Supplementary Materials

Confirmation of the Ech and HynAB deletion mutants by PCR and Southern-blot analyses

For confirmation of the deletion of the *echBC* and *hynAB* genes, Southern-blot analyses were performed using gDNA from *D. gigas* wild-type and the respective mutant strains extracted with Wizard Genomic DNA Purification Kit (Promega).

For the *ΔhynAB* deletion, *BciI* (Roche) was used for the digestion of ~3µg of *D. gigas* wild type and *ΔhynAB* strain gDNA. A probe for the ~1kb upstream region of the *hynA* gene was amplified using *Phusion* High-fidelity DNA polymerase (Thermo Scientific). Southern blotting, prehybridisation and hybridisation were carried out using the DIG High Prime DNA labeling and Detection Starter kit II (Roche), according to the manufactures protocol. The replacement of the *hynAB* genes for the *kanR* gene creates a new restriction site for *BciI* enzyme generating bands of different sizes. The results showed the expected higher size for the positive band in the *D. gigas* WT (4635bp) and a smaller size for the positive band in the Δ*hynAB* mutated strain (1623bp) as shown in Fig. S1.A.

In the case of the Ech mutation, ~2µg of *D. gigas* wild-type and Δ*echBC* gDNA was digested with *Nco*I (Fermentas). Specific probes for *echBC* and the *kanR* genes were amplified using *Taq* DNA polymerase (Invitrogen) and labeled with [32P]dATP. Southern blotting, prehybridisation and hybridisation were carried out as described in [1]. Hybridisation with the *echBC* probe resulted in a single positive band of the expected size (2752bp) only in the lane corresponding to *D. gigas* wild-type, whereas hybridisation with the kan^R probe

resulted in two positive bands of the predicted size (1230bp and 1623pb) only in the lane corresponding to *D. gigas* Δ*echBC,* as shown in Fig. S1.B.

Further confirmation was performed through PCR analyses using ~200ng of gDNA from *D. gigas* wild-type or the respective mutant strains as templates and specific oligonucleotides amplifying for the regions of interest (Table.S1). In the case of the Δ*echBC* mutant strain, PCR products of the expected size in *D. gigas* wild-type were obtained only with the primers for *echBC* (Fig.S2.A). In the *D. gigas* Δ*echBC* strainamplification was observed only with the primers for kan^R. Likewise, in the Δ*hynAB* mutant strain, amplification of the *hynAB* locus showed PCR products of the expected sizes in the *D. gigas* wild-type and in the *D. gigas* Δ*hynAB* strain (Fig.S2.B). The difference in size of the amplified bands is due to the fact that the kan^R gene has only ~1.5kb´s, whereas the *hynAB* genes have ~2.5kb´s. Furthermore, specific primers to amplify the kan^R genes presented PCR product of the expected size in the *D. gigas* Δ*hynAB* (1415bp), while for the wild type no amplification was observed (Fig.S2.B).

The results obtained by both PCR and Southern-blot confirmed the deletion of the *echBC* and *hynAB* genes and their replacement with the kan^R gene, creating two single mutant strains containing one hydrogenase each.

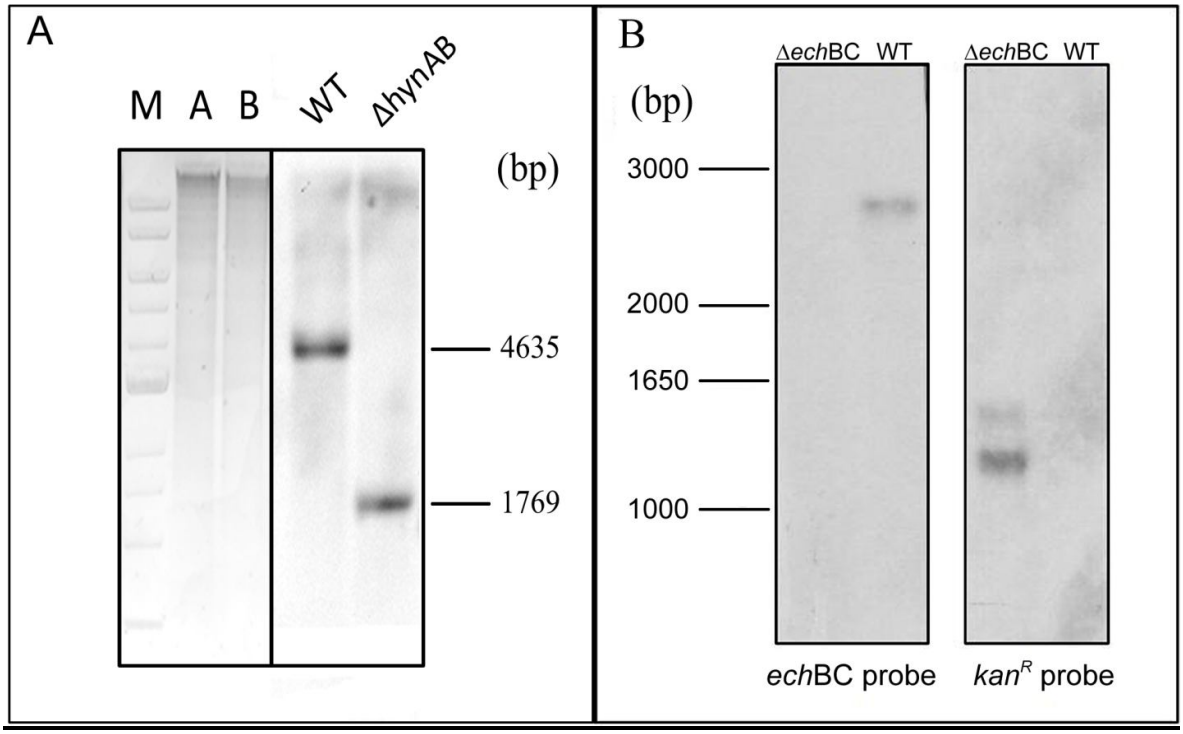

Fig.S1: Confirmation of *echBC* and *hynAB* deletions. A- Southern-blot analysis of *BciI*-digested genomic DNA from *D. gigas* wild type and Δ*hynAB* strains. Expected band sizes are as follows: 4635bp for WT and 1769bp for the Δ*hynAB*. B- Southern-blot analysis of *Nco*I digested genomic DNA from *D. gigas* wild-type and Δ*echBC* strains. Expected radio-labeled bands are as follows: Δ*echBC* (*echBC* probe), none; WT (*echBC* probe), 2752bp; Δ*echBC* (*kanR* probe), 1230bp and 1623bp; WT (*kanR* probe), none.

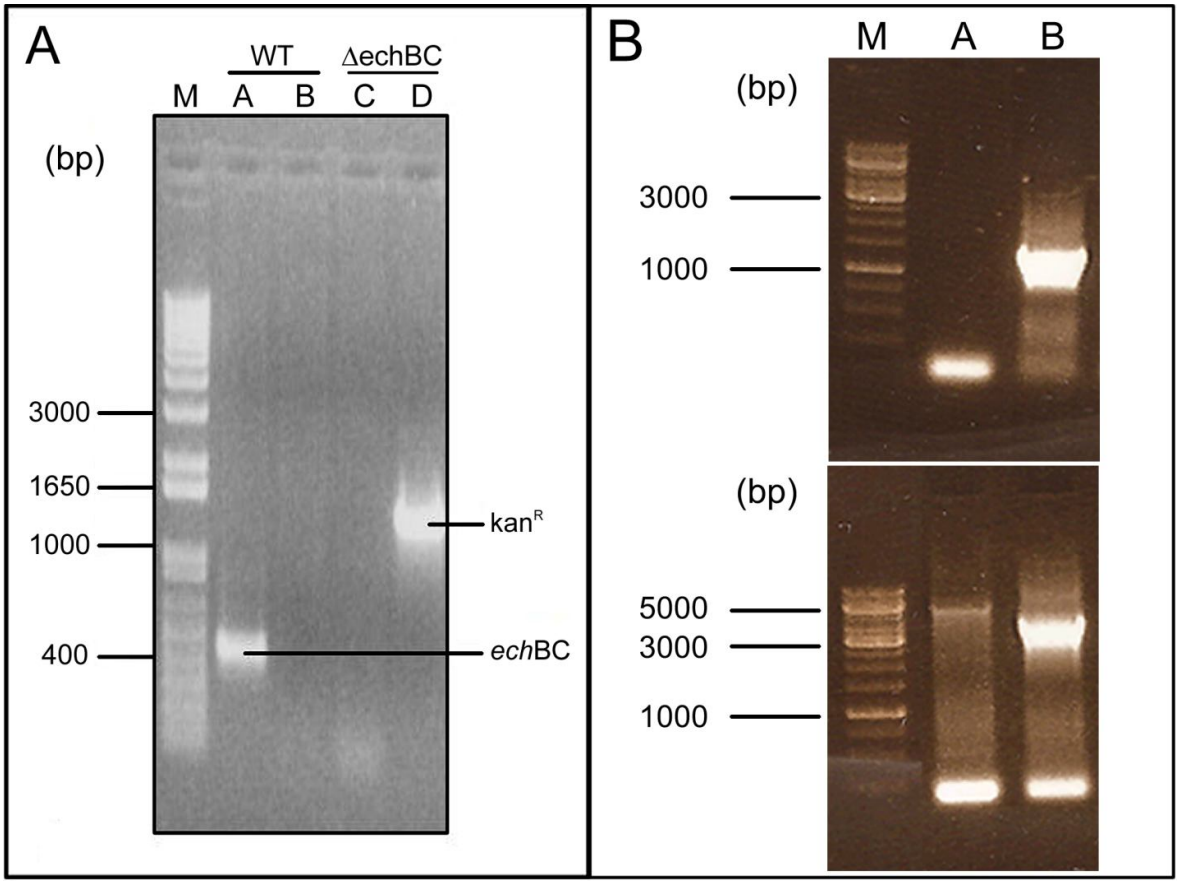

Fig.S2: Confirmation of *echBC* and *hynAB* deletion by PCR analysis: A- PCR products amplified from *D. gigas* wild-type and Δ*echBC* genomic DNA are as follows: Lane A- WT (EchBC-1/EchBC-2), 422bp; Lane B- WT (EchKan^{R}XbaI-fwd / EchKan^{R}XbaI-rev), none; Lane C- Δ*echBC* (EchBC-1/EchBC-2), none; Lane D- Δ*echBC* (EchKan^{R}XbaI-fwd / EchKan^{R}XbaI-rev), 1425 bp. B – PCR products amplified from *D. gigas* wild-type and Δ*hynAB* genomic DNA. Top image, amplification of kanR gene (HynABKan^{R}SalI_fwd / HynABKan^{R}BHI_rev): Lane A- WT, none; Lane B - Δ*hynAB*, 1415bp. Lower image, amplification of the deletion site (HynABFlankI_fwd/ HynABFlank II_rev): Lane A – WT, 4637bp; Lane B - Δ*hynAB*, 3416bp.

Table.S1: Oligonucleotides used for the creation and confirmation of *D. gigas* mutant strains.

Primers	**Sequence**
Δ***ech***	
EchFlankI_fwd	5´- GTA CGG ATC CGG TGC TGC GCA TGG CTC C - 3´
EchFlankI rev	5´- CAG CTC TAG AGA CCT CCC ACC ACC AGC AGC - 3´
Ech Flank II_fwd	5´- CGA GTC TAG AGG ATT GAG CCA TGC CCA TCC C - 3´
Ech Flank II_rev	5´- CTC AGG GCC CCG GTG TAG TCG TCC AGA ATG G – 3´
EchKan^{R}XbaI-fwd	5'- GAG TTC TAG ACA GCT ACT GGG CTA TCT G-3'
EchKan^{R}XbaI-rev	5'- GAG TTC TAG ACT GCA GTT CGG GGG CAT G-3'
EchBC-1	5´- GGG GGC CTC ATC ACC GGC G - 3´
EchBC-2	5´-GGG GCA GCT TGA ACA GCA TGG - 3´
Δ***hynAB***	
HynABFlankI_fwd	5´- TGA TTA CGC CAA GCT TTC AGG CGG AAG AAT TGG - 3´
HynABFlankI_rev	5´- CAG TAG CTG GTC GAC TTA CGT ACC CTC CGT CCT - 3´
HynABFlankII_fwd	5´- GAA CTG CAG GGA TCC GTT CCG GAC GCC AGA C - 3´
HynABFlank II_rev	5´- CAG TGA ATT CGA GCT CGA TTC TTC GGC TTC CTT G - 3´
HynABKan^{R}SalI_fwd	5´- GTC GAC CAG CTA CTG GGC TAT CTG - 3´
HynABKan^{R}BHI_rev	5´- GGA TCC CTG CAG TTC GGG GGC ATG - 3´

Table.S2: Oligonucleotides used in the qRT-PCR expression analyzes for *D. gigas* wild type and mutant strains.

Primers	**Sequence**
ech	
Ech_RT_fwd	5´- TCT GGT CCA CCA GCT TCG - 3´
Ech_RT_rev	5´- AGG CGG AAG ACA CCA TCC - 3´
hynAB	
HynAB_RT_fwd	5´- CCG ACG AAT ACG ATC TGA AC - 3´
HynAB_RT_rev	5´- TAC TTG ACG TGC TCC TCG AT - 3´
16S rRNA	
16S_RT_fwd	5´- CTC GTG CCG TGA GGT GTT - 3´
16S_RT_rev	5´- TCC CCA CCT TCC TCC TTG - 3´

Table.S3: HPLC quantification of substrates and products from of *D. gigas* wild-type (WT), Δ*echBC* and Δ*hynAB* strains from growth experiments in basal medium modified from [2] containing: Lactate 40mM/Sulfate 40mM, Pyruvate 40mM/Sulfate 40mM, Pyruvate 40mM/Sulfate* 5mM, Pyruvate 40mM and H_2/Sulfate 20mM. Quantifications where performed after inoculation (~4h) and at the end of stationary phase (~48h). Each data point is the average of three independent growth experiments. Standard deviation for each value is shown.

Growth Condition	Compound	Strains					
		WT		Δ*ech*		Δ*hynAB*	
		0h	48h	0h	48h	0h	48h
Lactate/Sulfate	Lactate	40.0±0.3	0.0±1.4	40.4±0.8	0.0±0.2	40.5±0.6	0.0±0.2
	Sulfate	42.2±5.8	25.7±5.3	40.0±0.3	21.0±3.4	43.6±1.5	23.6±3.9
	Acetate	1.7±0.5	42.0±2.9	1.2±0.7	39.5±1.5	2.0±1.0	42.3±1.5
Pyruvate/Sulfate	Pyruvate	45.0±1.7	0.0±0.2	43.3±5.2	0.0±0.4	41.1±3.9	0.0±0.2
	Sulfate	42.5±4.1	34.0±2.7	40.2±4.7	32.2±5.9	39.0±3.6	31.2±1.3
	Acetate	2.9±0.3	34.5±3.8	2.7±0.4	34.9±2.2	2.5±1.8	30.7±5.3
Pyruvate/Sulfate*	Pyruvate	44.6±6.4	0.0±0.2	45.3±2.9	0.0±0.4	42.0±3.8	0.0±0.2
	Sulfate	5.3±0.9	0.0±0.2	5.2±0.7	0.0±0.2	5.3±0.8	0.0±0.2
	Acetate	2.8±0.5	32.6±1.0	2.7±0.3	31.8±1.7	3.0±1.3	29.4±3.9
Pyruvate	Pyruvate	41.5±5.5	0.0±0.2	39.5±8.6	0.0±0.2	45.6±6.3	29.3±2.5
	Acetate	2.6±0.2	29.6±3.5	2.4±0.2	28.7±4.5	2.4±0.6	5.3±5.6
H_2/Sulfate	Acetate	9.9±0.5	9.2±0.7	10.9±0.5	12.6±1	-	-
	Sulfate	19.3±0.5	16.4±0.5	19.2±0.5	16.5±0.5	-	-

References

1. **Ausubel FM, B.R., Kingston RE, More DD, Seidman JG, Smith JA, Struhl K,** ed. 1995 Current protocols in molecular biology. Greene Publishing Associates and Wiley-Intersciences: N.Y.
2. **Malki, S., G. De Luca, M.L. Fardeau, M. Rousset, J.P. Belaich, and Z. Dermoun**. 1997. Physiological characteristics and growth behavior of single and double hydrogenase mutants of *Desulfovibrio fructosovorans.* Archives of Microbiology. **167**(1): 38-45.