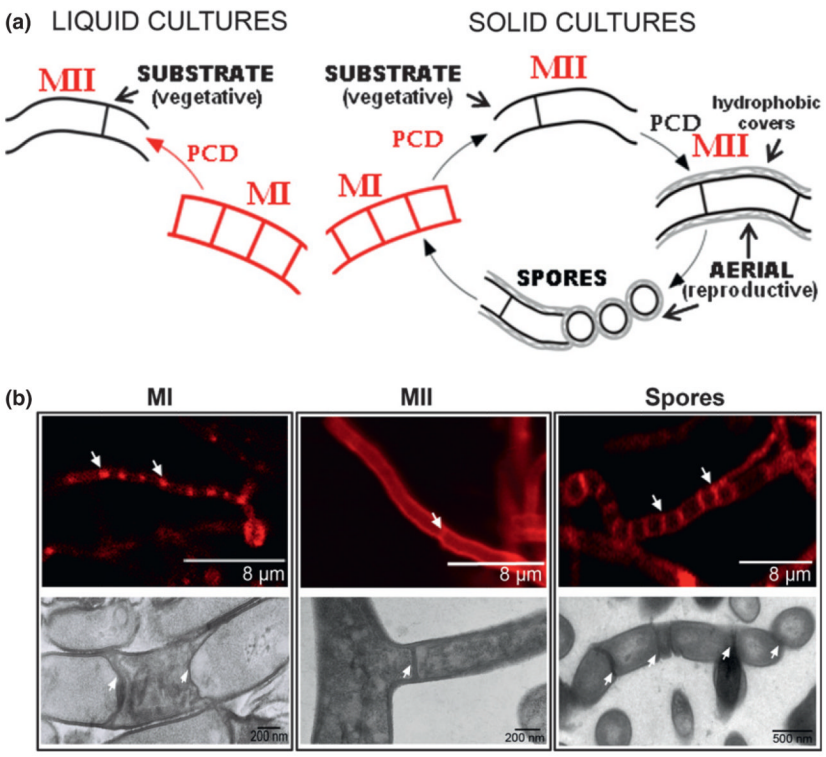

Fig. 1.

Streptomyces developmental cycle and mycelium differentiation. (a) *Streptomyces* developmental cycle in liquid (left) and solid (right) cultures. Newly described structures and the proposed nomenclature (Manteca *et al.*, 2005) are indicated in red: MI, first compartmentalized mycelium; MII, second multinucleated mycelium. Classical nomenclature (substrate and aerial mycelium) and hydrophobic layers are also indicated. PCD, programmed cell death. (b) Different types of mycelia observed under the confocal and electron microscopes. Upper panels, confocal images. Left, MI young compartmentalized hypha (notice the original spore in the right side) stained with membrane stain FM 4-64; mycelium is fully compartmentalized and compartments are separated by membranes (arrows). Centre, MII multinucleated hypha stained with the cell wall stain WGA. Right, sporulated hypha stained with WGA; notice thick cell wall septa separating spores. Some of the septa are indicated by arrows. Figure adapted from Manteca *et al.* (2006a, 2010b).

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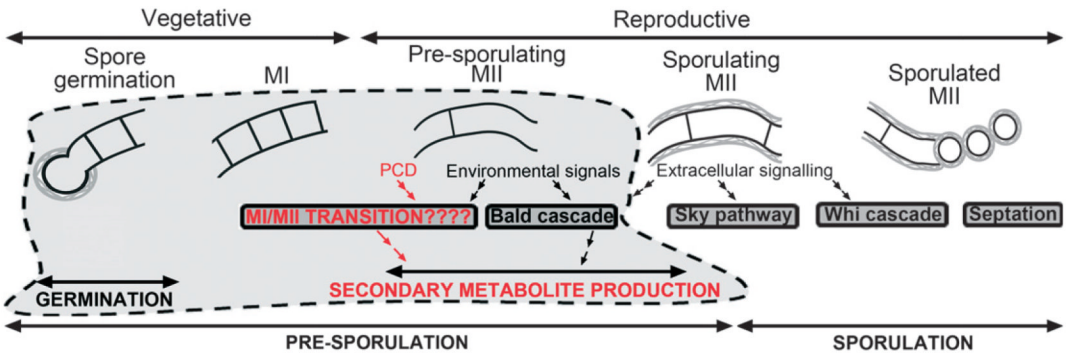

Fig. 2.

Biochemical pathways regulating *Streptomyces* differentiation. Pathways involved in hydrophobic covers formation (‘bald’, ‘sky’) and sporulation (‘whi’, ‘septation’) are illustrated. New developmental stages (MI/MII; Manteca *et al.*, 2005) and presporulation pathways (‘MI/MII transition’) switching on secondary metabolite production are indicated in red.

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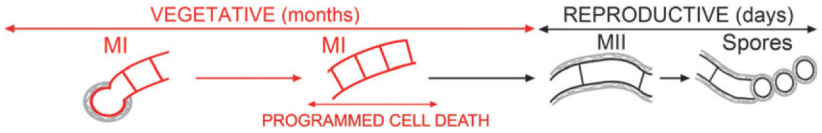

Fig. 3.

Proposed developmental model for *Streptomyces* growing in natural soils. Mycelial structures (MI, first mycelium; MII, second mycelium), vegetative and reproductive phases, and PCD are indicated. The vegetative phase is the predominant one. See text for details.

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Table 1

Genes harbouring eukaryotic type signalling domains in the *Streptomyces coelicolor* genome according to the Conserved Domain Database

Eukaryotic type domain	Genes						
Htra (cd00987)	SCO2171	SCO3977	SCO5149	SCO6074			
TIR (cl02060)	SCO0305	SCO2602	SCO2680	SCO5642	SCO5953		
AAA ATPases (cd00009)	SCO0025	SCO1506	SCO2094	SCO3373	SCO4067	SCO5587	SCO6529
	SCO1024	SCO1518	SCO2449	SCO3404	SCO4263	SCO6134	SCO6623
	SCO1306	SCO1648	SCO2617	SCO3661	SCO5270	SCO6394	SCO7523
	SCO1434	SCO1726	SCO3018	SCO3879	SCO5285	SCO6408	SCO7582
Ser/Thr kinases (smart00220)	SCO0239	SCO2244	SCO3277	SCO3860	SCO4507	SCO4820	
	SCO1278	SCO2450	SCO3344	SCO4192	SCO4775	SCO4911	
	SCO1468	SCO2666	SCO3360	SCO4377	SCO4776	SCO5192	SCO6951
	SCO1549	SCO2973	SCO3621	SCO4423	SCO4777	SCO6077	SCO7240
	SCO1551	SCO2974	SCO3820	SCO4481	SCO4778	SCO6085	SCO7251
	SCO1724	SCO3102	SCO3821	SCO4487	SCO4779	SCO6626	SCO7291
	SCO2110	SCO3234	SCO3848	SCO4488	SCO4817	SCO6681	
AP-ATPase (cl09099)	SCO0002	SCO1300	SCO2677	SCO3706	SCO5183	SCO5920	
	SCO0006	SCO1331	SCO2681	SCO3824	SCO5184	SCO5923	SCO6719
	SCO0132	SCO1433	SCO2737	SCO3876	SCO5188	SCO5973	SCO6720
	SCO0163	SCO1504	SCO2763	SCO3886	SCO5275	SCO6010	SCO6742
	SCO0166	SCO1621	SCO2767	SCO3934	SCO5277	SCO6047	SCO6814
	SCO0255	SCO1671	SCO2952	SCO3947	SCO5280	SCO6193	SCO6981
	SCO0322	SCO1707	SCO2969	SCO3958	SCO5339	SCO6259	SCO7008
	SCO0491	SCO1719	SCO2975	SCO4075	SCO5383	SCO6295	SCO7051
	SCO0493	SCO1742	SCO3005	SCO4116	SCO5387	SCO6366	SCO7173
	SCO0504	SCO1780	SCO3217	SCO4259	SCO5439	SCO6426	SCO7689
	SCO0700	SCO1798	SCO3235	SCO4316	SCO5448	SCO6512	SCO7690
	SCO0723	SCO1840	SCO3257	SCO4359	SCO5449	SCO6517	SCO7841
	SCO0742	SCO1848	SCO3261	SCO4405	SCO5451	SCO6633	SCO7845
	SCO0755	SCO1850	SCO3351	SCO4508	SCO5580	SCO6635	SCP1.110
	SCO0756	SCO1852	SCO3369	SCO4585	SCO5603	SCO6677	SCP1.136
	SCO0824	SCO1966	SCO3370	SCO4620	SCO5633	SCO6683	SCP1.169
	SCO1144	SCO2000	SCO3372	SCO4685	SCO5648	SCO6684	SCP1.205c
	SCO1147	SCO2257	SCO3418	SCO4797	SCO5668	SCO6719	SCP1.216Ac
	SCO1148	SCO2259	SCO3453	SCO4803	SCO5734	SCO6720	SCP1.290c
	SCO1152	SCO2324	SCO3526	SCO4909	SCO5750	SCO6742	SCP1.63
	SCO1183	SCO2463	SCO3541	SCO4963	SCO5802	SCO6814	SCP1.90c
	SCO1232	SCO2523	SCO3550	SCO5028	SCO5818	SCO6849	SCP2.05c
	SCO1246	SCO2532	SCO3556	SCO5166	SCO5835	SCO6865	
IL1 like (cl01077)	SCO2021						
DEADc (cd00046)	SCO3732	SCO4096					

Eukaryotic type domain	Genes	
Caspase (cl00042) + Ser/Thr kinases	SCO6861	
AP-ATPase + TIR (cl02060)	SCO4632	SCO5629

HtrA shock-induced-envelope-associated serine proteases (HtrA), Toll/IL-1 Receptor (TIR), AAA ATPases, Ser/Thr kinases, apoptotic ATPases (AP-ATPases), interleukin (IL)-1 receptor-associated kinase (IL1-like), DEAD-like helicases (DEADc), cysteine-aspartic proteases (caspases). Conserved domain database accession numbers are indicated in brackets.

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