COMPARISON OF THE CARBOHYDRATE COMPOSITION OF THE CELL WALLS OF STRAINS OF *STREPTOMYCES GRISEUS*

G. SZABÓ, G. BARABÁS, AND T. VÁLYI-NAGY

Pharmacological Institute of the Medical University, and Experimental Medical Research Institute, Antibiotic Department of the Hungarian Academy of Sciences, Debrecen, Hungary

Received for publication July 9, 1962

In our previous experiments (Arch. Microbiol. **40**:261, 1951), no correlation was found between any of the morphological or physiological characteristics of various *Streptomyces griseus* strains and their streptomycin-producing capacity. There was but one common feature of those strains which did not produce streptomycin: they all had a short life cycle, which was also characterized by the early appearance of short fragments of hyphae after a brief vegetative mycelial growth. This behavior was reminiscent of the development of the *Nocardia*. Avery and Blank (Can. J. Microbiol. **1**:140, 1954), Cummins and Harris (J. Gen. Microbiol. **15**:9, 1956), and Romano and Sohler (J. Bacteriol. **72**:865, 1956), studying the composition of isolated cell walls of *Streptomyces* and *Nocardia* strains, found differences between the genera and declared that the chemical composition of the cell wall was of taxonomic importance. *Streptomyces* strains contained hexose, whereas the cell walls of *Nocardia* had pentose in addition. However, Sohler, Romano, and Nickerson (J. Bacteriol. **75**:283, 1958) later found pentose in the cell wall of some *Streptomyces* strains. Those strains which were lysed by lysozyme contained only hexoses, whereas those *Streptomyces* which were not lysed contained both pentose and hexose.

We have studied the cell-wall composition of an *S. griseus* strain used industrially for the production of streptomycin (strain Ls-1; no. 52-1, according to the code number of our laboratory) and a stable mutant strain (no. 45) which did not produce streptomycin. This was done in the hope that the difference in the time of fragmentation of the hyphae between the two strains would be reflected in the carbohydrate composition of their cell walls.

The characteristics of the strains were described earlier (Arch. Microbiol. **40**:261, 1961). The strains were cultivated in shake culture in soybean medium for various periods of time. After harvesting, the cells were washed twice with distilled water and disintegrated with an ultrasonic disintegrator (Measuring & Scientific Equipment Ltd., London.). After centrifuging, the cell walls were washed with water, with 0.067 M phosphate buffer (pH 7.6), and then again with water several times. Cell-wall preparations were hydrolyzed with 1 N H_2SO_4 for 4 hr in boiling water. The method of cell-wall isolation is a slight modification of that used by Salton and Horne (Biochim. et Biophys. Acta **7**:177, 1951). Detailed results will be published elsewhere.

Hexose and pentose were present in the cell walls in both of our strains. Strain 52-1 contained 17% reducing substance, based on the hexose content determined by the anthrone reaction, and 2.6% pentose, as determined by the orcinol test (Meybaum, modified by Dische, *In* S. P. Colowick and N. O. Kaplan [ed.], *Methods in Enzymology*, vol. 3, p. 88, Academic Press, Inc., New York, 1955). The cell wall of strain 45 had 12.5% hexose and 3.5% pentose.

It is clear that the cell walls of both of our *S. griseus* strains contain pentose. This is in contrast to the finding of Romano and Sohler and in some respects to the data of Sohler, Romano, and Nickerson. Namely, both of our strains were susceptible to lysozyme action (Lysosim, Fluka AG) and, in spite of this, their cell walls contained pentose. We are not able to explain the pentose content by ribonucleic acid impurities because: (i) the pentose content did not decrease after ribonuclease treatment, and (ii) the pentose was proven by paper chromatography to be arabinose. Repetition of Romano and Sohler's experiments by cultivating our *S. griseus* strains in their media did not change our results concerning the pentose content. We think that the difference between their and our results is to be explained by strain specificity.

Microscopically, we found more and less lysozyme-susceptible parts along the hyphae.

This probably explains why lysozyme did not lyse completely our cell-wall preparations, some parts remaining undissolved even after a longer period of lysozyme digestion.

ILLUMINATION OF MICROMANIPULATOR TOOLS IN ULTRAVIOLET-FLUORESCENCE MICROSCOPY[1]

L. E. CASIDA, JR.

The Pennsylvania State University, University Park, Pennsylvania

Received for publication July 16, 1962

In recent years, the ultraviolet-fluorescence microscope has been used to identify microorganisms in cultures, tissues, and excretions (Beutner, Bacteriol. Rev. **25**:49, 1961; Mellors, *Analytical Cytology*, McGraw Hill Book Co., Inc., New York, 1959), in soil (Schmidt and Bankole, Science **136**:776, 1962), and in other materials. The living microorganisms of soil have been counted by staining the soil and its microflora with acridine orange before viewing with the ultraviolet-fluorescence microscope (Strugger, Can. J. Res. **26B**:188, 1948). The soil particles, including clay and humus, and dead microorganisms fluoresce an orange-to-red, whereas the living microorganisms fluoresce a bluish- to yellowish-green.

Casida (Can. J. Microbiol. **8**:115, 1962) presented a technique by which individual living soil microorganisms stained with acridine orange could be viewed with the ultraviolet-fluorescence microscope, and then isolated by micromanipulation directly from the soil to a growth medium. The organisms could then be cultured for morphological and physiological investigations. A problem was encountered in this technique in that the micromanipulator tool, which did not fluoresce, was difficult to locate in ultraviolet light. This problem was solved by utilizing a nichrome wire tool that was opaque to ultraviolet and visual light. Thus, by lowering the condenser, the shadow of the tool could be seen for its initial location in the microscopic field, and the tool itself could be observed as it was brought close to the organism to be isolated. In the latter instance, the tool was seen because it blocked a portion of the weak light emitted by the fluorescing microorganisms and soil particles.

It now has been found that visual light can be transmitted through a glass manipulation tool so that the point is easily observed in a field of ultraviolet light. This is accomplished by butting one end of a fiber optic (NA 0.56) with the back end of the tool, so that the light traveling through the fiber optic will continue through the tool and will be emitted at a point where the tool has been fire-polished and drawn to a point. Tools made of solid glass carry more light to their points than do those of capillary glass, although the latter can be seen in an ultraviolet field.

The fiber optic for this study was provided by the American Optical Co., and was of a diameter (approximately 0.7 mm) which would just fit into the back end of the manipulator-tool holder. Light was introduced into the fiber optic by mounting an exposed end in a cork held in the aperture of the iris diaphragm of a microscope illuminator, so that the optic was a few mm forward of the front lens of the illuminator.

Although this technique was developed for micromanipulation of organisms stained with acridine orange, it also should find use in studies where microorganisms, etc., have been stained with fluorescent antibody.

This study was supported by a grant from the Medical Sciences Research Foundation at Stanford University.

[1] Authorized for publication on 12 July 1962 as paper no. 2685 in the journal series of the Pennsylvania Agricultural Experiment Station.