

# THE ROLE OF THE HYDROGEN IN THE FORMATION OF METHANE IN METHANOGENS

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## Introduction

Hydrogen metabolism is one of the rate-limiting processes in anaerobic digestion of organic material. Several types of hydrogenases have been identified in methanogenic archaea. Hydrogenases are the components of the H<sup>+</sup>-translocating system in methanogens that is why they have an important role in the methane formation. The effect of hydrogen on the expression of hydrogenases and other genes has not been systematically examined yet. The exploration of the different environmental conditions, which affect the expression of the hydrogenases and other genes could improve our knowledge concerning molecular redox mechanisms in microbes.

## Experimental

Two hydrogenotrophic methanogen strains were examined, the hyperthermophile *Methanocaldococcus fervens* was grown at 85°C, the mesophile *Methanococcus voltae* at 37°C. The cells were cultivated in serum bottles in six parallels under hydrogen, than the gas phase was replaced by nitrogen. *M. fervens* grows solely in the hydrogenotrophic way, *M. voltae* can also utilize formiate as electron source. Samples were taken twice, after cultivation of the cells under hydrogen and 6 hours after flushing the bottles with nitrogen. The cells were centrifuged at 12.000 rpm for 10 minutes. RNA was isolated with Zymo Research Soil RNA MicroPrep and sequenced using Ion Torrent PGM with chip 316. The data were analyzed using KEGG and EggNOG databases.

## Results and discussion

The transcripts were mapped to the reference genomes of the examined methanogens. 1,682 genes were identify in *M. voltae* and 1,539 genes in *M. fervens*. They were classified into 19 KEGG categories. The average fold changes of the gene expressions in different categories in *M. voltae* calculated from the RPKM (reads per kilobase million) value are compiled in Figure 1. Practically all functional gene categories were expressed at higher level when hydrogen was present in the gas phase. This indicated that although the cells could utilize formiate as energy source, hydrogen was essential for most cell functions. Similar behavior was seen in the case of *M. fervens*.

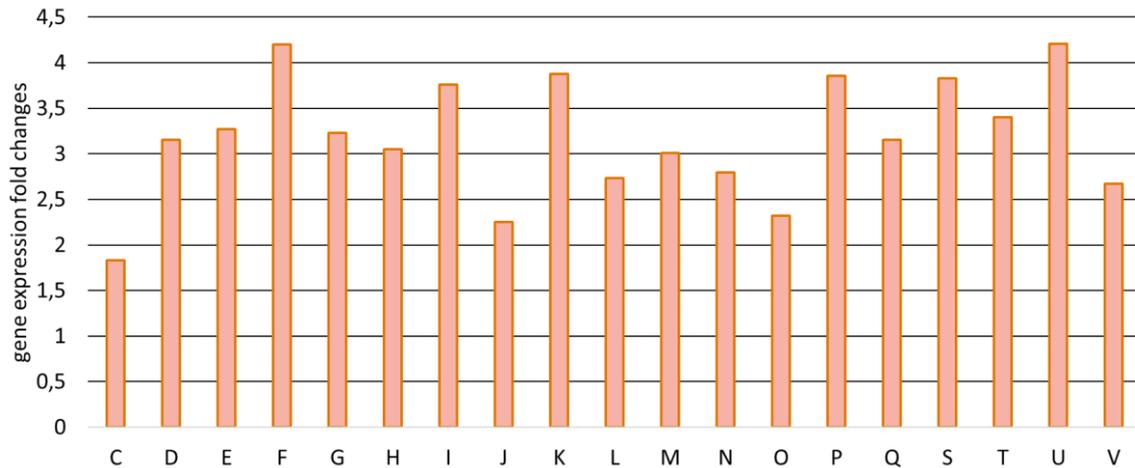


Figure 1. Genes classified in KEGG categories

C	Energy production and conversion	M	Cell wall/membrane/envelop biogenesis
D	Cell cycle control and mitosis	N	Cell motility
E	Amino Acid metabolism and transport	O	Post-translational modification, protein turnover, chaperone functions
F	Nucleotide metabolism and transport	P	Inorganic ion transport and metabolism
G	Carbohydrate metabolism and transport	Q	Secondary Structure
H	Coenzyme metabolism	S	Function Unknown
I	Lipid metabolism	T	Signal Transduction
J	Translation	U	Intracellular trafficking and secretion
K	Transcription	V	Defense mechanism
L	Replication and repair		

The fold changes of the individual genes was also examined and we have identified several genes to have elevated expression in the absence of hydrogen. Figure 2. indicates the genes which showed the most noticeable changes. These genes take part mainly in transcription (transcriptional regulator, ribosomal proteins) and in methanogenesis (subunit of formylmethanofuran dehydrogenase, coenzyme-B sulfoethylthiotransferase, coenzyme F430synthase).

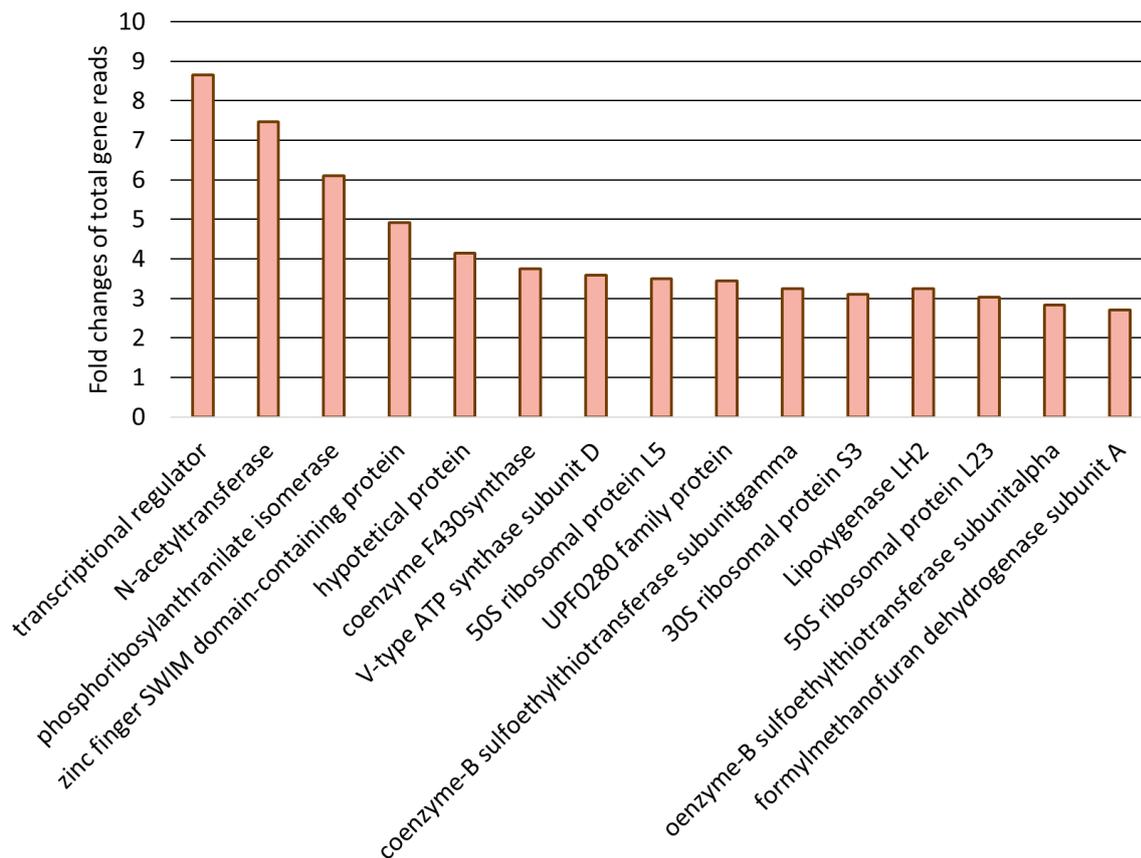


Figure 1. Genes having increased expression upon withdrawal of hydrogen in *M. voltae*

## Conclusion

The mRNA sequences were analyzed using the PATHVIEW visualization software. Although the expression of most enzymes involved in methanogenesis decreased under nitrogen, enzymes that catalyze the final step in the formation of methane and enzymes, which take part in the conversion of formiate and acetate significantly increased in *M. voltae*. This suggests the turn-on of an escape route for the surviving methanogens, the cells adopted to the new environment, the expression of the enzymes which utilize energy from formiate are increased. The cells were able to grow under these conditions. In contrast, the expression of all genes, which take part in the methanogenesis decreased the *M. fervens*. This archaeon can utilize only hydrogen and without hydrogen the cell turned into a decaying phase and even the stress gene transcriptions were not observed.

## Acknowledgements

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## References

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