

Table 1. Tandem mass spectrometric sequencing of a peptide containing the pyrrolysyl residue from recombinant MtmB1 synthesized by *E. coli* expressing *pylTSBCD*.

Sequence	b-series ions			a-series ions			y-series ions		
	Ion designation	Measured <i>m/z</i>	Δm between ion and preceding ion in series	Ion designation	Measured <i>m/z</i>	Δm between ion and preceding ion in series	Ion designation	Measured <i>m/z</i>	Δm between ion and preceding ion in series
Ala									
Gly	b-2	129.11							
Arg	b-3	285.16	156.05						
Pro	b-4	382.18	97.03				y-12	1281.63	
Gly									
Met	b-6	570.28	188.10 (G+M)						
Gly	b-7	627.30	57.02						
Val	b-8	726.38	99.08	a-8	698.37				
Pyl	b-9	963.53	237.15	a-9	935.54	237.17	y-7	840.44	237.12
Gly	b-10	1020.54	57.01	a-10	992.55	57.01	y-6	603.32	57.04
Pro	b-11	559.30 ⁺	97.06				y-5	546.28	226.10 (E+P)
Glu	b-12	1246.66	129.06	a-12	1218.64	226.09 (P+E)			
Thr	b-13	674.35 ⁺	101.04				y-3	320.18	101.08
Ser	b-14	717.87 ⁺	87.04				y-2	219.10	87.00
Leu							y-1	132.10	

In gel-chromatrypsin digestion of the urea-solubilized 50-kDa recombinant MtmB1 protein produced a peptide of $m/z=783.5$ for the doubly charged ion, which was then subjected to collision-induced dissociation (CID) mass spectrometry. The observed masses for the b-ion (N-terminal ion from peptide bond dissociation), y-ion (C-terminal ion from peptide bond dissociation), and a-ion (N-terminal ion from alpha and carbonyl carbon bond dissociation) series given above were all within 0.05 Da of the predicted masses for the MtmB peptide with sequence AGRPGMGVOGPETSL (where O=pyrrolysine). Bolded numbers highlight the individual masses of peptide residues as calculated by the difference in masses of an ion and the preceding ion within each series. Where an ion in a series

was not identified, the calculated Δm of the flanking ions reveals the summed mass of the two residues indicated in parenthesis. The observed mass of the pyrrolysyl residue calculated from the indicated a-, b-, and y-ions averaged 237.15 Da. The predicted mass of the pyrrolysine residue is 237.16 Da.