Occurrence of Polysaccharide Granules in Sporulating Hyphae of Streptomyces viridochromogenes

ALFREDO F. BRANA, MANUEL-BENJAMIN MANZANAL, AND CARLOS HARDISSON*
Departmento de Microbiologia, Universidad de Oviedo, Oviedo, Spain

Evidence of the presence of polysaccharide polymers is shown in Streptomyces sp. for the first time. Cytochemical methods revealed the occurrence of polysaccharide granules in sporulating hyphae of Streptomyces viridochromogenes. Onset of the sporogenesis coincided with the appearance of the granules, which reached a maximum number during the early stages of maturation. The later stages of maturation showed a decrease of these granules, and in mature spores no granules were observed.

A wide variety of bacterial species are able to accumulate lipid or polysaccharide polymers intracellularly as reserve material of carbon or energy or both (3). In several sporulating microorganisms, the storage and use of these reserve materials are closely related to sporulation (1, 6, 8, 17). The life cycle of Streptomyces was previously described by Wildermuth (21) in Streptomyces coelicolor, and the details of sporogenesis in Streptomyces viridochromogenes were studied by Rancourt and Lechevalier (13). In these works, as in other related papers carried out with different species of Streptomyces (2, 7, 10, 11, 18, 22), no reference was made to the occurrence of polysaccharide granules during the sporogenesis.

Different cytochemical methods have been used to localize polysaccharides in thin sections of bacteria (1, 4, 5, 9, 14, 15, 20). In this paper we show, by means of cytochemical techniques, the occurrence of polysaccharide polymers during the sporogenesis of S. viridochromogenes.

MATERIALS AND METHODS

Microorganisms, media, and culture conditions. S. viridochromogenes ATCC 14290 was used in this work. The media and culture conditions used were described in a previous paper (7).

Electron microscopy. Isolated colonies of 3 days of incubation were cut out of the medium, prefixed in 2% glutaraldehyde in 0.05 M cacodylate buffer at pH 6 for 2 h at room temperature (18 to 22°C), and postfixed in osmium tetroxide by the Ryter-Kellenberger method (16). Samples were dehydrated with acetone and embedded in Epon 812. Thin sections were cut on an LKB Ultratome III, mounted on Formvar-coated grids, and stained with uranyl acetate and lead citrate. Photographs of sections were taken with a Philips EM-300 electron microscope at 60 kV.

Staining of polysaccharides. For this study we selected the silver proteinate (SP) method of Thiery (19) because this method is specific for polysaccharides, and the chemical specificity of the reaction may be tested by several control reactions. Unmounted sections of pale gold interference color were transferred sequentially by means of plastic rings to the following reagents: 1% periodic acid (PA); 30 min; twice-distilled water, 30 min; 0.2% thiocarbohydrazide (TCH) (Fluka) in 20% acetic acid, 24 h; 10% acetic acid, 60 min; 5% acetic acid, 2 min; 2% acetic acid, 2 min; twice-distilled water, 60 min; 1% SP (Merck Sharp & Dohme), 30 min in the dark; and twice-distilled water, 30 min. Finally the sections were collected on Formvar-coated grids.

Because the fixatives used in electron microscopy frequently introduce unspecific staining, several investigators have reported the importance of control treatments to confirm the specificity of the reaction (14, 19). For this reason, the following control treatments were carried out by omitting various steps of the standard (PA-TCH-SP) method: PA, to verify the removal of the tissue-bound osmium from the sections; PA-SP, to demonstrate the absence of groups able to reduce SP after PA oxidation; and HP (3% hydrogen peroxide, 30 min)-TCH-SP, to demonstrate the absence of aldehyde groups before PA oxidation (HP removes the osmium from the sections and does not oxidize glycols to aldehydes).

RESULTS AND DISCUSSION

Thin sections of 3-day-old colonies of S. viridochromogenes exhibited all the stages of spore formation. After PA-TCH-SP treatment, thin sections of sporulating hyphae showed the presence of numerous round polysaccharide granules throughout the cytoplasm (Fig. 1 to 5). These highly stained granules were often arranged in clusters of 40 to 80 nm in diameter, which probably correspond to the electron translucent areas observed in thin sections of sporulating hyphae fixed by the Ryter-Kellenberger method, and stained with uranyl acetate and lead citrate (Fig. 6). The results of control reactions (Fig. 7 to 9) showed that the polysaccharide granules de-
Fig. 1-9. Sequential stages of spore formation in S. viridochromogenes showing the evolution of the polysaccharide granules during this process. The number of silver deposits increases progressively during the first stages of sporogenesis (Fig. 1, 2 and 3). During spore maturation these granules progressively disappear (Fig. 4), and in mature spores only slightly stained areas are observed (Fig. 5). Untreated section of a sporulating hypha at an intermediate stage of sporogenesis. Electron translucent areas (arrows) may be observed (Fig. 6). Thin sections of sporulating hyphae of S. viridochromogenes after different control treatments. As can be seen (Fig. 7), PA oxidation removes all the osmium bound to cell structures. After PA-SP (Fig. 8) and HP-TCH-SP (Fig. 9) treatments, no silver grains are observed. Bars indicate 200 nm.
tected in *S. viridochromogenes* are not due to unspecific staining, because no silver granules were present after PA-SP and HP-TCH-SP treatments.

The sequential development of polysaccharide granules during the sporulation process in *S. viridochromogenes* is shown in Fig. 1 to 5. Initially (Fig. 1), a small number of polysaccharide granules were detected in the cytoplasm of the aerial hyphae, and their presence was coincident with the initiation of the sporulation septum formation. Once the sporulation septum was completed (at this stage the spore wall is about 25 nm thick), the number of polysaccharide granules increased (Fig. 2), and the cytoplasm became filled with these granules. At the point of highest polysaccharide accumulation (Fig. 3), the thickness of the spore wall was about 35 nm, which corresponds to an intermediate stage of spore maturation. During later stages of spore maturation, the thickness of the spore wall increased, and the number of these granules progressively decreased (Fig. 4). In mature spores (thickness of wall about 50 nm), only small, slightly stained areas were visible, whereas the highly stained granules were not observed (Fig. 5). The slightly stained areas may correspond to the membranous vesicles that we had frequently observed near the cytoplasmic membrane in later stages of spore maturation (Fig. 10 and 11).

The polysaccharide was isolated and identified as a glycogen-like polymer. Its main features were: (i) complete digestion by amyloglucosidase; (ii) α- and β-amylolysis limit of 85% and 50%, respectively; (iii) maximum absorbance of the polysaccharide-iodine complex at 420 nm. The glycogen-like polymer was not detectable in mature spores (A. F. Braña, M.-B. Manzanal, and C. Hardisson, manuscript in preparation).

The appearance and evolution of these polysaccharide granules during the sporogenesis in *S. viridochromogenes* followed a pattern remarkably similar to that described in other sporulating microorganisms (1, 6, 8, 17). It is possible that this polymer plays the role of supplying a readily available source of carbon or energy or both for the synthesis of the spore components required for spore maturation in *S. viridochromogenes*. On the other hand, the initiation of synthesis of sporulation septum may be correlated with the appearance of polysaccharide granules, because the depletion of free glucose may facilitate the derepression of sporulating genes, as has been suggested for endospore-forming bacteria (1, 12).

**ACKNOWLEDGMENTS**

We thank Antoinette Ryter and Keith F. Chater for helpful discussions and critical reading of the manuscript. A.F.B. was the recipient of a predoctoral fellowship from the Ministry of Education of Spain.

**LITERATURE CITED**