Coating of single living cells in micro-organized polyelectrolyte shells using layer-by-layer method

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INTRODUCTION AND OBJECTIVE

Saccharomyces cerevisiae is an eukaryotic model which is largely used for fundamental studies and which has a key rule for oxidative stability of fermented products. During the fermentative phase, yeasts are subjected to a variety of stresses (O_2, pH, ethanol…) toxic for the cells (Gibson 2007). In order to protect cells against these stresses, the modification of the surface architecture of biological cells while retaining the internal working properties of the native system is a great challenge. The objectives of this study were to coat the living cells in organized ultrathin shells using Layer by Layer (LbL) assembly. This method uses alternating charged layers of polyionic polymers (Ai 2003) in order to form a protective barrier around the cells. Exploiting scanning electron microscope, we provided evidence of the shell and cell integrity after the coating procedure.

MATERIALS AND METHODS

Materials

Yeast strain CBS 8066 of *Saccharomyces cerevisiae* used in this study was obtained at Centrum Voor Schimmelcultures (Baarn & Delft, Holland). Sodium alginate (SA) was provided by Fisher Scientific. It was used after purification and then freeze-dryed as previously described (Mession 2012). Commercial whey powder rich in β-lactoglobulin (BLG) content was provided by Danisco (BIPRO: BLG, 90.5 wt % of the total protein content; 14.4 % α-lactalbumin, 4.1 % BSA and 72 % β-lactoglobulin).

Encapsulation procedure

The yeast cells (10^8 cells per mL) were suspended in acetate buffer at pH 3.8. The sample was kept under weak stirring. BLG solution was added to yeast dispersion and held for 20 min at room temperature for adsorption completion. Polycation (BLG 0.1 % w/v) and polyanion (SA 0.001 % w/v) were adsorbed sequentially, washed with acetate buffer between steps, and yeast cells separation from supernatant was achieved by centrifugation.

Zeta potentiometer measurements

The electrophoretic mobilities, μ, of the yeast cells and the polyelectrolytes were measured with a ZetaCompact ® from CAD instruments, in a fused rectangular quartz channel (5 x 2 x 70 mm) by measuring their travelling velocities at the stationary level under a microscope. Instrument description was presented in previous study (Senée 2001). Three measurements for each sample were made to ensure data reliability. In the model solvent for which the ion valency is well known, the ζ-potential of the particles was deduced from the mobility μ by application of Smoluchowski's law.

Transmission Electron Microscopy (TEM)

TEM was used to visualize the encapsulated yeast cells. Concentrated yeast coated samples were fixed for 2 h at 4 °C with 2 % paraformaldehyde and 0.5 % glutaraldehyde in acetate buffer for control cells. After washing, cells were dehydrated progressively in 30 %, 50 %, 70 %, 90 % and 100 % ethanol, impregnated with LRWhite, and polymerized under UV. Ultrathin sections (90 nm) were obtained using an Ultracut E ultramicrotome (Reichert, Depew, NY, USA) and contrasted with uranyl acetate. Observations were performed on a Hitachi 7500 transmission electron microscope (operating at 80 kV) equipped with an AMT camera driven by AMT software (AMT, Danvers, MA, USA).

Field Emission Gun Scanning Electron Microscopy (FE-SEM)

FE-SEM was used also to image the encapsulated yeast cells. Concentrated yeast coated samples were fixed for 2 h at 4 °C with 2.5 % glutaraldehyde in acetate buffer. Cells were dehydrated progressively in 30 %, 50 % and 70 % ethanol. Then a critical-point was performed. Samples were then sputter coated with gold and observed in a FE-SEM JOEL 6500.

RESULTS AND DISCUSSION

Ionic strength, pH, and concentration of the polyion solution affect LbL assembly, film thickness and stability. For the LbL process, the pH of the polyelectrolyte solutions should be selected to maintain a high degree of polyion ionization (Ai 2003). Figure 1 showed that the charge of yeast cells and polyelectrolytes are opposites and strong enough for electrostatic interaction between pH 3.8 and 4.0. The ζ-potential measurements indicated that the surface potential of washed *S.cerevisiae* cells is -22 mV (Figure 2). Therefore, the LbL assembly proceeded with the alternate adsorption of cationic polymer (BLG) and anionic polymer (SA), which resulted in symmetric changes in the cell potential to positive and negative, characteristic of the LbL process (Balkundi 2009).

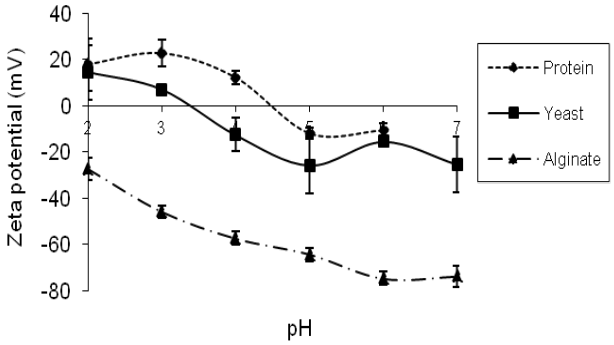

Figure 1 : ζ-potential of *S.cerevisiae* and polyelectrolytes as a function of pH.

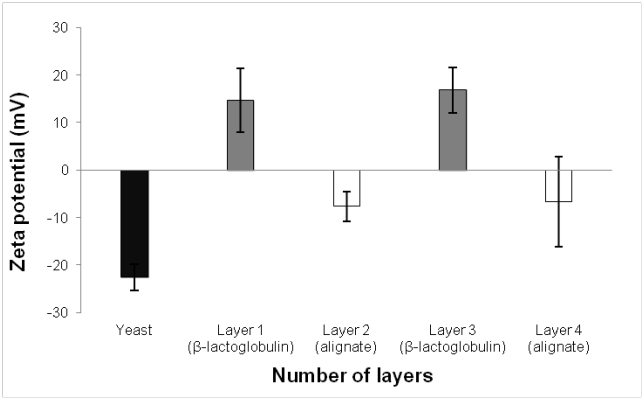

Figure 2 : ζ-potential of *S.cerevisiae* cells in acetate buffer at pH 3.8, showing alternation with successive deposition of cationic and anionic polyelectrolytes (polycation : β-lactoglobulin and polanion : alginate).

The surface morphology of yeast coated by polyelectrolyte layers was investigated by TEM and SEM. Figure 3a showed FE-SEM images of a yeast cell before and after coating by a protein layer in 10 mM acetate buffer at pH 3.8. The yeast cells are coated by cationic polymer (BLG). Indeed, its surface is smooth while the cell is covered by a rough « carpet » of protein after coating. Figure 3b showed TEM images of a yeast cell coated by a protein layer. A black layer of β-lactoglobulin is observed around the yeast cells. The thickness of this layer is about 20-40 nm.

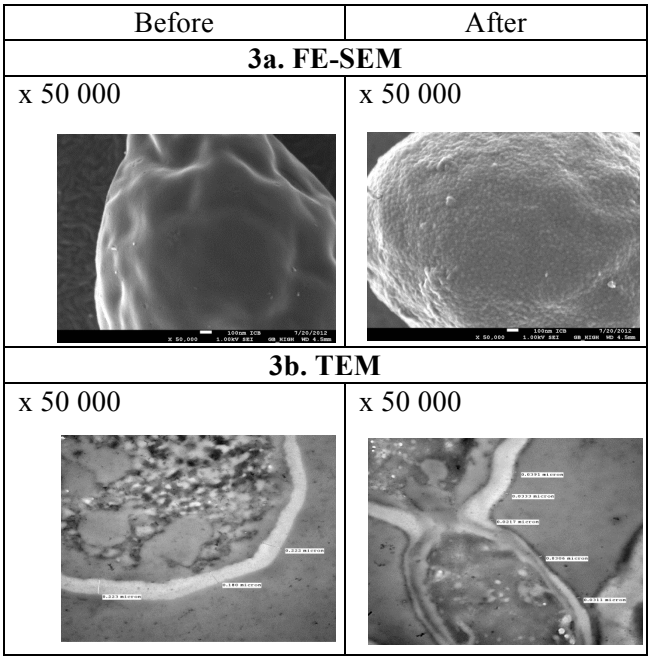

Figure 3 : Surface of yeast cells before and after coating in Scanning Electron Microscopy

CONCLUSIONS

Saccharomyces cerevisiae was coated at pH 3.8-4.0 in defined conditions : 10^8 ¢/mL using Layer-by-Layer method in acetate buffer with ionic strength of 10 mM with twopolyelectrolytes ([β-lactoglobulin] = 0.1 % (w/v) and [Sodium alginate] = 0.001 % (w/v)). The first layer of β-lactoglobulin has been observed around the yeast cell by microscopy method (SEM and TEM). Zeta potential of *S. cerevisiae* in acetate buffer indicated alternative deposition of cationic and anionic polyelectrolyte layers around the yeast cell. Further work will investigate the protective role of this multilayer structure.

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