Single-cell fingerprint dynamics of antibody fragment secreting Bacillus megaterium cells

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Abstract

Recombinant antibodies and antibody fragments (ABF), being indispensable tools for research, diagnostics and therapy, are traditionally produced either in mammalian cells or in recombinant microbial strains like Escherichia coli. A promising alternative is the use of the Gram positive Bacillus megaterium, which is an expression host with high secretion capacities. To gain a better understanding and deeper insights into the production process, studies at single cell level are most desirable. The evaluation of single cell performances leads, with the use of appropriate methods like flow cytometry, to the discrimination and physiological characterization of particular bacterial populations. In this work the production and secretion of the model ABF anti-lysozyme (scFv D1.3) was investigated and culture heterogeneities in cell morphology, membrane potential (MP) and productivity were experimentally analysed by means of flow cytometry. A first approach focussed on the characterization of the MP, since the membrane properties play a very important role in the cell physiology and also influence the product secretion. The MP directly reflects the characteristics of the proton motive force which is involved in generation of ATP, sugar transport, chemotaxis and survival at low pH. Therefore it is a most sensitive parameter enabling state estimation and stability monitoring of the underlying bioprocess. The MP estimation was done by means of a staining method based on the fluorescent dye DiOC2. Another process relevant parameter is the current ABF production status of cells. Therefore an appropriate assay based on fluorophor coupled detection antibodies was developed. The particular single-cell fluorescence data from stained cells was normalized to their fluorescence surface density. Based on this approach it became possible to explicitly distinguish between high productive and less productive cells. By these means, production and secretion heterogeneities with different pattern of productivity were revealed for ABF secretion with Bacillus megaterium. Both approaches were used to deeply characterize bioprocesses from shaking flask to bioreactor scale. Batch and oscillating fed-batch processes were analysed and the dynamics in membrane potentials and ABF production were studied. The methods revealed distinct cell heterogeneities under conditions of late induction and high initial carbon source concentrations. The underlying multidimensional data sets were clustered into sub populations according to a model-based clustering approach (bioconductor© software). In a further study a novel single cell analysis technique based on electrical cell properties, called impedance flow cytometry (IFC)

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(Axetris©), was compared to the previously established fluorescence based methods. Viability and MP measurements were shown to give qualitative and quantitative comparable results. Its simplicity and rapidness makes this new, microfluidic chip-based technique a promising tool for prospective online monitoring of bioprocesses on single cell level. David F, Berger A, Hansch R, Rohde M, Franco-Lara E (2011) Single cell analysis applied to antibody fragment production with Bacillus megaterium: development of advanced physiology and bioprocess state estimation tools. Microb. Cell Fact. 10:23. David F, Steinwand M, Hust M, Bohle K, Ross A, D´ubel S, Franco-Lara E (2011) Antibody production in Bacillus megaterium: strategies and physiological implications of scaling from micro titer plates to industrial bioreactors. Biotechnology J.

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