Genome based Metabolic Flux Analysis (MFA) of *Methanococcus maripaludis* for improved methane productivity

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Rising levels of CO_2 in the atmosphere is one of the major problems around the world. At present, CO_2 emissions in atmosphere are minimized by storing or converting the emitted CO_2, which demands compression and is expensive. Recent progress shows that microbial conversion of CO_2 to methane is a novel biocatalytic approach for carbon capture and conversion to fuel that seems relatively economical.

Hydrogenotrophic methanogens are archaebacteria that utilize CO_2 and convert it to methane for ATP synthesis. *Methanococcus maripaludis,* a hydrogenotrophic methanogen, appears to be a highly promising methanogen for large-scale utilization in industries due to its inherent ability to utilize CO_2 and N_2, which are major components of flue gas. In addition, it's short doubling time of 2h under mesophilic conditions and availability of tools for genetic manipulations offer additional advantages. Even though the microbe has been thoroughly studied, the studies on improved methane productivity are lacking in the literature.

In order to study genetic and environmental parameters affecting methanogenesis, this work present a first constraint-based genome-scale metabolic model of *M. maripaludis* S2. This model consists of 570 reactions, 556 distinct metabolites, and 518 genes across 52 pathways and covers 30% of open reading frames (ORFs). The draft model was constructed with the help of online databases and further refined using literature and annotated or proteomic evidences. The model was successfully validated using biomass growth data and phenotypic studies present in the literature. Using the experimentally measured CO_2 uptake rate as input, the model predicts specific growth rate, methane evolution rate, hydrogen uptake rate in addition to intracellular fluxes across all metabolic pathways under different media conditions. Based on the model findings, we were able to propose a hypothesis that different carbon and nitrogen sources influence growth and methanogenesis significantly. The comparison of fluxes with *Methanosarcina barkeri*, *Methanosarcina acetivorans,* and *Sulfolobus solfataricus* P2 showed *M.*

maripaludis S2 to be a better organism for producing methane from CO_2. *In silico* flux analysis of metabolic network using various metabolic engineering approaches such as gene(s) knockouts, over expression of certain gene(s), etc. helped us in identifying potential genes targets for improved methane productivity. This model also provides a framework for the comprehensive study of existing or new metabolic processes, effects of media components, effect of inhibitory metabolites etc. in different strains of *M. maripaludis* and in other hydrogenotrophic methanogens. Experimental studies are underway to validate our model predictions.

Keywords: Biocatalysis, Flue gas, Hydrogenotrophic methanogens, *M. maripaludis*, Genome-scale model, Flux balance analysis