Optimization of H2-sensing regulatory hydrogenase production from *Ralstonia eutropha* H16 in *Escherichia coli*

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Concerns about exhaustion of fossil fuels and global warming have led to increasing attention to clean and renewable energy. Here, biohydrogen is a very attractive alternative. In nature, the most efficient biohydrogen producers are hydrogenases, which are abundant metalloenzymes showing tremendous potentials as biocatalysts in hydrogen production, fuel cells and H_2-driven cofactor regeneration. The apo-enzymes are not active unless they are modified by a complicated maturation process responsible for the assembly and incorporation of the required cofactors. Over the past two decades, important achievements have been made in the understanding of these highly complex enzymes. However, due to the complex structure and maturation process of hydrogenases, their heterologous production has been a challenging task and their sensitivity to ambient O_2, CO seriously limits the potential industrial applications[1].

The β-proteobacterium *Ralstonia eutropha* H16 hosts four different O_2-tolerant [NiFe]-hydrogenases (MBH, SH, AH and RH)[2]. In the present study, the *R. eutropha* RH was selected as a model to develop a heterologous hydrogenase production system in *Escherichia coli*, owing to its relatively simple architecture and widely used spectroscopic studies.

After reconstruction of structural RH subunit expression, we investigated relevant cultivation parameters and obtained an initial production yield of 14 mg/L of purified RH in the initial batch *E. coli* shake-flask cultures. A 18-fold improvement of the production yield was achieved in EnPresso-based fed-batch-like shake-flask cultures[3]. This RH productivity was further enhanced by the simple and highly efficient IPTG/lactose based autoinduction with shorter cultivation time[4], corresponding to several 100-fold increase in the amount of RH purified from the native host *R. eutropha*. However, different spectroscopic assays showed partially matured RH (~99% inactive) production indicating the incapability of *E. coli* indigenous maturation system for full cofactor maturation. Subsequently, co-expression of the native maturation genes (*hypA1B1F1CDE*, *hoxN1*, *hypX*) resulted in active RH production in *E. coli* and cofactor maturation efficiency was further increased by nickel addition and lowering the expression temperature. Additionally, spectroscopic investigations clearly indicated that the catalytic properties of the cofactors of RH produced from *E. coli* were in good accord with the data available on the native RH isolated from *R. eutropha*[5].

Our results lay a good basis for the future production of functional hydrogenases for basic as well as applied science.

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