

Figure 1. Multilocus sequence typing tree where *Streptomyces hygroscopicus* NRRL 30439 resides in a clade of *Streptomyces* and adjacent to *S. lydicus* A02.

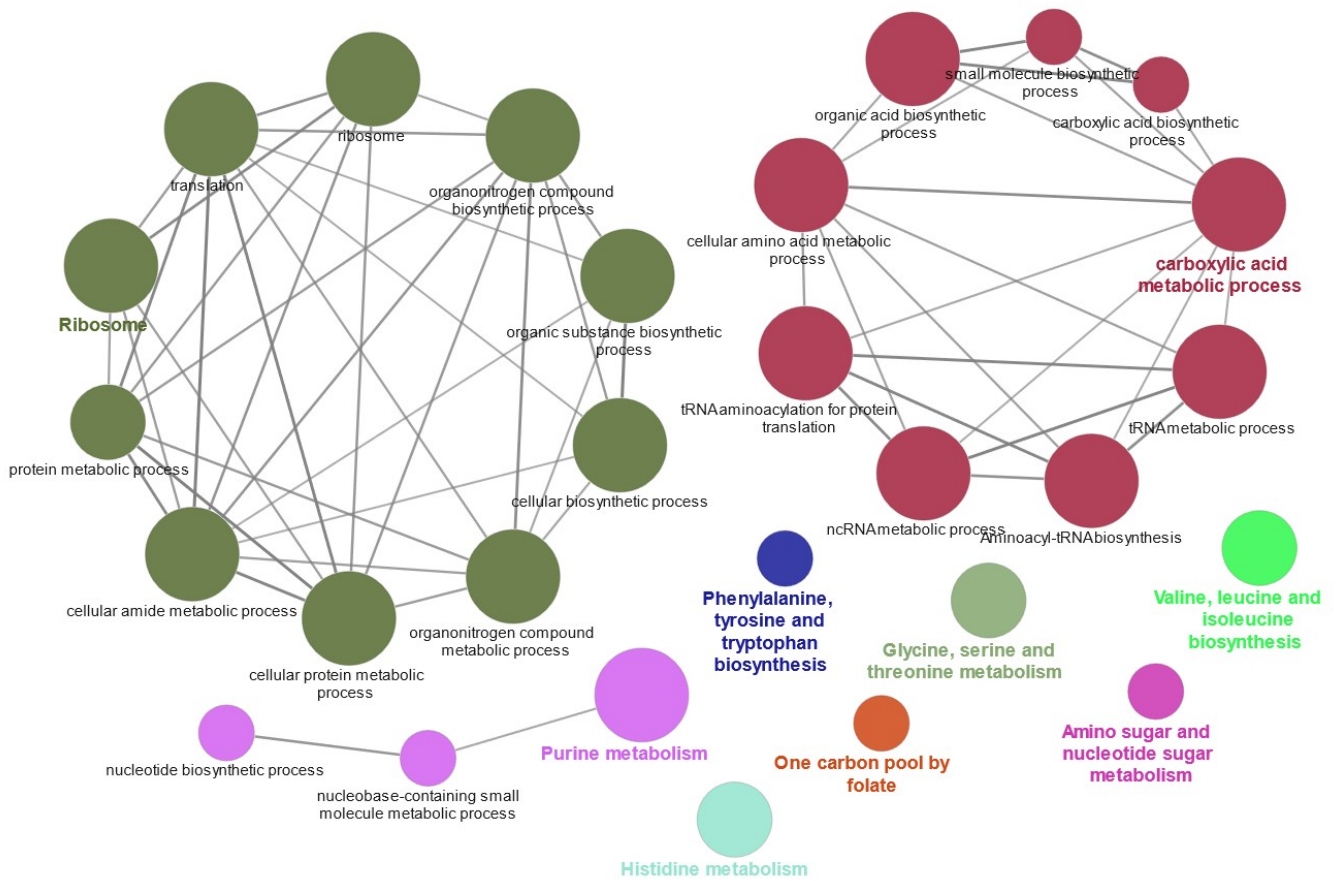


Figure 2. GO and KEGG pathway enrichment of proteins belonging to k-means cluster 1. The size of the nodes is representative of term enrichment significance (Bonferroni step down correction).

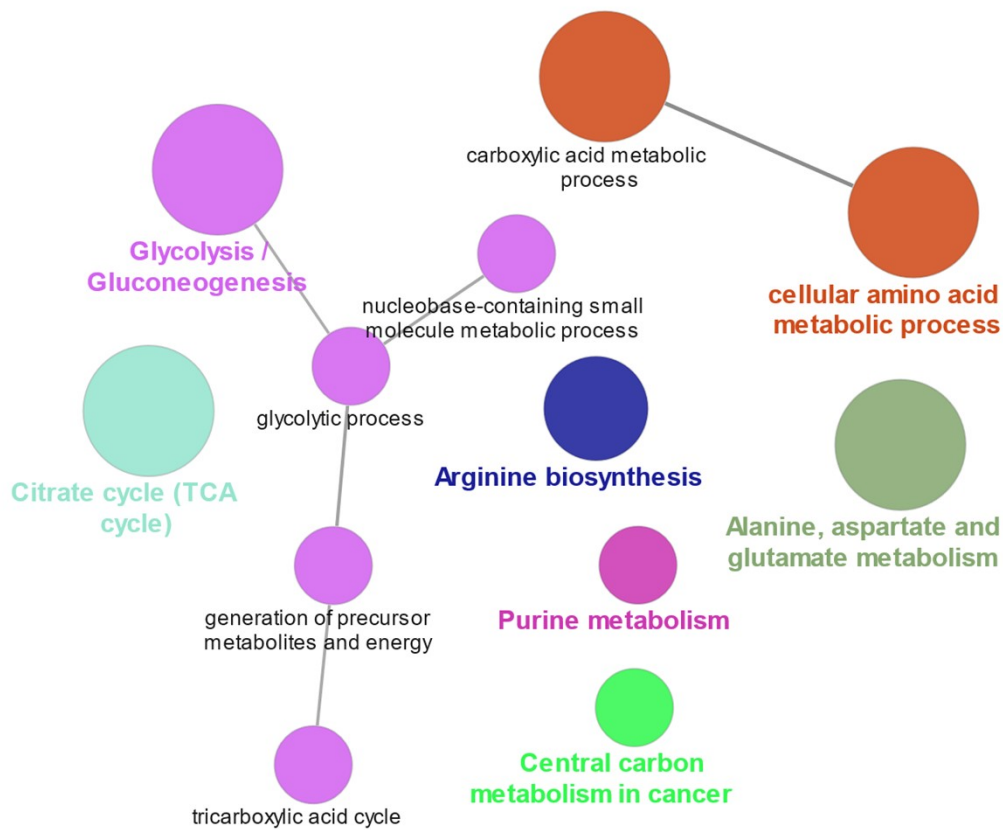


Figure 3. GO and KEGG pathway enrichment of proteins belonging to k-means cluster 2. The size of the nodes is representative of term enrichment significance (Bonferroni step down correction).

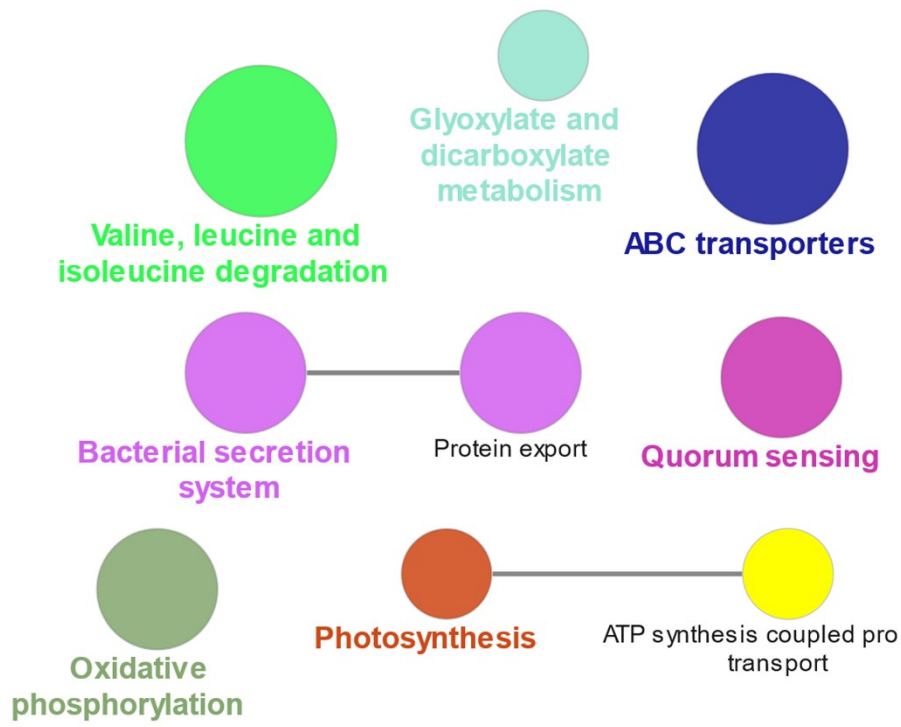
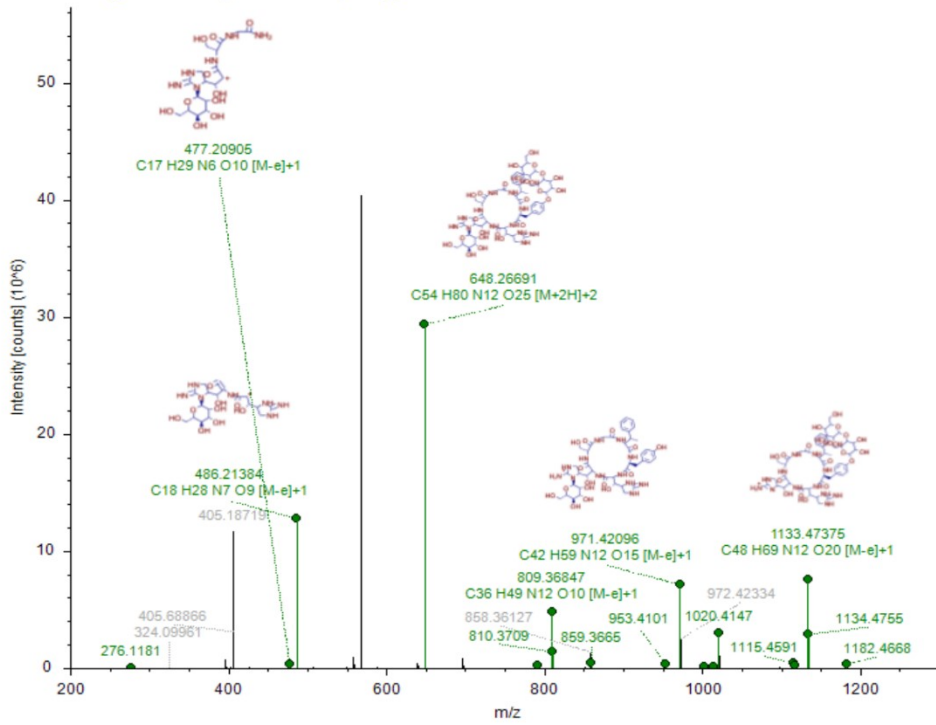


Figure 4. GO and KEGG pathway enrichment of proteins belonging to k-means cluster 4. The size of the nodes is representative of term enrichment significance (Bonferroni step down correction).

a

20200817_T4_016 (F11) #16344, RT=21.877 min, MS2, FTMS (+), (HCD, DDA, 648.2668@28, +2)
 alpha-Mannopectimycin C54 H78 N12 O25, MW: 1294.52011, Area: 12467986806
 FISh Coverage: 18 Matched, 24 Unmatched, 9 Skipped

**b**

20210915_T4_057_MPP_Std (F20) #11632, RT=37.814 min, MS2, FTMS (+), (HCD, DDA, 648.2404@28, +2)
 alpha-Mannopectimycin C54 H78 N12 O25, MW: 1294.52011, Area: 2660851535
 FISh Coverage: 14 Matched, 25 Unmatched, 2 Skipped

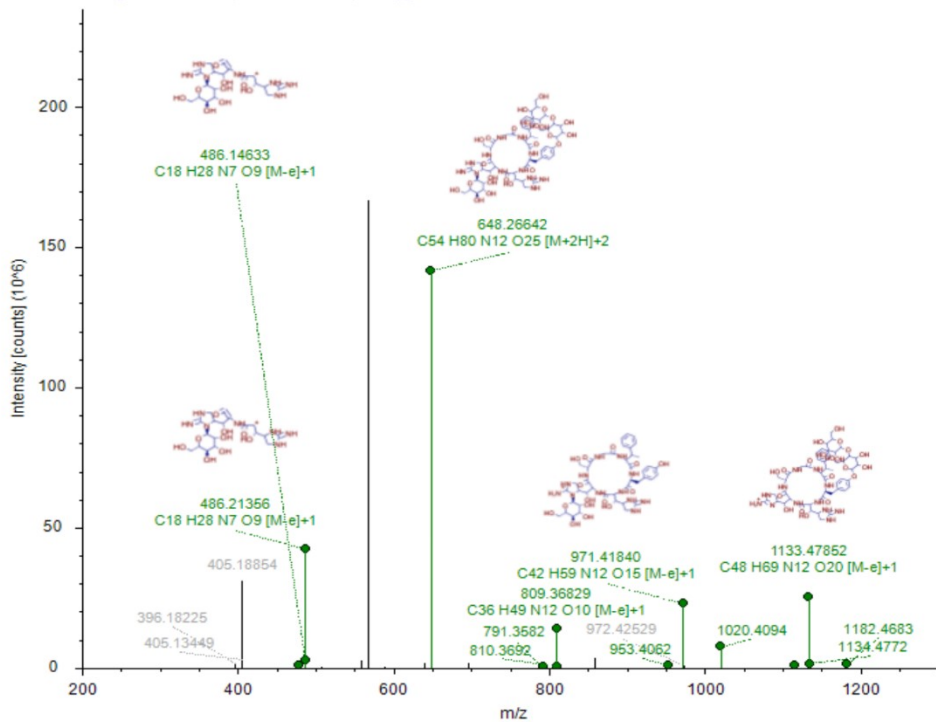


Figure 5. Mannopectimycin (MPP) fragmentation spectra, annotated using Compound Discoverer v3.1. a) Fragmentation spectra of MPP found in fermentation supernatant, with a FISh coverage of 38.6. b) Fragmentation spectra of a purified standard of alpha-mannopectimycin, with a FISh coverage of 35.9. Fragment spectra of the supernatant sample is strongly supported by the spectra of the alpha-mpp standard.

20200817_T4_009 (F8) #17992, RT=22.360 min, MS2, FTMS (+), (HCD, DDA, 486.2142@28, +2)
 beta-Mannoheptimycin C42 H58 N12 O15, MW: 970.41446, Area: 541431662
 FISH Coverage: 32 Matched, 111 Unmatched, 77 Skipped

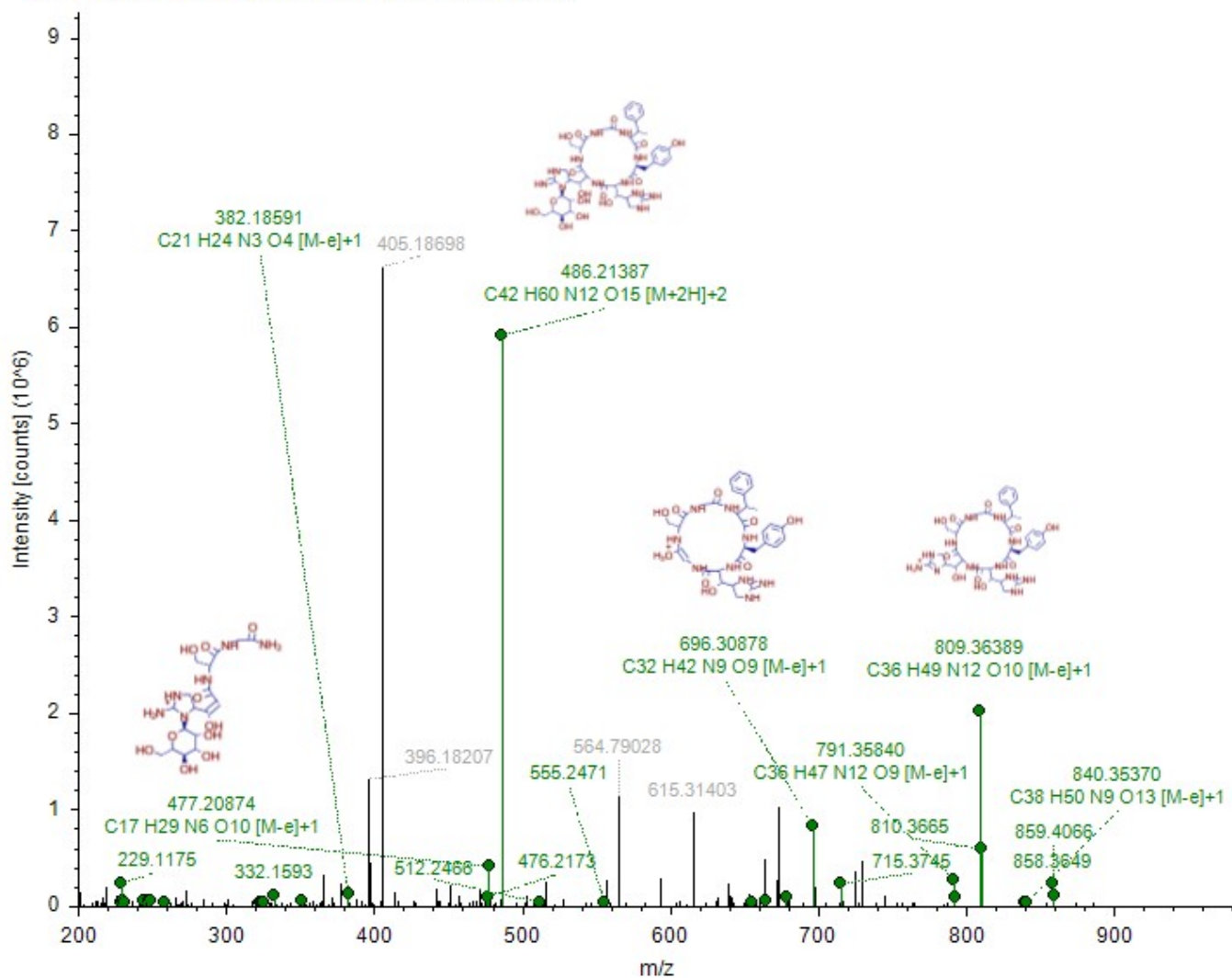


Figure 6. Proposed fragment spectra of beta-mannoheptimycin from fermentation supernatant. The parent ion (486.21387, [M+2H]) was found in supernatant samples, and assigned to beta-mannoheptimycin with a FISH score of 17.5. Annotation was done using Compound Discoverer v3.1. Coisolation of primary ions of similar mass eluting at the same time as beta-mannoheptimycin is evident, with a considerable number of unmatched peaks.

20200817_T4_016 (F11) #37243, RT=41.316 min, MS2, FTMS (+), (HCD, DDA, 690.2959@28, +2)
 gamma-Mannoheptimycin C59 H86 N12 O26, MW: 1378.57762, Area: 1674559669
 FISh Coverage: 22 Matched, 33 Unmatched, 19 Skipped

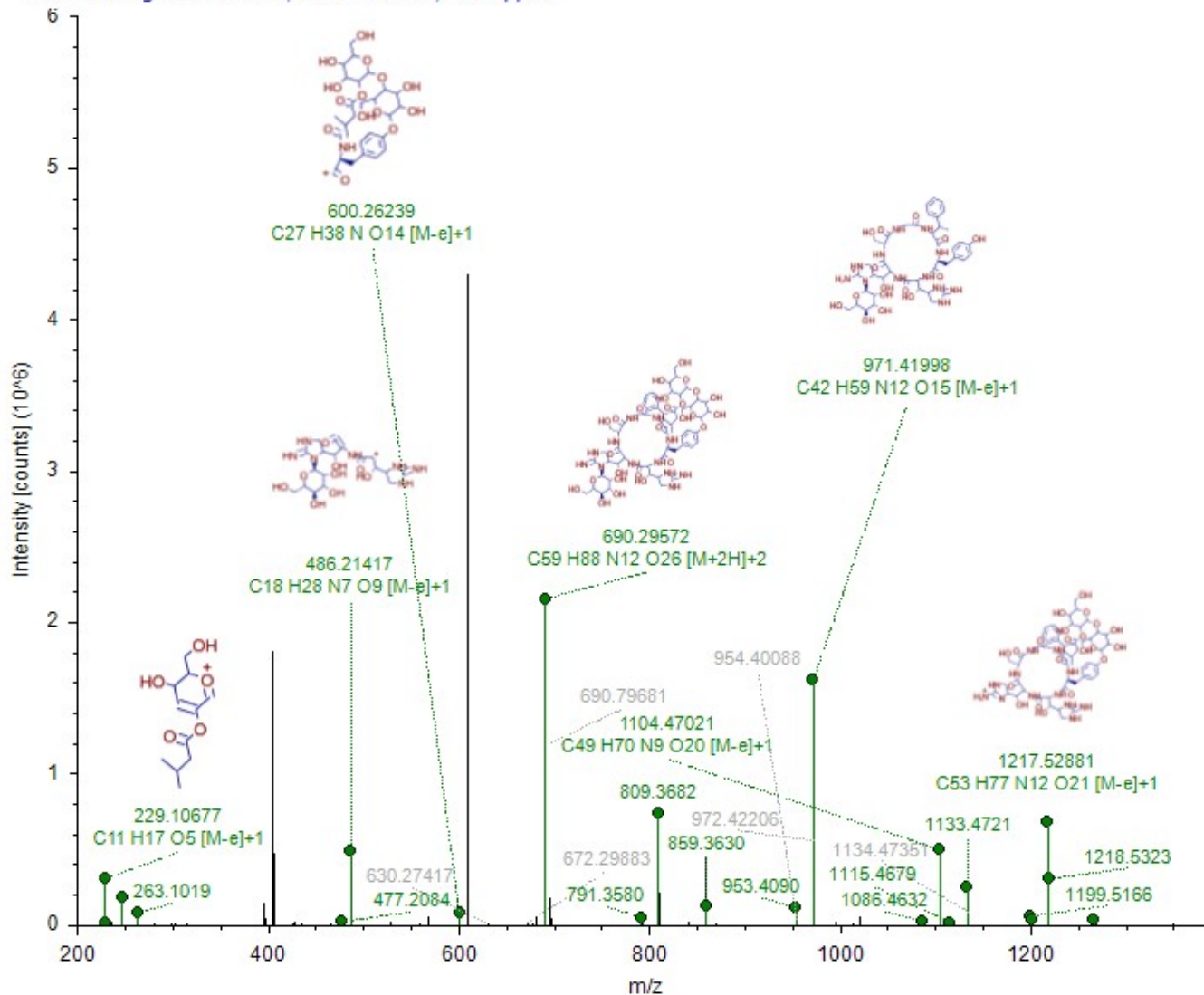


Figure 7. Proposed fragment spectra for gamma/delta/epsilon-mannoheptimycin from supernatant samples with a FISh coverage of 32.2.

20200817_T4_011 (F9) #42977, RT=42.656 min, MS2, FTMS (+), (HCD, DDA, 809.3687@28, +2)
 Mannopeptimycin-aglycone C36 H48 N12 O10, MW: 308.36164, Area: 4557795168
 FISH Coverage: 61 Matched, 68 Unmatched, 65 Skipped

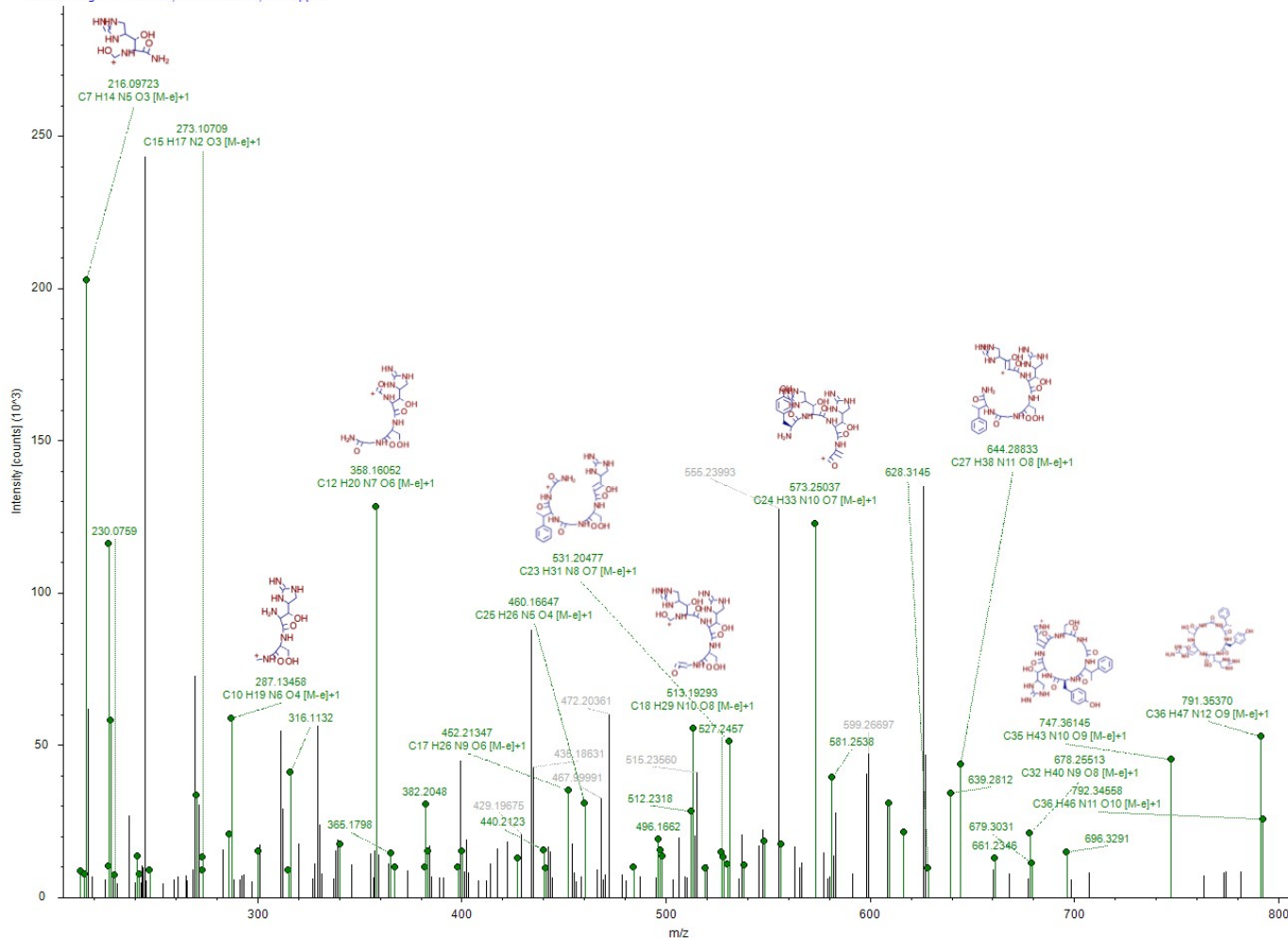


Figure 8. Proposed fragment ion spectra for the aglycone variant of mannopeptimycin with a FISH coverage of 48.7.

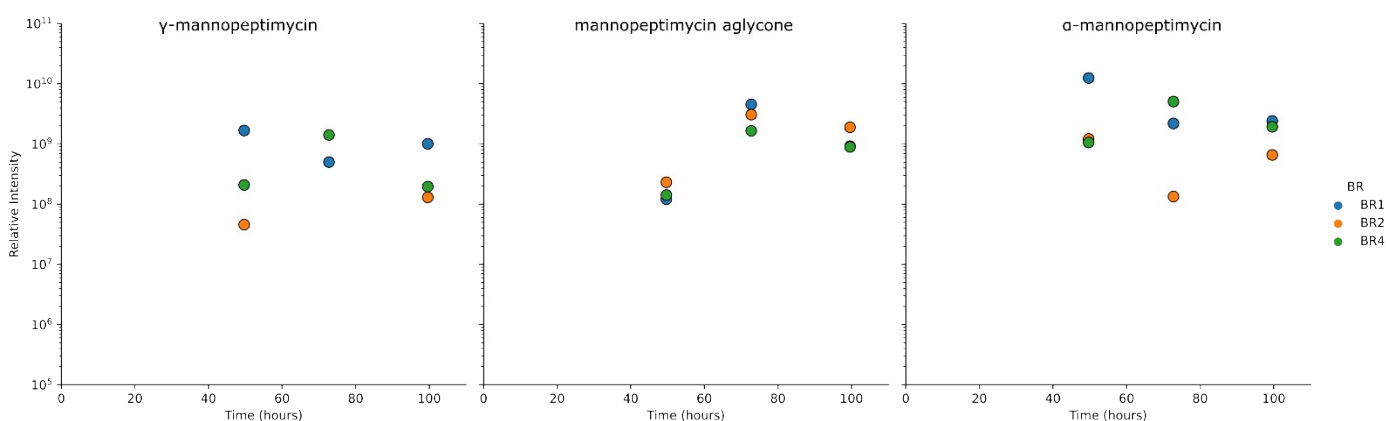


Figure 9. Relative intensities (MSMS) of mannopeptimycin variants detected in supernatant samples taken from 3 replicate bioreactors. Samples were taken at 7.75, 25.75, 49.67, 72.75, and 99.6 hours into the fermentation. None of mannopeptimycin variants were detected in the first 2 supernatant samples. Beta-mannopeptimycin was excluded from the analysis due to low FISH scoring compared to alpha, gamma/delta/epsilon, and aglycone variants.