

Improvement of Amorpha-4,11-diene Production by a Yeast-Conform Variant of *Vitreoscilla* Hemoglobin

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Amorpha-4,11-diene is the precursor of the antimalarial compound artemisinin. The effect of *Vitreoscilla* hemoglobin (VHb) and its yeast-conform variant (VHbm) on amorpha-4,11-diene production in engineered *Saccharomyces cerevisiae* was investigated. First, the *VHb* gene was mutated to the yeast-conform variant *VHbm* based on step-by-step extension of a short region of the gene through a series of polymerase chain reactions (PCR). The artificial *VHbm* gene contained codons preferred by the yeast translation machinery. Two yeast expression vectors containing *VHb* or *VHbm* gene were constructed and introduced into the amorpha-4,11-diene-producing strain *S. cerevisiae* WK1 to form WK1[VHb] and WK1[VHbm], respectively. Western blot and CO-difference spectrum absorbance assay showed that *VHb* and *VHbm* were successfully expressed. In shake flasks, VHbm expression conferred higher cell growth than VHb expression. GC-MS results indicated the amorpha-4,11-diene production in WK1[VHbm] and WK1[VHb] was 3- and 2-fold higher than that in WK1, respectively. This suggests that VHb might improve the amorpha-4,11-diene production in engineered *S. cerevisiae*.

Key words: Amorpha-4,11-diene, Engineered *Saccharomyces cerevisiae*, *Vitreoscilla* Hemoglobin