Theresa McHugh
15 August 2016
Microbial Diversity

Comparative Microscopy of an Actinomycete Isolate

Actinobacteria exhibit the greatest degree of morphological differentiation among Gram-positive bacteria, and morphological characteristics remain a basic index for the characterization and classification of these taxa (Li *et al*. 2016). Microscopes continue to be invaluable tools for exploring actinobacterial diversity. This project aimed to use a variety of microscopic approaches to investigate the morphological differentiation of an Actinomycete isolate believed to be a member of the genus *Streptomyces*.

Stereomicroscopy

Examinations under a stereoscope revealed the secretion of droplets in a seven-day-old culture (Figure 1). Morphological differentiation in actinomycetes is known to coincide with the production of secondary metabolites, including antibiotics and enzyme inhibitors (Fiedler *et al*. 2005). Morphogenesis and antibiotic production in *Streptomyces* are initiated when nutrient limitation is encountered, with molecular signaling in substrate hyphae resulting in aerial hyphal growth and secondary metabolite secretion in response to starvation (Li *et al*. 2016).

Light microscopy

The transplantation embedding method (Figure 2) was used according to Xu *et al*. 2007. Briefly, a rectangular hole was dug out of an agar plate, the edges of the hole were inoculated, and sterile cover slips were placed over this area. Plates were incubated at 30 ℃, and cover slips were removed at multiple time points for microscopic observations. Spore chains were visible with both the inverted light microscope (Figure 3) and traditional light microscope

(Figure 4). It is believed that an earlier part of the differentiation process prior to spore formation was also observed, with the specimen showing morphological changes resembling apical growth in *Stroptomyces* (Li *et al*. 2016; Figure 5). The transplantation embedding method proved superior to the approach of colony scraping to make a wet mount, as the latter resulted in the fine image structure being obscured by the out-of-focus light.

Environmental scanning electron microscopy

A single colony was excised from an agar plate, placed on a stub covered with carbon tape, and viewed in the environmental scanning electron microscope (ESEM). An ESEM is a scanning electron microscope (SEM) that allows the user to visualize samples that are "wet," and/or uncoated. As with conventional SEM, an ESEM utilizes a scanned electron beam and electromagnetic lenses to focus and direct the beam on the surface of the specimen. The major benefit of EMEM is that samples can be examined faster and more easily, without modifying the specimen. This method allowed for an in-depth examination of actinomycete mycelia (Figure 6).

Scanning electron microscopy

For SEM, a specimen is required to be completely dry. Fixation was performed to prevent shape deformation. This was achieved by incubating entire colonies and cover slips in a solution of 3% paraformaldehyde for 2 hours. The fixed tissue was then washed with distilled water and dehydrated. Because air-drying causes collapse and shrinkage, dehydration was achieved by replacement of water in the cells with ethanol: a stepwise progression of 30, 50, 70, 90, and 100% ethanol, with 20-minute submersions in each. The solvent was then replaced with liquid carbon dioxide by critical point drying, and ultimately the carbon dioxide was

removed while in a supercritical state. The dry specimen was sputter-coated with platinum (10 nm), and mounted on a stub with carbon tape before examination in the microscope. This method revealed an abundance of spores (Figure 7). In some cases, the spores remained in chains, held together by the proteinaceous sheath of the hyphal wall (Durie and Frey 1955; Figure 8). At very high magnification, it was possible to observe degradation of the sheath (Figure 9), as well as the texture of the spore wall (Figure 10).

It was noted that fixation and sputter coating vastly improved the quality of the images obtained with the ESEM (Figure 11). Whenever possible, these extra preparation methods are recommended. The added time investment is approximately five hours.

Acknowledgements

I am thankful for the support of the Microbial Diversity instructors and teaching assistants, particularly Kurt Hanselmann, whose suggestions and encouragement were invaluable to me. Kyle and Jim from the SEM facility in Lillie also provided tremendous assistance.

References

Durie EB and Frey D. (1955) Spore formation in the Genus Streptomyces. Nature 176: 935–936.

Fiedler HP, Bruntner C, Bull AT, Ward AC, Goodfellow M, Potterat O, Puder C, and Mihm G. (2005) Marine actinomycetes as a source of novel secondary metabolites. Antonie van Leeuwenhoek 87: 37–42.

Li Q, Chen X, Jiang Y, Jiang C. (2016) Morphological Identification of Actinobacteria. In: Actinobacteria – Basics and Biotechnological Applications. http://dx.doi.org/10.5772/61461.

Xu LH, Li WJ, Liu ZH, Jiang CL. (2007) Actinomycete Systematics – Principles, Methods, and Practice. Science Press: Beijing.

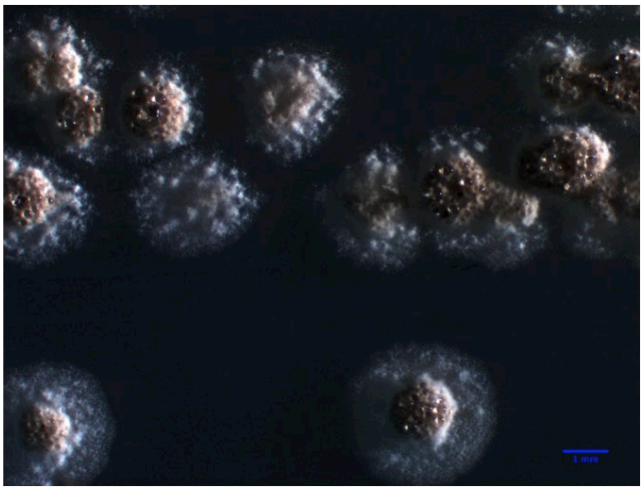

Figure 1. Droplet secretion, 7x magnification.

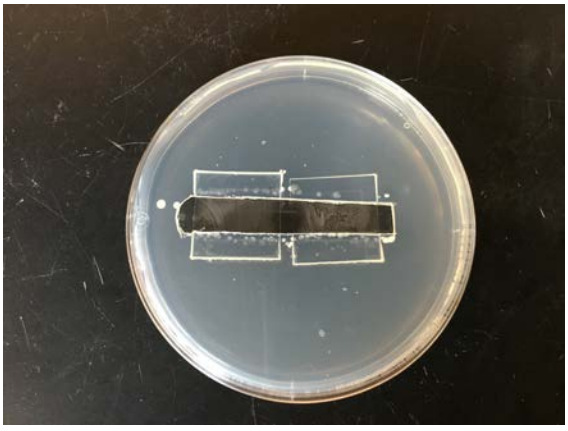

Figure 2. Transplantation embedding method.

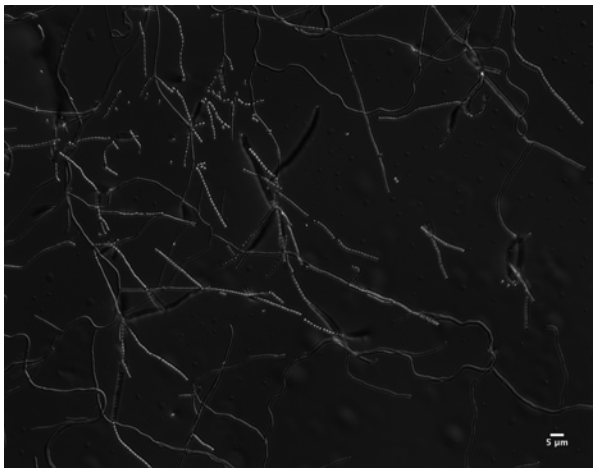

Figure 3. Spore chains observed with the inverted light microscope, 40x objective, DIC.

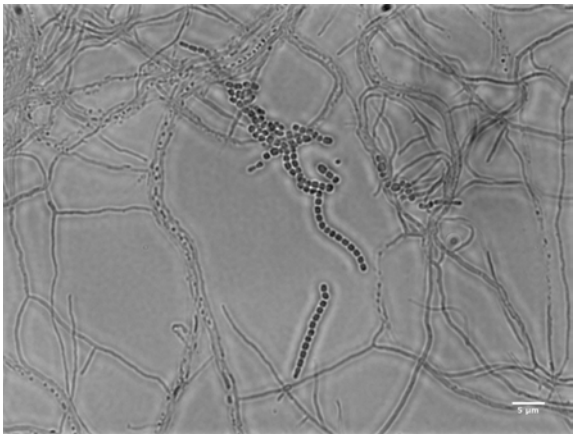

Figure 4. Spore chains observed with the light microscope, 100x objective, phase contrast.

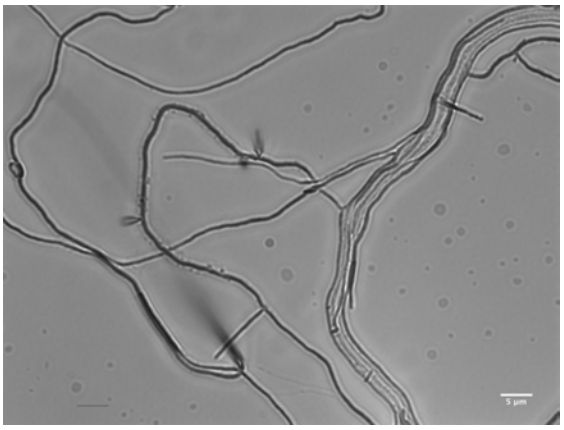

Figure 5. Apical growth observed with the inverted light microscope, 100x objective, phase contrast.

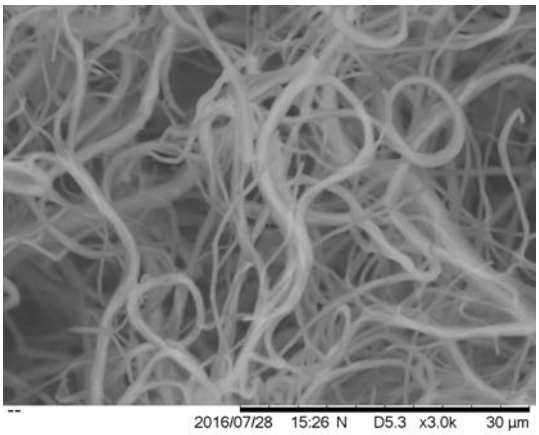

Figure 6. Mycelium observed with ESEM.

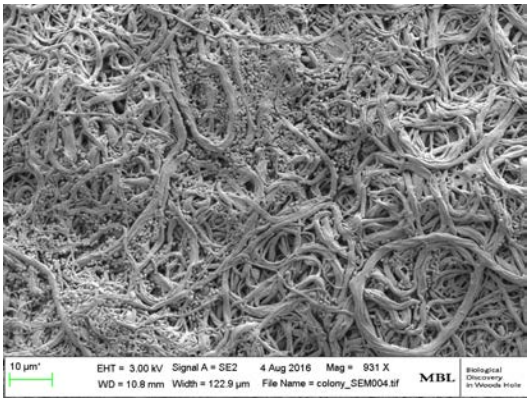

Figure 7. Spores observed with SEM.

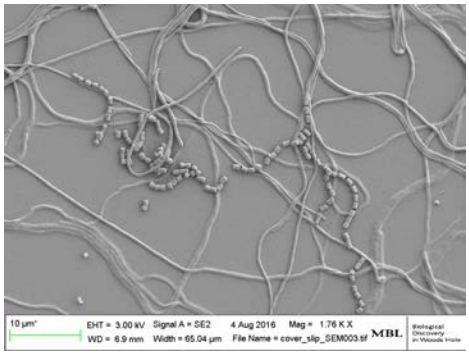

Figure 8. Chains of spores observed with SEM.

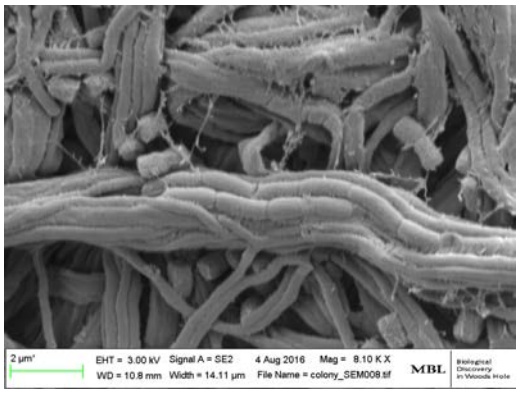

Figure 9. Hyphal sheath degradation. observed with SEM.

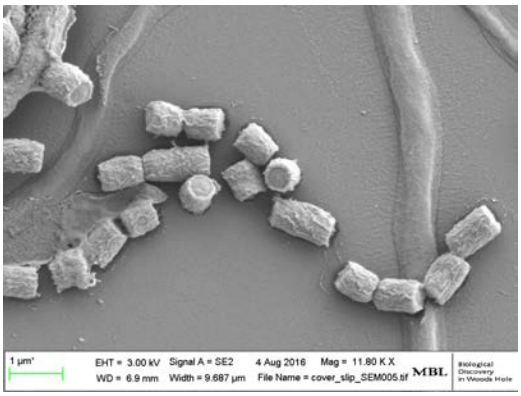

Figure 10. Texture of spore walls observed with SEM.

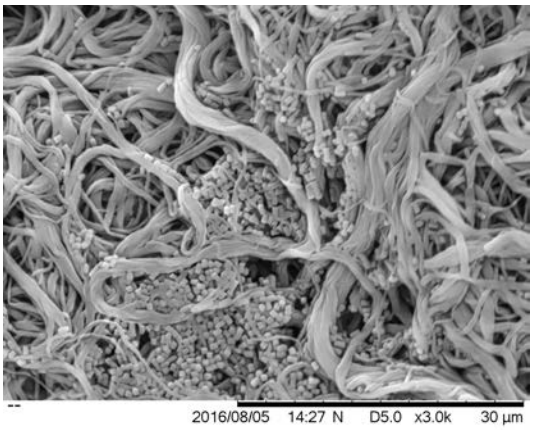

Figure 11. Improved ESEM image quality after fixation and coating of sample.