

## Metabolic engineering of *Methylobacterium* sp. DH-1 for lactic acid production from methane

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To produce D-lactic acid (D-LA) from methane, we engineered *Methylobacterium* sp. DH-1, a methanotroph which can use methane as a sole carbon and energy source. Since *Methylobacterium* sp. DH-1 does not produce LA naturally, L- or D-LA can be produced by introducing stereospecific lactate dehydrogenase gene. LA toxicity is one of the limiting factors for high-level production of LA. Therefore, we first performed adaptive laboratory evolution of *Methylobacterium* sp. DH-1, generating a LA-tolerant strain Ev8.0. Ev8.0 also showed resistance to other weak acids such as formic acid, acetic acid, and propionic acid. Genome sequencing of Ev8.0 revealed a causal gene encoding a novel transcription factor, whose overexpression is responsible for the increase in LA tolerance. When D form-specific lactate dehydrogenase was introduced into the genome, Ev8.0 produced about 8-fold higher level of D-LA from methane compared to wild-type strain, suggesting that LA tolerance is a critical limiting factor for LA production in this host.