**Table S1**. Effect of overexpression of *ptsG* and *mtlA* on PEP-dependent phosphorylation of seven PTS sugars by crude extract preparations of recombinant *E. coli* strains BW25113-pMAL-*ptsG* and BW25113-pMAL-*mtlA* as compared to the control strain, BW25113-pMAL. The experiments were conducted in quadruplicate as indicated (columns 2-4), expressed as ratios (columns 5 and 8) and averaged (columns 6 and 9) with standard deviations (SD) (columns 7 and 10). The results of this and other experiments are summarized in Figure 2.

|  |  |  |
| --- | --- | --- |
| **PTS sugar** | **Specific Activity** **(CPM/μg)** | **Relative activity** |
| **WT-pMAL** | **WT-pMAL-*ptsG*** | **WT-pMAL-*mtlA*** | **OE *ptsG*/WT** | **OE *mtlA*/WT** |
| **Value** | **Aver-age** | **SD** | **Value** | **Aver-age** | **SD** |
| **Fructose** | 7 | 12 | 17 | 1.6 | 1.5 | 0.3 | 2.4 | 3 | 0.7 |
| 8 | 11 | 20 | 1.3 | 2.4 |
| 6 | 10 | 21 | 1.8 | 3.8 |
| 7 | 8 | 23 | 1.2 | 3.5 |
| **Mannitol** | 22 | 29 | 474 | 1.3 | 1.3 | 0.1 | 21.8 | (22.3) | 0.8 |
| 20 | 25 | 455 | 1.2 | 22.9 |
| **N-Acetylglucosamine** | 52 | 64 | 25 | 1.2 | 1.2 | 0.1 | 0.5 | 0.5 | 0.1 |
| 45 | 57 | 21 | 1.3 | 0.5 |
| 42 | 50 | 25 | 1.2 | 0.6 |
| 45 | 49 | 20 | 1.1 | 0.4 |
| **Methyl alpha glucoside** | 58 | 514 | 32 | 8.9 | (8.4) | 1.3 | 0.6 | 0.5 | 0.0 |
| 52 | 492 | 28 | 9.5 | 0.5 |
| 59 | 391 | 28 | 6.6 | 0.5 |
| 51 | 432 | 26 | 8.5 | 0.5 |
| **2-Deoxyglucose** | 19 | 147 | 17 | 7.8 | 7.9 | 1.3 | 0.9 | 0.8 | 0.1 |
| 19 | 136 | 13 | 7.2 | 0.7 |
| 16 | 160 | 14 | 9.8 | 0.8 |
| 18 | 126 | 11 | 6.9 | 0.6 |
| **Trehalose** | 37 | 180 | 19 | 4.9 | (4.9) | 0.5 | 0.5 | 0.6 | 0.1 |
| 30 | 166 | 16 | 5.5 | 0.5 |
| 28 | 120 | 19 | 4.2 | 0.7 |
| 31 | 158 | 16 | 5.1 | 0.5 |
| **Galactitol** | 142 | 42 | 22 | 0.3 | 0.3 | 0.0 | 0.2 | 0.2 | 0.0 |
| 141 | 48 | 25 | 0.3 | 0.2 |
| 135 | 44 | 27 | 0.3 | 0.2 |
| 139 | 47 | 24 | 0.3 | 0.2 |

**Table S2**. Effect of overexpression of *manY* or *manYZ* on PEP-dependent phosphorylation of seven PTS sugars by crude extract preparations of recombinant *E. coli* strains BW25113-pMAL-*manY* and BW25113-pMAL-*manYZ* as compared to the control strain, BW25113-pMAL. The table format is as described for Figure S1.

|  |  |  |
| --- | --- | --- |
| **PTS sugar** | **Specific Activity** **(CPM/μg)** | **Relative activity** |
| **WT-pMAL** | **WT-pMAL-*manY*** | **WT-pMAL-*manYZ*** | **OE *manY*/WT** | **OE *manYZ*/WT** |
| **Value** | **Aver-age** | **SD** | **Value** | **Aver-age** | **SD** |
| **Fructose** | 6 | 7 | 12 | 1.2 | 1 | 0.26 | 1.9 | 1.7 | 0.35 |
| 8 | 7 | 11 | 0.9 | 1.4 |
| **Mannitol** | 22 | 17 | 21 | 0.8 | 0.9 | 0.2 | 0.9 | 0.9 | 0.05 |
| 22 | 23 | 19 | 1 | 0.9 |
| **N-Acetylglucosamine** | 48 | 37 | 68 | 0.8 | 0.8 | 0.03 | 1.4 | 1.5 | 0.06 |
| 42 | 35 | 64 | 0.8 | 1.5 |
| **Methyl alpha glucoside** | 58 | 69 | 55 | 1.2 | 1.2 | 0.03 | 0.9 | 1 | 0.05 |
| 51 | 63 | 51 | 1.2 | 1 |
| **2-Deoxyglucose** | 31 | 23 | 57 | 0.7 | 0.7 | 0.01 | 1.8 | (1.9) | 0.05 |
| 29 | 22 | 56 | 0.8 | 1.9 |
| **Trehalose** | 36 | 35 | 38 | 1 | 1 | 0.01 | 1.1 | 1.1 | 0.01 |
| 34 | 33 | 35 | 1 | 1.1 |
| **Galactitol** | 98 | 73 | 36 | 0.7 | 0.7 | 0.01 | 0.4 | 0.4 | 0.01 |
| 96 | 71 | 34 | 0.7 | 0.4 |

# Table S3. Strains and plasmids used in this study

|  |  |  |
| --- | --- | --- |
| **Strains or plasmids** | **Genotype or description** | **Reference or source** |
| **Strains** |  |  |
| BW25113 | Wild type, *lacI*q *rrnB*T14 Δ*lacZ*WJ Δ*hsdR*514 Δ*araBAD*AH33 Δ*rhaBAD*LD78 | Wanner (2000) |
| BW\_P*mtlA*-*lacZ* | BW25113 in which P*mtlA* is fused to *lacZ* at the *lac* locus | This study |
| BW\_P*man*-*lacZ* | BW25113 in which P*manXYZ* is fused to *lacZ* at the *lac* locus | This study |
| BW\_P*gatY*-*lacZ* | BW25113 in which *wt* P*gatY* is fused to *lacZ* at the *lac* locus | This study |
| **Plasmids** |  |  |
| pMAL | pMAL-p2x in which the *BglI*I/*BamH*I flanked region containing *malE* is deleted  | This study |
| pMAL-*ptsG* | Ptac driving *ptsG* in pMAL | This study |
| pMAL-*galP* | Ptac driving *galP* in pMAL | This study |
| pMAL-*mtlA* | Ptac driving *mtlA* in pMAL | This study |
| pMAL-*manY* | Ptac driving *manY* in pMAL | This study |
| pMAL-*manYZ* | Ptac driving *manYZ* in pMAL | This study |
| pMAL-*ansP* | Ptac driving *ansP* in pMAL | This study |
| pKDT | Insertion of an *rrnB* terminator downstream of km gene in pKD13 | Klumpp et al.(2009) |
| pKDT\_P*mtlA* | P*mtlA* cloned downstream of the *rrnB* terminator in pKDT | This study |
| pKDT\_P*man* | P*man* cloned downstream of the *rrnB* terminator in pKDT | This study |
| pKDT\_P*gatY* | P*gatY* cloned downstream of the *rrnB* terminator in pKDT | This study |

**Table S4. Oligonucleotides used in this study**

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence** | **Use** |
| mtlAp-Xh-F | atactcgagacgattgtatgacgaaggcataacatg | Cloning P*mtlA* in pKDT |
| mtlAp-Bm-R | aatggatccaccatgttgctgaggaaacgaccaaag | Cloning P*mtlA* in pKDT |
| manXp-Xh-F | atactcgagaccttcctttgcaaacgaatgtgacaag | Cloning P*manX* in pKDT |
| manXp-Bm-R | ttaggatcctgccgttttaagcaactgctctgcag | Cloning P*manX* in pKDT |
| gatYp-Xh-F | atactcgagtgcctacatagcactgccacgtatg | Cloning P*gatY* in pKDT |
| gatYp-Bm-R | attggatccttagttgttcagcatctgctttgtcgatac | Cloning P*gatY* in pKDT |
| mtlAp-Z-P2 | cgacggccagtgaatccgtaatcatggtcatagctgtttcctgtgtgaaattacatgttgctgaggaaacgaccaaagc | P*mtlA*:*lacZ* transcriptionalfusion |
| manXp-Z-P2 | cgacggccagtgaatccgtaatcatggtcatagctgtttcctgtgtgaaattaaagcaactgctctgcagcccaaccatg | P*manX*:*lacZ* transcriptionalfusion |
| gatYp-Z-P2 | cgacggccagtgaatccgtaatcatggtcatagctgtttcctgtgtgaaattagttgttcagcatctgctttgtcg | P*gatY*:*lacZ* transcriptionalfusion |
| ptsGhis-Nd-F | ttacatatgtttaagaatgcatttgctaacctgc | Cloning *ptsG* into pMAL |
| ptsGhis-Sal-R | aatgtcgacttagtgatgatgatgatgatggtggttacggatgtactcatccatctc | Cloning *ptsG* into pMAL |
| galP-Nd-F | atacatatgcctgacgctaaaaaacaggggcggtc | Cloning *galP* into pMAL |
| galPhis-Sal-R | aatgtcgacttagtgatgatgatgatgatgacctccaccatcgtgagcgcctatttcgcgcag | Cloning *galP* into pMAL |
| mtlA-Nd-F | aatcatatgtcatccgatattaagatcaaagtgc | Cloning *mtlA* into pMAL |
| mtlAhis-Sa-R | aatgtcgacttagtgatgatgatgatgatgcttacgacctgccagcagttccagcac | Cloning *mtlA* into pMAL |
| manY-Nd-F | tatcatatggagattaccactcttcaaattgtgc | Cloning *manY* into pMAL |
| manYhis-Sa-R | aatgtcgacttagtgatgatgatgatgatggtccagttcgttatcgagatcgttgttac | Cloning *manY* into pMAL |
| manZ-Sal-R | attgtcgacttacagtcccagcaggccgcaagcgtaac | Cloning *manYZ* into pMAL |
| pMAL-ver-F | tcgtataatgtgtggaattgtgagc | Verifying DNA cloning in pMAL |
| ptsG-ver-F1 | taaccgtttctaccgtattaagctg | Verifying *ptsG* cloning in pMAL |
| ptsG-ver-F2 | tatgatctccgcggcgctgacctc | Verifying *ptsG* cloning in pMAL |
| galP-ver-F1 | tgatctcgatgtatcagttgatgatc | Verifying *galP* cloning in pMAL |
| galP-ver-F2 | tgaccaacgtacttgccacctttatcg | Verifying *galP* cloning in pMAL |
| mtlA-ver-F1 | tcgctattctggcattcctcggcattg | Verifying *mtlA* cloning in pMAL |
| mtlA-ver-F2 | acaccaaaaggtgcttacttcgctaac | Verifying *mtlA* cloning in pMAL |
| mtlA-ver-F3 | atttcgctgaccaacttcctcgacagc | Verifying *mtlA* cloning in pMAL |
| manY-ver-F | tactattaccgttgctttccagcac | Verifying *manY* cloning in pMAL |
| manZ-ver-F2 | tggttgatacaactcaaactaccac | Verifying *manYZ* cloning in pMAL |
| manZ-ver-F3 | tggtgcgtctggcaacccgttactac | Verifying *manYZ* cloning in pMAL |