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Colony development in *Streptomyces carpinensis*: a streptomycete with substrate mycelium spores

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Abstract

In this paper we present an ultrastructural study of spore formation in aerial vs. substrate mycelia of *Streptomyces carpinensis*. Both mycelia initiated spore formation at nearly the same time of colony development but exhibited different patterns of spatial localization of sporulation: spore formation took place throughout the aerial mycelium whereas in the substrate mycelium was confined to a narrow zone at the bottom of the colony. The ultrastructural changes leading to spore formation, however, were quite similar in both mycelia, differing only with respect to the outer components of the sporal wall. Spores formed in the aerial mycelium were covered by a thin sheath whereas the spores formed in the substrate mycelium were covered by an amorphous electron-dense material.

Keywords: *Streptomyces carpinensis*; Sporulation; Colony development

1. Introduction

The streptomycetes are distinguished by their ability to carry out a complex developmental cycle which culminates in the formation of a highly structured colony containing two different types of mycelia: a substrate mycelium that grows on and into the culture medium and an aerial mycelium which develops into the air and forms chains of spores [1–3]. Differentiation processes occurring in the aerial mycelium have been the subject of several ultrastructural studies (reviewed in [2,3]). Differentiation processes occurring in the substrate mycelium, although reported in several *Streptomyces* species [4–6], have received less attention and have not yet been examined in detail. In the present work, electron microscopic techniques were used to investigate the behavior of the substrate mycelium and the ultrastructural changes which it undergoes during colony development and sporulation in *Streptomyces carpinensis*, a microorganism which typically forms spores in both the substrate and the aerial mycelium.

2. Materials and methods

Streptomyces carpinensis ATCC 27116 (formerly known as *Elytrosporangium carpinense* [7]) was grown as lawns on glucose-asparagine-yeast extract medium [8]. Plates (containing 30 ml of culture medium) were inoculated by spreading 0.2 ml of a spore

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Fig. 1. Vertical section through a 2-day-old colony of *S. carpinensis*. The arrowheads indicate the agar surface. The bar represents 2 μm.

suspension (10^8 spores ml^{-1}) and incubated at 28°C. The developmental stage of the lawns was monitored by observing the changes in coloration of the cultures. At various times of incubation, samples of the lawns (exhibiting uniformity of development) were cut out from the cultures and fixed overnight in 1% (w/v) osmium tetroxide. After being washed in 0.5% (w/v) uranyl acetate for 3 h and dehydrated through increasing concentrations of acetone, the samples were embedded in Epon 812 resin. Before polymerization, samples were properly positioned to facilitate vertical sectioning of the whole mycelium. Ultrathin sections obtained from selected fields of the mycelium were stained with uranyl acetate and lead citrate and observed in a Philips EM 300 electron microscope.

3. Results and discussion

During the first 2 days of development the colony consisted of two populations of hyphae: closely packed hyphae growing on the surface of the culture medium, and a large network of hyphae which develop within the culture medium (Fig. 1). These colonies contained only substrate hyphae since sheathed hyphae growing away from the surface of the colony were not seen. Colonies of 3 days of incubation appeared as a complex and heterogeneous network of hyphae that follow different trends of development depending on their position in the colony. At the top

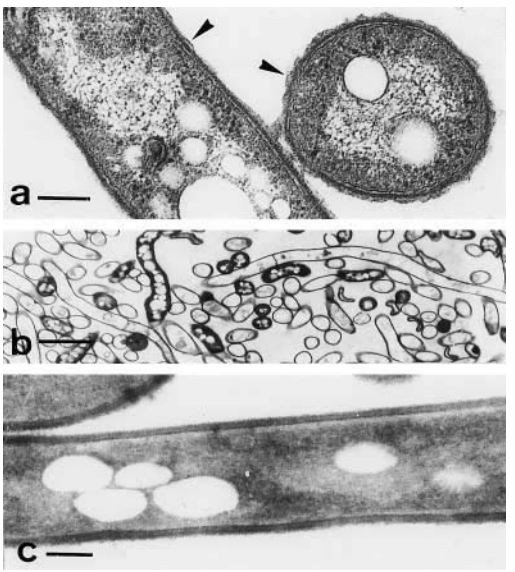

Fig. 2. Sections of selected parts of a 3-day-old colony of *S. carpinensis*. a: Sheathed hyphae of the aerial mycelium in the upper region of the colony (arrowheads indicate the sheath). b: Lysed hyphae of the substrate mycelium close to the agar surface. c: Substrate hypha in the bottom of the colony. The bars represent 0.2 μm (a, c) and 2 μm (b).

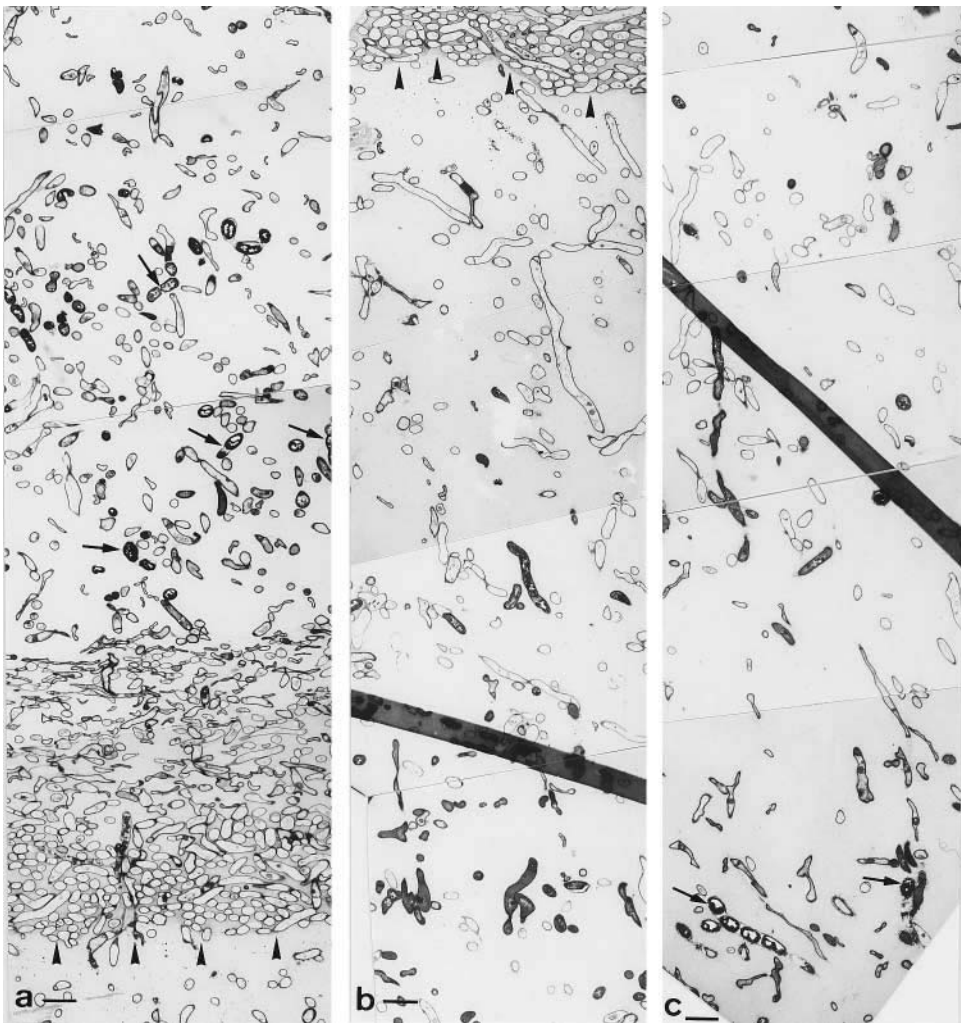

Fig. 3. Vertical sections through a 5-day-old colony of *S. carpinensis*. a: The aerial mycelium and lysed hyphae of the substrate mycelium above the agar surface (bottom). b, c: The substrate mycelium within the culture medium from the agar surface (top of panel a) to the bottom of the colony (bottom of panel c). The arrowheads indicate the agar surface and the arrows the spores. The bars represent 2 μm.

of the colony the substrate mycelium differentiates into sheathed hyphae (Fig. 2a) which grow upwards into the air forming the aerial mycelium. Close to the agar surface the substrate mycelium undergoes lysis (Fig. 2b). Within the culture medium the substrate mycelium does not form sheathed hyphae and remains morphologically undifferentiated (Fig. 2c).

After 5 days of incubation, the colonies developed chains of spores in both substrate and aerial mycelia. Colonies with spores only in the aerial mycelium or colonies with spores only in the substrate mycelium were never found in sections obtained from cultures between 4 and 7 days of incubation, which indicates that both mycelia initiated sporulation at nearly the same time of development. Four mycelial zones could be recognized along the vertical axis of these colonies. (i) The aerial mycelium (Fig. 3a), which contains a mixture of sporulating, entire and lysed hyphae. (ii) Below the aerial mycelium (bottom of Fig. 3a) there was a compact mass of empty lysed hyphae corresponding to the population of substrate hyphae that had previously developed above the surface of the agar. (iii) Within the culture medium (Fig. 3b,c), the substrate mycelium was largely com-

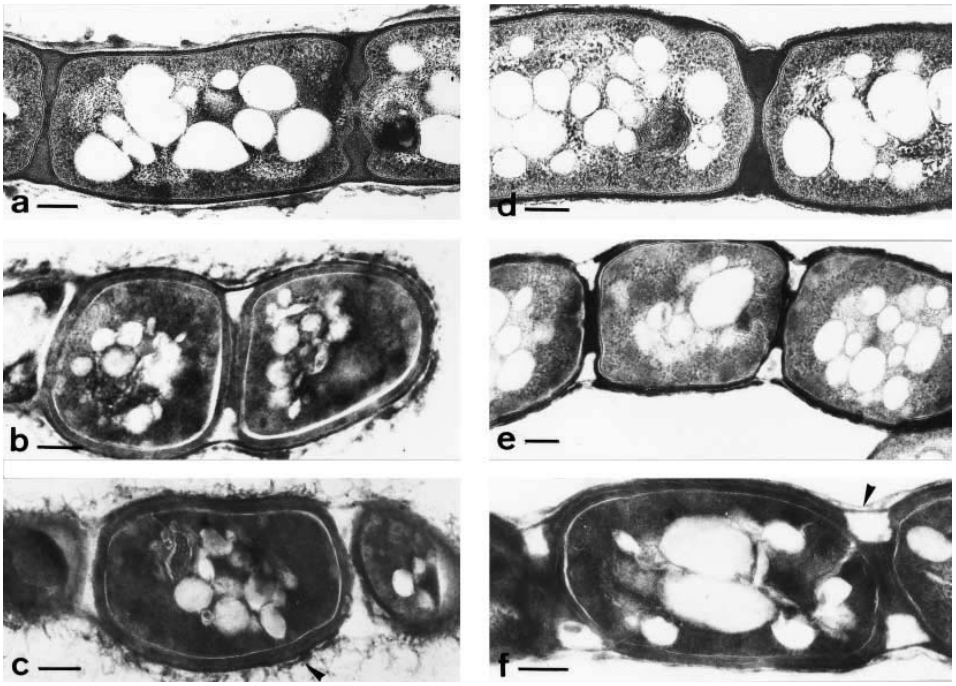

Fig. 4. Longitudinal sections of substrate (a,b,c) and aerial (d,e,f) sporulating hyphae of *S. carpinensis* at different stages of development. In both mycelia the sporulating hyphae are subdivided into compartments by sporulation septa consisting of two components: a thick ring and a thin cross-wall which develops from the inner edge of the ring and closes the septum (a,d). After septation the sporal wall thickens gradually, the sporulation septum undergoes hydrolysis and the sporal compartments separate from each other (b,e). Mature spores are covered by an amorphous material in the substrate mycelium (c, arrowheads) or by a thin sheath in the aerial mycelium (f, arrowheads). The bars represent 0.2 μm.

posed of lysed hyphae. (iv) At the bottom of the colony (approximately 120 μm below the agar surface, bottom of Fig. 3c), the substrate mycelium consisted of lysed hyphae and hyphae at different stages of sporulation. Microscopic observation of sections obtained from samples of cultures of 5 days of incubation did not reveal changes in the overall organization of the colony nor in the ultrastructural features of the hyphae existing at each of the zones that constitute it.

The ultrastructural changes leading to spore formation (summarized in Fig. 4) were quite similar in both mycelia, differing only with respect to the outer components of the sporal wall: spores formed in the substrate mycelium were covered by an amorphous electron-dense material (of unknown chemical nature), whereas those formed in the aerial mycelium were covered by a thin sheath. Deposition of the amorphous material around the substrate hyphae was a sporulation-specific event since: (i) it was only detected in sporulating hyphae, and (ii) sporulating hyphae lacking amorphous material were not seen.

The spatial distribution of sporulating hyphae, however, was not the same in both mycelia. Chains of spores were apparent throughout the aerial mycelium with no preferential localization to any particular zone (Fig. 3a). In the substrate mycelium, however, spores were seen in only a deep narrow zone at the bottom of the colony (Fig. 3c). This observation is interesting because it implies that spore formation is subjected to spatial control in the substrate mycelium. In this regard, experiments carried out with *S. coelicolor* [9,10] revealed that genes necessary for sporulation are under the control of a sporulation-specific σ factor (σ^{whiG}). It has also been suggested [6,10] that, in non-sporulating hyphae, an anti-σ factor might bind to σ^{whiG} thus preventing its development towards spore formation. Taking this into account, absence of spore formation in substrate

hyphae located at or near the surface of the culture medium in *S. carpinensis* might result from modulation of such hypothetical anti-σ activity by some type of extracellular signal excreted by the hyphae and that would accumulate in large amounts in the high-hyphal-density zones of the colony. Such a control mechanism would restrict spore formation to hyphae that develop far enough from the agar surface (bottom of the colony) or that are no longer in contact with it as those that form the aerial mycelium.

Acknowledgments

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