Electronic Supplementary Material (ESI) for RSC Advances.

Electronic supplementary information

Title. The cold way for glutathione biosynthesis in the psychrophile Pseudoalteromonas haloplanktis. Redundancy and reaction rates

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Table S1 Primers for the amplification of a DNA segment containing the coding sequence of the *Ph-gsh*A I gene

	Oligonucleotide sequences [a]
Direct primer	5′d-T_{-15}CGAGGAAGTATCATATG•GCA•ACA•CAT•GAT_{15}-3′
Reverse primer	5′d-C_{1593}TTAAAACTAACTATCTC•GAG•AGG•ATC•AAA•ATA•AGC_{1558}-3′

[a] Numbering begins from starting codon, italicized; underlined letters indicate mismatches for creating the *Nde*I and *Xho*I restriction sites.

Table S2 Primers for RT-PCR amplification of gene segments from *P. haloplanktis* [a]

Gene	Direct primer	Reverse primer
*Ph-gsh*A I	5’d-ACG•GCA•ACC•TTG•ACG•ATT•AC-3’	5’d-CAT•CTG•TTG•GTG•TTT•CAC•CG-3’
*Ph-gsh*A II	5’d-CAC•ACT•TAT•CGT•CGT•GGG•CT-3’	5’d-ACC•CCT•GCC•AAA•AGC•TAT•CC-3’
*Ph-gsh*B	5’d-AGA•TCC•CCC•TTT•TGA•TAC•CG-3’	5’d-AAA•GTG•TCG•GGC•GTG•TAT•TC-3’
Ph-sod	5’d-AAT•GGT•TTG•ATC•CCA•GGC•ACT-3’	5’d-TAA•ACA•CGC•CAC•CGT•CTG•AT-3’
Ph-r*RNA* 16S	5’d-GGT•AAT•ACG•GAG•GGT•GCG•AG-3’	5’d-TCT•CGC•TTA•ACA•AAC•CGC•CT-3’

[a] Primer Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome) and Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/) softwares were used for primer designing. Each primer was controlled by http://eu.idtdna.com/calc/analyzer software for avoiding dimer and hairpin formation.

Fig. S1

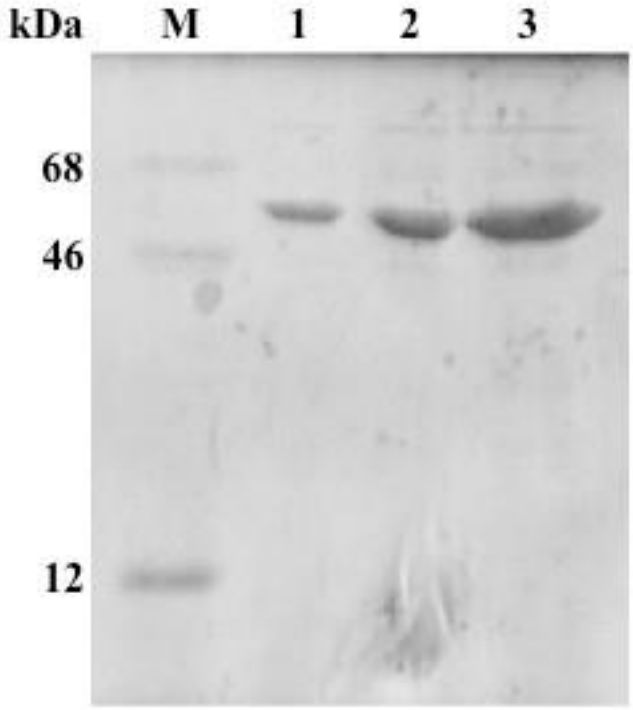

Figure S1 SDS-PAGE of r*Ph*GshA I

Increasing amounts (1, 2.5 and 5 µg) of purified r*Ph*GshA I (*lanes* 1-3) were analysed on a 12% polyacrylamide gel. Migration of molecular mass protein standards (*lane* M) is reported on the left.

Fig. S2

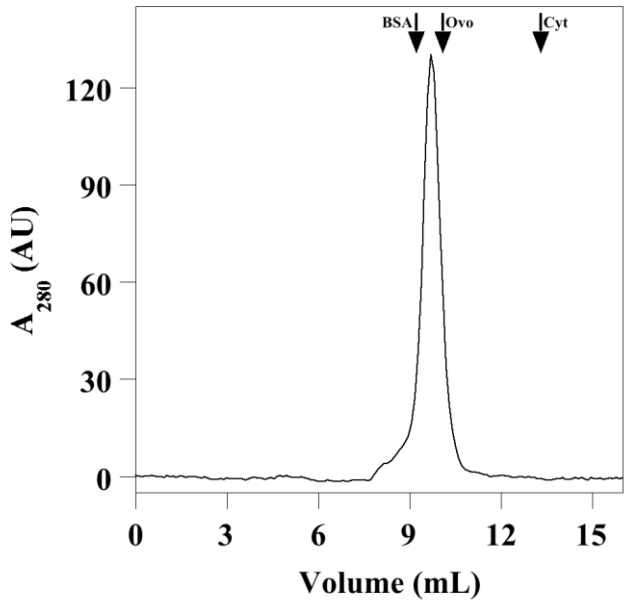

Figure S2 Size exclusion chromatography profile of r*Ph*GshA I under non-denaturing conditions

A sample of r*Ph*GshA I (100 µg in 200 µl) was loaded on a SuperdexTM 75 10/300 GL column. Elution was realised with 20 mM Tris•HCl buffer, pH 7.8, containing 100 mM KCl at a flow rate of 0.5 mL min^{-1}. The column was calibrated with bovine serum albumin (BSA, Mr 68,000), ovalbumin (Ova, Mr 46,000) and cytochrome *c* (Cyt, Mr 12,400); the corresponding elution positions are indicated by arrows on the top. Absorbance at 280 nm (A_{280}) was continuously monitered and expressed as arbitrary units.

Fig. S3

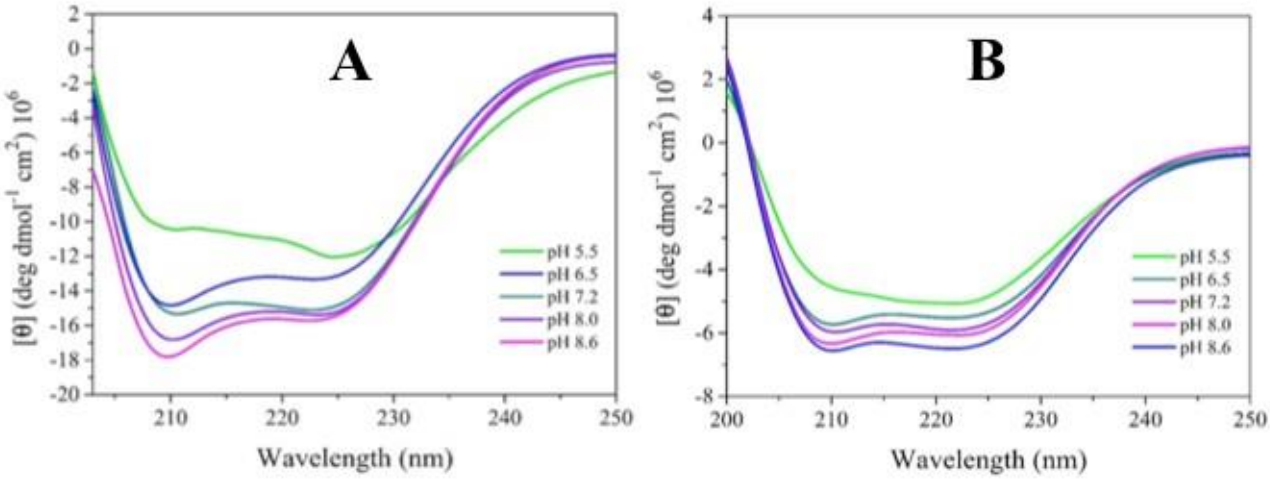

Figure S3 Far-UV CD spectra of r*Ph*GshA I (A) and r*Ph*GshA II (B) at different pHs

Spectra were collected at 20°C; enzymes were dissolved at 0.2 mg mL^{-1} in 10 mM buffers (sodium phosphate, pH range 5.5 – 8.0 or Tris•HCl, pH 8.6) supplemented with MKT solution.