Crystal structure of Rv3717 reveals a novel amidase from *Mycobacterium tuberculosis*

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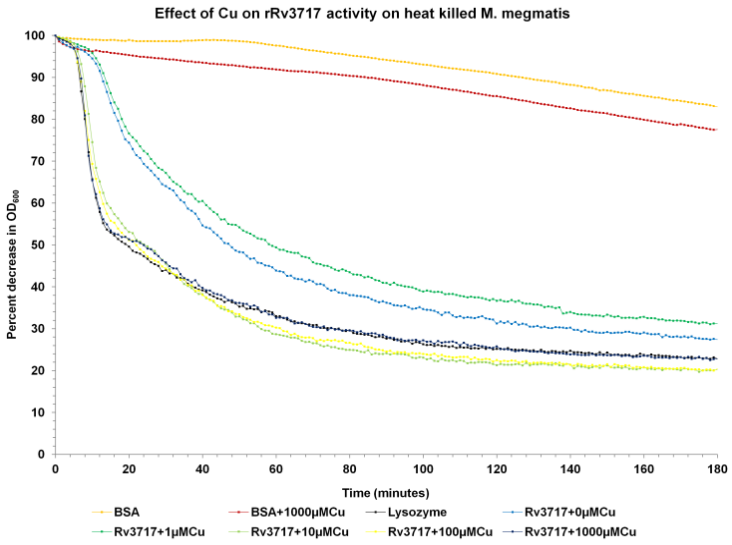
Supplementary data

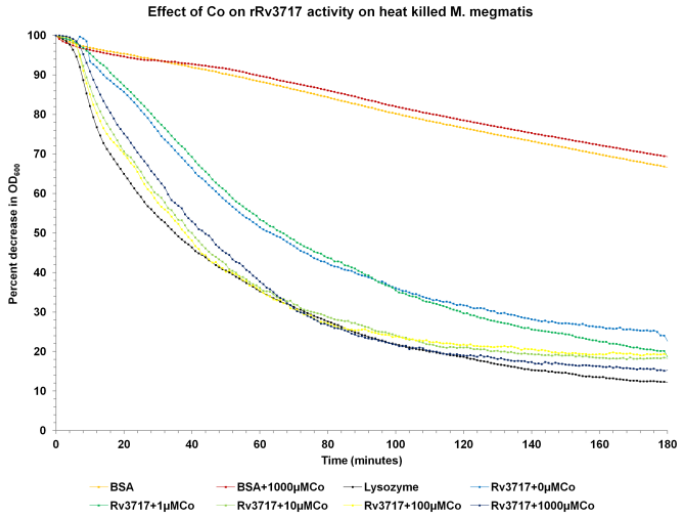
Supplementary Figure S1: Activity of rRv3717-A on heat killed *M. smegmatis* in presence of different metal ions

Activity of rRv3717-A was estimated with 0 (blue), 1(sea green), 10 (light green), 100 (yellow) or 1000 μm (brown) of (A) Cu^{2+}, (B) Co^{2+}, or (C) Mg^{2+} at 37°C. BSA (orange) or BSA+ 1000 μm of respective cation (red) was used as negative control; while lysozyme (black) served as positive control for the reaction.

A.



B.


Effect of Co on rRv3717 activity on heat killed M. megmatis
Percent decrease in OD600
Time (minutes)
BSA
BSA+1000µMCo
Lysozyme
Rv3717+0µMCo
Rv3717+1µMCo
Rv3717+10µMCo
Rv3717+100µMCo
Rv3717+1000µMCo

C

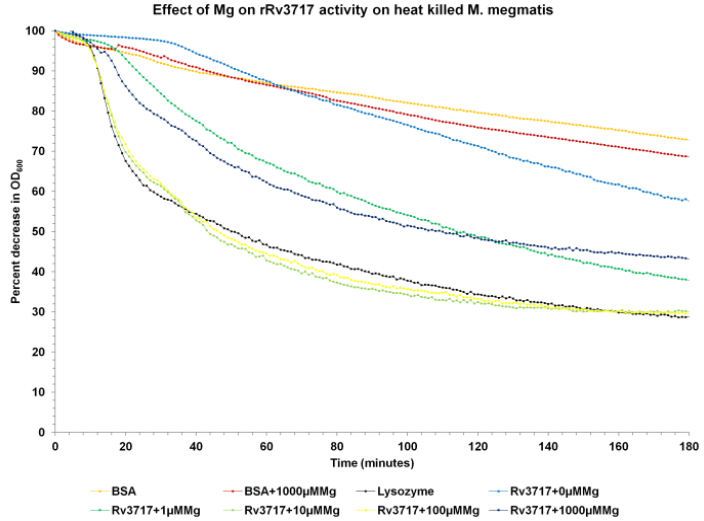
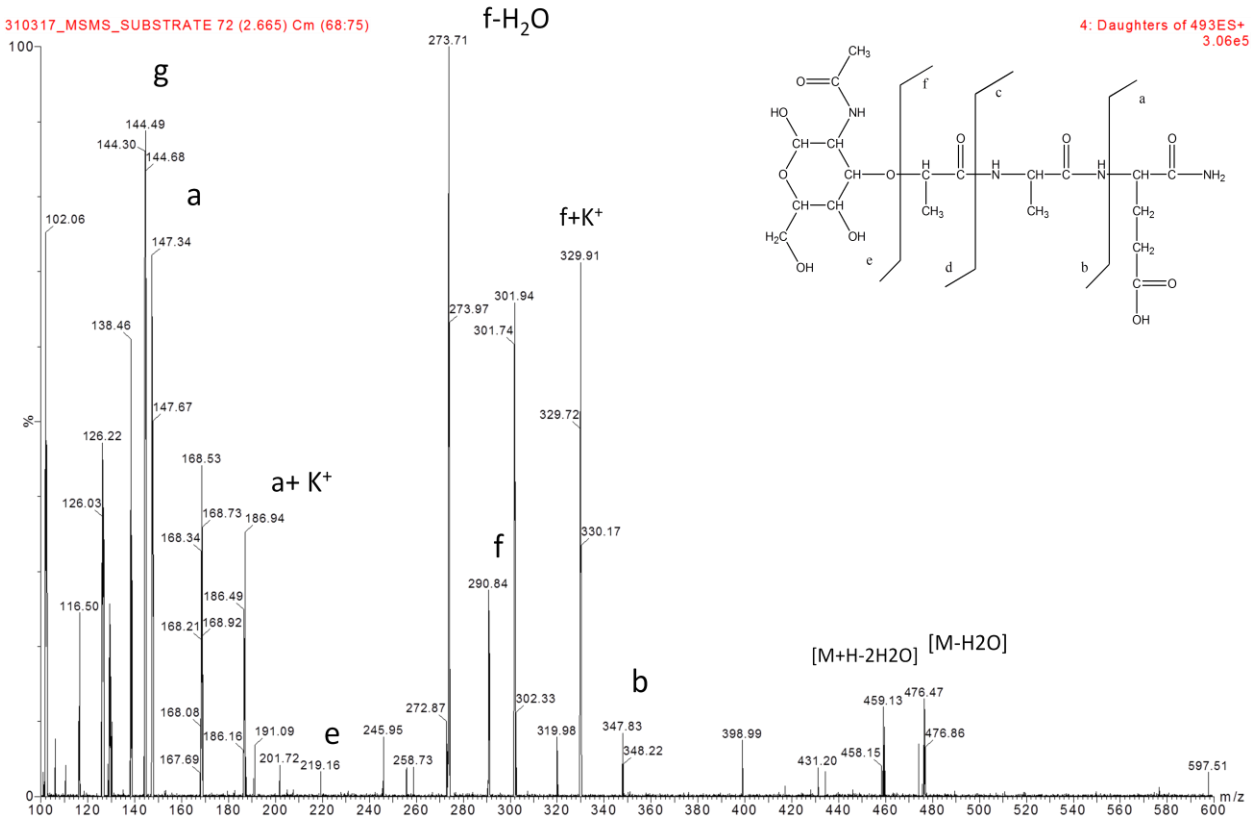
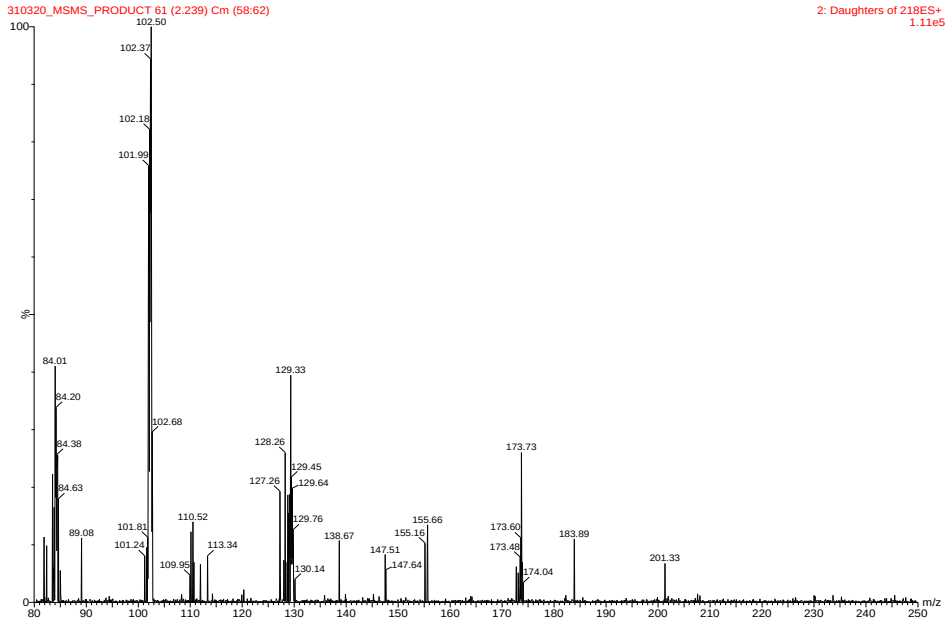

Effect of Mg on rRv3717 activity on heat killed M. megmatis
Percent decrease in OD600
Time (minutes)
BSA
BSA+1000µMMg
Lysozyme
Rv3717+0µMMg
Rv3717+1µMMg
Rv3717+10µMMg
Rv3717+100µMMg
Rv3717+1000µMMg

Figure S2: MS/MS analysis of (A) substrate, N-acetylmuramoyl-L-alanyl-D-isoglutamine **(B) dipeptide,** L-alanyl-D-isoglutamine **and (C) N-acetyl muramic acid**

A



B


310320_MSMS_PRODUCT 61 (2.239) Cm (58:62)
2: Daughters of 218ES+
1.11e5
100
%
0
102.50
102.37
102.18
101.99
84.01
84.20
84.38
84.63
89.08
102.68
101.81
101.24
110.52
109.95
113.34
128.26
129.33
129.45
129.64
127.26
129.76
130.14
138.67
147.51
147.64
155.16
155.66
173.73
173.60
173.48
174.04
183.89
201.33
80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250
m/z

C


310320_MSMS_PRODUCT 71 (2.627) Cm (68:75)
4: Daughters of 294ES+
6.01e5
100
%
0
138.55
138.36
138.23
126.65
126.40
126.21
144.40
144.85
98.10
98.23
84.43
97.59
99.18
109.55
124.11
168.51
168.83
186.78
80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300
m/z

Figure S3: MS analysis of N-acetylmuramoyl-L-alanyl-D-isoglutamine in the control reaction (no protein) after 4 hours at 37ºC