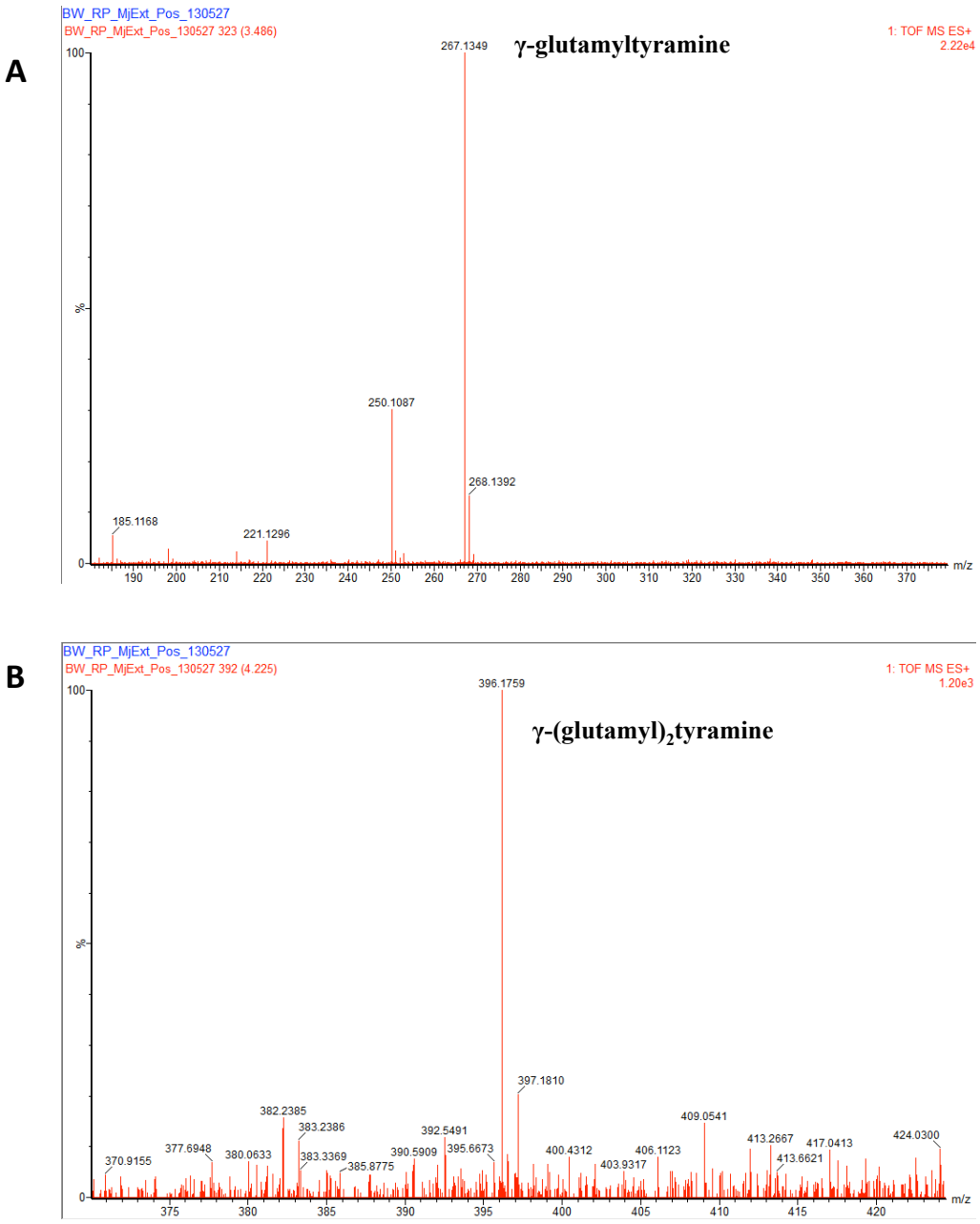
SUPPLEMENTAL MATERIALS

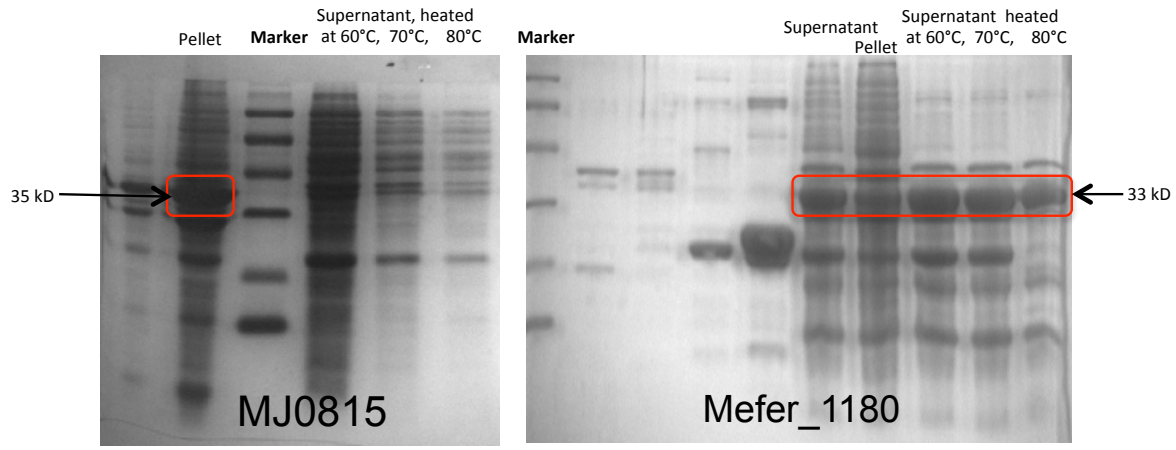
Preparation and High-Resolution Mass Spectral Analysis of Cell Extracts. Frozen cell pellets (1 g) of *M. jannaschii* were added to a screw capped test tube and suspended in 5 ml water. Six ml of methanol was added to this suspension and the sample was sealed and heated with shaking for 5 min at 100 °C. The heating was to ensure that all proteins were denatured so that any bound cofactors would be released. After cooling, the samples were centrifuged and the pellets re-extracted in the same manner. The resulting extracts were combined and evaporated to 1 mL using a stream of nitrogen. Large molecules were removed from the sample by passing through an Amicon Ultra 3K centrifugal filter. The final sample was concentrated to 0.15 mL for analysis. The cell extracts were then analyzed directly on a Waters SYNAPT G2-S high definition mass spectrometer connected to a Waters Acquity UPLC I-class system with an Acquity UPLC BEH C18 column (Waters, 2.1 mm x 75 mm, 1.7 um particle size). Solvent A was water with 0.1% formic acid and solvent B was acetonitrile. The flow rate was 0.2 mL/min and gradient elution was employed in the following manner (t (min), %B): (0.01, 5), (6, 15), (21, 35), (23, 65). Ten μL of the sample was injected. The mass spectral data were collected in high resolution MSe continuum mode. A lock spray scan (Function 3) was collected every 20 seconds for calibration and the lock spray analyst used was leucine-enkephalin. Parameters were: 2.8 kV capillary voltage, 125 °C source temperature, 350 °C desolvation temperature, 35 V sampling cone, 50 l/h cone gas flow, 500 l/h desolvation gas flow, and 6 l/h nebulizer gas flow. The collision energies for the low energy scans (Function 1) were 4V and 2V in the trap and the transfer regions, respectively. Collision energies for the high energy scans (Function 2) were ramped from 25 to 45 V in the trap region and 2V in the transfer region.

Analysis of Tyramine in Cell Extracts of Organisms containing Methanofuran. Cell extracts of *M. jannaschii*, *Methanothermobacter thermautotrophicus*, *Methanosarcina thermophia*, *Methanococcus vanniellii, Methanosarcina barkeri*, and *Archaeoglobus fulgidus* were prepared as described for the *M. jannaschii* cells. The proteins were precipitated by the addition of 20 μl of 2 M trichloroacetic acid (TCA) to 0.1 ml of each extract and the protein precipitate was separated by centrifugation (16000*g* for 10 min). The resulting clear extract was diluted 3-fold with water and applied to a small (2 ×10 mm) Dowex 50W-8X-H^+ column. The column was washed with 400 μL of water and the amino containing compounds were eluted with 400 μL 6 M NH_4OH. The eluted amino acids were converted into their trifluoroacetyl methyl ester (TM) derivatives and assayed by gas chromatography-mass spectrometry (GC-MS) as previously described (5).

GC-MS analysis of the amino acid fraction of cell extracts from five different methanogens as well as *A. fulgidus* all demonstrated the presence of free tyramine. The molar ratios of free tyramine to free tyrosine obtained from the following organisms were: *Methanothermobacter thermautotrophicus* (8.0), *Methanosarcina thermophia* (2.3), *Methanococcus vanniellii* (0.9*), Methanosarcina barkeri* (1.0), *M. jannaschii* (0.2), and *Archaeoglobus fulgidus* (1.4). Tyramine was not detected in similarly prepared extracts of *E. coli* or yeasts. The electron impact mass spectra (70 eV) of the ditrifluoroacetyl derivative of a known sample of tyramine and of tyramine isolated from *Methanosarcina barkeri* and confirmed the presence of tyramine in each of these organisms.



Supplemental Figure 1. Positive ion mode data from UPLC-ESI-HR-MS obtained from a cell extract of *M. jannaschii.* The components of the peak $MH^+ = 267.13$ *m/z* (A) and $MH^+ = 396.18$ *m/z* (B) represent γ-glutamyltyramine and γ-(glutamyl)$_2$tyramine, respectively.



Supplemental Figure 2. Overexpression of the Gene Products of MJ0815 and Mefer_1180. Frozen *E. coli* cell pellets (~0.4 g wet weight from 200 mL of media) were suspended in 3 mL of extraction buffer (50 mM TES, 10 mM $MgCl_2$, 20 mM DTT at pH 7.0) and lysed by sonication. After sonication, cell lysates were centrifuged to separate the supernatant from the lysate pellet. The supernatant was then heated for 10 min at 60, 70, and 80 °C followed by centrifugation (16000*g* for 10 min) to test the protein stability. The insoluble protein from the lysate pellet was suspended and solubilized by 8 M urea. All the resulting samples were loaded to SDS-gel.

Supplemental Table 1. The peptides identified by MALDI-MS from in-gel trypsin degestion.

Protein Hit	Identified peptides	Observed molecular weight $[M + H]^+$	Calculated molecular weight [M]
Mefer_1180			
	QYIDER	823.3430	822.3872
	IYPLSLNR	975.6118	974.5549
	YLTYLTIK	1014.5035	1013.5797
	GFVGGEVNIK	1019.4811	1018.5448
	KIYPLSLNR	1103.5906	1102.6498
	LKDEIFNEAIK	1319.6206	1318.7132
	DFADDYKDFENLK	1619.5977	1618.7151