

Supplemental

Figure S1. Organization of the *agaSK* gene cluster. The arrows represent the different genes and their orientation shows the transcriptional direction. P1 to P4 are putative promoters; \Uparrow are potential stem-loop structures that might act as rho-independent transcriptional terminators. The free energy of the thermodynamic ensemble is given on top, expressed as kcal.mol⁻¹. MM: theoretical molecular mass and F: putative function.

Figure S2. Expression profile of transcripts originating from the *agaSK* gene. (A) RT-PCR amplifications using mRNA obtained from *in vivo* conditions (see Experimental Procedures). (1) amplification of 16S; (2) amplification of *agaSK*; (3) negative control of 16S; (4) negative control of *agaSK* (B) PCR amplification from genomic DNA (5) amplification of 16S; (6) negative control of 16S; (7) amplification of *agaSK*; (8) negative control of *agaSK*.

Figure S3. Amino acid sequence of AgaSK. The α -galactosidase domain is underlined with bars of the same colour hues as in Fig. 3. The kinase domain is underlined with an orange bar. Secondary structure elements are shown above the sequence. The Walker A-motif is underlined in green.

Figure S4. HPAEC analysis of melibiose (A) and raffinose (B) hydrolysis by AgaSK. Dark lines represent the sample medium with inactivated enzyme and dashed lines represent the sample medium with AgaSK.

Figure S5. Binding of ATP to AgaSK. The effect of ATP on AgaSK and NATA fluorescence spectra is followed. Increasing concentrations of ATP were added to a 2 ml assay containing 0.1 μ M AgaSK or N-acetyl-tryptophanamide (NATA). Fluorescence intensity was recorded between 310 and 380 nm upon excitation at 282 nm after each addition of ligand. Each curve was corrected for the fluorescence of the buffer alone containing the same ligand concentration. From the upper to the lower curves, the concentration of ATP was 0, 25, 50, 75, 100, 125, 175, 200, and 250 μ M respectively. The AgaSK fluorescence spectrum is centered at 340 nm and NATA spectrum at 357 nm.

Figure S6. MS spectra of phosphorylated sugar. (A) Positive MALDI-MS mass spectrum of control sample. Sodiated ion of raffinose is annotated at m/z 527.144. ATP ions are indicated with a black star ($[M+H]^+$ m/z 507.999; $[M+Na]^+$ m/z 529.983; $[M+2Na-H]^+$ m/z 551.967; $[M+3Na-2H]^+$ m/z 573.949; $[M+4Na-3H]^+$ m/z 595.931). (B) Positive MALDI-MS mass spectrum of sample from enzymatic reaction. Sodiated ion of phosphorylated sucrose is annotated at m/z 445.059 ($[M+Na]^+$) and 467.038 ($[M+2Na-H]^+$). ADP ions are indicated with a black star ($[M+H]^+$ m/z 428.026; $[M+Na]^+$ m/z 450.008; $[M+2Na-H]^+$ m/z 471.990; $[M+3Na-2H]^+$ m/z 493.970).