Elemental maps of low phosphorus content in *Corynebacterium glutamicum* ΔmcbR

M. Epping[1], R. Reichelt[2] and H. Kohl[1]

1. Physikalisches Institut und Interdisziplinäre Centrum für Elektronenmikroskopie und Mikroanalyse (ICEM), Westfälische Wilhelms-Universität Münster, Wilhelm-Klemm-Straße 10, 48149 Münster, Germany
2. Institut für Medizinische Physik und Biophysik, Universitätsklinikum, Westfälische Wilhelms-Universität Münster, Robert-Koch-Str. 31, 48149 Münster, Germany

m.epping@uni-muenster.de
Keywords: elemental maps, EFTEM, phosphorus, C. glutamicum, Corynebacterium

The *Corynebacterium glutamicum* is of immense industrial importance. By genetic engineering, the fermentative production of nearly every amino acid is possible. One mutant is the *C. glutamicum* ATCC 13032 ΔmcbR. A regulator (McbR) was removed in order to reach a higher biosynthesis of L-methionine and L-cystein. L-cystein is an amino acid, which up to now can only be produced by chemical synthesis. Instead of L-cystein and L-methionine, a higher amount of Adenosine triphosphate (ATP) has been found. X-ray microanalysis has shown small granules with higher phosporus- and sulfur-signal than the surrounding part of the bacterium [1]. The phosphorus can be found as constituent of ATP and DNA. It should be possible to map both by EFTEM. In its wildtype, *C. glutamicum* contains 1,4% up to 2,2% phosphorus, depending on the growing medium [2].

In our work, the *C. glutamicum* was examined by EFTEM and we calculated phosphorus maps. The specimen - *C. glutamicum* ΔmcbR - was embedded in Spurr and was not stained. Staining with uranyl acetate or lead citrate would disturb the creation of phosphorus maps, because of their overlapping energy loss edges. The recordings in the 200 keV Zeiss Libra 200 were made with 50 nm thick sections. They were placed on 1000 mesh. To calculate the phosphorus signal, a program by Gralla [3] was used. In order to calculate the SNR, it was necessary to record three pre-edge images and one post-edge image. Phosphorus has a delayed edge starting at 132 eV and reaching its maximum at 153 eV. A slitwidth of 18 eV was used. The energy-windows were placed at 71-89 eV, 91-109 eV, 111-129 eV and 146-164 eV. The exposure time for all four energy loss images was 10 s and the recording order was from higher to lower energy loss. We used a low beam current, to reduce mass loss. To calculate the phosphorus map, the program corrected the sample drift and used the power law model to subtract the background signal.

Figure 3 shows the calculated phosphorus signal. The granule which should contain ATP shows the highest signal. The linescan in Figure 5 shows, that the signal is more than twice as high as in the surrounding part of the bacterium. The calculated SNR is shown in Figure 4. The linescan at the marked position is shown in Figure 2. The Rose-Criterion requires a SNR higher then 3 to prove the presence of an element in an elemental map. At all positions of high phosphorus signal, this criterion is met. The granule even shows a increased SNR of 5 to 9.

We found granules of higher phosphorus content and confirmed the result of X-ray microanalysis. Our next aim is to acquire sulfur maps from the same specimen [4].

1. J. O. Krömer et al., Microbiology (2008); 154: 3917-3930.
2. L. Eggeling, Handbook of *corynebacterium glutamicum* (2005)

DOI: 10.3217/978-3-85125-062-6-312,

3. B. Gralla, personal communication, Universität Münster
4. The provision of the specimen by C. Bolten is gratefully acknowledged, Technische Universität Braunschweig
For excellent specimen preparation we thank Mrs. U. Malkus, Universität Münster

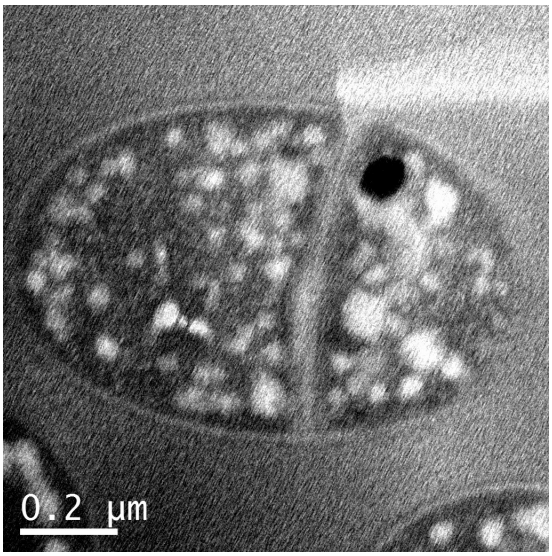

Figure 1. Image of elastic-scattered electrons. Energy-window at 0 eV with an energy-slit of 18 eV. The dark spot is one of the granules containing ATP.

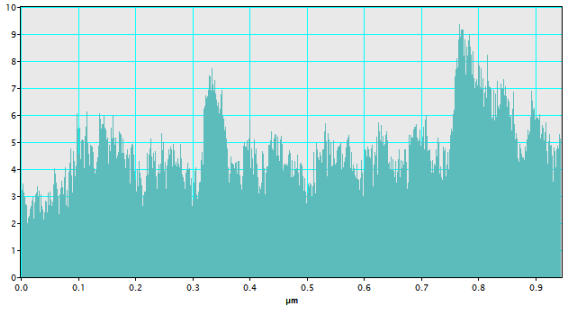

Figure 2. Linescan at the marked position in Figure 4.

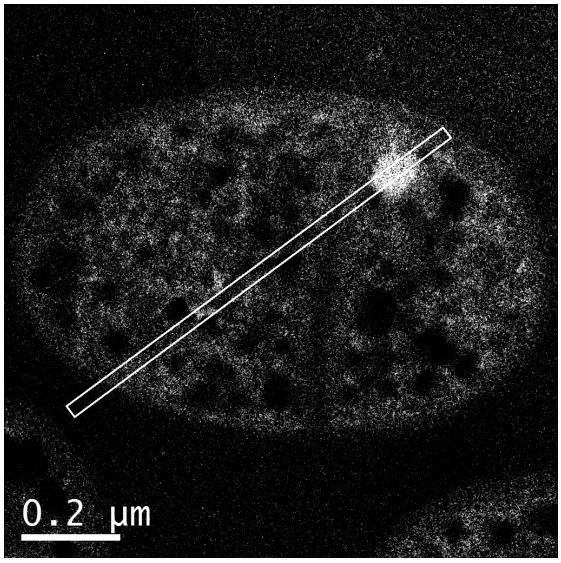

Figure 3. Calculated phosphorus-signal and position of linescan (Figure 5.)

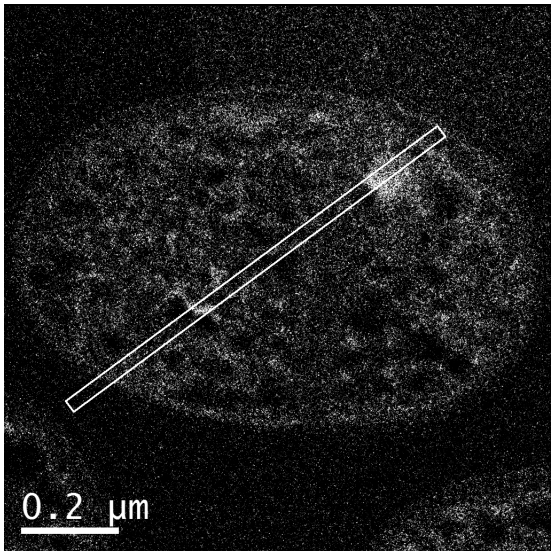

Figure 4. Calculated SNR (contrast limits: 5 -10)

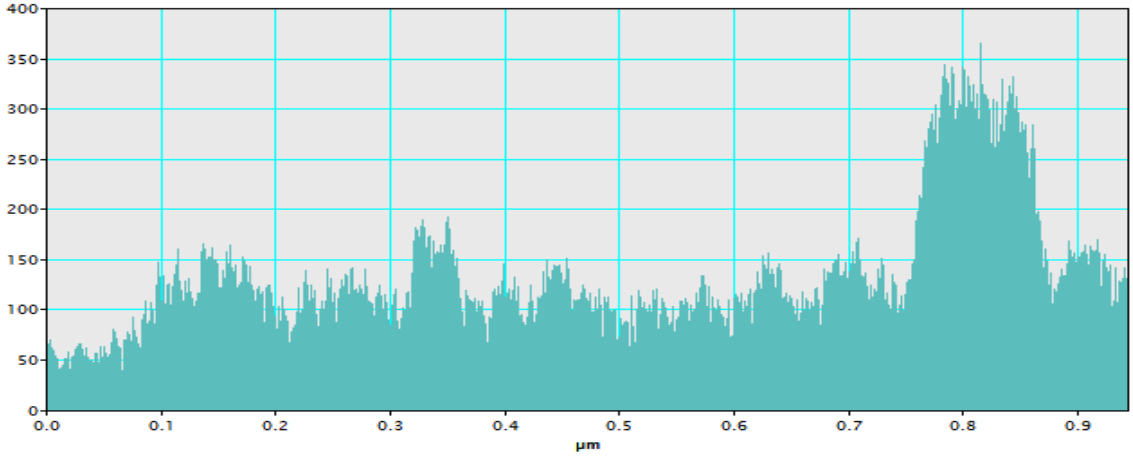

Figure 5. Linescan at the marked position in Figure 3.

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-312, © Verlag der TU Graz 2009