Biosensor based analysis of microbial production strains

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Abstract

Metabolite detection and quantification in single bacterial cells is one of the great challenges of current research in the field of Industrial Biotechnology. Here, we report the development of a biosensor which enables the intracellular detection of L-methionine or branched-chain amino acids and transforms this information into an optical readout. The described sensor is based on the transcriptional regulator Lrp of Corynebacterium glutamicum, which was recently shown to activate expression of the amino acid exporter BrnFE in response to increased levels of L-methionine or the branched-chain amino acids L-leucine, L-isoleucine and L-valine (1). For this purpose the genomic region containing the open reading frame of lrp and the intergenic region of lrp-brnF were cloned in front of eyfp, thereby setting eyfp expression under control of PbrnF. Due to the specificity characteristics of Lrp, this sensor is suitable for the detection of L-methionine and the branched-chain amino acids L-leucine, L-valine and L-isoleucine. In further experiments, this sensor technology was successfully implemented in an ultra high-throughput FACS screening (10,000 cells per second) for the isolation of cells after random mutagenesis which exhibited increased intracellular concentrations of the effector amino acids. Several mutants excreting significant amounts of these amino acids could already be obtained in a first screen. Given that this sensor technology allows the analysis of bacterial production strains at single-cell resolution, we studied isogenic microcolonies of a C. glutamicum strain (ATCC13032 aceE) in a microfluidic device under conditions supporting L-valine production. In fact, heterogeneity of single-cell fluorescence was observed for cells of microcolonies originating from a common ancestor. These findings consequently suggest significant variability of these cells with respect to valine production. Altogether, these results demonstrate the versatile applications of this sensor technology, such as high-throughput screening or the analysis of cell-to-cell variability of industrial production strains, and hence, emphasize it as a highly valuable tool for efficient strain development in Industrial Biotechnology. 1) Lange, C, Mustafi, N, Frunzke, J, Kennerknecht, N, Wesel, M, Bott, M, Wendisch, VF. (2011) Lrp of Corynebacterium glutamicum controls expression of the brnFE operon encoding the export system for L-methionine and branched-chain amino acids. J. Biotechnol., in press

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