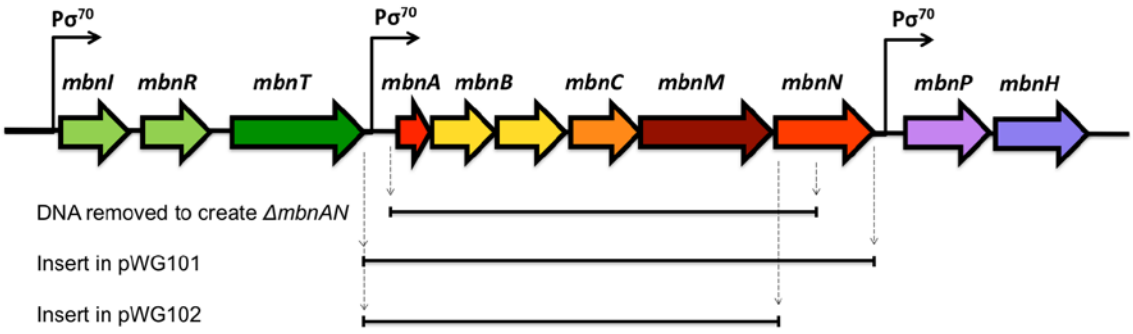

Figure S1. DNA removed to construct *M. trichosporium* OB3b Δ*mbnAN* mutant and inserts used to construct pWG101 and pWG102.

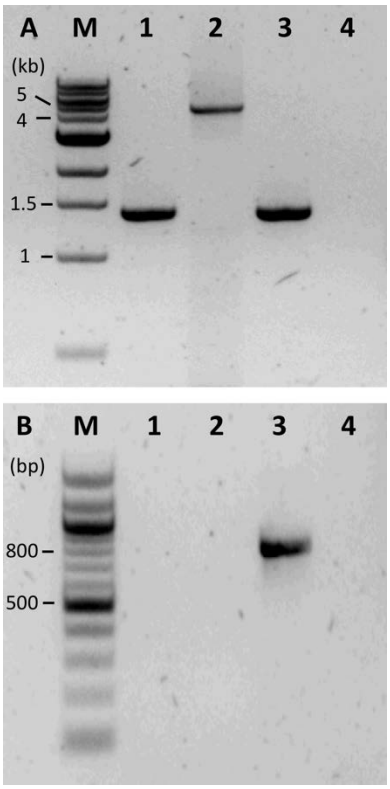

Figure S2. Confirmation of construction of *M. trichosporium* OB3b Δ*mbnAN* mutant. Figure S1A - PCR of *mbnABCMN* using primers mbn21/22. M - molecular weight markers. Lanes 1 – 4 are PCR of *mbnABCMN* from: 1 - *M. trichosporium* OB3b *mbnAN* mutant (expected product of 1.4 kb); 2 - *M. trichosporium* OB3b wildtype (expected product of 4.8 kb); 3 – pWG012 plasmid (positive control; expected product of 1.4 kb); 4 - distilled deionized water (negative control). Figure S1B - PCR of pK18mob*sacB* plasmid backbone using primers pK18-bb-F/R. M - molecular weight markers; Lanes 1 – 4 are PCR of pK18mob*sacB* plasmid backbone from: 1 - *M. trichosporium* OB3b *mbnAN* mutant; 2 - *M. trichosporium* OB3b wildtype; 3 - pWG012 plasmid (positive control; expected product of 800 bp); 4 - distilled deionized water (negative control).

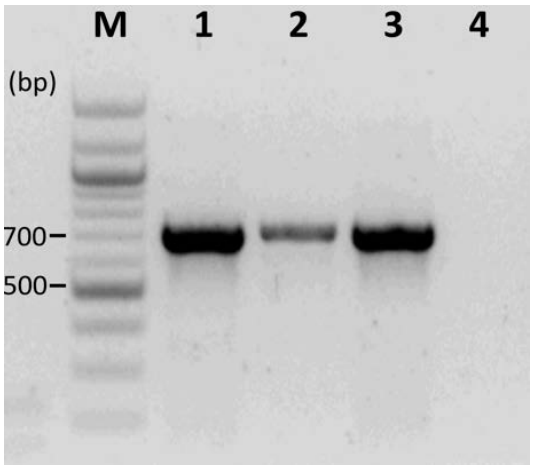

Figure S3. Confirmation of expression of *mbnPH* in Δ*mbnAN* mutant using primers mbnPH-F/R (expected product size of 700 bp) M: molecular weight markers. Lanes 1 – 4 are RT-PCR of *mbnPH* from: 1 - *M. trichosporium* OB3b Δ*mbnAN* grown in the absence of copper; 2 - *M. trichosporium* OB3b Δ*mbnAN*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

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

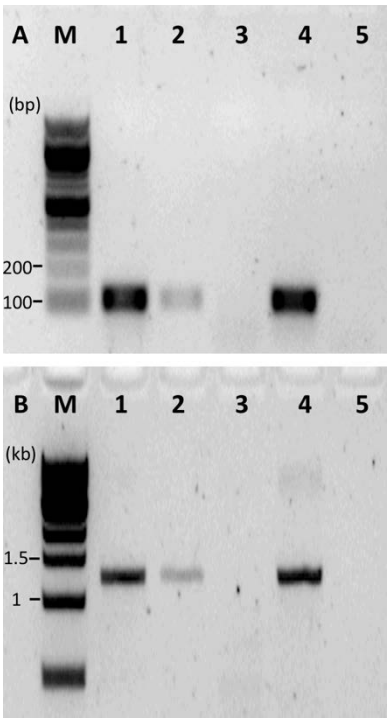

Figure S5. Expression of *mbnA* and *mbnN* in Δ*mbnAN* + pWG101. Figure S3A - RT-PCR of *mbnA* using primers qmbnA_FO/RO (expected product of 100 bp). M - molecular weight markers. Lanes 1 -5 are RT-PCR of *mbnA* from: 1 - *M. trichosporium* OB3b Δ*mbnAN* + pWG101 grown in the absence of copper; 2 - *M. trichosporium* OB3b Δ*mbnAN* + pWG101 grown with 1 μM copper; 3 - *M. trichosporium* OB3b Δ*mbnAN* grown in the absence of copper (negative control); (4) *M. trichosporium* OB3b wild type grown in the absence of copper (positive control); (5) distilled deionized water (negative control). Figure S3B - RT-PCR of *mbnN* using primers mbnN-F/R (expected product size of 1.2 kb). M molecular weight markers . Lanes 1 – 5 are RT-PCR of *mbnN* from: 1 - *M. trichosporium* OB3b Δ*mbnAN* + pWG101 grown in the absence of copper; 2 - *M. trichosporium* OB3b Δ*mbnAN* + pWG101 grown in the presence of 1 μM copper; 3 - *M. trichosporium* OB3b Δ*mbnAN* grown in the absence of copper (negative control); 4 - *M. trichosporium* OB3b wild type grown in the absence of copper (positive control); 5 - distilled deionized water (negative control).

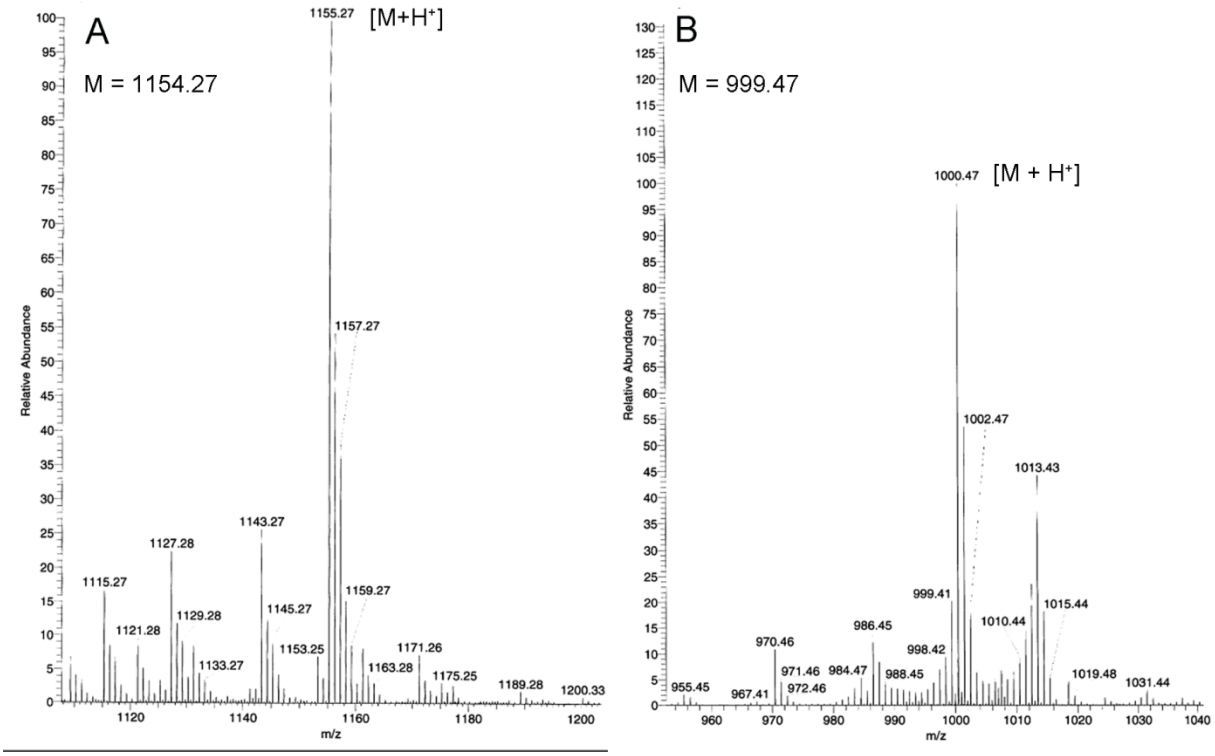

Figure S6. LC-MS/MS mass spectra of methanobactin from (A) Δ*mbnAN* + pWG101 (B)Δ*mbnAN* + pWG102 (B).

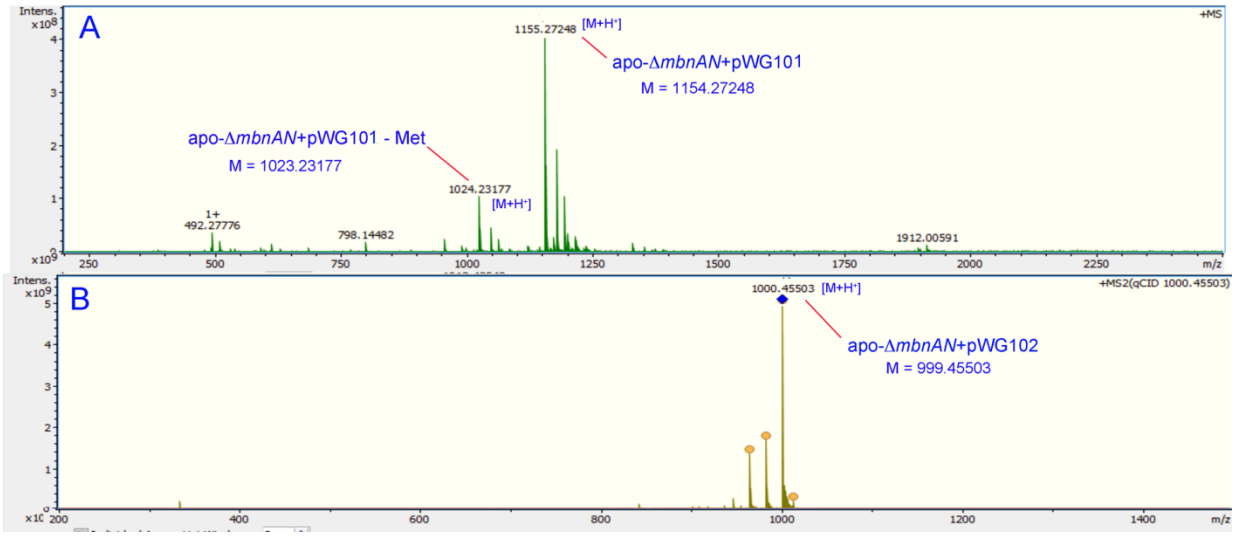

Figure S7. FT-ICR mass spectra of methanobactin from (A) Δ*mbnAN* + pWG101 and (B) Δ*mbnAN* + pWG102. Samples were collected from a Dianion HP-20 column.

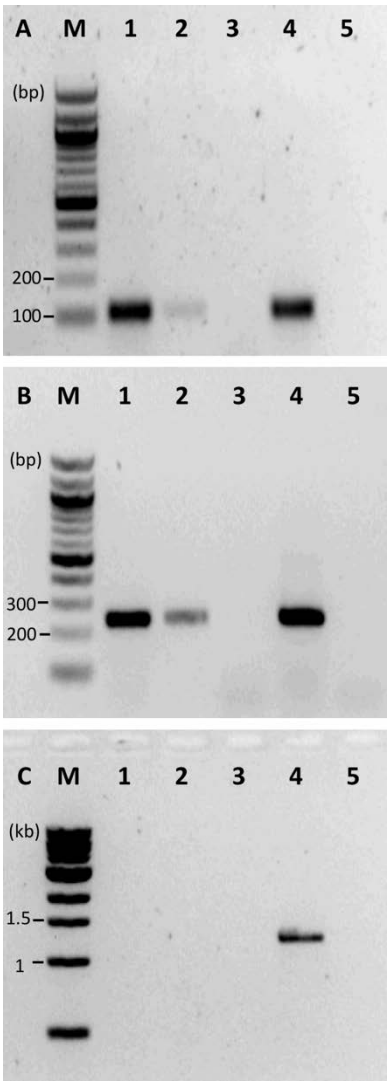

Figure S8. Expression of *mbnA*, *mbnM* and *mbnN* in Δ*mbnAN* + pWG102. Figure S4A – RT-PCR of *mbnA* using primers qmbnA_FO/RO (expected product size of 100 bp). M - molecular weight markers. Lanes 1 -5 are RT-PCR of *mbnA* from: 1 - *M. trichosporium* OB3b Δ*mbnAN* + pWG102 grown in the absence of copper; 2 - *M. trichosporium* OB3b Δ*mbnAN* + pWG102 grown with 1 μM copper; 3 - *M. trichosporium* OB3b Δ*mbnAN* grown in the absence of copper (negative control); (4) *M. trichosporium* OB3b wild type grown in the absence of copper (positive control); (5) distilled deionized water (negative control). Figure S4B – RT-PCR of *mbnM* using primers mbnM-F/R (expected product size of 220 bp). M - molecular weight markers. Lanes 1 -5 are RT-PCR of *mbnM* from: 1 - *M. trichosporium* OB3b Δ*mbnAN* + pWG102 grown in the absence of copper; 2 - *M. trichosporium* OB3b Δ*mbnAN* + pWG102 grown with 1 μM copper; 3 - *M. trichosporium* OB3b Δ*mbnAN* grown in the absence of copper (negative control); (4) *M. trichosporium* OB3b wild type grown in the absence of copper (positive control); (5) distilled deionized water (negative control). Figure S4C – RT-PCR of *mbnN* using primers mbnN-F/R (expected product size of 1.2 kb). M - molecular weight markers. Lanes 1 -5 are RT-PCR of *mbnN* from: 1 - *M. trichosporium* OB3b Δ*mbnAN* + pWG102 grown in the absence of copper; 2 - *M. trichosporium* OB3b Δ*mbnAN* + pWG102 grown with 1 μM copper; 3 - *M. trichosporium* OB3b Δ*mbnAN* grown in the absence of copper (negative control); (4) *M. trichosporium* OB3b wild type grown in the absence of copper (positive control); (5) distilled deionized water (negative control).

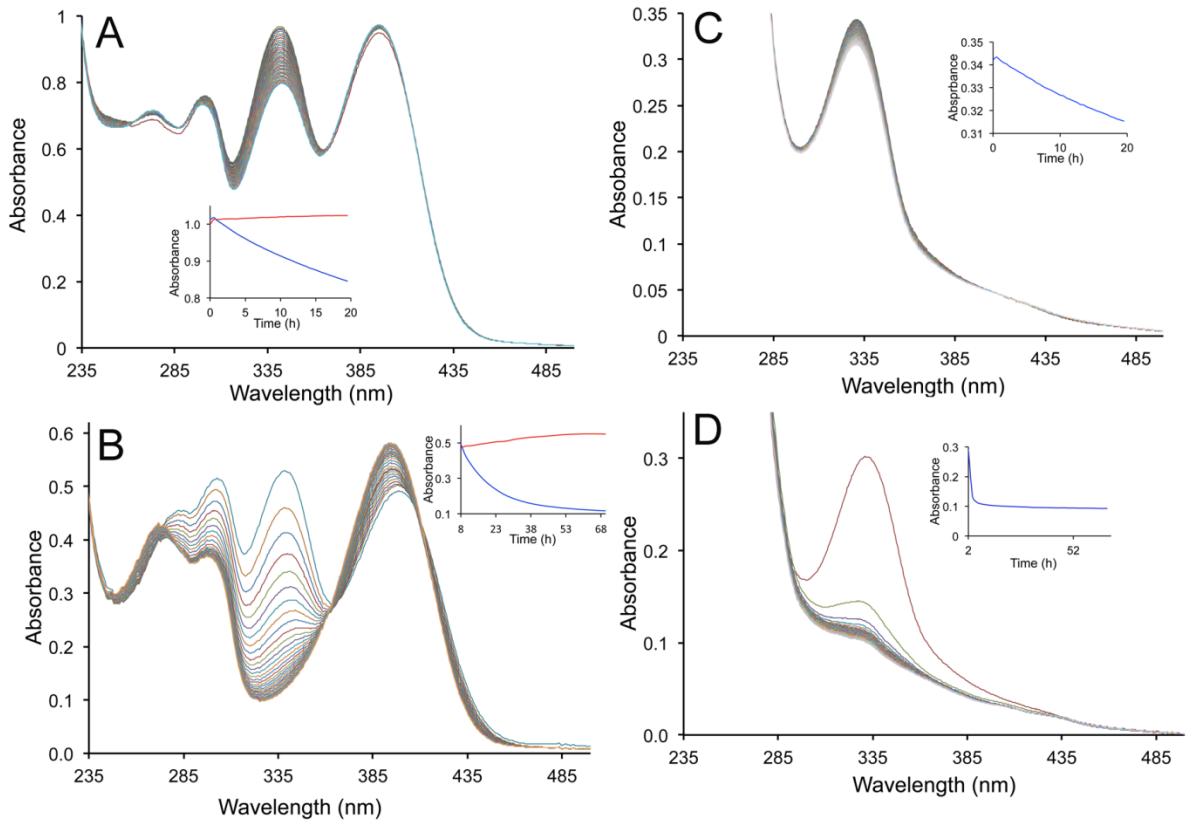

Figure S9. UV/visible absorption spectra of methanobactin from Δ*mbnAN* + pWG101 (A and B) and Δ*mbnAN* + pWG102 (C and D) of samples incubated in either 10 mM HCl (A and C) or 100 mM HCl (B and D). Spectra were taken either at 20 min (A and C) or at 120 min (B and C) intervals. Insets A and B, absorbance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

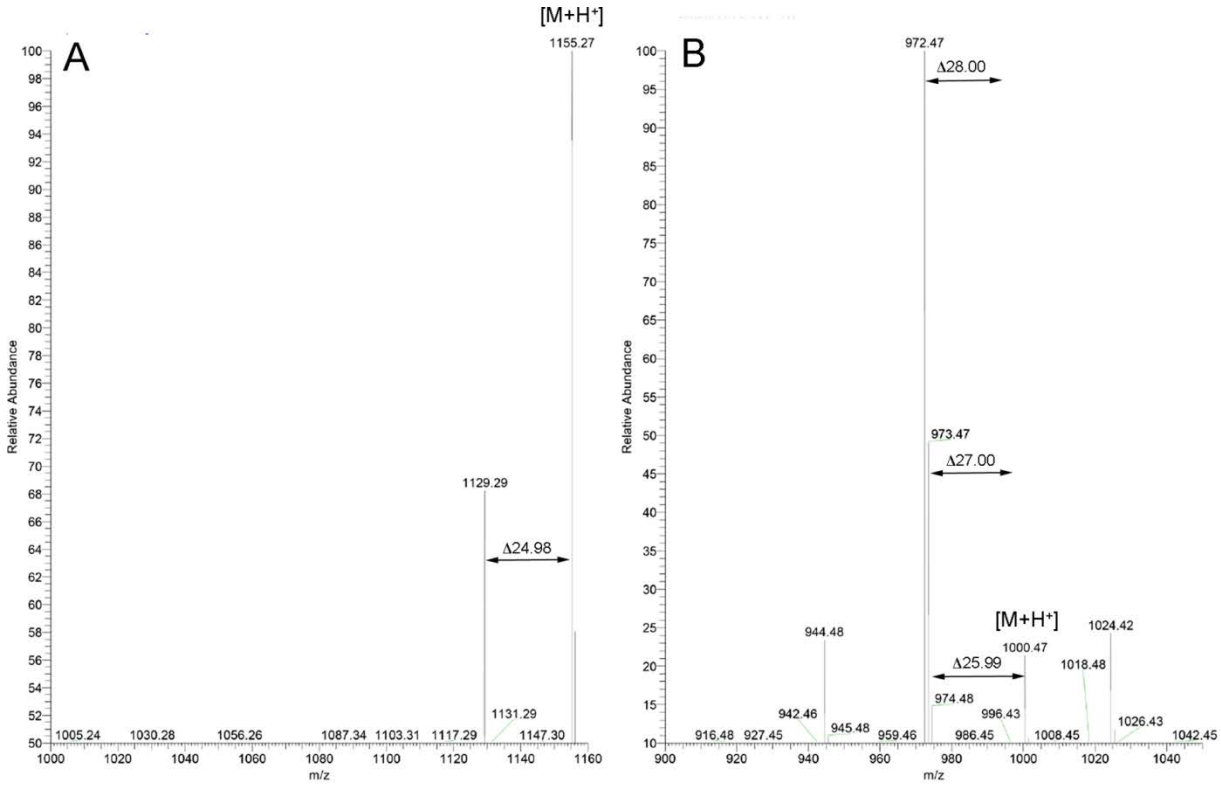

Figure S10. LC-MS of methanobactin from (A) Δ*mbnAN* + pWG101 (A) and (B) Δ*mbnAN* + pWG102 following a 12 h incubation in 100 mM HCl.