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Early Stages of Arthrospore Maturation in *Streptomyces*

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In the sporogenesis of *Streptomyces*, two basic stages can be considered: sporulation septum synthesis and arthrospore maturation. Most of the information about the ultrastructural changes accompanying the sporogenesis refer to the first stage of the process, but nothing has been published about the evolution of the sporulation septum during maturation. In a previous paper, proposed three basic types of sporulation septum formation in *Streptomyces*. Our ultrastructural study on the evolution of the sporulation septum during the early stages of arthrospore maturation in seven species of *Streptomyces* indicates correlation between the sporulation septum type and its evolution during the arthrospore maturation. In types I and II the material of the annuli was incorporated into the lateral walls of the arthrospore, whereas in types II and III the deposits were lysed during the maturation. Only in type III was the arthrospore wall synthesized de novo. In type I there was total integration and in type II there was partial integration of the septum into the arthrospore wall.

The ultrastructural changes that accompany sporogenesis in *Streptomyces* have been studied by several authors (1, 6-10) and have been recently reviewed by Kalakoutskii and Agre (5). In a previous paper (4) we proposed three types of sporulation septum formation in *Streptomyces*. The completion of the sporulation septum and the delimitation of the mycelium into spore-sized compartments mark the beginning of a series of partially known changes, resulting in the formation of mature arthrospores. Wildermuth and Hopwood (8) and McVittie (6) have described some modifications that accompany this process of maturation, mainly the rounding off of the corners, that is, the change from the cylindrical hyphal shape to ellipsoidal or spherical forms of the arthrospore and the thickening of the wall, but nothing has been published about the evolution of the sporulation septum.

In this paper we present the results of an ultrastructural study on the evolution of the sporulation septum during the early stages of arthrospore maturation in seven species of *Streptomyces*.

MATERIALS AND METHODS

Microorganisms, media, and culture conditions. The microorganisms, media, and culture conditions used were described previously (4).

Electron microscopy. Single colonies containing mature arthrospores, after 3 to 5 days of incubation, were fixed with osmium tetroxide as previously described (4) and embedded in Araldite. Thin sections were cut with glass knives on an LKB III ultramicrotome and picked up on Formvar-coated grids. The specimens were examined in a Philips EM 300 electron microscope at an accelerating voltage of 60 to 80 kV. Photographs were taken at instrument magnifications of 20,000 to 30,000.

RESULTS AND DISCUSSION

The ultrastructural study of sporulation septum formation in *Streptomyces* suggests, as we have previously stated (4), the existence of three basic types of sporulation septa. Results obtained in this work indicate a correlation between a sporulation septum type and its evolution during arthrospore maturation. In type I the septum is formed from the beginning by two separate cross walls, which grow centripetally until fusing (i.e., *Streptomyces albus, S. erythraeus,* and *S. aureofaciens*). Once the septum is completed, each cross wall thickens evenly during the maturation process until it forms the lateral wall of the mature arthrospore (Fig. 1 to 5).

In type II, distinguished by a deposit of material before synthesis of the double annulus, which completes the septum (i.e., *S. flaveolus, S. ambofaciens,* and *S. antibioticus*), only the central electron-dense zone of each annulus is incorporated into the lateral wall of the arthrospore, but the deposits are completely lysed. The lysis starts in the septum zones near the hyphal walls (Fig. 6 to 11).

Type III is represented only by *S. cinnamonensis* (Fig. 12 to 18), in which the sporulation septum is formed by a single deposit of electron-dense material. After the septum is completed

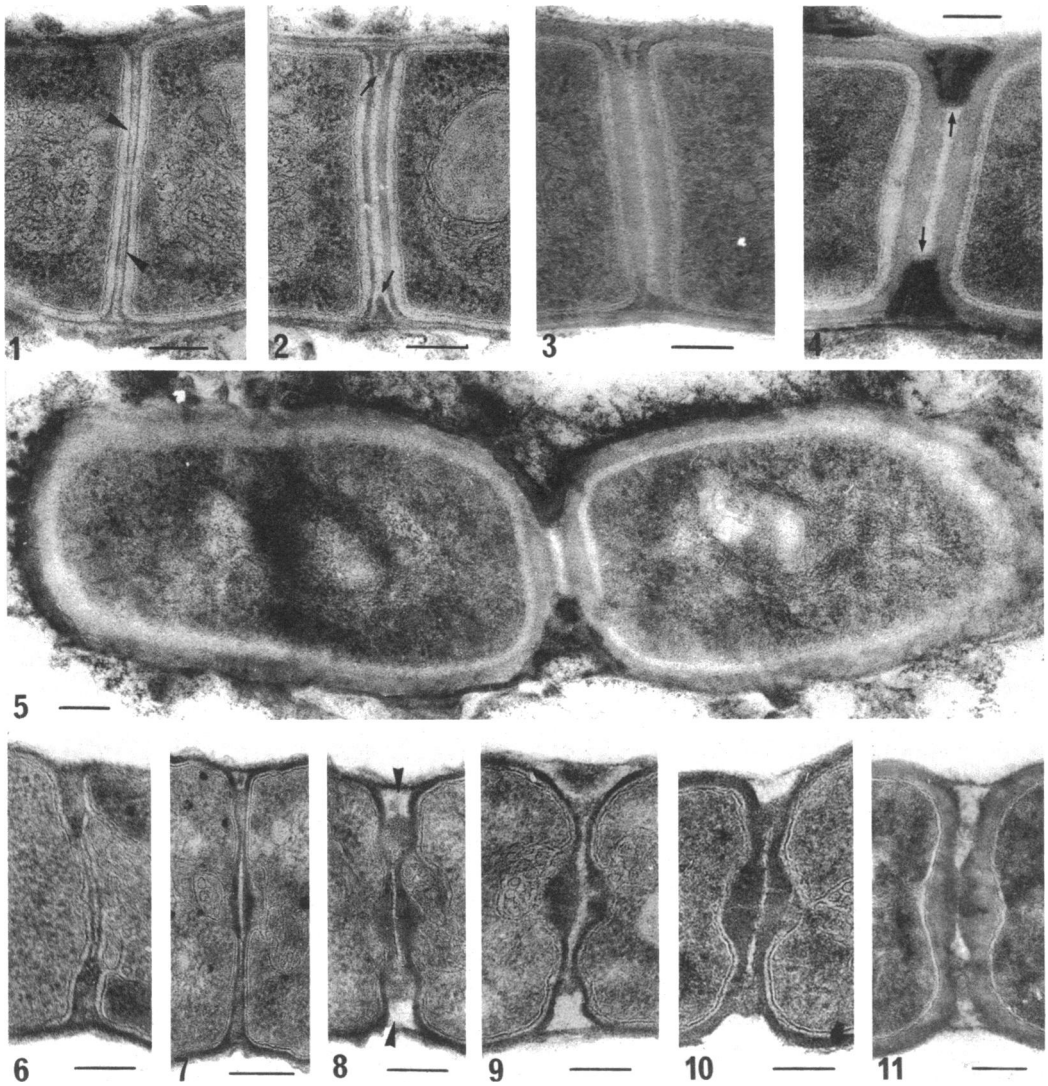

FIG. 1-11. *Sequential stages of arthrospore maturation in type I and II Streptomyces, showing the evolution of the sporulation septum. Bar = 100 nm in all figures.*

FIG. 1-5. *Successive early stages of arthrospore maturation in S. erythraeus. Note that the two separate cross walls of the sporulation septum (Fig. 1, arrowheads) thicken evenly (Fig. 2-4), causing the formation of the lateral wall of the arthrospore (Fig. 5). This process was similar for S. albus and S. aureofaciens, but only in S. erythraeus was the deposit of an extremely electron-dense, wedge-shaped material between the two arthrospores and the hyphal wall (arrows) observed; this material may play a role in the separation of mature arthrospores.*

FIG. 6-11. *Successive early stages of arthrospore maturation in S. flaveolus. The newly formed sporulation septum (Fig. 6) shows a double cross wall between the deposit material. Note that the deposit material is lysed while the material of the double annulus is integrated into the lateral wall of the arthrospore. The lysis starts in the septum zones near the hyphal wall (Fig. 8, arrowhead). This process was similar for S. ambofaciens and S. antibioticus.*

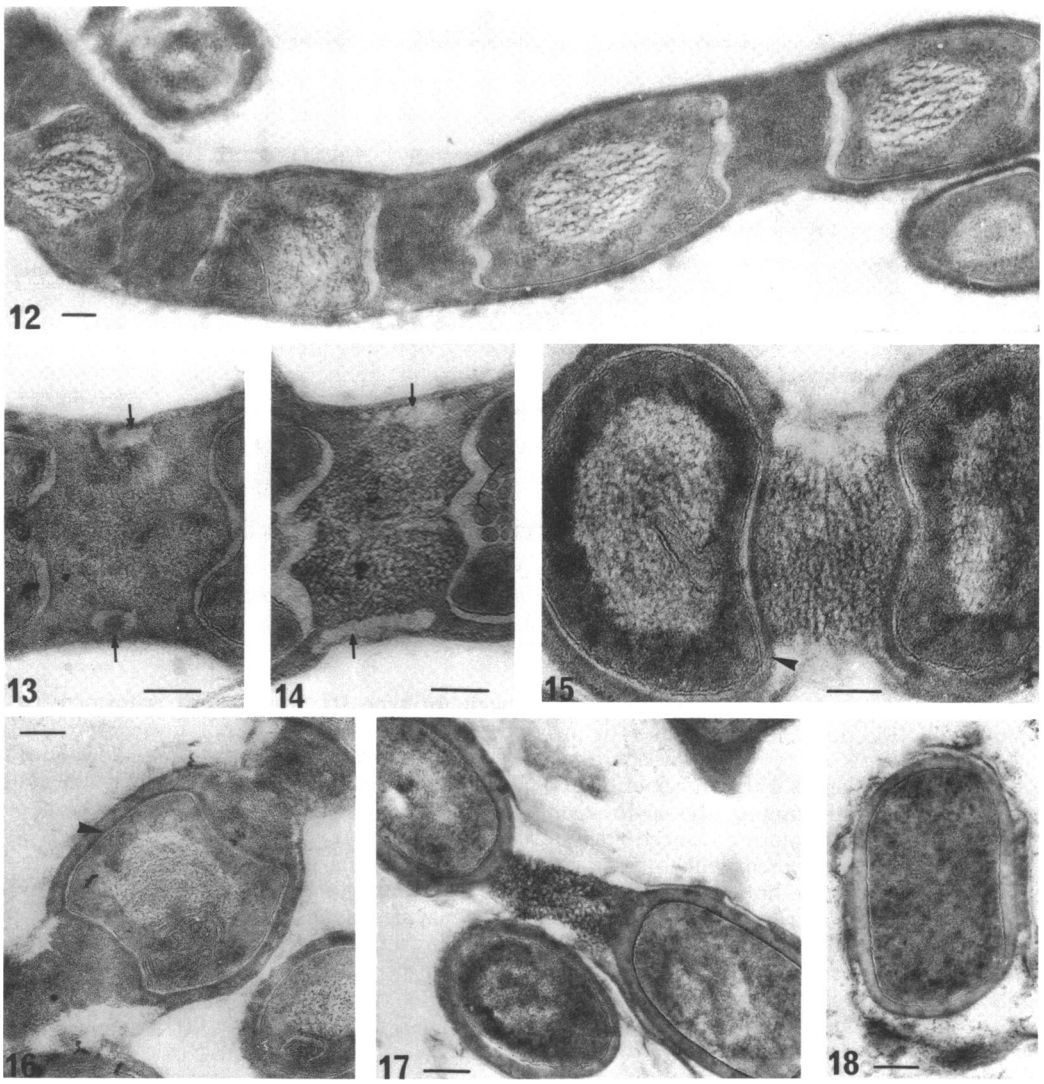

FIG. 12–18. *Sequential stages of arthrospore maturation in type III Streptomyces (S. cinnamonensis) showing the evolution of the sporulation septum. Note (Fig. 12) that the septum is formed by a single, thick cross wall. At an initial stage of arthrospore maturation (Fig. 13 and 14), the lysis begins in the septum zones near the hyphal wall (arrows). At a later stage (Fig. 15 and 16) the lysis of the septum continues and starts the synthesis of the arthrospore coat, which is seen as a thin layer of greater electron density surrounding the future arthrospore (arrowheads). In a more advance stage (Fig. 17) the arthrospores remain attached by remnants of the septum material. Finally, in mature arthrospores (Fig. 18) the material of the septum has been lysed.*

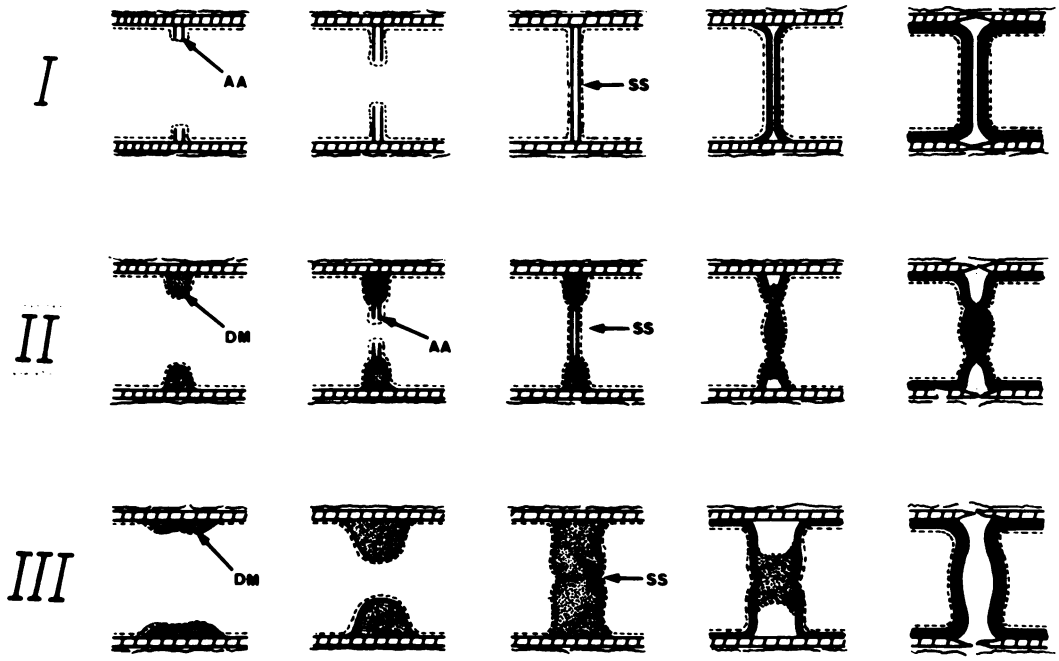

FIG. 19. *Diagrams showing sequential stages of sporogenesis in the three basic types of septum formation in Streptomyces. Abbreviations: AA, Double edge of ingrowing annulus; DM, deposit of septum material; SS, Sporulation septum. Type I: S. erythraeus, S. albus, and S. aureofaciens. Type II: S. flaveolus, S. ambofaciens, and S. antibioticus. Type III: S. cinnamonensis.*

(Fig. 12), it starts to lyse in the zones near the hyphal walls (Fig. 13 and 14). As the lysis proceeds, the synthesis of a typical arthrospore coat occurs, which is seen as a thin layer of greater electron density surrounding the arthrospore (Fig. 15 and 16). In a more advanced stage of maturation the arthrospores, which now have a well-developed wall, are linked by remnants of the sporulation septum (Fig. 17), and finally the septum is completely lysed in mature arthrospores (Fig. 18). Dorokhova et al. (3) describe a similar process of lysis of the sporulation septum during the sporogenesis of *Micropolyspora rectivirgula*. It seems, therefore, that in this type there is no incorporation of the septum into the arthrospore wall, which is completely synthesized de novo.

Figure 19 shows the sequential stages of the sporogenesis in the three basic types. In type I, the septum is formed by two separate cross walls incorporated into the lateral walls of the arthrospore. In type II, the septum is formed by two deposits near the hyphal wall, which are completed by two annuli. Only the annuli are incorporated into the lateral walls of the arthrospore while the deposits are lysed. In type I there is total integration and in type II there is partial integration of the septum into the arthrospore wall. In type III, the septum is formed by a single deposit, which is not incorporated into the lateral wall of the arthrospore. Only in this type is the arthrospore wall synthesized de novo.

This study comfirms, as previously proposed by other authors (2, 5), that the role of controlled autolysis during arthrospore maturation must be very important. Probably the regulation of the enzymes involved in wall synthesis and lysis will play a fundamental role in the entire maturation process.

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