Lawrence Berkeley National Laboratory
Recent Work

Title

The Ech Hydrogenase is Important for Growth of D vulgaris with Hydrogen

Permalink

https://escholarship.org/uc/item/51k0d97h

Authors

Stolyar, S.M.
Wall, J.
Stahl, D.A.

Publication Date

2007-02-12

The Ech Hydrogenase is Important for Growth of *Desulfovibrio vulgaris* with Hydrogen

S.M. Stolyar[1], J. Wall[2], and D.A. Stahl[1]

[1] Department of Civil and Environmental Engineering, [2] Department of Biochemistry, University of Missouri-Columbia, Columbia, MO

INTRODUCTION

One objective of the Virtual Institute for Microbial Stress and Survival (VIMSS) and the Environmental Stress Pathway Project (ESPP) is to determine the genetic and physiological bases for cooperative and competitive interactions among environmental microbial populations of relevance to the DOE. The ESPP Applied Environmental Core (AEC) and Functional Genomics Core (FGC) have identified a number of genes that may participate in cooperative interactions between sulfate reducers and methanogens under low sulfate conditions.

The Deltaproteobacterium *D. vulgaris* is able to grow in the absence of an electron acceptor via syntrophy with hydrogenotrophic organisms. Despite decades of research, energy conservation in *D. vulgaris* is not well understood. The presence of multiple hydrogenases, including many located in the periplasm in all studied *Desulfovibrio* strains - and the observation that hydrogen is produced and then consumed during growth with lactate and sulfate (Tsuji&Yagi, 1980) - lead to the formulation of the hydrogen cycling hypothesis as a mechanism for energy conservation (Odom & Peck, 1981). The completed genome sequence of *D. vulgaris* Hildenborough has since revealed genes for at least six different hydrogenases: four periplasmic and two cytoplasmic. Although several have been partially characterized biochemically and genetically, their roles in *D. vulgaris* under different growth conditions remain mostly undefined.

One of the membrane-bound hydrogenases, Ech, is very similar to a proton pumping hydrogenase from *Pyrococcus furiosus DSM 3638* (Sapra *et. al.,* 2004) and *Thermoanaerobacter tengcongensis* (Soboh *et.al.,* 2004). It was suggested that a role for the Ech of DvH is hydrogen production using ferredoxin as a redox partner (Pohorelic *et al.,* 2002; Rodrigues *et al.,* 2003).

In this work we examined the growth and metabolite production of an *echA* (DVU0434) *D. vulgaris* Hildenborough mutant under three different growth conditions: i) in medium amended with lactate and sulfate and ii) in medium amended with acetate, hydrogen and sulfate, and iii) in coculture with the hydrogenotrophic methanogen *Methanococcus maripaludis*, lacking an electron acceptor.

MATERIALS and METHODS

D. vulgaris was grown on a B3o medium in 25 ml Balch tubes at 30 psi with either a 80%N_2:20%CO_2 or 80%H_2:20% CO_2 gas mixture in the headspace volume of approximately 15 ml. The basal B3o medium (pH 7.2) contained (per liter): 0.25g NaCl, 5.5 g $MgCl_2$•$6H_2O$, 0.1g $CaCl_2$•$2H_2O$, 0.5g NH_4Cl, 0.1g KCl, 1.4g Na_2SO_4, 25mM $NaHCO_3$, 5.75mM K_2HPO_4, 0.001g resazurine, 0.078g Na_2S · 9 H_2O, 1ml Thauer's vitamins of (containing per liter 0.02g biotin, 0.02g folic acid, 0.1g pyridoxine HCl, 0.05g thiamine HCl, 0.05g riboflavin, 0.05g nicotinic acid, 0.05g DL pantothenic acid, 0.05g *p*-aminobenzoic acid, 0.01g vitamin B12), 1ml of trace minerals (per liter: 1.0g $FeCl_2$•$4H_2O$, 0.5g $MnCl_2$•$4H_2O$, 0.3g $CoCl_2$•$4H_2O$, 0.2g $ZnCl_2$, 0.05g Na_2MoO_4•$4H_2O$, 0.02g H_3BO_3, 0.1g $NiSO_4$•$6H_2O$, 0.002g $CuCl_2$•$2H_2O$, 0.006g Na_2SeO_3•$5H_2O$, 0.008g Na_2WO_4•$2H_2O$). This basal medium was amended with lactate and sulfate for growth in mono-culture or co-culture.

The concentration of organic acids and inorganic ions (sulfate, phosphate) in culture fluids were determined using a Dionex 500 system equipped with an AS11HC column. In some cases the concentration of organic acids was also measured on an HPLC equipped with a HPX 78 (Bio-Rad) column. Hydrogen concentrations were determined with a RGD2 Reduction Gas Detector (Trace Analytical) with 60/80 MOLE SIEVE 5A column (6' X 1/8'') with N_2 as carrier gas. The concentration of methane and carbon dioxide was measured on a GC equipped with a TCD and "80/100 HAYESEP Q" column (6' X 1/8'') with helium as carrier gas.

The *echA* was deleted, generating the mutant JW380.

Figure 1.

echABCDEF operon in wild type *D. vulgaris*

0434 | 0433 | 0432 | 0431 | 0430 | 0429

echA deletion mutant (JW380)

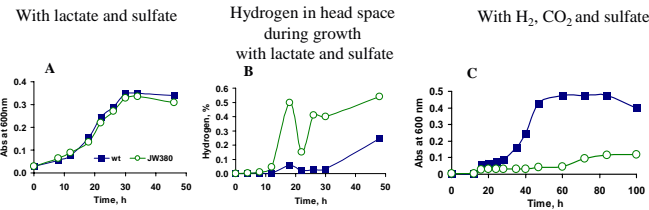Km R | 0433 | 0432 | 0431 | 0430 | 0429

Genes coding for membrane proteins are shown in blue
Genes coding for soluble cytosolic proteins are in green

RESULTS

Growth of *D. vulgaris* JW380 monoculture

Figure 2.

With lactate and sulfate

Hydrogen in head space during growth with lactate and sulfate

With H_2, CO_2 and sulfate

RESULTS

Growth of *D. vulgaris* JW380 in syntrophic association with *M. maripaludis* without sulfate

Figure 3.

Figure 4.

Growth

Lactate consumption and acetate production
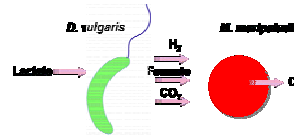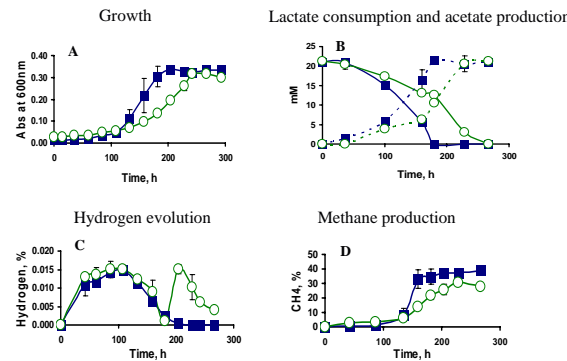
Hydrogen evolution

Methane production

REFERENCES

Meuer *et al.* 2002. Genetic analysis of the archaeon *Methanosarcina Barkeri* Fusaro reveals a central role for Ech hydrogenase and ferredoxin in methanogenesis and carbon fixation. Proc. Natl. Acad. Sci. **99**:5632-5637.
Odom and Peck. 1981. Hydrogen cycling as a general mechanism for energy coupling in the sulfate-reducing bacteria *Desulfovibrio* sp. FEMS. Microb. Lett. **12**:47-50.
Pohorelic *et al.* 2002. Effects of deletion of genes encoding Fe-only hydrogenase of *Desulfovibrio vulgaris* Hildenborough on hydrogen and lactate metabolism. J. Bacteriol. **184**:679-686.
Porat *et al.* 2006. Disruption of the operon encoding Ehb hydrogenase limits anabolic CO2 assimilation in the archaeon *Methanococcus maripaludis*. J. Bacteriol. **188**:1373-1380.
Rodrigues *et al.* 2003. A novel membrane bound Ech [NiFe] hydrogenase in *Desulfovibrio gigas*. BBRC **306**:366-375.
Sapra *et al.* 2003. A simpleenergy-conserving system;proton reduction coupled to pr0ton translocation. PNAS **100**:7545-7550.
Soboh *et al.* 2004. A multisubunit membrane-bound [NiFe] hydrogenase and NADH-dependent Fe-only hydrogeanse in fermenting bacterium Thermoanaerobacter tengcongensis. Microbiology **150**:2451-2463.
Tsuji and Yagi. 1980. Significance of the hydrogen burst from growing cultures of *Desulfovibrio vulgaris* Miyazaki, and the role of hydrogenase, and cytochrome c3 in energy production system. Arch. Microbiol. **125**:35-43.

SUMMARY

On lactate, the mutant demonstrated a growth rate and yield comparable to the wild type strain, but evolved more hydrogen as measured by its accumulation in the headspace during growth in batch culture (Figure 2A and B). A coculture consisting of the mutant strain and a hydrogenotrophic methanogen (*M. maripaludis*) demonstrated only slightly reduced growth rate and increased hydrogen accumulation in stationary phase when lactate was consumed relative to the wild type (Figure 4). This suggested a minor role of Ech in energy conservation during syntrophic growth. The hypothetical mechanism of hydrogen oxidation under these two growth conditions are shown on Figure 5.

Figure 5. Growth of *D. vulgaris* on Lactate and Sulfate or in syntrophy with methanogen
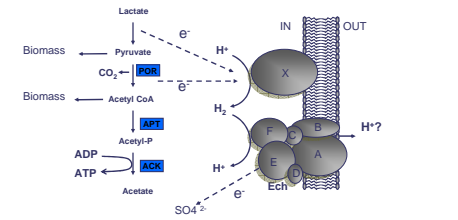
X – unknown cytoplasmic hydrogenase; POR – pyruvate oxidoreductase; APT – acetyl phosphotransferase; ACK – acetate kinase

In a medium containing acetate and an atmosphere of H2/CO2, growth of the mutant was severely impaired relative to the wild type (Figure 2C). Thus, the available data suggest that the primary role of the Ech hydrogenase is oxidation of hydrogen during sulfate respiration, possibly also contributing to the production of reduced ferredoxin required for conversion of Acetyl CoA to pyruvate by pyruvate oxidoreductase, as was previously demonstrated for the homologous hydrogenases in *M. barkeri* and *M. maripaludis* (Meuer *et al.,* 2002; Porat *et al.,* 2006). The hypothetical mechanism of hydrogen oxidation under this growth condition are shown on Figure 6.

Figure 6. Growth of *D. vulgaris* on Hydrogen and Sulfate
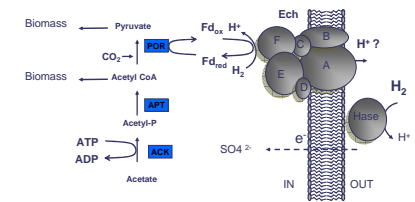
Hase – periplasmic hydrogenase(s)

Additional information on function of another hydrogenases in *D. vulgaris* you can find on Poster #59

ACKNOWLEDGEMENT