

Enhancement of apparent O₂ tolerance in hydrogen production by co-expressing *Vitreoscilla* Hemoglobin

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Vitreoscilla Hemoglobin (VHb) is the most famous, widely used bacterial hemoglobin, which has a potential of broad biotechnological application. This protein exerts many positive effects on cellular events such as protein synthesis, cell growth in hypoxic condition. We exploited VHb in hydrogen production where oxygen sensitivity of hydrogenase is one of the most troublesome problems. With recombinant *Escherichia coli* BL21 over-expressing its own [NiFe] hydrogenase 1, which has hydrogen producing ability as previously shown, we tested the hypothesis that VHb may enhance O₂ tolerant hydrogen production by serving as an O₂ diffusion barrier. We showed that VHb co-expressing recombinant strain produced more H₂ gas compared with the negative control and this effect was a composite result of both O₂-dependent and O₂-independent processes. The possibility that the increased hydrogen production was due to the promoted synthesis of recombinant [NiFe] hydrogenase 1 by VHb was strongly excluded by the additional experiments. In conclusion, VHb can be applied to biohydrogen production, giving the enhanced 'apparent' oxygen tolerant property.