Protein thiocarboxylate-dependent methionine biosynthesis
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Supplementary data

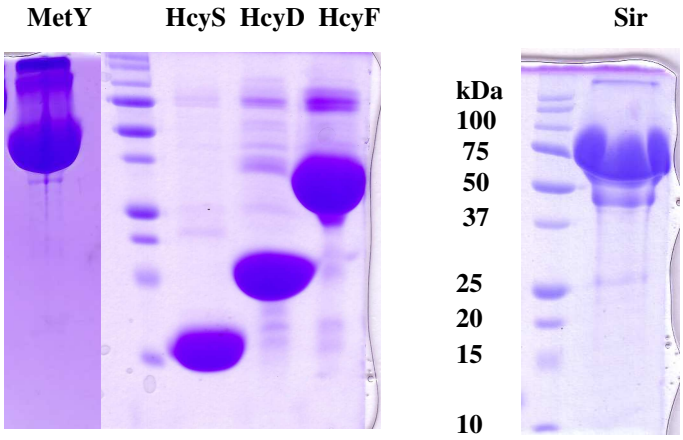

Figure 1s. SDS-PAGE of some of the Ni-NTA purified His-tagged proteins, HcyS, HcyD, HcyF, Sir and MetY.

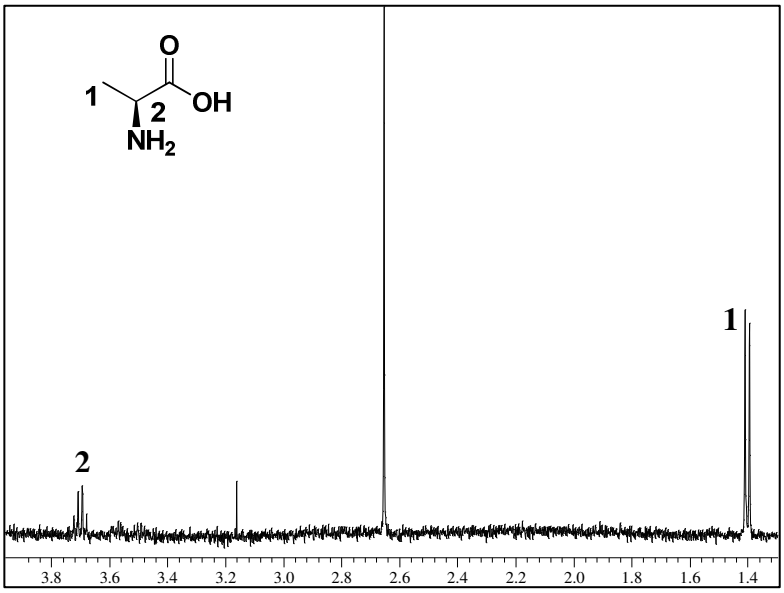

Figure 2s. ^{1}H-NMR of L-alanine released from HcyS-Ala upon treatment with HcyD.

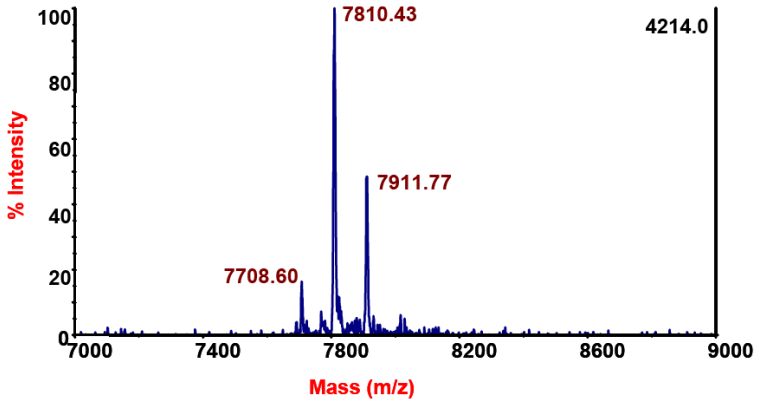

Figure 3s. HcyS-COSH (observed mass: 7708.6 Da, expected mass: 7704.82 Da, error: 0.05%) in the presence of MetY and O-acetyl-L-homoserine, 1 h incubation time, is converted to HcyS-homocysteine (observed mass: 7810.43 Da, expected mass: 7805.96 Da, error: 0.06%) and a higher molecular-weight adduct (7911.77 Da) whose identity is not known but could possibly be the HcyS-homolanthionine adduct.

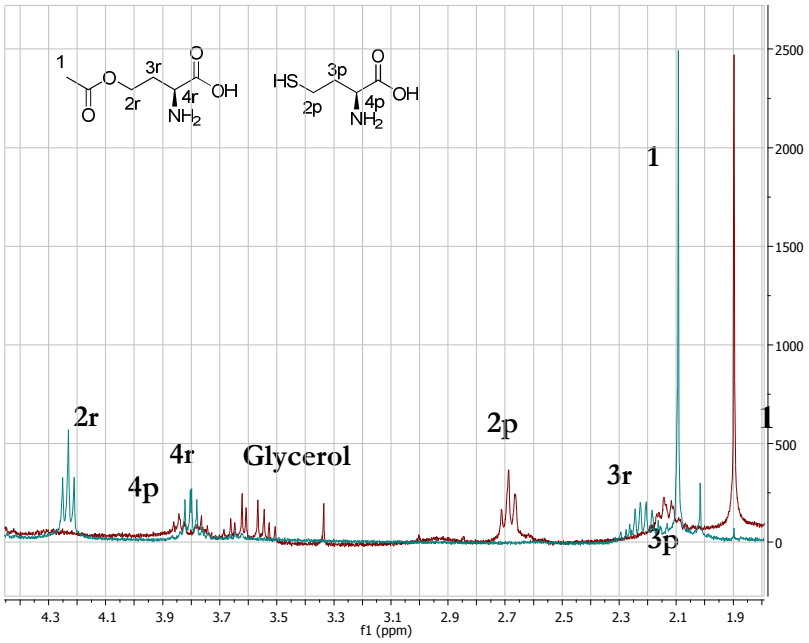

Figure 4s. [1]H-NMR of L-homocysteine formed by the MetY-catalyzed addition of sulfide to O-acetyl-L-homoserine (red), 1 h incubation time. No homocysteine is formed in the absence of the enzyme (blue).

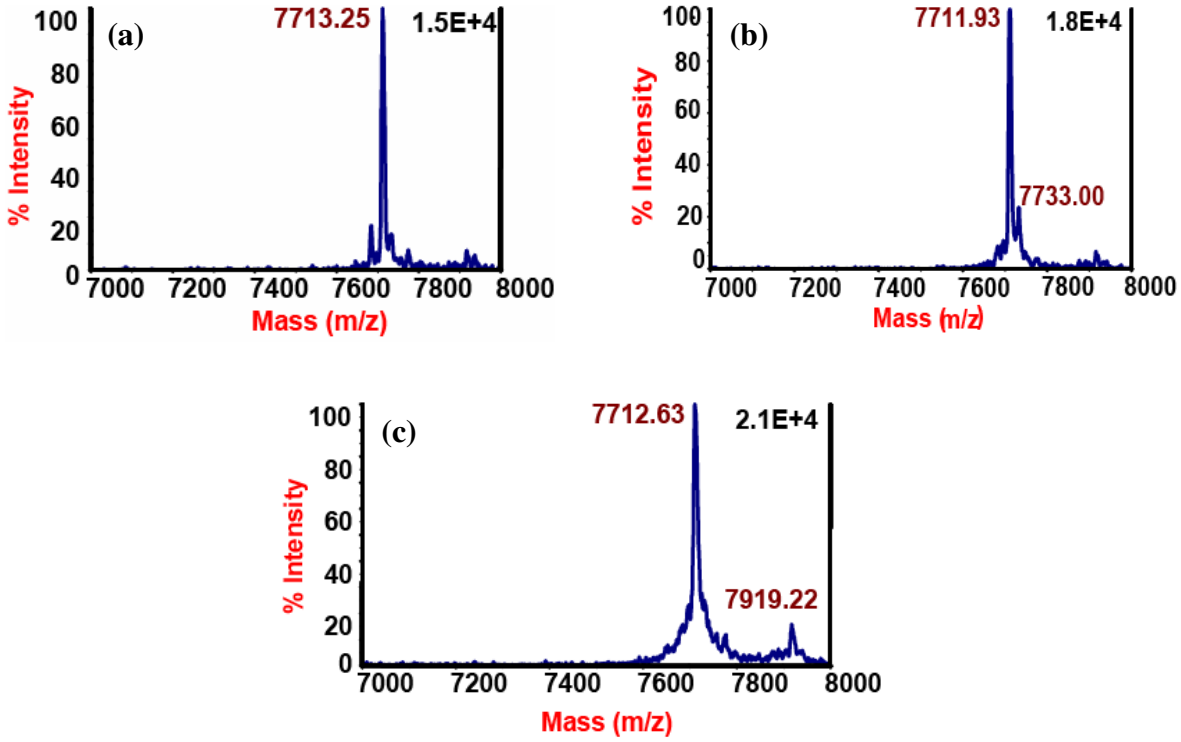

Figure 5s. MALDI-MS analysis of (a) HcyS-COSH (observed mass: 7713.25 Da, expected mass: 7704.82 Da, error: 0.1%) in the presence of MetZ and O-acetyl-L-serine, (b) HcyS-COSH (observed mass: 7711.93 Da, expected mass: 7704.82 Da, error: 0.09%) in the presence of MetZ and O-acetyl-L-homoserine (c) HcyS-COSH (observed mass: 7712.63 Da, expected mass: 7704.82 Da, error: 0.1%) in the presence of MetZ and O-succinyl-L-homoserine. No 7805.96 Da adduct corresponding to HcyS-Homocysteine adduct is formed in any of these cases.

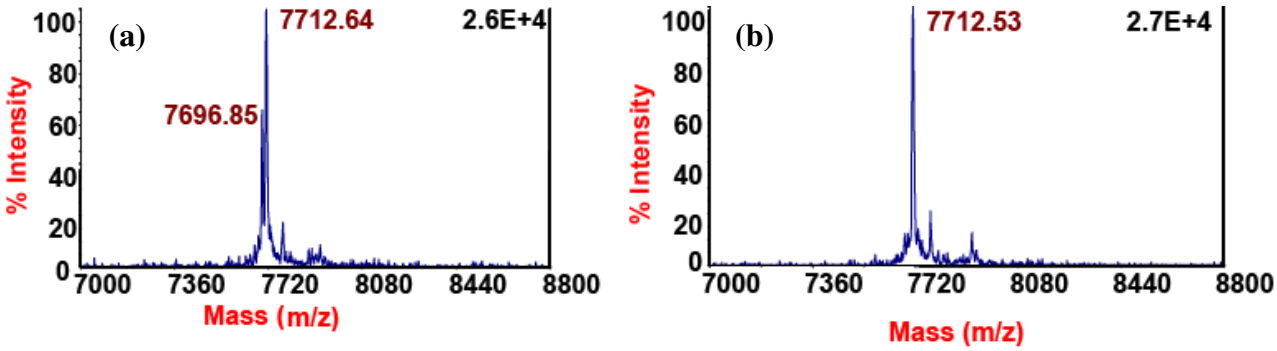

Figure 6s. MALDI-MS analysis (a) HcyS-COSH (observed mass: 7712.64 Da, expected mass: 7704.82 Da, error: 0.1%) in the presence of HcyD forms HcyS-COOH (observed mass: 7696.85 Da, expected mass: 7688.82 Da, error: 0.1%, observed mass change: 15.79 Da) (b) HcyS-COSH (observed mass: 7712.53 Da, expected mass: 7704.82 Da, error: 0.1%) in the absence of HcyD.

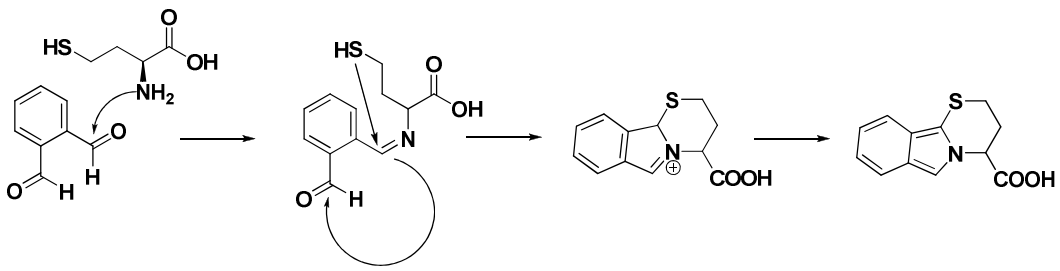

Figure 7s. o-Phthalaldehyde derivatization of homocysteine .

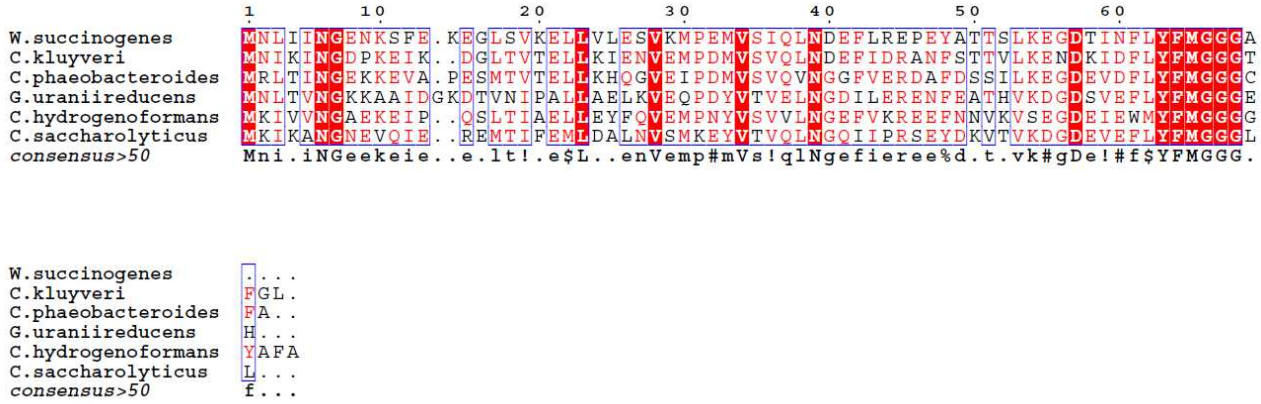

Figure 8s. Sequence alignment of putative HcyS-like proteins from *Wolinella succinogenes*, *Clostridium kluyveri* DSM 555, *Chlorobium phaeobacteroides* BS1, *Geobacter uraniireducens* Rf4, *Carboxydothermus hydrogenoformans* Z-2901 and *Caldicellulosiruptor saccharolyticus* DSM 8903. All these organisms have HcyS-like proteins clustered with HcyD-like metalloprotease and O-acetyl/O-succinylhomoserine sulfhydrylase.

Table 1s. Primers used for cloning genes in this study.

Genes	Restriction sites	Vector	Primers
sir	NdeI/XhoI	THT	Forward primer: 5'-GGG TAG CAT ATG AGC CAC TAC ACC CTA CCC CCC TCC GTC G-3' Reverse primer: 5'-CCC TAC TCG AGT TAT CGT TTT TGA ATC CTC ACG CGC C-5'
hcyD	NdeI/XhoI	THT	Forward primer: 5-GGG TAG CAT ATG CTC AAA ATC CCT AAA GCG CTC TTT G-3' Reverse primer: 5'-CCC TAC TCG AGT TAG ATC ACC TCG ATA TTT TCG GGA G-3'
hcyF	NdeI/XhoI	THT	Forward primer: 5'-GGG TAG CAT ATG AGA GAG TTT AGC GAA GAG GAG CTA G-3' Reverse primer: 5'-CCC TAC TCG AGT TAG GGA TTT TGA GCA TGA TTC ACC TCG CAG ATG GGT TGT TC-3'
hcyS	NdeI/XhoI	THT	Forward primer: 5'-GGG TAG CAT ATG AAT CTC ATC ATC AAC GGA GAG AAT AAG-3' Reverse primer: 5'-CCC TAC TCG AGT TAT GCG CCC CCT CCC ATG AAA TAT AAA AAG-3'
metY	NdeI/XhoI	THT	Forward primer: 5'-GGG TAG CAT ATG AGG GGA TTC ACC ACG AGG GCG C-3' Reverse primer: 5'-CCC TAC TCG AGT TAA CAT AGC GCT TGC AAA ATA TCC TC-3'
metZ	NdeI/XhoI	THT	Forward primer: 5'-CAGCACATGCATATGCC AGCCCACAAAGATGAGACT-3' Reverse primer: 5'-TTATTCCGCTCGAGTTAA GCTTTGGCTAGGGCTTG-3'
250	NdeI/XhoI	THT	Forward primer: 5'-CAG ATT CAC ACC CAC ATG TGC TAT TCC GTC-3' Reverse primer: 5'-GAC GGA ATA GCA CAT GTG GGT GTG AAT CTG-3'
Salmonella typhimurium cysG	NcoI/XhoI	pACYCDuet	Forward primer: 5'-GGG TAG CCA TGG ACC ATT TGC CTA TAT TTT GTC AAT TAC G-3' Reverse primer: 5'-CCC TAC TCG AGT TAA TGA TTA GAG AAC CAA TTT AAT TTA TC-3'
Truncated *hcyS* (with C-terminal alanine removed)	NdeI/SapI	pTYB1	Forward primer: 5'-CAGCACATGCATATGAAT CTCATCATCAACGGAGAGAATAA-3' Reverse primer: 5'-AATGTTTGCTCTTCCGCA GCCTCCTCCCATGAAATATAAA-3'