Supplementary Figure 2 Analysis of expression of selected dehydrogenases in glucose grown *G. oxydans* cultures:

**Materials and methods:**
For expression analysis of selected dehydrogenases, a medium containing 75 mM glucose, 5.0 g/l yeast extract and 3.0 g/l tryptone at pH 6.0 was used. Cells were grown in baffle shake flasks to mid log phase and harvested at an OD600 of 0.6. The pellet was washed with cold saline, frozen in liquid nitrogen and disrupted by using a ball mill (Braun, Melsungen). Total RNA was prepared using RNeasy Kit (Qiagen, Hilden) according to guidelines of the manufacturer and digested for 45 min with 100 U DNAses to remove contaminating chromosomal DNA. Total absence of DNA was checked after digestion by real time PCR using the primer pairs that were also employed as positive control. Quantitative real-time PCR was done on a iCycler (BioRad, Hercules, CA) using QuaniTect SYBR green RT-PCR Kit (Qiagen, Hilden) according to the instructions of the manufacturer in 20 µl volume with 400 ng total RNA as template. Primer with melting points of 63.0 °C - 65.0 °C and a length of 22 - 23 nt were deduced for each ORF under investigation to amplify products of 100 to 150 bp length. The bars indicate CT values, which represent the cycle when the PCR product passed the threshold fluorescence value. 1-2. Subunits of PQQ dependent alcohol dehydrogenase (GOX1067 & 1068); 3-5. Subunits of membrane-bound acetaldehyde dehydrogenase (GOX0585-0587); 6. Lactate dehydrogenase (GOX1170); 7. Lactate dehydrogenase (GOX2071); 8. PQQ-dependent glucose dehydrogenase (GOX0265); 9-11. Subunits of membrane-bound gluconate-2-dehydrogenase (GOX1230-1232); 12-13. Subunits of sorbitol dehydrogenase (GOX0854 & 0855); 14-16. Subunits of sorbitol dehydrogenase (fructose forming) (GOX2094-2097); 17. Uncharacterized PQQ-containing oxidoreductase (GOX0516); 18. Uncharacterized PQQ-containing oxidoreductase (GOX1441); 19. Uncharacterized PQQ-containing oxidoreductase (GOX1857); 20. Soluble glucose dehydrogenase (GOX2015); 21. Soluble sorbitol dehydrogenase (GOX1432); 22. Soluble L-sorbose reductase (GOX0849); 23. Soluble acetaldehyde dehydrogenase (GOX2018); 24. Soluble alcohol dehydrogenase (GOX0313); 25-27. positive controls: Glutamate synthase (GOX1852), α-chain of RNA polymerase (GOX0356) and DnaA (GOX0001); 28-31. Negative controls using total RNA from *Bacillus licheniformis* as template. The corresponding primers derived from uncharacterized PQQ-containing oxidoreductases (GOX0516, 1441, 1857) and soluble glucose dehydrogenase (GOX2015). The data shown represent the average Ct-values of four Real-time-PCR experiments with RNA templates prepared from at least two independent cultures.

**Results:**
As mentioned before, *G. oxydans* contains a large number of genes encoding oxidoreductases with unknown function and substrate spectrum. To prove whether these genes are expressed we analyzed a selected subset by RT-real time PCR. This approach included genes encoding proteins known to be functional from biochemical studies as well as genes encoding uncharacterized oxidoreductases. For transcription analysis total RNA was prepared from cells grown in batch culture on a complex medium containing glucose as major carbon source. As evident form Supplementary Fig. 2 all dehydrogenases tested are expressed under the given growth condition with some transcripts in relatively high concentration. Most prominent were the PQQ dependent alcohol dehydrogenase (GOX1067 & 1068) and the glycerol/sorbitol dehydrogenase (GOX0854 & 0855). This finding also suggests that the organism expresses a large number of its dehydrogenases simultaneously, presumably to cope with a multitude of substrates and their incomplete oxidation.