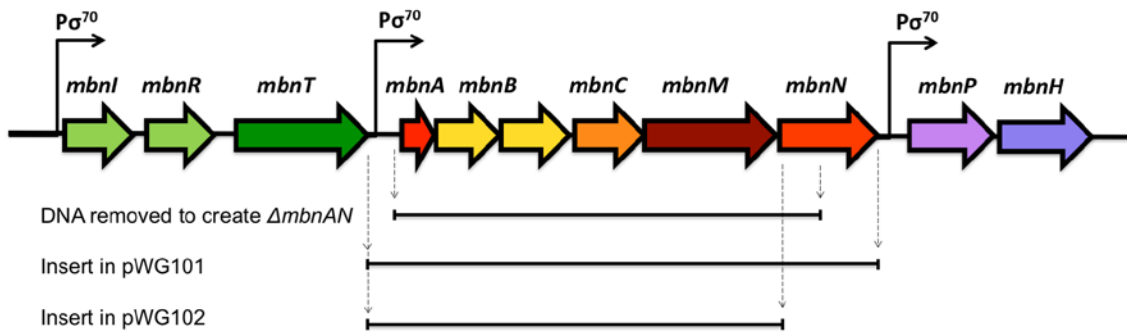


1



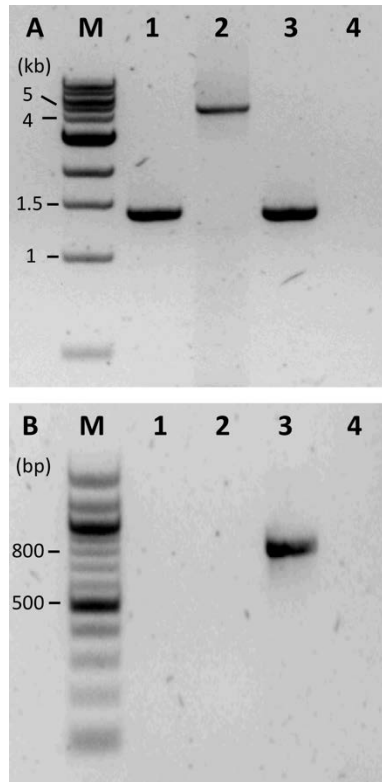
2

3 **Figure S1.** DNA removed to construct *M. trichosporium* OB3b $\Delta mbnAN$ mutant and inserts used to
4 construct pWG101 and pWG102.

5

6

7



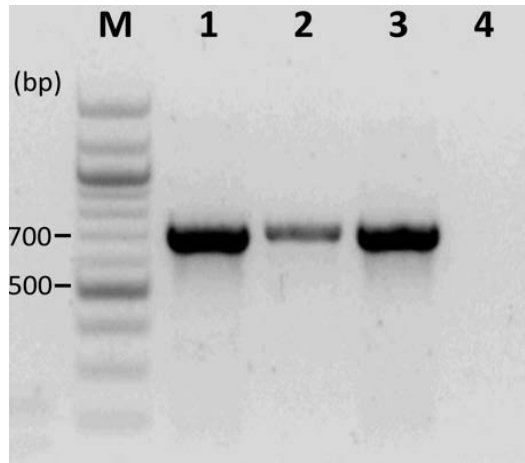
8

9

10 **Figure S2.** Confirmation of construction of *M. trichosporium* OB3b Δ *mbnAN* mutant. Figure S1A - PCR of
11 *mbnABC MN* using primers *mbn21/22*. M - molecular weight markers. Lanes 1 – 4 are PCR of
12 *mbnABC MN* from: 1 - *M. trichosporium* OB3b *mbnAN* mutant (expected product of 1.4 kb); 2 - *M.*
13 *trichosporium* OB3b wildtype (expected product of 4.8 kb); 3 - pWG012 plasmid (positive control;
14 expected product of 1.4 kb); 4 - distilled deionized water (negative control). Figure S1B - PCR of
15 pK18mobsacB plasmid backbone using primers pK18-bb-F/R. M - molecular weight markers; Lanes 1 – 4
16 are PCR of pK18mobsacB plasmid backbone from: 1 - *M. trichosporium* OB3b *mbnAN* mutant; 2 - *M.*
17 *trichosporium* OB3b wildtype; 3 - pWG012 plasmid (positive control; expected product of 800 bp); 4 -
18 distilled deionized water (negative control).

19

20



21

22

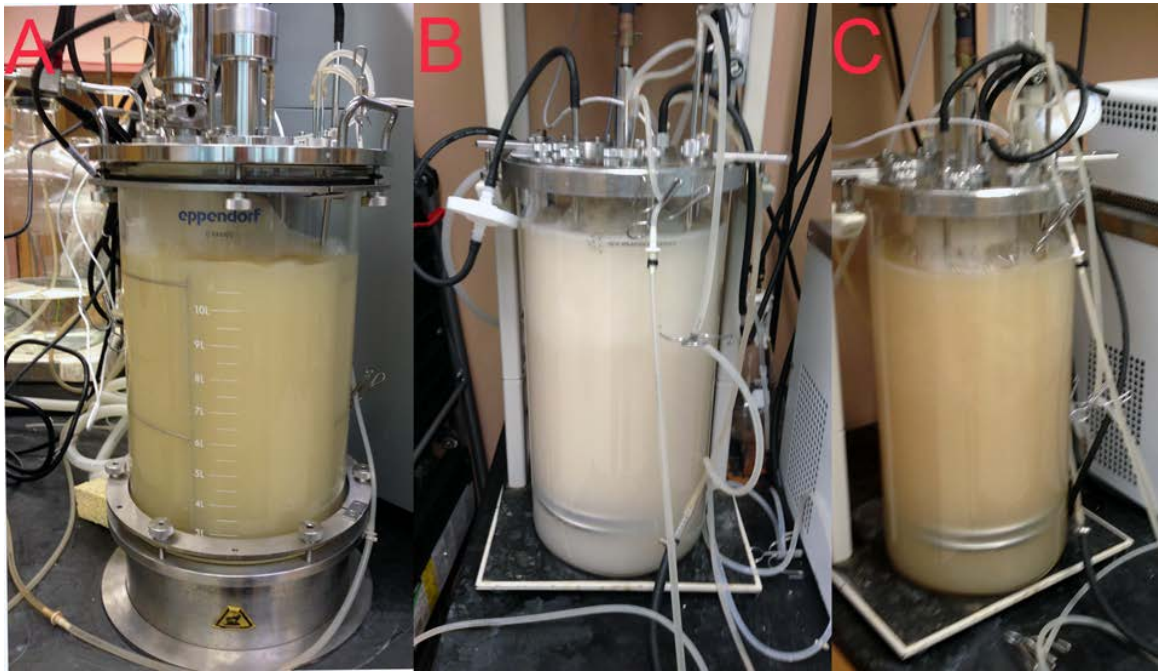
23 **Figure S3.** Confirmation of expression of *mbnPH* in $\Delta mbnAN$ mutant using primers *mbnPH*-F/R
24 (expected product size of 700 bp) M: molecular weight markers. Lanes 1 – 4 are RT-PCR of *mbnPH* from:
25 1 - *M. trichosporium* OB3b $\Delta mbnAN$ grown in the absence of copper; 2 - *M. trichosporium* OB3b
26 $\Delta mbnAN$ mutant grown in the presence of 1 μ M copper; 3 - *M. trichosporium* OB3b wild type grown in
27 the absence of copper (positive control); (4) distilled deionized water (negative control).

28

29

30

31

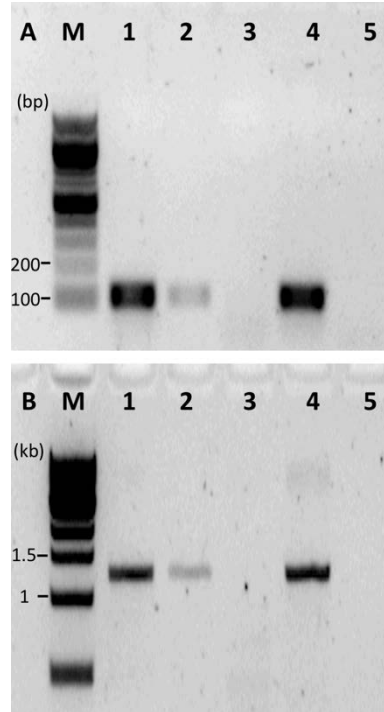


32

33

34 **Figure S4.** Fermenters of (A) wild-type *M. trichosporium* OB3b, (B) $\Delta mbnAN$, and (C) $\Delta mbnAN$ +
35 pWG101 cultured in NMS media amended with 0.2 μM CuCl_2 .

36



37

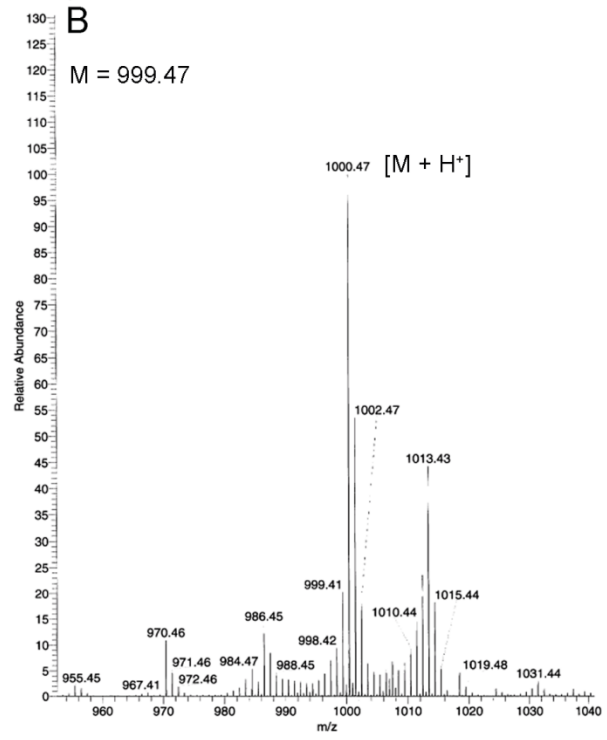
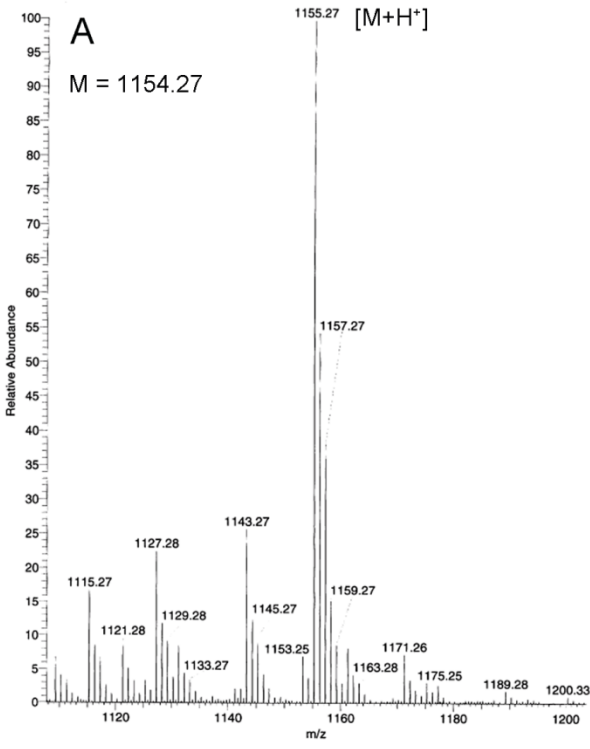
38

39 **Figure S5.** Expression of *mbnA* and *mbnN* in $\Delta mbnAN$ + pWG101. Figure S3A - RT-PCR of *mbnA* using
 40 primers qmbnA_FO/RO (expected product of 100 bp). M - molecular weight markers. Lanes 1 -5 are RT-
 41 PCR of *mbnA* from: 1 - *M. trichosporium* OB3b $\Delta mbnAN$ + pWG101 grown in the absence of copper; 2 -
 42 *M. trichosporium* OB3b $\Delta mbnAN$ + pWG101 grown with 1 μ M copper; 3 - *M. trichosporium* OB3b
 43 $\Delta mbnAN$ grown in the absence of copper (negative control); (4) *M. trichosporium* OB3b wild type grown
 44 in the absence of copper (positive control); (5) distilled deionized water (negative control). Figure S3B -
 45 RT-PCR of *mbnN* using primers mbnN-F/R (expected product size of 1.2 kb). M molecular weight
 46 markers . Lanes 1 – 5 are RT-PCR of *mbnN* from: 1 - *M. trichosporium* OB3b $\Delta mbnAN$ + pWG101 grown
 47 in the absence of copper; 2 - *M. trichosporium* OB3b $\Delta mbnAN$ + pWG101 grown in the presence of 1 μ M
 48 copper; 3 - *M. trichosporium* OB3b $\Delta mbnAN$ grown in the absence of copper (negative control); 4 - *M.*
 49 *trichosporium* OB3b wild type grown in the absence of copper (positive control); 5 - distilled deionized
 50 water (negative control).

51

52

53



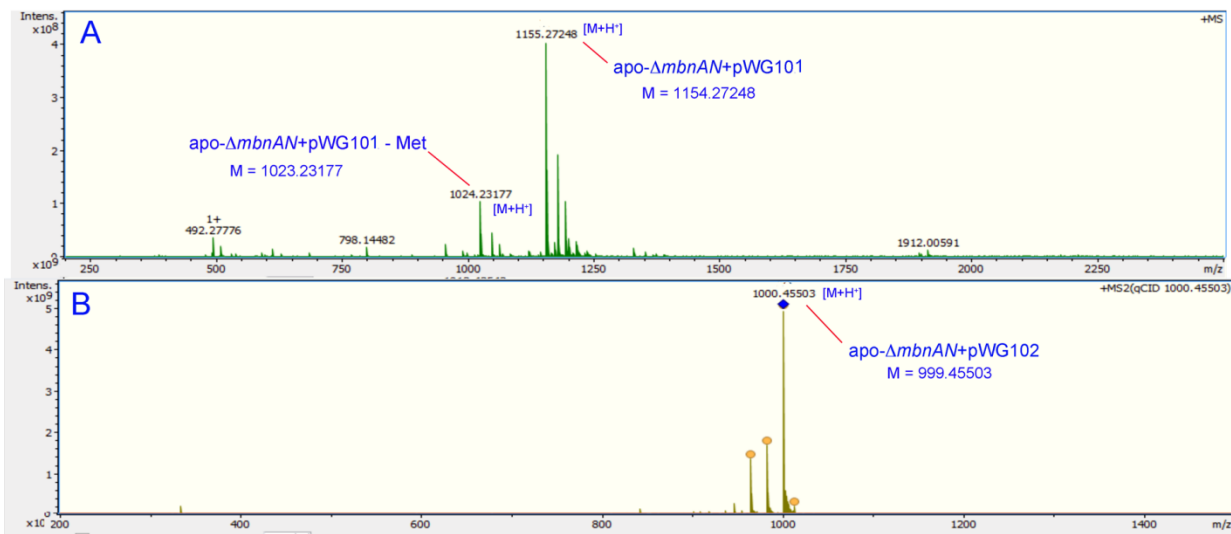
54

55

56 **Figure S6.** LC-MS/MS mass spectra of methanobactin from (A) $\Delta mbnAN$ + pWG101 (B) $\Delta mbnAN$ +
 57 pWG102 (B).

58

59

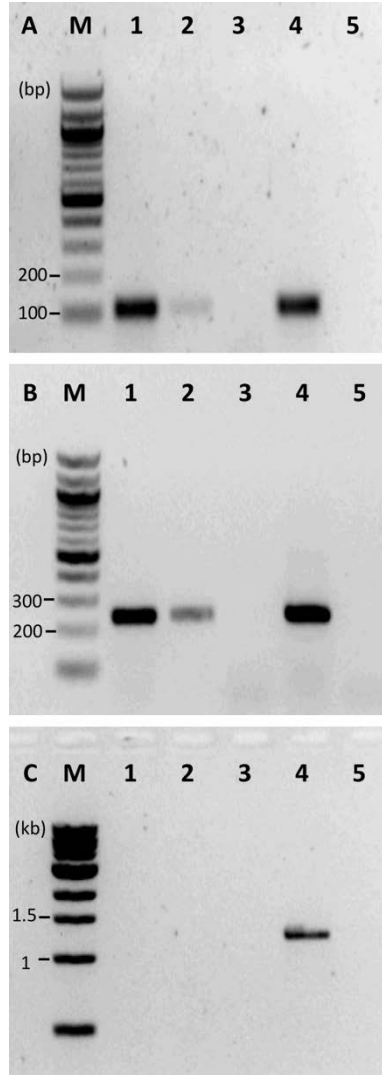


60

61 **Figure S7.** FT-ICR mass spectra of methanobactin from (A) *ΔmbnAN* + pWG101 and (B) *ΔmbnAN* +

62 pWG102. Samples were collected from a Dianion HP-20 column.

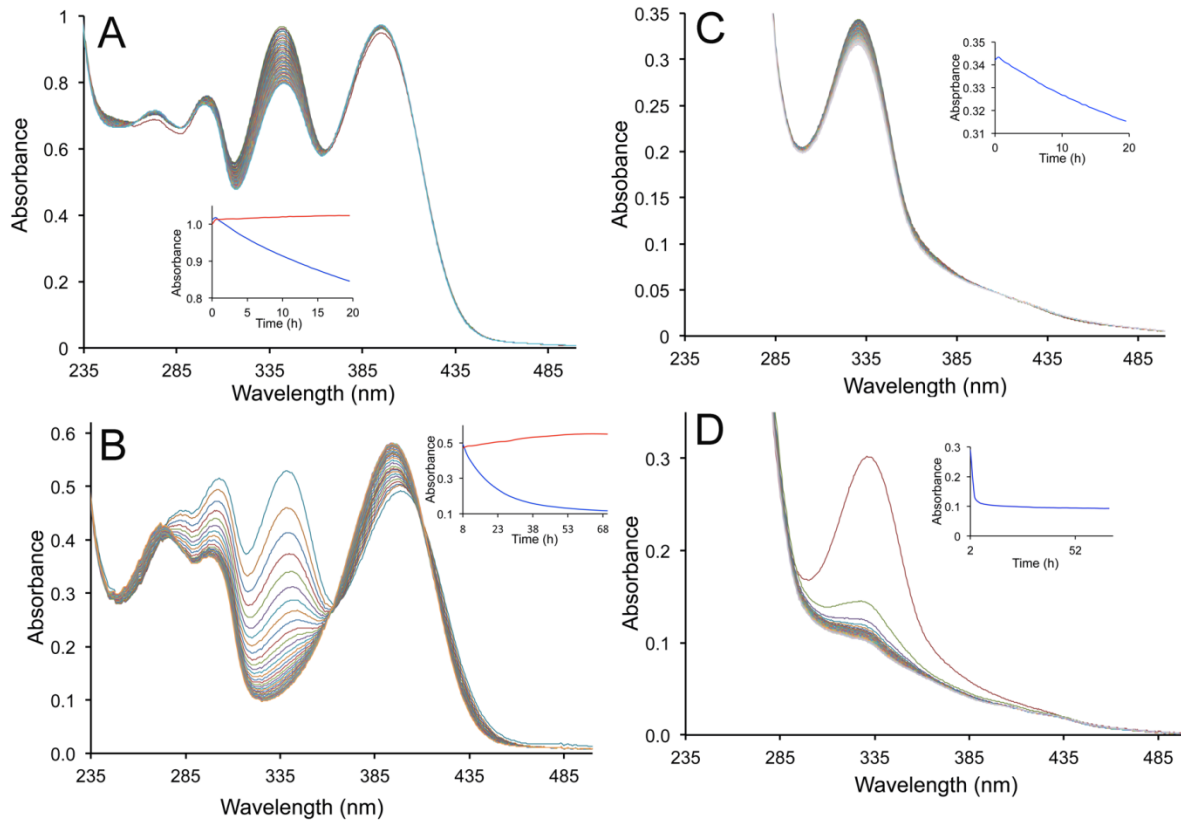
63



64

65 **Figure S8. Expression of *mbnA*, *mbnM* and *mbnN* in $\Delta mbnAN$ + pWG102.** Figure S4A – RT-PCR of *mbnA*
66 using primers qmbnA_FO/RO (expected product size of 100 bp). M - molecular weight markers. Lanes 1
67 -5 are RT-PCR of *mbnA* from: 1 - *M. trichosporium* OB3b $\Delta mbnAN$ + pWG102 grown in the absence of
68 copper; 2 - *M. trichosporium* OB3b $\Delta mbnAN$ + pWG102 grown with 1 μ M copper; 3 - *M. trichosporium*
69 OB3b $\Delta mbnAN$ grown in the absence of copper (negative control); (4) *M. trichosporium* OB3b wild type
70 grown in the absence of copper (positive control); (5) distilled deionized water (negative control). Figure
71 S4B – RT-PCR of *mbnM* using primers mbnM-F/R (expected product size of 220 bp). M - molecular
72 weight markers. Lanes 1 -5 are RT-PCR of *mbnM* from: 1 - *M. trichosporium* OB3b $\Delta mbnAN$ + pWG102
73 grown in the absence of copper; 2 - *M. trichosporium* OB3b $\Delta mbnAN$ + pWG102 grown with 1 μ M
74 copper; 3 - *M. trichosporium* OB3b $\Delta mbnAN$ grown in the absence of copper (negative control); (4) *M.*
75 *trichosporium* OB3b wild type grown in the absence of copper (positive control); (5) distilled deionized
76 water (negative control). Figure S4C – RT-PCR of *mbnN* using primers mbnN-F/R (expected product size
77 of 1.2 kb). M - molecular weight markers. Lanes 1 -5 are RT-PCR of *mbnN* from: 1 - *M. trichosporium*
78 OB3b $\Delta mbnAN$ + pWG102 grown in the absence of copper; 2 - *M. trichosporium* OB3b $\Delta mbnAN$ +
79 pWG102 grown with 1 μ M copper; 3 - *M. trichosporium* OB3b $\Delta mbnAN$ grown in the absence of copper
80 (negative control); (4) *M. trichosporium* OB3b wild type grown in the absence of copper (positive
81 control); (5) distilled deionized water (negative control).

82



83

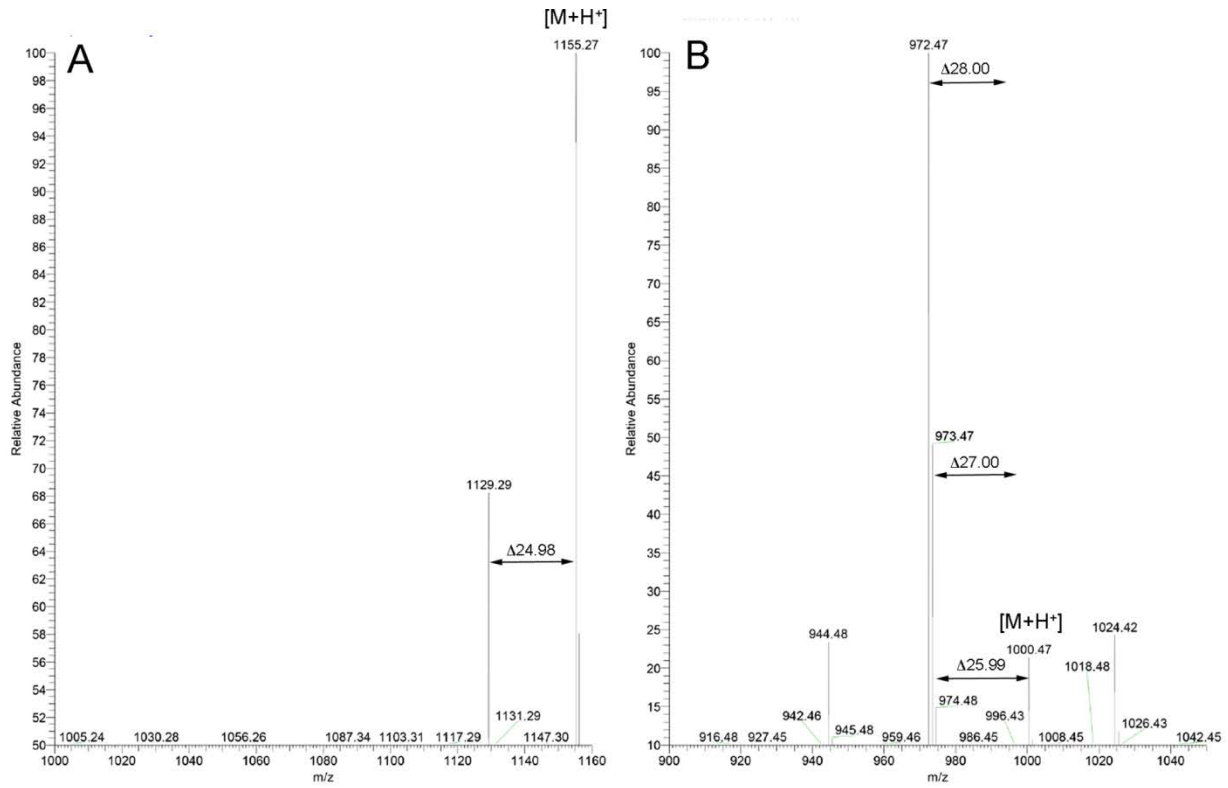
84

85 **Figure S9.** UV/visible absorption spectra of methanobactin from $\Delta mbnAN + pWG101$ (A and B) and
86 $\Delta mbnAN + pWG102$ (C and D) of samples incubated in either 10 mM HCl (A and C) or 100 mM HCl (B and
87 D). Spectra were taken either at 20 min (A and C) or at 120 min (B and C) intervals. Insets A and B,
88 absorbance changes at 340 nm (blue trace) and 394 nm (red trace). Insets C and D, absorbance
89 changes at 337 nm (blue trace).

90

91

92



93

94

95 **Figure S10.** LC-MS of methanobactin from (A) $\Delta mbnAN$ + pWG101 (A) and (B) $\Delta mbnAN$ + pWG102

96 following a 12 h incubation in 100 mM HCl.

97

98