Summary

Methanogenic archaea are a diverse group of strictly anaerobic microorganisms able to convert specific growth substrates into methane as part of their metabolism. Methanosarcina acetivorans is considered one of the model organisms for genetic engineering given its metabolic versatility. Moreover, the genes involved in methanogenesis involve transcriptional and translational regulatory mechanisms that will determine the microorganism's cellular functions and responses. Protein engineering and regulation is a field that needs further studies to fully understand the molecular processes that control gene expression. In this study, twelve promoter-RBS complexes from diverse methanogens were selected to test the strength and relevant response in different growth environments. Two substrates were selected, and gene expression levels were quantified with the help of a β-glucuronidase reporter system. This activity was assessed, and the expression levels of this gene were engineered by trying different promoter and RBS combinations from four selected candidates. Our results reveal alterations in gene expression in response to the modifications made (up to a 10-times increased activity), which indicates a regulation in the transcriptional and translational level in M. acetivorans. These insights offer a wider perspective on the available tools for metabolic engineering of methanogens.