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Postprint: Study on the Relationship between IrrE Expression from Deinococcus radiodurans and Organic Solvent Tolerance in Arthrobacter simplex and Its Regulatory Mechanism

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Abstract

Steroid hormone drugs currently represent the second largest class of drugs used clinically, surpassed only by antibiotics. The C1,2 dehydrogenation of steroid compounds is a typical example of microbial transformation employed in the industrial production of steroid pharmaceuticals. Among the microorganisms utilized, Arthrobacter simplex has become a commonly employed industrial strain due to its high specificity and rapid reaction rate. Transformation reactions typically employ organic solvents (usually ethanol at 4% concentration) to enhance the solubility of hydrophobic substrates; however, the amount of organic solvent is strictly limited due to its detrimental effects on microorganisms, which severely restricts substrate loading in the transformation system and consequently impacts yield. To overcome this bottleneck, there is an urgent need to construct microbial strains with high organic solvent tolerance suitable for industrial applications.

In this study, the global transcription factor IrrE from Deinococcus radiodurans was introduced into Arthrobacter simplex. The results demonstrated that IrrE expression did not significantly affect the strain' s growth, metabolism, or catalytic enzyme activity, but markedly enhanced its organic solvent tolerance, thereby improving production efficiency in transformation systems containing high concentrations of organic solvents and substrates. The regulatory mechanism through which IrrE expression enhances the organic solvent tolerance of A. simplex was elucidated through analysis of general stress response-related metabolite levels, key enzyme activities, and transcription levels of relevant genes. Promoter engineering was employed to obtain A. simplex strains with differential IrrE expression, which further revealed that IrrE expression level positively correlates with strain tolerance. Finally, the steroid C1,2 dehydrogena-

tion capability of the IrrE-expressing strain PM588, mediated by an optimized promoter mutant, was evaluated. This strain produced 20.3 g/L of prednisone acetate in a transformation system containing 70 g/L of cortisone acetate substrate and 10% ethanol, representing a 62.4% increase compared to the strain expressing IrrE under the original promoter (12.5 g/L). Under these conditions, the control strain harboring an empty plasmid exhibited no significant transformation activity. These findings provide a novel strategy for constructing highly efficient steroid-transforming strains and hold significant scientific importance for elucidating the global regulatory mechanism of IrrE in hosts under stress conditions.

Full Text

Preamble

Title: Study on the Relationship Between IrrE Expression from *Deinococcus radiodurans* and Organic Solvent Tolerance in *Arthrobacter simplex* and Its Regulatory Mechanism

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Abstract: Steroid drugs represent the second largest class of pharmaceuticals in clinical use, surpassed only by antibiotics. The microbial C1,2 dehydrogenation of steroid compounds is a typical example of industrial biocatalysis for steroid drug production. *Arthrobacter simplex* has become the predominant industrial strain due to its high substrate specificity and rapid reaction rate. However, the biotransformation process typically requires organic solvents (usually ethanol at 4% concentration) to enhance the solubility of hydrophobic substrates. The dosage of these solvents must be strictly controlled due to their detrimental effects on microbial cells, which severely limits substrate loading in the transformation system and ultimately constrains product yield. To overcome this bottleneck, there is an urgent need to construct robust microbial strains with high organic solvent tolerance for industrial applications.

This study introduced the global transcription factor IrrE from *Deinococcus radiodurans* into *A. simplex*. The results demonstrated that IrrE expression had no significant effect on cell growth, metabolism, or catalytic enzyme activity, but substantially enhanced organic solvent tolerance, thereby improving production efficiency in systems containing high concentrations of organic solvents and substrates. The regulatory mechanism underlying the improved solvent tolerance was elucidated through analysis of general stress response-related metabolites, key enzyme activities, and transcriptional levels of relevant genes. Furthermore,

promoter engineering was employed to generate *A. simplex* strains with differential IrrE expression levels, revealing that higher IrrE expression correlated with enhanced solvent tolerance.

Finally, the steroid C1,2 dehydrogenation capability of the engineered strain PM588, which harbors an optimized promoter mutant driving IrrE expression, was evaluated. In a transformation system containing 70 g/L cortisone acetate and 10% ethanol, the prednisone acetate titer reached 20.3 g/L, representing a 62.4% increase over the strain expressing IrrE under the original promoter (12.5 g/L). Under these conditions, the control strain carrying an empty plasmid showed negligible conversion activity. These findings provide a novel strategy for constructing highly efficient steroid-transforming strains and offer important scientific insights into the global regulatory mechanisms of IrrE in host cells under stress conditions.

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