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Novel approaches to light-up solventogenic and acetogenic *clostridia* the FAST way

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As the number of metabolic engineering tools for solventogenic and acetogenic bacteria increases, so does the need for fluorescent reporter proteins, which show bright fluorescence even in anoxic environments. Most often used fluorescent reporters such as GFP are oxygen-dependent, thus their use is limited in anaerobic clostridia. Moreover, the oxygen-independent fluorescence of FMN-binding fluorescent proteins often only shows poor cyan-green fluorescence. These problems can be overcome by making use of the fluorescence-activating and absorption-shifting tag (FAST) as its fluorescence is strong and oxygen-independent.

FAST was used to construct C- or N-terminally tagged fusion proteins and respective fusion genes were heterologously expressed in the acetogen *Eubacterium limosum*. The production of the FAST-tagged bifunctional aldehyde/alcohol dehydrogenase or the acetoacetate decarboxylase originating from *Clostridium acetobutylicum* was monitored during the growth of *E. limosum* cells. Production of the fusion proteins resulted in brightly fluorescent cells and the production of the for *E. limosum* non-native products butanol as well as acetone indicated that the functionality of respective fusion proteins was not negatively affected.

In addition, the dynamics of synthetic *C. acetobutylicum* and *C. saccharoperbutylacetonicum* co-cultures were investigated by establishing the reporter proteins greenFAST and redFAST, which show orthogonal green and red fluorescence, respectively. Moreover, greenFAST and red-FAST were used to construct a tightly regulated inducible two-plasmid system and produced both in *C. saccharoperbutylacetonicum*. Hence, orthogonal green and red fluorescence during growth were determined at single-cell level.