Microbial Cell Factories

Oral Presentation

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Monitoring of stress responses

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from The 4th Recombinant Protein Production Meeting: a comparative view on host physiology
Barcelona, Spain. 21–23 September 2006

Published: 10 October 2006

Microbial Cell Factories 2006, **5**(Suppl 1):S23 doi:10.1186/1475-2859-5-S1-S23

Background

The consideration of bacterial stress and starvation responses is of great importance for the successful establishment of an industrial large scale fermentation process. Suitable analysis techniques for stress and starvation specific genes are therefore particularly interesting for the monitoring and control of such processes. The combined methods of transcriptome analysis, high resolution two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and mass spectrometry have been extensively applied for the description of general and specific stress and starvation responses of industrial microorganisms.

Results

By means of proteomics and transcriptome analyses we identified marker genes of the gram-positive bacteria *Bacillus subtilis* and *Bacillus licheniformis*. The expression of such marker genes is specifically regulated by distinct stress and starvation conditions. For both bacteria, which represent important industrial hosts with a long history in industrial enzyme production, we have filtered a set of marker genes, which could be used as indicators for process-relevant stress situations during protein production fermentation processes. For example, in Figure 1 starvation specific marker proteins for nitrogen, phosphate and glucose limitation of *B. licheniformis* are summarised.

Such process-critical genes/proteins can be used as biomarkers in order to control the fitness and productivity of these industrial bacterial hosts during fermentation processes. DNA- and protein-chips specific for such process-relevant marker genes would be valuable diagnostic tools for the monitoring of cellular physiology. In this

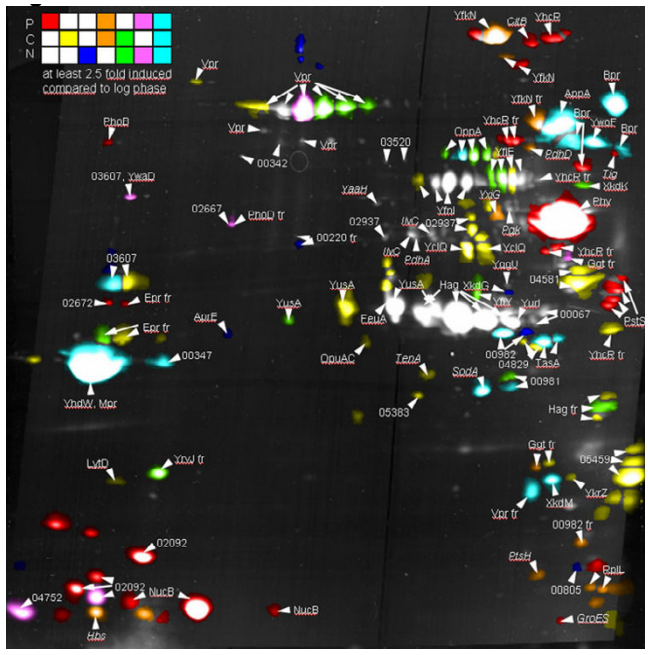

Figure 1
Colour coding of extracellular marker proteins of *B. licheniformis* for phosphate, glucose and nitrogen starvation conditions. Colour coding was done with the Delta 2D software http://www.decodon.com. (P: phosphate starvation, C: glucose starvation, N: nitrogen starvation). [1]

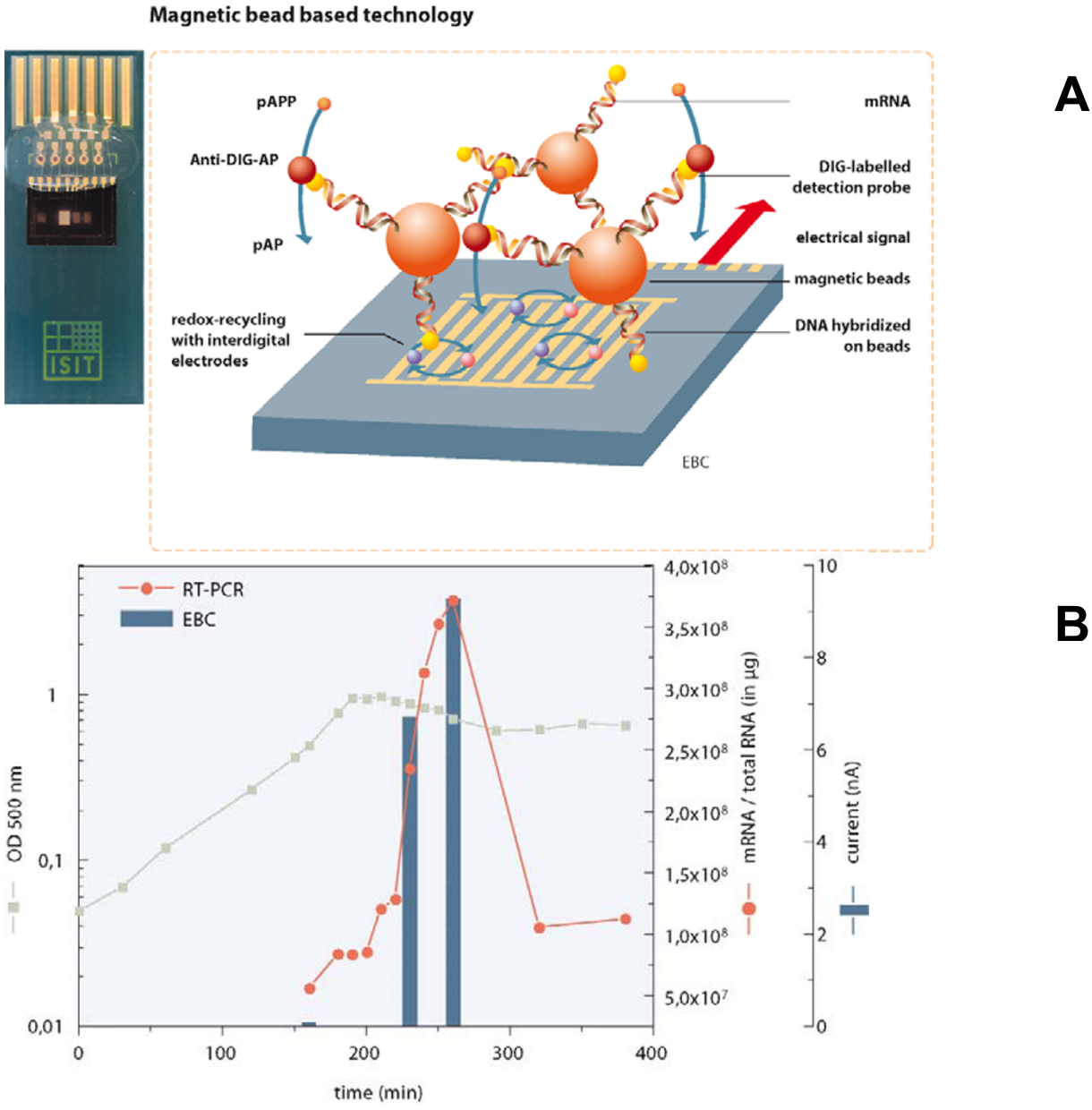

Figure 2
A Schematic presentation of the electric chip principle and **B** analysis of a *B. subtilis* glucose-starvation marker gene with an electric DNA-chip during a glucose-limited fermentation process. EBC = electric DNA-chip, RT-PCR = real time RT-PCR

respect fast mRNA and protein analytical techniques for an *at-line* monitoring of gene expression during bioprocesses are required. The electric chip technique fulfills these requirements [2]. This technique allows a fast and reproducible expression analysis of process-relevant marker genes (see Figure 2).

Conclusion

It is demonstrated that electric chips loaded with mRNA specific DNA-probes or with marker protein specific antibodies represent a suitable alternative for gene expression analyses in competition with the real time RT-PCR during fermentation processes. The electric chip technique is easy

to automate and could be cheaper in the handling than the established gene expression analysis techniques. The electric biochip combined with an automated sample preparation establishes a basis for continuous *at-line* monitoring of host cell physiology during industrial bioprocesses.

Acknowledgements

This study was financially supported by the Ministry of Education, Science and Culture of Mecklenburg-Vorpommern (grant number: 0202120) and the Federal Ministry of Education, and Science (BMBF) (grant number: 031U213A).

References

1. Voigt B, Schweder T, Sibbald MJ, Albrecht D, Ehrenreich A, Bernhardt J, Feesche J, Maurer KH, Gottschalk G, Van Dijl JM, Hecker M: **The extracellular proteome of *Bacillus licheniformis* grown in different media and under different nutrient starvation conditions.** *Proteomics* 2006, **6:**268-281.
2. Jürgen B, Barken KB, Tobisch S, Pioch D, Wümpelmann M, Hecker M, Schweder T: **Application of an Electrical DNA-Chip for the Expression Analysis of Bioprocess-Relevant Genes of *Bacillus subtilis*.** *Biotechnol Bioeng* 2005, **92:**299-307.