**Supplementary Information**

**Enzymes of an alternative pathway of glucose metabolism in obligate methanotrophs**

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**Supplementary Table S1.** The effects of K+, NH+4, Na+ cations (50 mM) and divalent metals (1mM) on the activity of GDHs from methanotrophs

|  |  |
| --- | --- |
| Cations | Residual activity, % |
| MtmGDH | MbGDH | MtmGntK\* | MbGntK\* |
| Without cations | 100± 1 | 100± 1 | <0.01 | <0.01 |
| K+ | 103 ± 1 | 131 ± 1 | 72 ± 1 | 99 ± 1 |
| Na+ | 79 ± 2 | 128 ± 1 | 100± 2 | 135± 1 |
| NH4+ | 166 ± 5 | 136 ± 1 | 70 ± 1 | 117 ± 1 |
| Mg2+ | 95 ± 1 | 117 ± 1 | 100± 2 | 100 ± 2 |
| Co2+ | 92 ± 1 | 14 ± 1 | 26 ± 1 | 127 ± 2 |
| Cu2+ | 92 ± 2 | 59 ± 1 | 2± 0,6 | 5 ± 0,5 |
| Mn2+ | 82 ± 1 | 54 ± 1 | 102± 2 | 135 ± 1 |
| Ni2+ | 87 ± 1 | 10 ± 0.2 | 10± 1 | 66 ± 1 |
| Cd2+ | 89 ± 1 | 3 ± 0.5 | 4 ± 0,6 | 18 ± 1 |
| Zn2+ |  62 ± 0.5 | 6 ± 0.2 | 2± 0,2 | 30 ± 1 |
| Ba2+(1) | n.d. | n.d. | 2± 1 | 75 ± 1 |
| Ca2+(1) | n.d. | n.d. | 43± 1 | 115 ± 1 |
| Sn2+(1) | n.d. | n.d. | 82 ± 1 | 80 ± 1 |

\*All reactions were carried out in the presence of 5 mM MgCl2, except for “without cations”.

n.d. – not determined

|  |  |
| --- | --- |
| Effector (Concentration) | Relative activity (%) |
| *Mtm. alcaliphilum* | *Mb. luteus* |
| Without effector | 100± 2 | 100± 1 |
| Glucose-6-phosphate: 2.5 mM | 102 ± 1 | 81 ± 1 |
|  5 mM |  | 67 ± 1 |
|  10 mM |  | 60 ± 1 |
| Glucose-1-phosphate(5 mM) | 103 ± 1 | 103 ± 1 |
| Fructose-6-phosphate:2.5mM |  | 93 ± 1 |
|  5 mM | 101 ± 1 | 85 ± 1 |
|  10 mM |  | 81 ± 1 |
| Fructose-1,6-bisphosphate (5 mM) | 103± 1 | 94 ± 1 |
| Phosphoenolpyruvate (1 mM) | 94 ± 1 | 99 ± 2 |
| Pyruvate (1 mM) | 96 ± 1 | 104 ± 1 |
| Oxaloacetate (1 mM) | 107 ± 3 | 108 ± 1 |
| Isocitrate (1 mM) | 140 ± 4 | 105 ±1 |
| Citrate (1 mM) | 119 ± 1 | 111 ± 3 |
| Malate (1mM) | 95 ± 5 | 113 ± 2 |
| α-Ketoglutarate (1 mM)  | 104 ± 1 | 108 ± 1 |
| Succinate (1 mM) | 99 ± 1 | 115 ± 3 |
| Lactate (1 mM) | 116 ± 1 | 100 ± 1 |
| Serine (1 mM) | 108 ± 3 | 110 ± 2 |
| ATP (1 mM) | 93± 2 | 112 ± 1 |
| ADP (1mM) | 87 ± 1 | 110 ± 1 |
| AMP (1mM) | 109 ± 1 | 108 ± 2 |
| PPi(1 mM) | 100 ± 2 | 109 ± 1 |

**Supplementary Table S2.** The effects of various metabolites on the activity of GDHs from methanotrophs

**Supplementary Table S3.** The effects of various metabolites on the activity of methanotrophic GntKs

|  |  |
| --- | --- |
| Effector (Concentration) | Relative activity (%) |
| *Mtm. alcaliphilum* 20Z | *Mb. luteus* |
| Without effector | 100 ± 1 | 100 ± 1 |
| Pyruvate (3) | 95 ± 2 | 105 ± 1 |
| ADP (5) | 71 ± 1 | 97 ± 1 |
| AMP (4) | 98 ± 1 | 111 ± 2 |
| 6-Phosphogluconate (5) | 81 ± 1 | 84 ± 2 |
| Phosphoenolpyruvate (5) | 84 ± 1 | 85 ± 1 |
| α-Ketoglutarate (1) | 90 ± 2 | 113 ± 1 |
| Oxaloacetate (1) | 101 ± 1 | 111 ± 1 |
| Glucose 1-Phosphate (5) | 96 ± 2 | 110 ± 1 |
| Glucose 6-Phosphate (5) | 96 ± 1 | 101 ± 2 |
| PPi (2) | 100 ± 1 | 105 ± 1 |

**Supplementary Table S4.** The primers used in the work. The restriction endonuclease sites are underlined

|  |  |  |
| --- | --- | --- |
| Primers | Target | Sequence (5’-3’) with indication of restriction endonuclease sites |
| GluDH-20Z-F (pET28)  | Cloning of the *Mtm. alcaliphilum gdh* gene in pET28 His6-tag at the N-terminus | TTCATATGCCGCTTATTATCGAGAA (NdeI) |
| GluDH-20Z-R (pET28) | TAAGCTTACCCGCCTTCGCGAAAGC (HindIII) |
| Ub-F | Cloning of the gene encoding ubiquitin in pET28 – resultant vector - pET28Ub-shHis | TTCCATGGGCCATCATCATCATCATCACCATATGCAAATCTTTGTG (NcoI) |
| Ub-R | TTCCGCGGAGGCGCAACACCAGGT (SacII) |
| GDH-20Z-F(SacII) | Cloning of the *Mtm. alcaliphilum gdh* gene in pET28Ub-shHis - His6-tag and ubiquitin at the N-terminus | TCCGCGGTGGAATGCCGCTTATTATCGAG(SacII) |
| GDH(Mlut98)-F | Cloning of the *Mb. luteus gdh* gene in pET28Ub-shHis - His6-tag and ubiquitin at the N-terminus | TCCGCGGTGGAATGCCATTACTGATCGAAAAGAC (SacII) |
| GDH(Mlut98)-R | TGAATTCAGCCGCCCTCGCGAAATC (EcoRI) |
| MtmGntk-F | Cloning of *Mtm. alcaliphilum gntk* gene in pET30  | TCATATGGATGGCGCGCAAGAAAATCGC (NdeI) |
| MtmGntk-R | TAAGCTTCGGCGAAAGACGGGTCGAGC (HindIII) |
| MbGntk-F | Cloning of *Mb. luteus gntk* gene in pET30 | TCATATGAATAACGAATTTGATGAC (NdeI) |
| MbGntk-R | TAAGCTTCAGGCCTAGATAAACCATTAT (HindIII) |
| dGDH2\_f1 | Upper flanking region of the *gdh* gene in *Mtm. alcaliphilum* | ATAGATCTGTTGCACTGCGACGAAATG (BglII)  |
| dGDH2\_r1 | AAGGTACCGGCATCGGACATATAATTGA (Acc65I) |
| dGDH2\_f2 | Lower flanking region of the *gdh* gene in *Mtm. alcaliphilum* | TACCGCGGAAGCCGATTTACTGAA (SacII)  |
| dGDHr | TT*GAGCTC*TATCCGTTGGCTGCCGA (SacI) |
| dGnKinF  | Cloning of the *gntk-gnl* DNA locus | ATGAATTCGGTCGATGAAACGGTCT (EcoRI) |
| dGnKinR | TTACGCGTTCTTTTCCGCATTCCAGT (MluI) |
| PamyF  | Cloning a DNA locus containing a putative promoter region of the *amy* gene | ATTCTAGATCCGCTCGTTGTCCAAA (XbaI) |
| PamyR | ATTTGACTCGTCAGTACTGAGTTGTGTTTTCGT |
| PglnF | Cloning a DNA locus containing a putative promoter region of the *gntk* gene | TATCTAGACTGGCGCGTTTTCTCGA (XbaI) |
| PglnR | ATTTGACTCGTCAGTCCGGC |
| SDglnK+CmF  | Cloning of the *cat* gene | ACTGACGAGTCAAATATGGAGAAAAAAATC |
| CmR  | AA*GCATGC*ATTAATCAGGCGTAGCAACCAGGCGT (SphI) |



**Supplementary Figure 1S**. Oligomeric state determination of the recombinant enzymes from *Mm. alcaliphilum* and *Mb. luteus*.Molecular mass determination using gel filtration chromatography. The proteins were detected by monitoring their absorbance at 280 nm.

1.6

1.4

1.2

1.0

0.8

0.6

0.4

0.2

1.6

1.4

1.2

1.0

0.8

0.6

0.4

0.2

**Supplementary Figure 2S**. The growth of *Methylotuvimicrobium alcaliphilum* 20Z (blue line), *gdh*-/*glk*- (green line) and *gntk*-/*glk*- (red line) mutant cells in the presence of 3% NaCl

 1 50 95

B. megaterium(1) ----------------MYTDLKDKVVVITGGSTGLGRAMAVRFGQEEAKVVINYYNNEEEALDAKKEVEEAGGQAIIVQGDVTKEEDVVNLVQTA

Mtm. alcaliph(1) ------------MPLIIEKSLAGQRALVTGSSSGIGAAIAVALAEAGARVAINYMSDAEGAGEIVDQIQALGGEAIAVQADVSQEDQVIAMFQQV

Mb. luteus (1) ------------MPLLIEKTLSGQRALITGGSSGIGAAVARAMAEAGARVAINYVSDAEDAQKLVDEIQAGGGEAFAVQADVSCEEEVKLMFEIV

Ms. fibrata (1) MSNSEPLPDVVMPFCPVRQMLRGQKALVTGAVSGIGRAVAIHMAQEGASVVINYASNDAGARDLVEEIEAKGGAAIAVKADVSDEEQVQAMFRRA

Mm. agile (1) ---------------------MNKVLVITGGSRGIGAATALLAAAREYTVCINYRKNQAAAEAVVDEIKSKGGNAIAVAADVSLESEVKNLFKVI

Mv. miyakonen(1) ------------MIPEQNLDLTGKVAFISGAAVGIGRAVALRLGKAGATIGLHYYSSSDAANALLAELAAANIKTLLLPADLTQEGQANAAIDKL

Mm. denitrify(1) -----------MNTATAAQPLQGRTALVVQANAGIGAAVAEAFAAAGARVMINYLHENAGADQVAQRIRIKQGQTMIFQADISQESQVKSMFDVL

Consensus (1) I L GK ALIT**G**ASS**G**I**G**AAVAL LA AGARV

 96 150 \* \* \*\* \*\* \* 188

B. megaterium (80) IKEFGTLDVMINNAGV-ENPVPSHELSLDNWNKVIDTNLTGAFLGSREAIKYFVENDIK--------GNVINMSSVHEMIPWP-LFVHYAASK

Mtm. alcaliph (84) IGTWGSLDILVNNAGI-QLDAPFVEMTLKQWETVMAVNLTGQFLCAREAVKEFLRRGVV-PELSCSAGKIICNSSVHDIIPWA-GHVNYSASK

Mb. luteus (84) IKRWGSLDILVANAGI-QLDSPVAEMTLAQWNKVLAVNLTGQFLCAREAVKEFLRRGLIP-ELSSAMGKIICMSSVHDVIPWA-GHINYAASK

Ms. fibrata (96) IAEFGTIDILVNNAGL-QKDSPFEEMTLSQWNTVLGVNLTGQFLCAREAVREFKRRGVVP-AVSCAAGKIISMSSVHEVIPWA-GHVNYAASK

Mm. agile (75) DEQLGPISALVNNAGILEKQMRVEDIDAARLHRIFAANVVGSFLCAREAVRRMSTKHGG------HGGAIVNVSSAASRLGSAGEYVDYAASK

Mv. miyakonen (85) VAETGRLDIVVNNSGGLVKRAKIEDCLLENWNKSLDINLTSAFLVTKRAIPHLRASGN---------GSIVNILSLSVQTGGANGGGAYAAAK

Mmn. denitrif (86) VAYWGGLDILVTNAHL-EIDNSALDINRKPSQTVLTQNLVGHFLCVRQAMHEFSRQGCQS-ALPNSAGNIICVSSLRNGLSQE-GQINYTVCK

Consensus (96) IA WGSLDILVNNAGI DAP EMTL QWNKVLAV**N**LTG FLCAREAVKEF RRGV L AAG II MSSV**H**DVIPWA GHVN**Y**AA**SK**

 189 \* \* \* \* 250 281

B. megaterium(163) GGMKLMTETLALEYAPKGIRVNNIGPGAMNTPINAE**K**FADPVQRADVESMIPMGYIGKPEEVAAVAAFLASSQASYVTGITLFADGGMTKYPS

Mtm. alcaliph(174) GGLLMFMKSLAQEVAHDKIRVNAVSPGAIKTPINRSAWETPEAEA**D**LLKLIPYERVGESADIARAVVWLASDASDYVVGTTLYIDGGMTLYPG

Mb. luteus (174) GGVLMFMKSLAQEVAHQRIRVNAISPGAIRTPINRSAWETSEAER**E**LLKLIPYERIGEPMDIGHAAVWLASDASDYVVGTTLYIDGGMTLYPG

Ms. fibrata (186) GGVMLMTKSIAQEVAPYRIRINSVCPGAIRTPINRDAWGTQEAYAELMKLVPYKRIGEPEDVARAVVWLASDDADYVHGVSLFVDGGMTLYPG

Mm. agile (162) GAIDTLTIGLSQEVAEEGIRVNAVRPAFIYTDMHADGGEPGR-VDRIKESLPMKRGGQPAEVANAILWLLSEEASYTTGTFIDLAGGK-----

Mv. miyakonen(168) GGLMVFTHTLAKELAP-LVRTNSVMPGTVETQHHEIHSTPEMMEN-YRKQTPLGRNTYAEEVASSVLFLSSNMASHINGALIDISGGRFLR--

Mmn. denitrif(175) PSMSSLLQDFAAALGQANTNID-LASADV--PSLAEYWGTNQ-----------------EDIANAAVGLAAAAYADPISNNLKIGGS------

Consensus (189) GGLLLLT SLAQEVA IRVNAV PGAI TPI**N** DAW T **D**L KLIP RIG PEDVA AVVWLASD ASYV G TLFIDGGMTLYP

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B. megaterium(256) FQAGRG

Mtm. alcaliph(267) FREGG-

Mb. luteus (267) FREGG-

Ms. fibrata (279) FETGG-

Mm. agile (249) ------

Mv. miyakonen(257) ------

Mmn. denitrif(242) ------

Consensus (282) F G

**Supplementary Figure 3S**. Multiple alignment of the primary structure of glucose 1-dehydrogenases. The square denotes Rossmann fold and amino acid residues forming hydrogen bonds with D-glucose. The asterisk indicates amino acid residues forming active site.