thermo scientific

CASE STUDY

Institute for Molecular Science, NINS Japan

Phenom ProX Desktop SEM for microbiology research

Determining cell sizes of rod-shaped bacteria in biofilms

Many bacteria grow on surfaces and form aggregates called biofilms. Since biofilms are found everywhere and show strong resistance to antibiotics, the mechanism of biofilm formation and regulation has been an attractive area of research. Dr. Shiro Yoshioka is using a soil bacterium, *Pseudomonas fluorescens*, as a model organism to understand how biofilm formation is regulated.

Transposon mutagenesis is often used to identify the genes that are required for biofilm formation. If the transposon is inserted into a gene involved in this process, the mutant will show increased or reduced biofilm formation depending on its role. Using this technique, Yoshioka's team identified a gene responsible for the synthesis of a bacterial second messenger, c-di-GMP, whose loss caused reduction in biofilm formation compared to the wild type strain. During this research, Yoshioka's team also found that disruption of one of the genes in *de novo* purine nucleotide biosynthesis resulted in reduced biofilm formation compared to the wild type. Since the importance of the genes in this pathway has been repeatedly reported for various bacteria, these mutants were investigated to clarify the relationship between this biosynthesis pathway and biofilm formation in *P. fluorescens.*

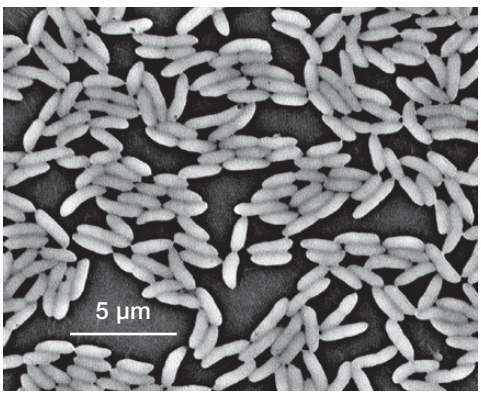

Figure 1. An example SEM image of the biofilm formed by *P. fluorescens* cells on a polyvinyl chloride surface (magnification: 12,000x).

The Phenom ProX Desktop SEM for biofilm research

While biofilm formations by the mutants were reduced to less than half of the wild type, the cell counting experiments suggested that the number of mutant cells in the biofilms was comparable to that of the wild type. To investigate this result, Yoshioka's team hypothesized that the cell size of the mutants in the biofilms became smaller than the size of the wild type cells. In other words, the mutations caused the reduction in cell size for the biofilm cells. This assumption is not so strange because the mutants cannot synthesize both AMP and GMP, which may lead to slower growth.

The scanning electron microscopy (SEM) is one of the best methods to examine the cell size of bacteria in biofilms. The Yoshioka team employed a Thermo Scientific™ Phenom ProX Desktop SEM, which allowed them to obtain a SEM image within a minute of sample setting. "This fast speed allowed us to prepare several micrographs for one strain, enabling further detailed analyses using the Thermo Scientific ParticleMetric Software," Yoshioka noted (Figure 1).

ThermoFisher SCIENTIFIC

thermo scientific

The power of ParticleMetric Software

A comparison of the SEM images of the wild type with those of the mutants clearly indicated that a size reduction occurred in the mutants. However, it was difficult to emphasize the difference in cell sizes between wild type and mutants without numerical comparisons.

The ParticleMetric Software for the Phenom ProX Desktop SEM is able to extract various parameters for each particle observed in the SEM images. Because *P. fluorescens* is rod-shaped, the circumscribed circle diameter could be a good approximation for the length of the cells. Using ParticleMetric Software, Yoshioka's team collected the circumscribed circle diameters of more than 1,500 individual cells between several SEM images of the same strain. This resulted in a histogram and median diameter (see Figure 2).

The ParticleMetric Software performed this procedure automatically. The only point of attention for the team was the automatic particle detection sometimes treating the aggregated cells as a single cell. ParticleMetric Software allows users to intuitively remove such cells for reliable calculations.

The results of the calculations revealed that the lengths of the mutant cells were reduced by 25~30% compared to that of the wild type. Thus, the evidence obtained by the Phenom ProX Desktop SEM and ParticleMetric Software successfully proved Yoshioka's hypothesis that the biofilm's mutant cells became smaller than the wild type cells.

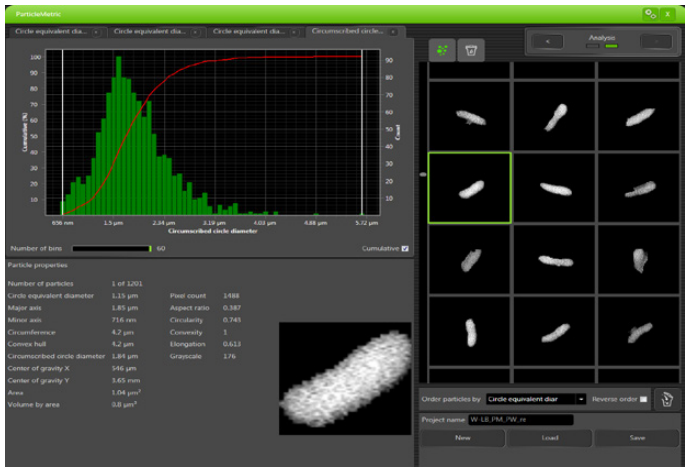

Figure 2. A histogram generated by the ParticleMetric Software for the biofilm cells of *P. fluorescens* after collecting the circumscribed circle diameters for more than 1,500 individual cells. The median of the circumscribed circle diameter was calculated to be 1.79µm. Besides the histogram, the properties of one selected cell are being displayed.

Conclusion

Application of the Phenom ProX Desktop SEM and ParticleMetric Software for biofilm research provided valuable information on bacterial cell size in biofilms. Yoshioka concludes, "Since many bacteria change their size and morphology with nutrient availability, or in the presence of antibiotics, knowledge of cell size and morphology will be necessary for future studies in biofilm research. The speed of the Phenom ProX Desktop SEM and user-friendly ParticleMetric Software will provide great advantages for researchers investigating microorganisms."

Institute for Molecular Science

Shiro Yoshioka is PhD Assistant Professor at the Institute for Molecular Science in Japan. It is their mission to enhance the progress of molecular science by covering broader research areas, mutual exchange of human resources among all the universities in Japan and international cooperation with worldwide scientific societies.

Find out more at **thermofisher.com/phenom**

ThermoFisher SCIENTIFIC