Single cell-scale metabolite sensor-based system for rapid isolation of bacterial metabolite producers

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Abstract

Whereas flow cytometry for the counting and evaluation of surface properties of bacterial cells is well established, this is not yet the case for the analysis of intracellular features of them. To do this, we have established intracellular metabolite sensors converting a specific cytosolic metabolite in a concentration dependent manner into an optical output. This achievement offers us the unique possibility to detect among individual cells those with increased metabolite level, and increased production performance. Since increase of metabolite formation is a major issue in biotechnology our technology bridges the gap between HT-techniques of generation genetic diversity and HT-techniques for product analysis by supplying an extrem rapid and efficient technique substituting current laborious screening procedures which are based on cultivation and characterisation of clones. With the aim to select for L-lysine producers, the entire methodology consisting of i) sensor design, ii) genome mutation, iii) FACS selection, iv) gene sequencing and v) genome sequencing was applied to the wild type of Corynebacterium glutamicum which does not excrete L-lysine. As a result, out of 1.8x107 mutagenised and sensor-carrying cells 580 cells were selected within 30 min via FACS, of which 270 grew up in cultures, and 95 were proven to be L-lysine producers. Of these 40 were further analysed in more detail by partial sequencing, and 1 analysed by whole-genome sequencing, revealing new mutations and targets to improve L-lysine accumulation which is currently made with bacteria in an amount of 1.300.000 tons per year.

Keywords: FACS, cytosolic metabolite sensor, strain development, visualisation of pool concentrations

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