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Standardizing the methodology for transformation of anaerobic bacteria as hosts for recombinant enzyme production and development of fast activity screening for tungsten-dependent aldehyde oxidoreductases

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PURPOSE OF THE ABSTRACT

The development of new techniques for the expression of various enzymes is a critical task in biotechnology. Not all valuable enzymes are easy to produce in standard bacterial hosts such as E. coli, due to their complex structures and strict requirements for folding and post-translational modification or special requirements for activation or cofactor insertion. The aim of this research was to overcome the challenges of expressing and producing enzymes from anaerobic metabolic pathways that are typically hard to obtain using traditional expression hosts. Our approach involved standardizing the methodology for the transformation of the bacterial cells, optimizing growth conditions, and selecting appropriate vectors for gene expression.

For this study, we have chosen the facultatively anaerobic bacterium Aromatoleum evansii KB 740 as a host strain for the expression of three different enzymes from related species, i.e., benzylsuccinate synthase (BSS) from Thauera aromatica K172, tungsten-dependent aldehyde oxidoreductases (AOR) from Aromatoleum aromaticum and molybdenum steroid C25 dehydrogenase (S25DH) from Sterolibacterium denitrificans. BSS requires activation to a glycine radical state by a special activating enzyme, while both AOR and S25DH require the synthesis and correct incorporation of tungsten or molybdopterin cofactors, respectively.

Compared to T. aromatica and A. aromaticum, A. evansii has a shorter generation time, can switch its metabolism from anaerobic to aerobic swiftly, and grows better on solid agar media. However, no molecular biology protocols for the genetic transformation of A. evansii have been developed, leaving conjugation as the only functional way of recombinant enzyme engineering.

We have compared classical conjugation and direct transformation of A. evansii for developing recombinant enzyme expression systems with a broad-host plasmid pASG3_mob+ [1]: i) conjugation was achieved with a 2,6-diaminopimelic acid (DAP)-auxotrophic donor strain, E. coli WM3064 mixed with A.evnasii, and ii) direct transformation conditions of A. evansii with plasmid DNA were developed by electroporation.

The former strategy, albeit time-consuming, yielded recombinant systems for AOR and BSS and enabled mutational studies of both enzymes. On the other hand, electroporation turned out to be much faster than conjugation. It was carried out under aerobic conditions, with 1 mM MOPS buffer, but using lower voltage and significantly increased

incubation times compared to other established protocols. This improved methodology for electroporation significantly increased our success rate of obtaining recombinant A. evansii, while simultaneously cutting down the amount of time necessary for obtaining and purifying the desired clones. Comparing electroporation to conjugation outcomes, we can state that electroporation had a clear advantage on conjugation, providing recombinant clones faster and limiting the possibility of getting a contaminated culture. Additionally, we have developed a fast-screen colorimetric assay on Petri dishes for the early detection of AOR production. Overproduced enzyme activity was visualised through a reaction of benzaldehyde oxidation with benzyl viologen.
Our findings demonstrate the feasibility of using non-standard bacteria as expression systems for difficult enzymes, providing a potential avenue for the development of novel biocatalysts. Also, the assay developed for the fast detection of enzyme activity proved to be cost-effective and useful for high-throughput screening while making a plasmid library.

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

transformation | enzyme expression | Aromatoleum evansii | activity assay

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