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Scanning Electron Microscopy as a Useful Tool for Monitoring Cell Disruption of Recombinant *Escherichia coli* SHuffle T7

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HPV16 full-length-, HPV16-truncated- and HPV16 His-tagged L1 proteins were produced in *E. coli* SHuffle T7 as insoluble proteins within inclusion bodies (IBs). Recovery of active protein from IBs is a difficult task, which requires separation of IBs from other component of bacteria, as the first step [1].

The aim of this work was to evaluate the cell disruption by high pressure homogenization for IBs isolation by Scanning Electron Microscopy (SEM).

The bacterial cells integrity was evaluated before and after the first, third and fifth lysis cycles. Samples for SEM were fixed with 2% phosphate glutaraldehyde, 16 h, 4°C and post-fixed in 1% osmium tetroxide, 1 h and dehydrated with increasing concentrations of acetone in water (30-100%) by consecutive washing steps. Samples were sputter coated with a 13 nm gold layer by SBC-12 small size ion sputter coater and imaged in a TESCAN Vega 3 SEM. The IBs average diameter was evaluated by measuring 10 IBs on three SEM pictures (10 000 X) using the Image J program. Cell disruption was also evaluated by viability analysis using classical microbiology techniques.

SEM micrographs revealed intact *E. coli* rods before lysis with IBs observed as bulges at the cells poles (Fig. 1 A-C). After the first cycle (Fig. 1 D-F), most of the cells were broken and IBs released, but several *E. coli* bacilli remained intact and some IBs were trapped inside partially broken cells. At the end of the third cycle (Fig. 1 G-I), spherical IBs prevailed, with a size average about 500 nm for the three evaluated strains. These values were consistent with the size of IBs at the stationary-phase cultures [2]. Similar results were obtained after the fifth cycle (Fig. 1 J-L).

These results agreed with those obtained by cell viability analysis, which showed that three cycles were enough to disrupt 99.9% of cells. Thus, SEM was a useful tool to evaluate cell disruption of recombinant *E. coli* SHuffle.

References:

[1] E. Chapman-Davis et al. World J. Obstet. Gynecol. 5 (2016), p. 5.
[2] L. Zhai, et al. Gardasil-9: Antivir. Res. 6 (2016), p.101.

HPV16 full-length L1 protein **HPV16-truncated -L1 protein** **HPV16 His-tagged L1 protein**

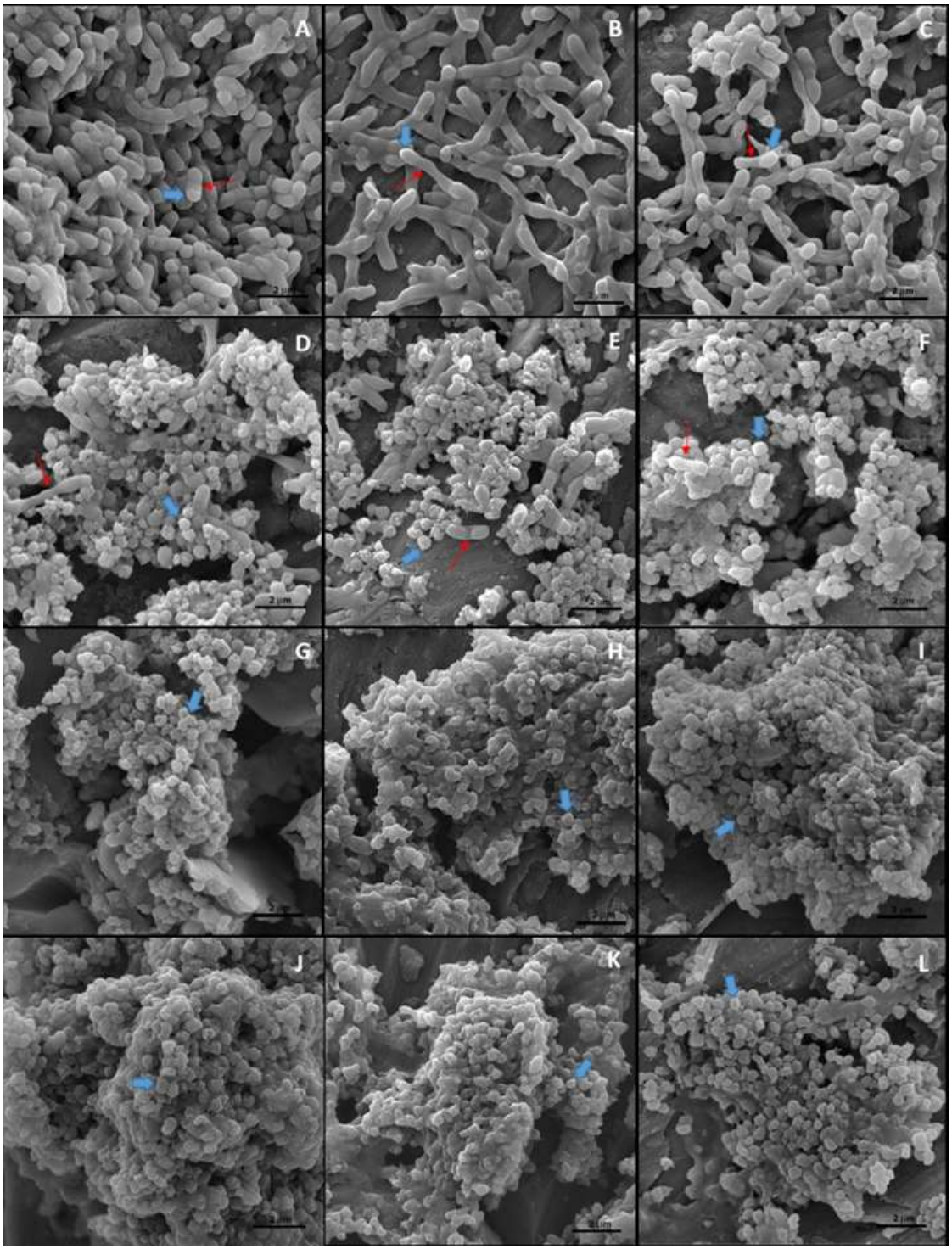

Figure 1. SEM micrographs of representative cell lysates images from *E. coli* SHuffle T7 harboring the pETHPV16L1 (A, D, G, J), pETHPV16ΔNCL1 (B, E, H, K) and pETHPV16L1myc-6xHis (C, F, I, L) plasmids, before (A-C) and after 1 (D-F), 3 (G-I) and 5 (J-L) disruption cycles. Narrow red arrows: *E. coli* Bacillus, thick blue arrows: IBs. Bars 2 µm.

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