

Supplementary Information

Culturable diversity and biological properties of bacterial endophytes associated with the medicinal plants of the *Vernonia anthelmintica* (L.) Willd

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Abstract: As one of the important traditional medicinal plants listed in the Chinese pharmacopoeia, *Vernonia anthelmintica* has been shown to possess various biological activities. In this study, we characterized culturable endophytic bacteria associated with the medicinal plant *V. anthelmintica* (L.) Willd collected from Hotan, within the Xinjiang autonomous region of China. Bacterial endophytes were identified by 16S rRNA gene sequence analysis and compared with similar sequences from the GenBank. Isolated strains exhibited 99.08-100% similarity to *Bacillus haynesii* XJB-5, *Bacillus proteolyticus* XJB-16, *Bacillus halotolerans* XJB-35, *Bacillus safensis* XJB-71, *Pseudomonas punonensis* XJB-7, *Lysinibacillus fusiformis* XJB-17, *Streptococcus lutetiensis* XJB-66, *Leclercia adecarboxylata* XJB-12, *Paenibacillus alvei* XJB-14 and *Pantoea agglomerans* XJB-62. The ethyl acetate extracts of the bacterial isolates demonstrated various biological activities such as antimicrobial, cytotoxic, antidiabetic, antioxidant and melanin content assay and tyrosinase activity in murine B16 cells. A crude extract of *B. halotolerans* XJB-35 showed higher biological activities than other bacterial strains; therefore, this strain was studied further in order to select the optimized parameter for enhancing the production of bioactive compounds. The optimal culture medium was found to be nutrient broth (NB)

medium using peptone as its carbon source and yeast extract as its nitrogen source. A 24 h incubation time produced the optimal conditions for the maximum growth of *B. halotolerans* XJB-35 and the production of bioactive compounds. Moreover, we investigated the non-polar chemical composition from the dichloromethane fraction using GC-MS analysis. Our findings provide valuable information regarding the production of bioactive secondary metabolites by *B. halotolerans* XJB-35, for use by the medicinal and pharmaceutical industry.

Keywords: Bacterial endophytes, antimicrobial, cytotoxic, antidiabetic, antioxidant activity, bioactive secondary metabolites

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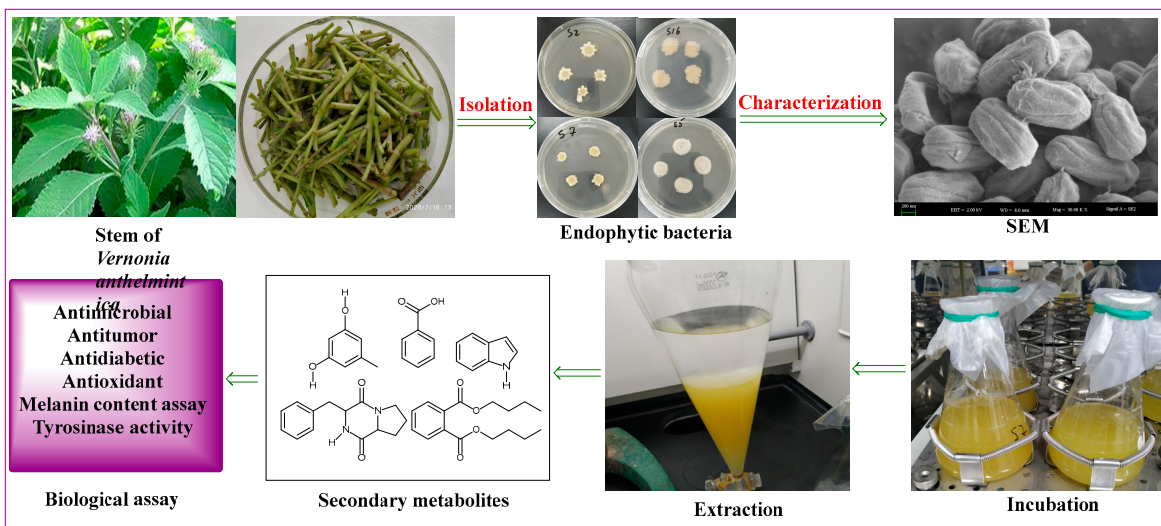


Figure S1. Main approach of this work

Gene sequences of endophytic bacteria

Bacillus haynesii XJB-6

TCTGTCCACCTTCGCGCGGTGGCTCCAAAAGGTTACCTCACCAGCTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTACAAAGCCCGGGAACGTATTCACCGCGG
CATGCTGATCCGCGATTACTAGCGATTCCAGCTTCACGCAGTCGAGTTGACAGACTGCGATCCGAAGTGAAGAACAGATTGTGGGATTGGCTTAGCCTCGCGGCTTCGCTGCCCTT
TGTTCTGCCCATTTGTAGCAGCTGTGTAGCCACAGGTCAATAGGGGATGATGATTGACGTCATCCCCACCTTCCTCCGGTTTGTACCCGGCAGTCACCTTAGAGTGCCTCACTGA
ATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCAACATCTCAGCAGACGAGCTGACGACAACCATGACCACCTGTCACTCTGCCCCCGAAGGGGAAG
CCCTATCTAGGGTTGTCAGAGGATGCAAGACCTGGTAAGGTTCTTCGGGTTGCTTCGAATTAACACCATGCTCCACCGCTTGTGCGGGGCCCGGTCAATTCCTTTGAGTTT
CAGTCTTCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTTGTGTCAGCACTAAAGGGCGGAACCCCTTAACACTTAGCACTCATCGTTACGCGGTGGACTACACGGGT
ATCTAATCCTGTTGCTGCCCGACGCTTTCGGCGCTCAGCGTCAGTTACAGACAGAGAGTCGCTTCGCGACTGGTGTTCCTCCACATCTCTACGCATTTACCCGCTACACGTGG
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GGCGGTGTCTCAGTCCAGTGTGGCGGATCACCTCTCAGGTCCGGTACGCATCGTTGCTGGTAGGCGGTATCTCACCACCTAGTAAATGCGCCGCGGGTCCATCTGTAAGT
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CACCCGTCCGCGCTAACATCAGGGAGCAAGTCCCATCTGTCGCTCGACTTGATGTATTAGGCACGCCGCGAGCTTCTGCTGAGCCAGATCCAACTT

Bacillus proteolyticus XJB-16

TGCTATACATGCAAGTCGAGCGAATGGATTGAGAGCTTGCTCTTATGAAGTTAGCGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAGACTGGGATAACTCCGGGA
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Bacillus halotolerans XJB-35

TGCTATACATGCAAGTCGAGCGAAGAGAGTTGCTCCCGGATGTTAGCGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAA
CCGGGGCTAATACCGGATAGTTCTTGAACCGCATGGTTCAAGGATGAAGAGCGGTTTCGGCTGTCACTTACAGATGGACCCCGCGGCATTAGCTAGTTGGTGGGTAATGGC
TCACCAAGGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCGAGCAGTAGGGAATCTCCGCAATGGAC
GAAAGTCTGACGGAGCAACGCGCGGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCCTTCAATAGGGCGGTACCTTGACGGTACCTA
ACCAGAAAGCCACGGCTAATACGTGCGCAGCAGCGCGGTAAATACGTAGGTGGCAAGCGTTGTCGGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTCTTAACTGATG
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CACCAGTGGCGAAGGCGACTCTGTGTTGTAAGTACGCTGAGGAGCGAAAGCGTGGGAGCGAAGCAGGATTAGATACCTGGTAGTCCACCGCTAAACGATGAGTGCTAA
GTGTTAGGGGTTTCCGCCCTTAGTGCTGCTAGCTAACGCAATTAAGCACTCCGCTGGGAGTACGGTCCGAAGACTGAAACTCAAAGGAATTGACGGGGGCCGCAAGCG
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Bacillus safensis XJB-71

TGCTATACATGCAAGTCGAGCGAAGAGAGTTGCTCCCGGATGTTAGCGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAA
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CCAGGAAGCAACGCGCTAAGTACGTGCGCAGCAGCGCGGTAAATACGTAGGTGGCAAGCGTTGTCGGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGCTTCTTAAGTCTGATGTG
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Pseudomonas punonensis XJB-7

CAGGCTACACATGCAAGTCGAGCGGTTGACGGGAGCTTGCTCCCTGATTACGCGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTATTAGTGGGGGACAACGTTTCGAAA
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Lysinibacillus XJB-17

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Streptococcus lutetiensis XJB-66

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Leclercia adecarboxylata XJB-12

CGGCAGGCCTACACATGCAAGTCGAGCGGTAGCACAGGGAGCTTGCTCCTGGGTGACGAGCGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACT
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Paenibacillus alvei XJB-14

TGCTATACATGCAAGTCGAGCGGACTTGATGGAAGTCTTGCACTCCTGATGGTTAGCGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCATAAAGACTGGGATAACCCAG
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CCTGAGAAGAAAGCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAAGCGGCAATGTAAGTT
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AATCCTTAAAGAGCGGATCTCAGTTCGGATTGCAAGGTCGAACCTCGCCTGACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGACACACCG
TACACACCGCCGTCACACCACGAGAGTTTACAACACCCGAAGTCGGTGAGGTAACCCGAAGGAGCCAGCCGCGGAAGGTGGGTAGA

Pantoea agglomerans XJB-62

CGGCAGGCTACACATGCAAGTCGAACGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTA
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CATAAAGTGCCTGCTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGACAC
ACCGCCCGTCAACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACCTTTGGATGCA

HPLC Analysis of secondary metabolites produced by endophytic bacteria

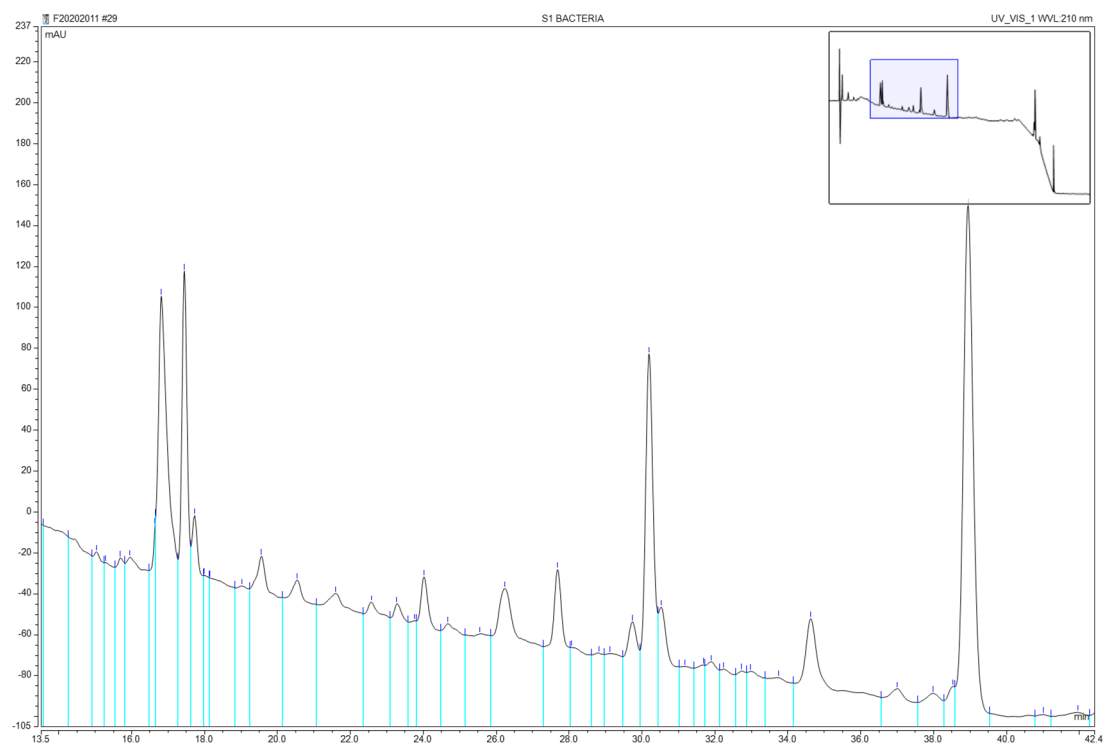


Figure S2. HPLC Analysis of natural products synthesized by endophytic bacteria *B. haynesii* XJB-5

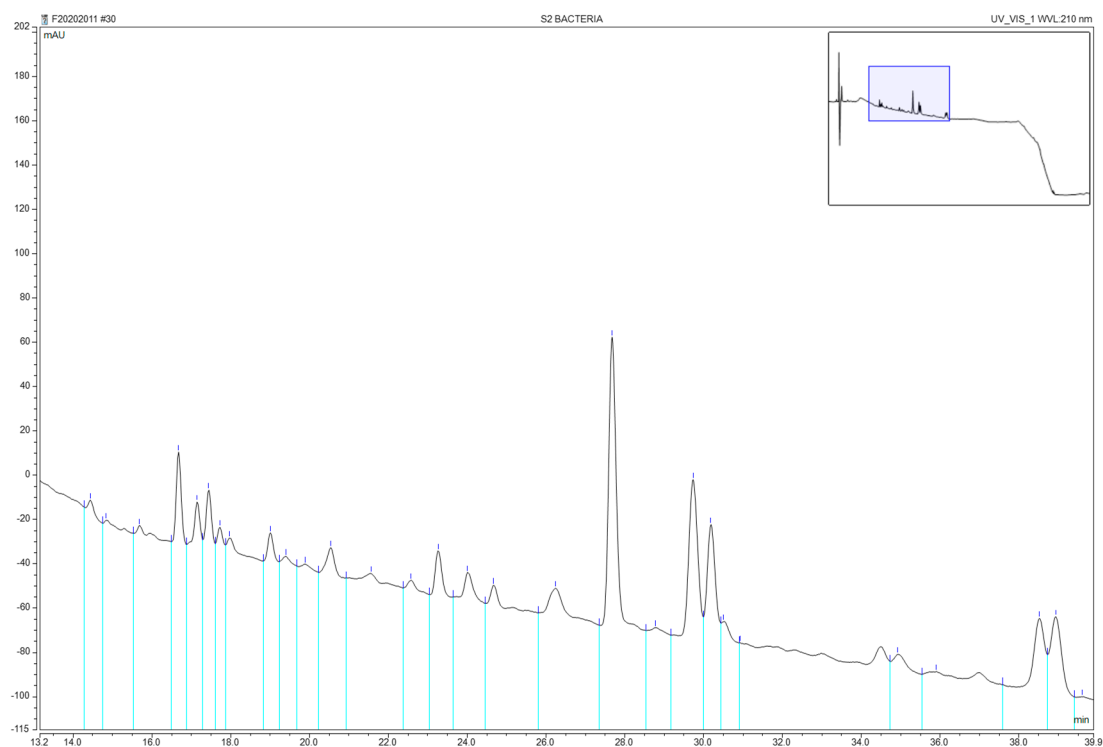


Figure S3. HPLC Analysis of natural products synthesized by endophytic bacteria *P. punonensis* XJB-7

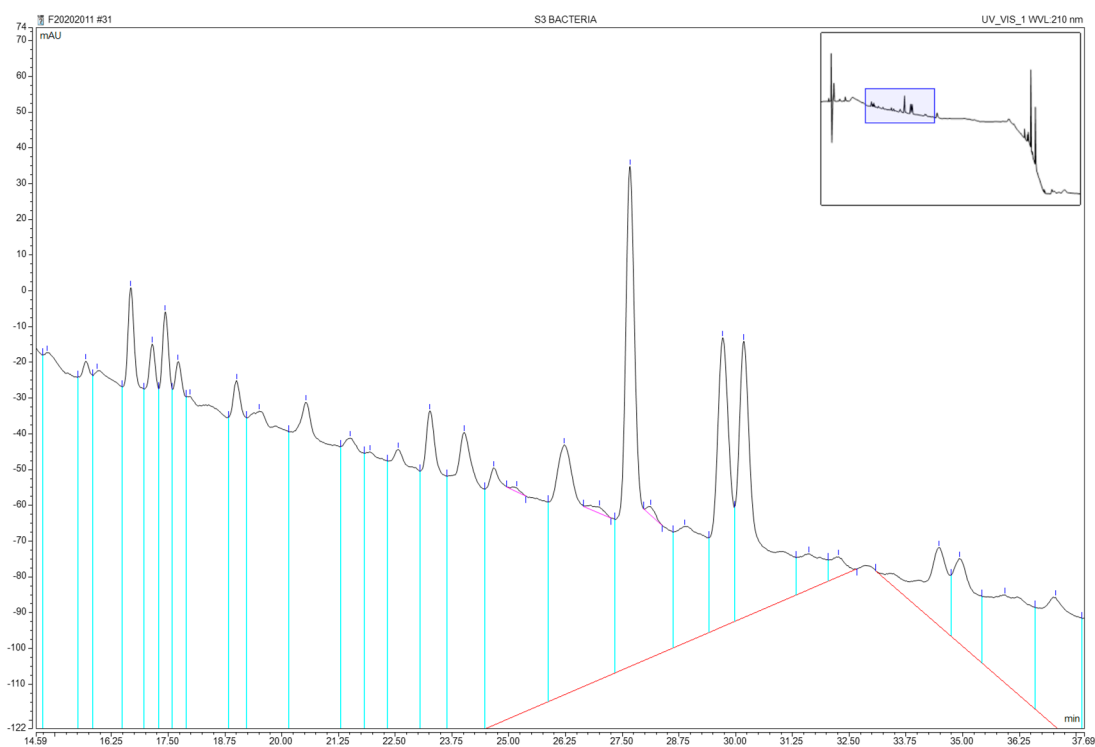


Figure S4. HPLC Analysis of natural products synthesized by endophytic bacterium *L. fusiformis* XJB-17

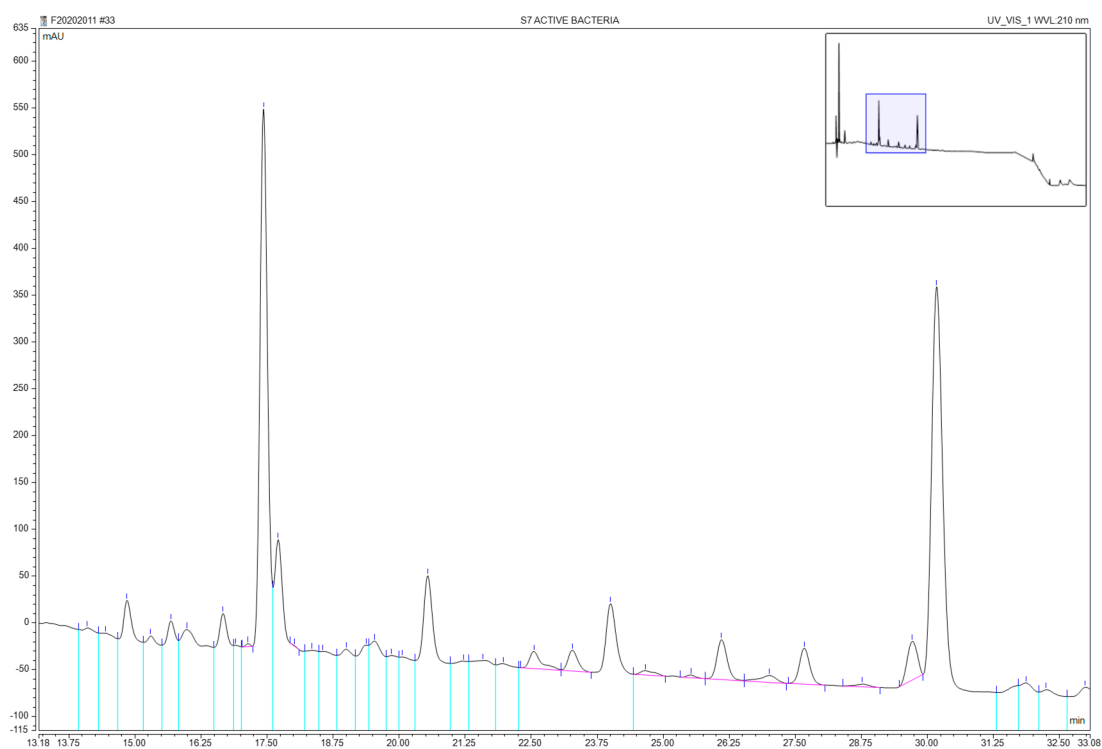


Figure S5. HPLC Analysis of natural products synthesized by endophytic bacterium *B. halotolerans* XJB-35

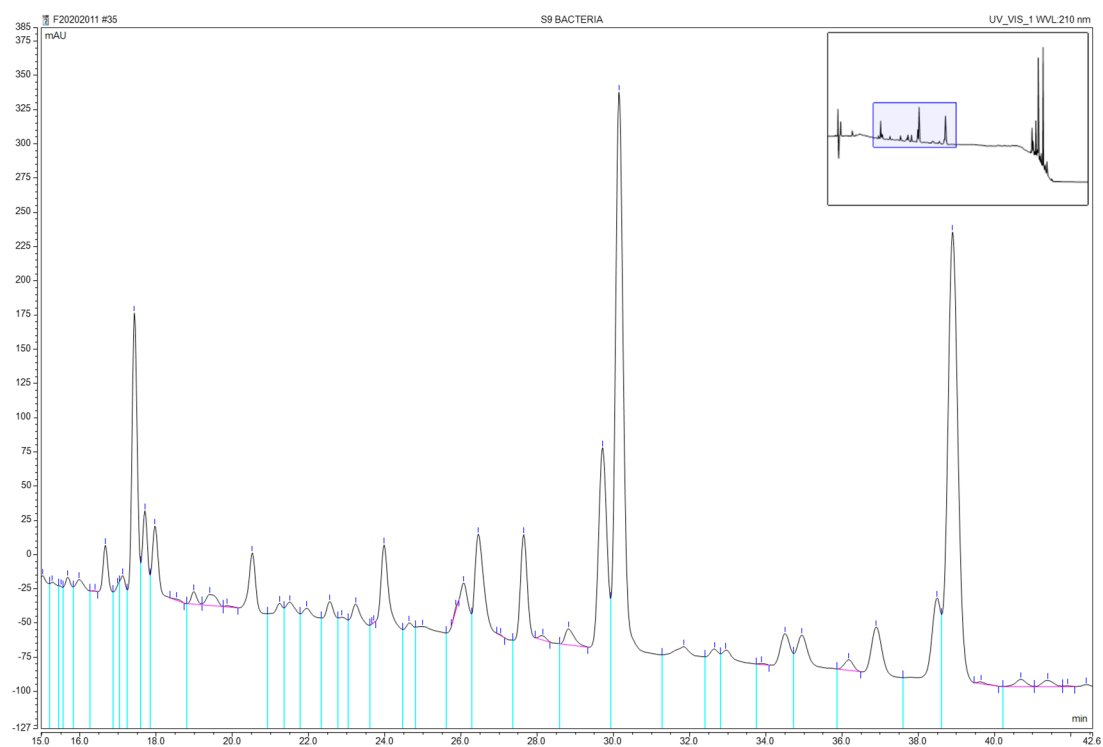


Figure S6. HPLC Analysis of natural products synthesized by endophytic bacteria *B. safensis* XJB-71

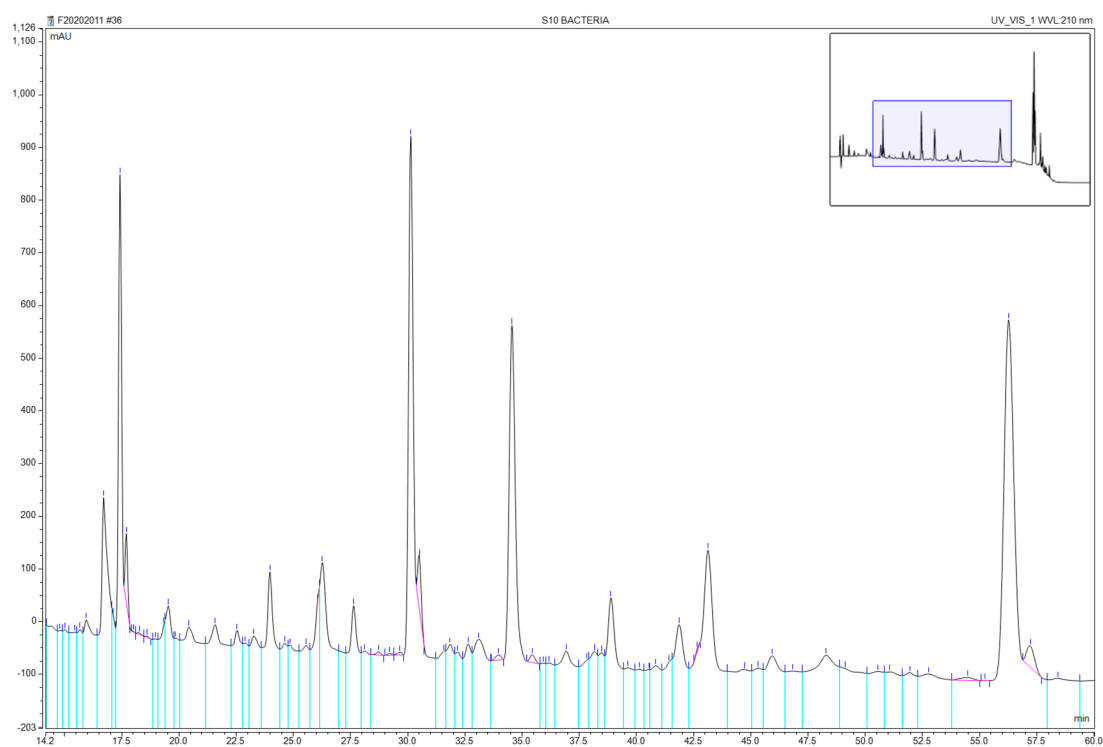


Figure S7. HPLC Analysis of natural products synthesized by endophytic bacteria *S. lutetiensis* XJB-66

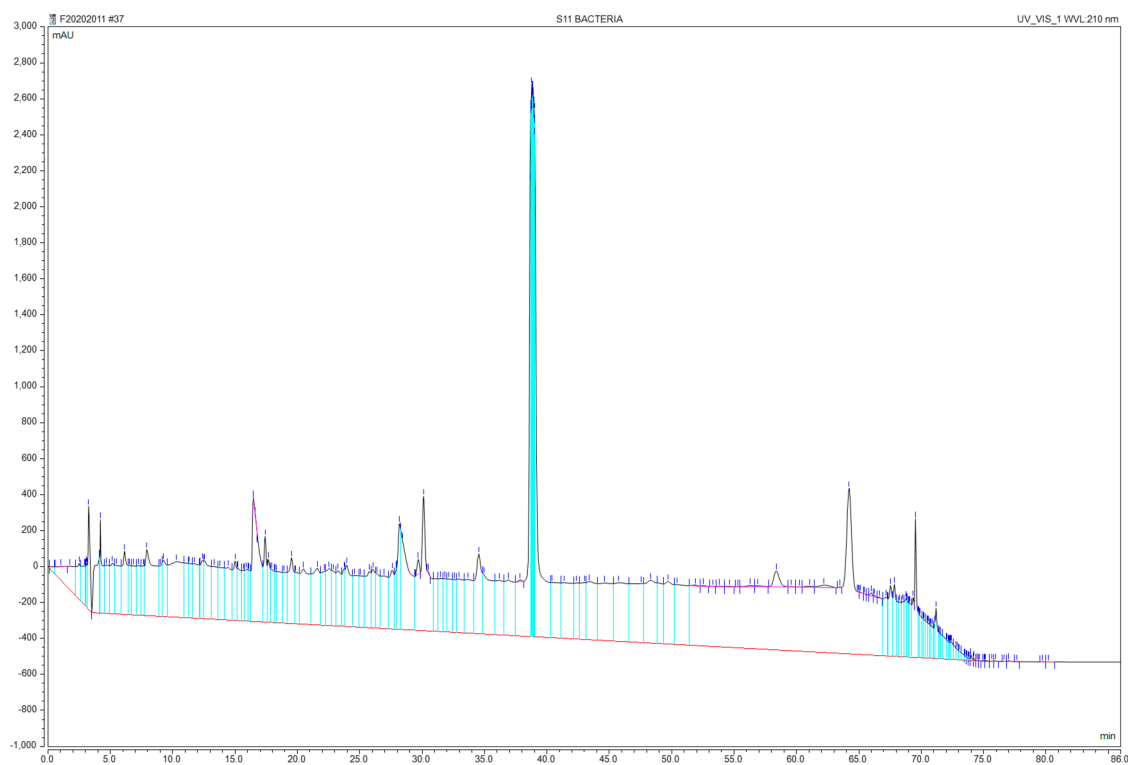


Figure S8. HPLC Analysis of natural products synthesized by endophytic bacteria *L. adecarboxylata* XJB-12

HPLC Analysis of natural products synthesized in different incubation time by most active endophytic bacteria *B. halotolerans* XJB-35

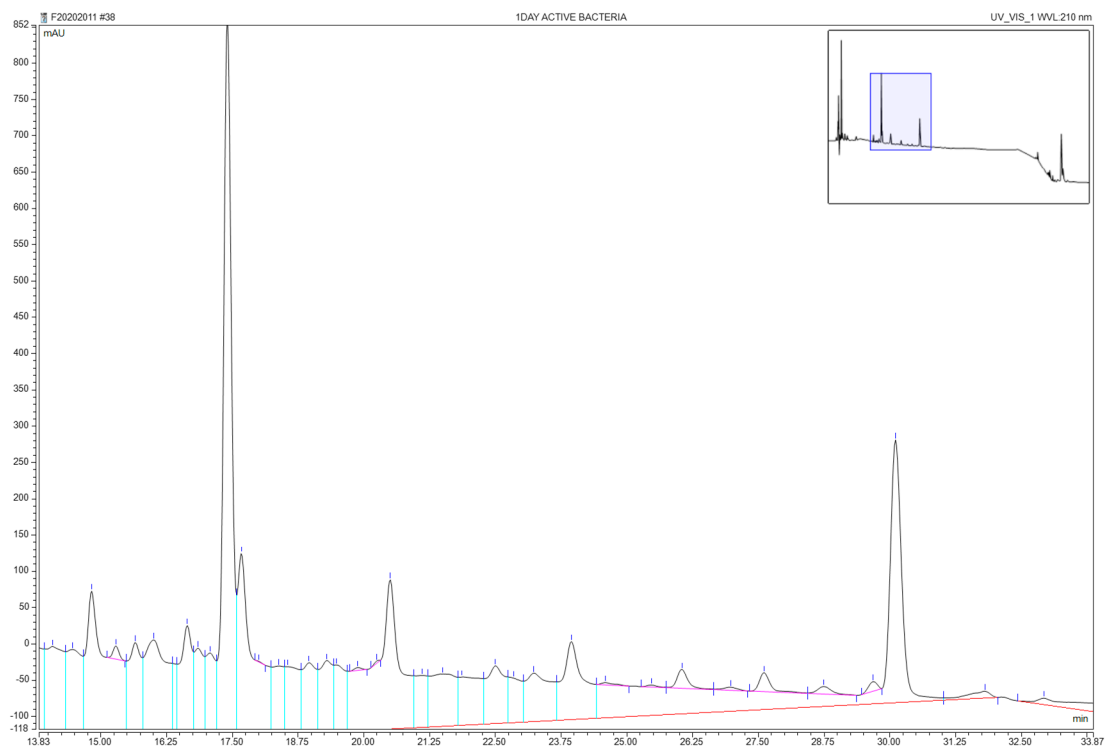


Figure S9. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on 24 hours

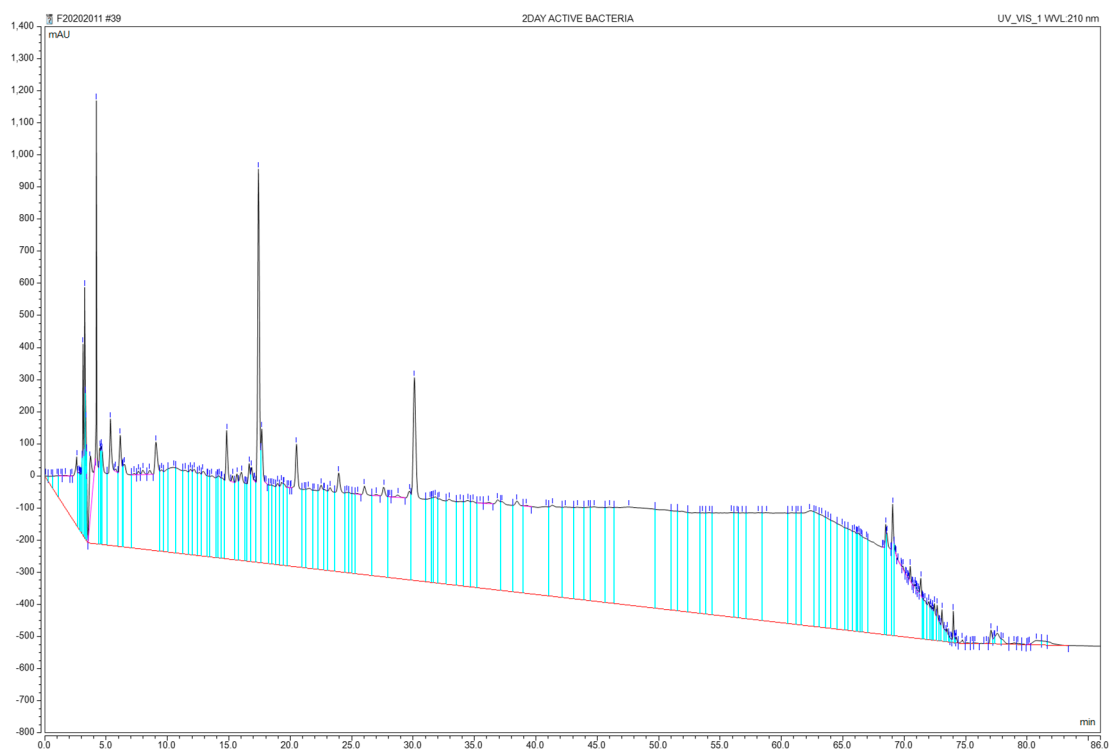


Figure S10. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on 48 hours

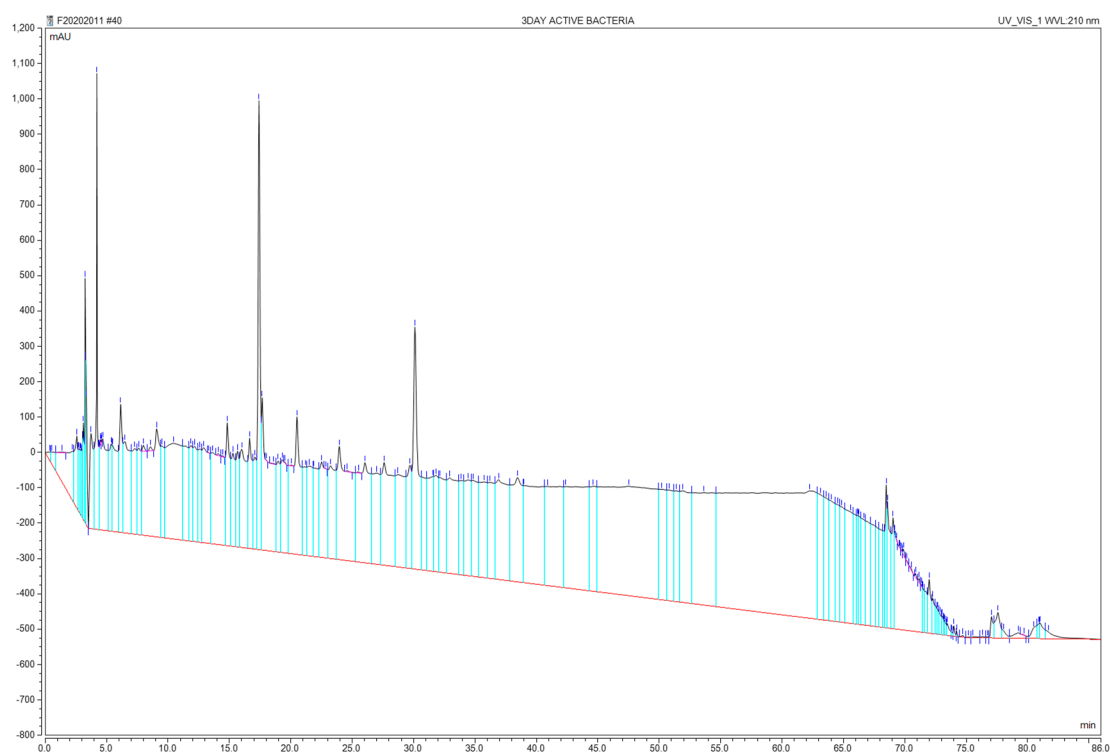


Figure S11. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on 72 hours

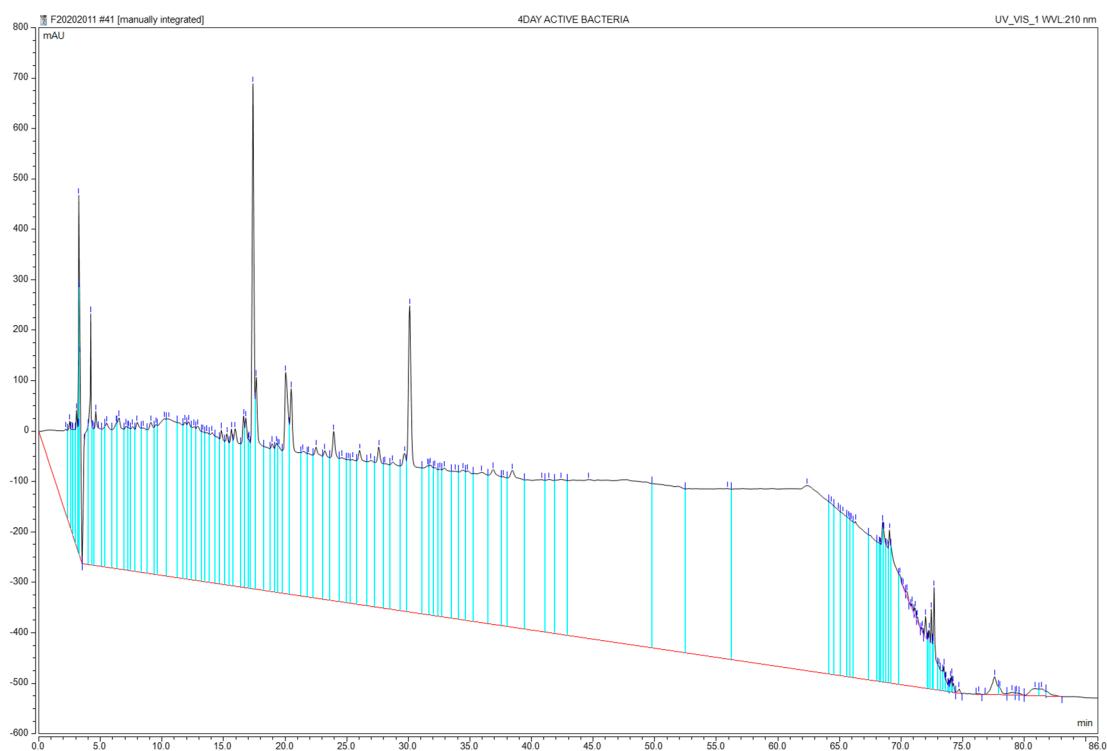


Figure S12. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on 96 hours

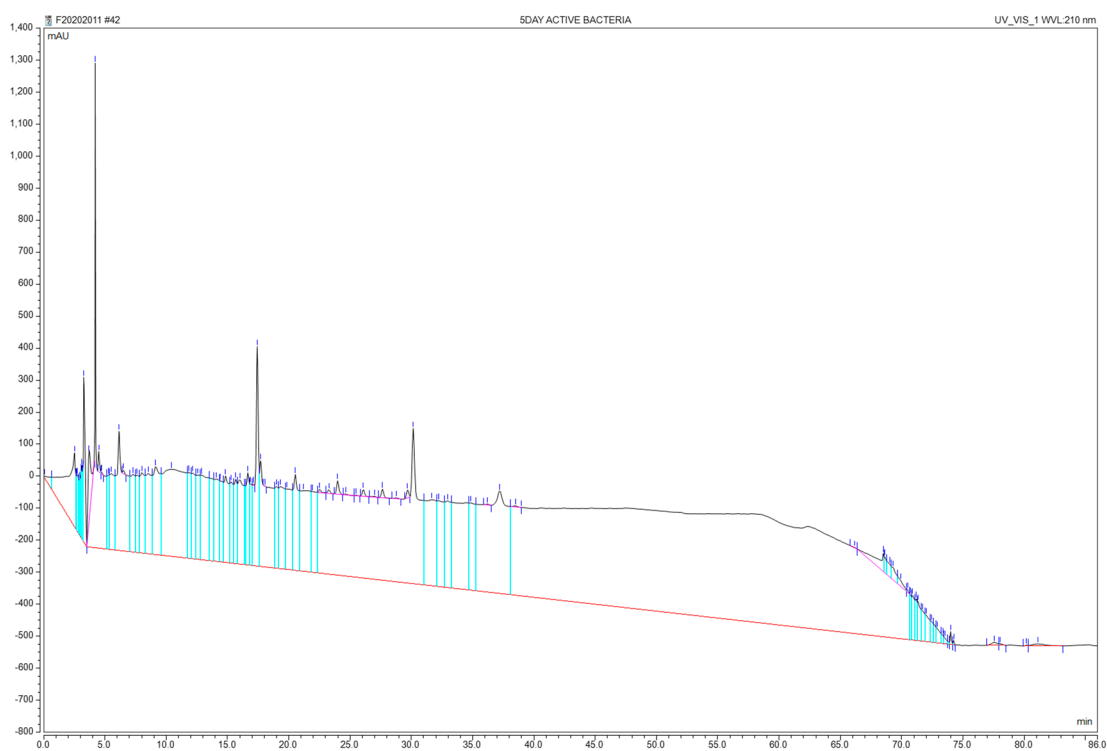


Figure S13. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on 120 hours

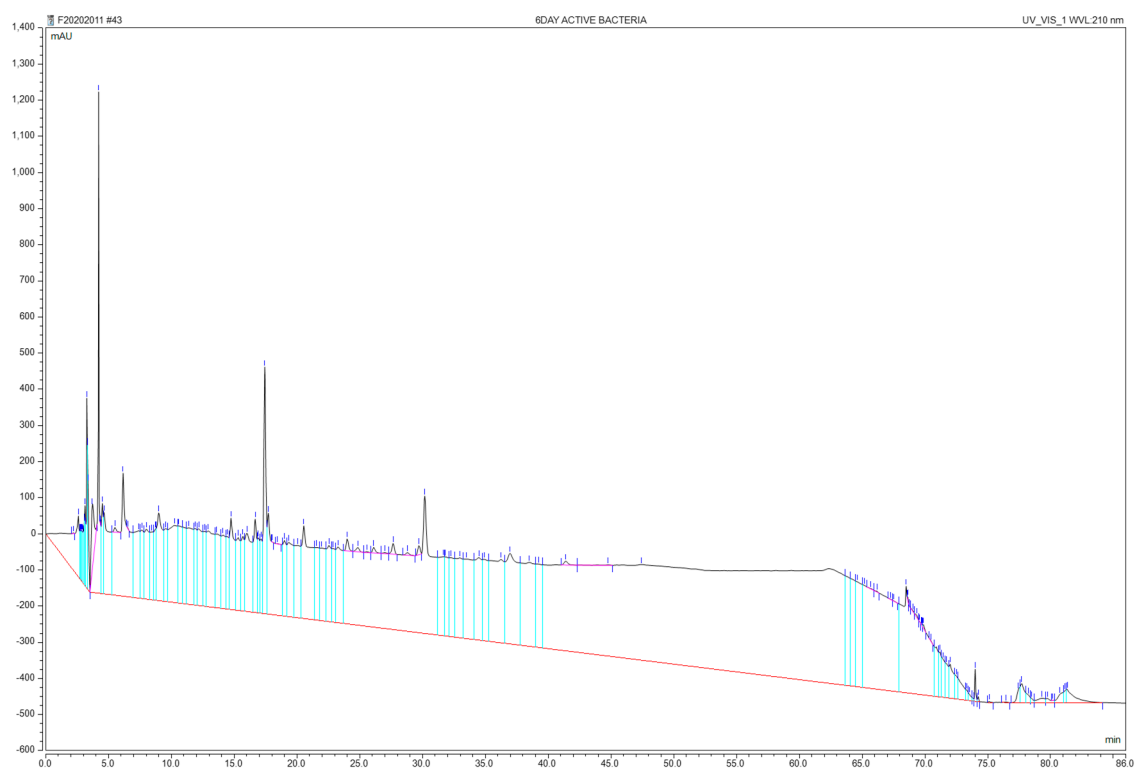


Figure S14. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on 144 hours

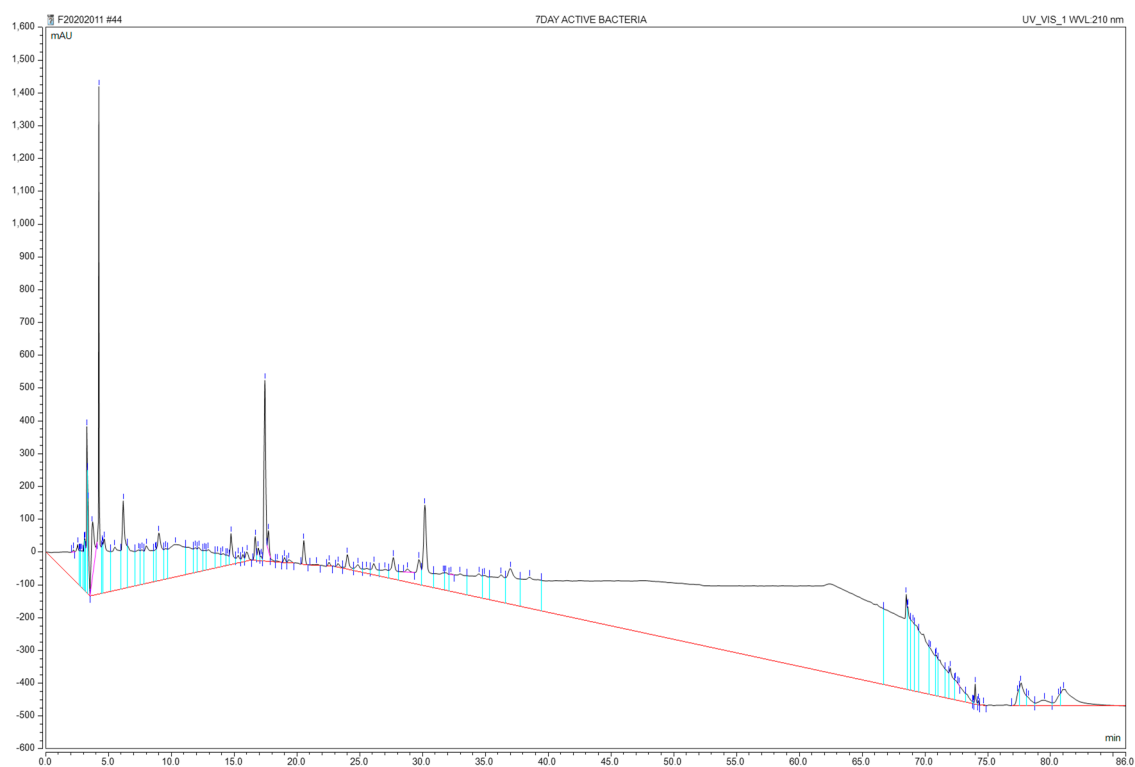


Figure S15. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on 168 hours

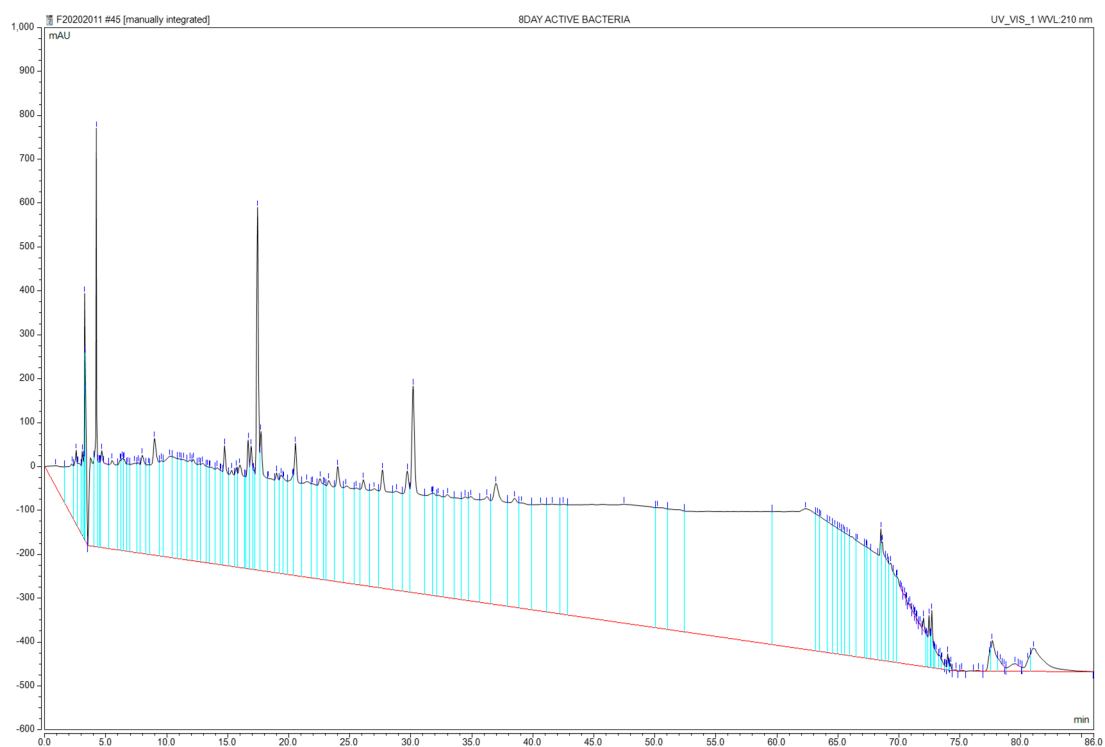


Figure S16. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on 192 hours

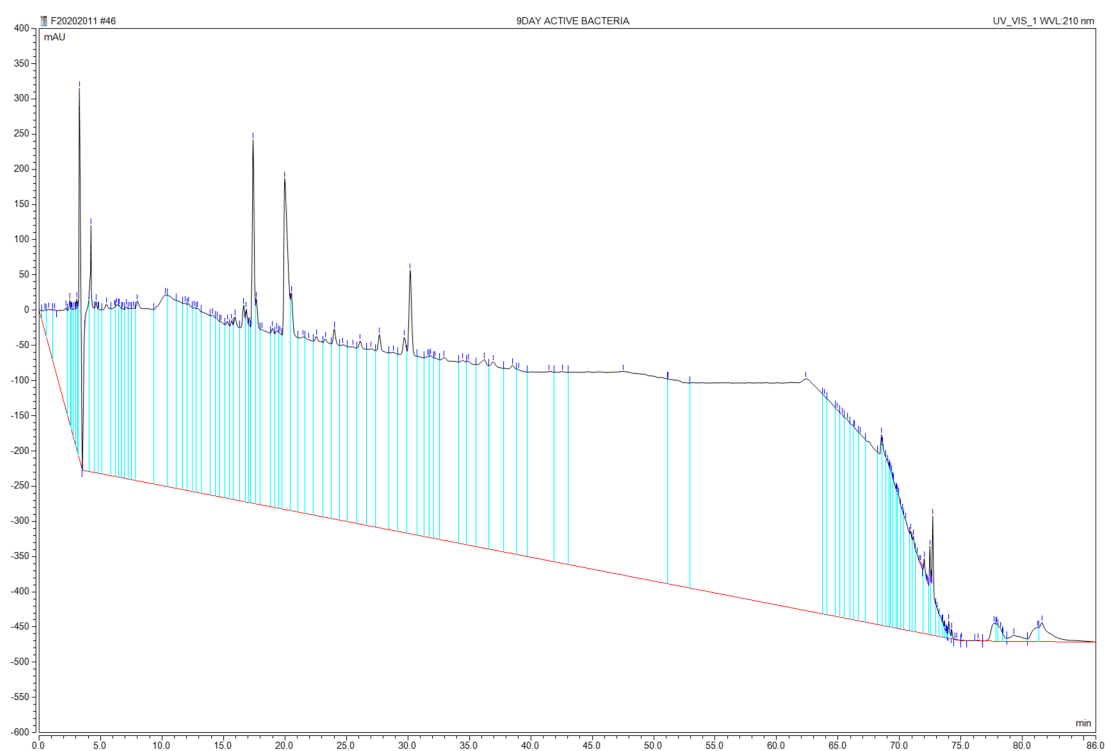


Figure S17. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on 216 hours

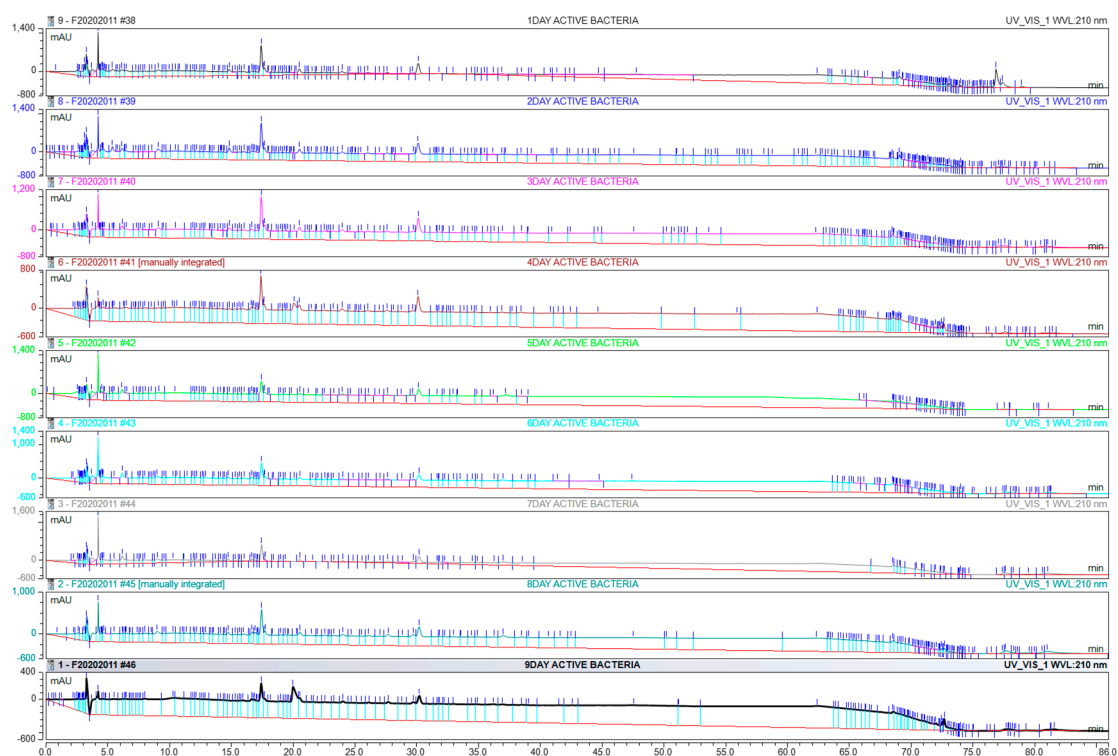


Figure S18. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on different incubation times

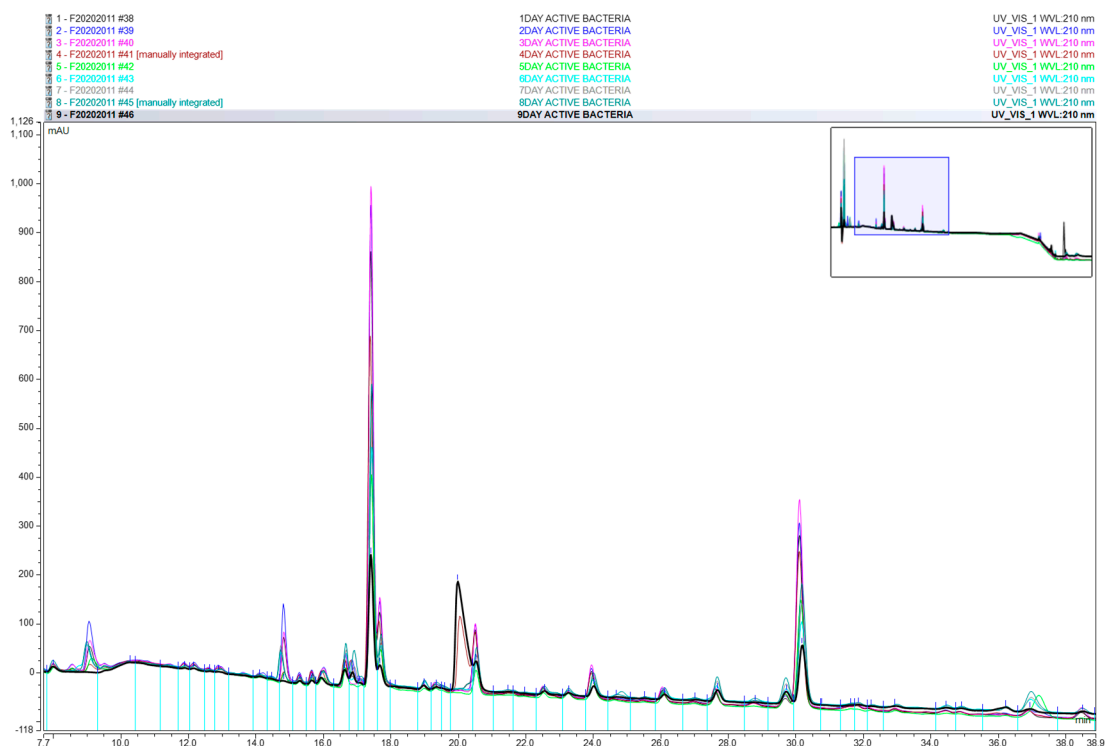


Figure S19. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on different incubation times

HPLC Analysis of natural products synthesized in different medium by most active endophytic bacteria *B. halotolerans* XJB-35

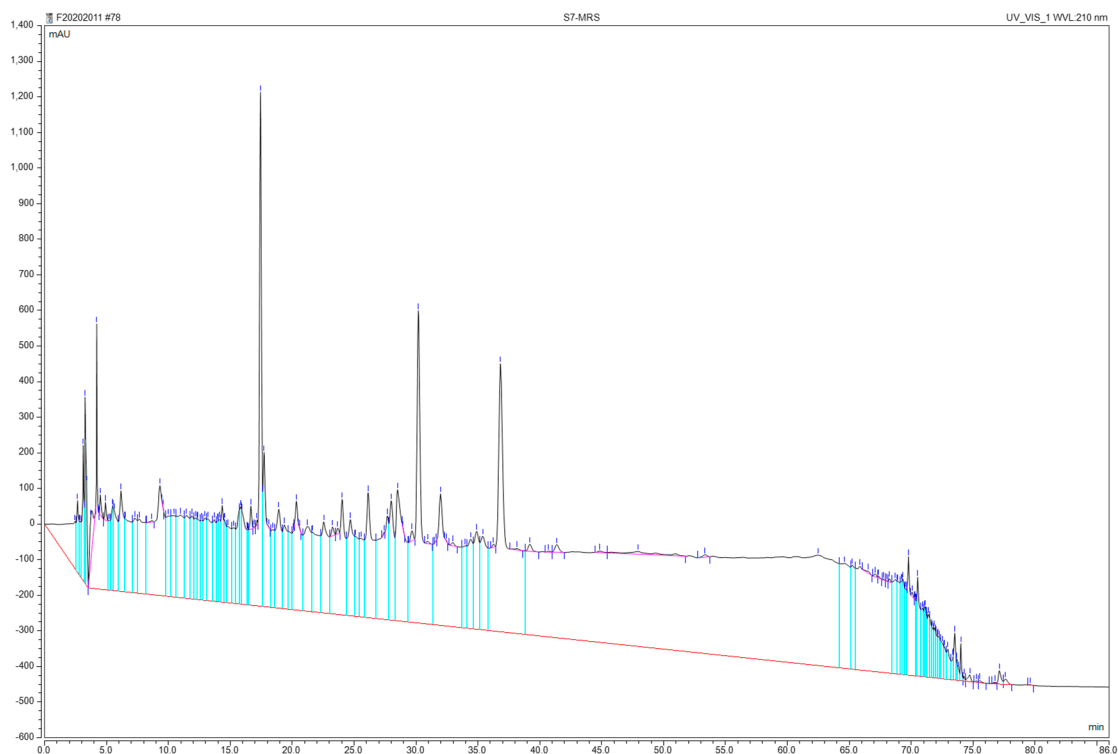


Figure S20. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on MRS media

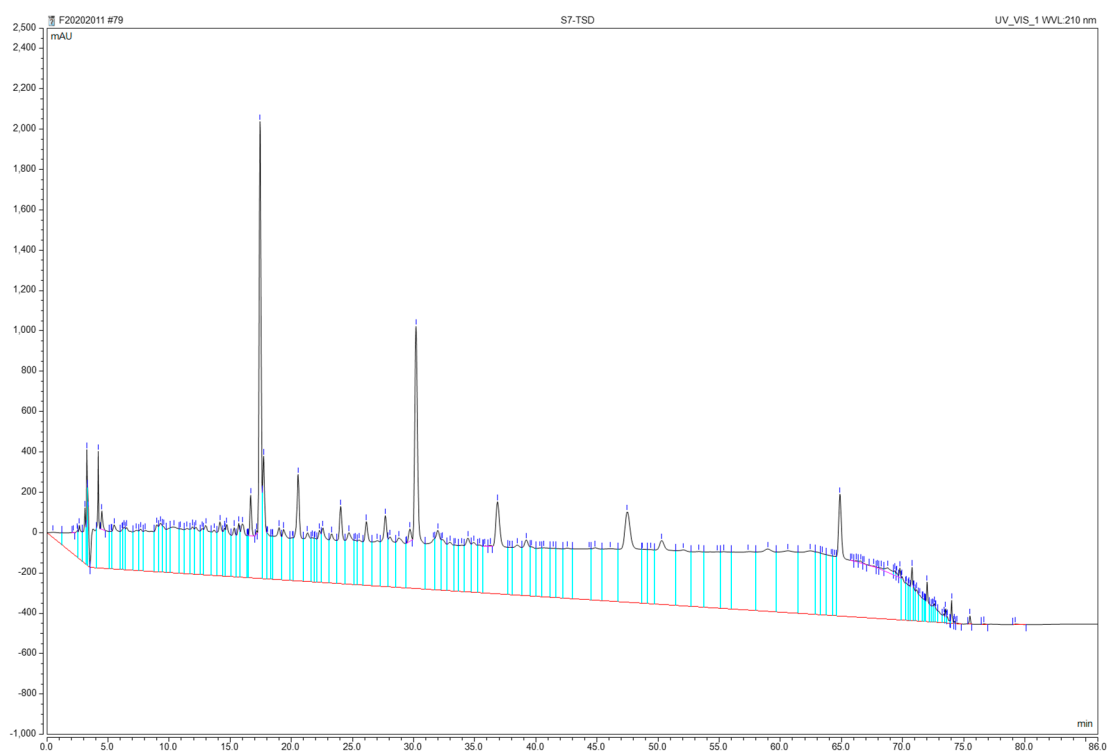


Figure S21. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on TSD media

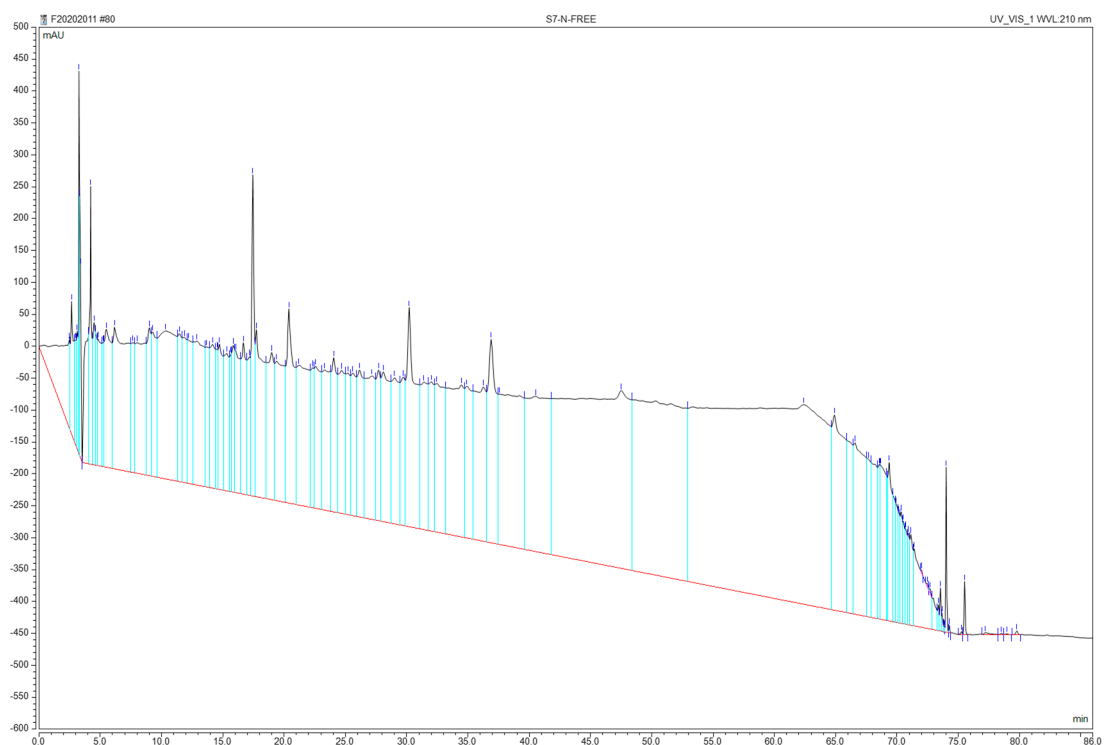


Figure S22. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on N-Free media

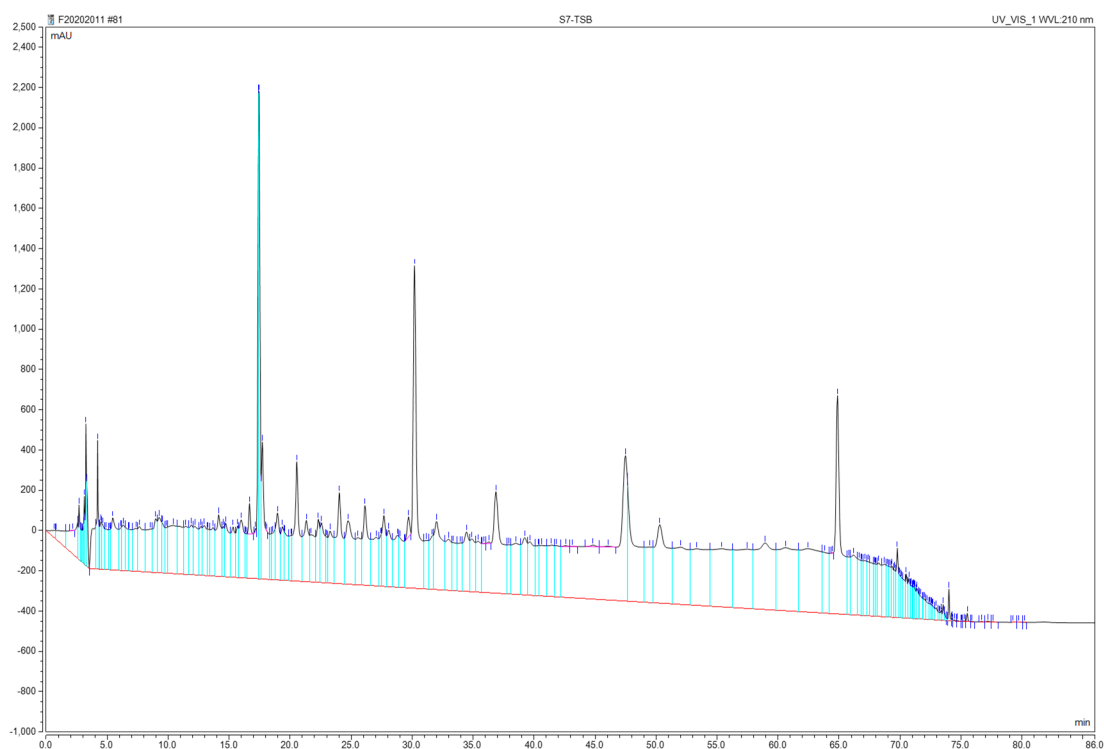


Figure S23. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on TSB media

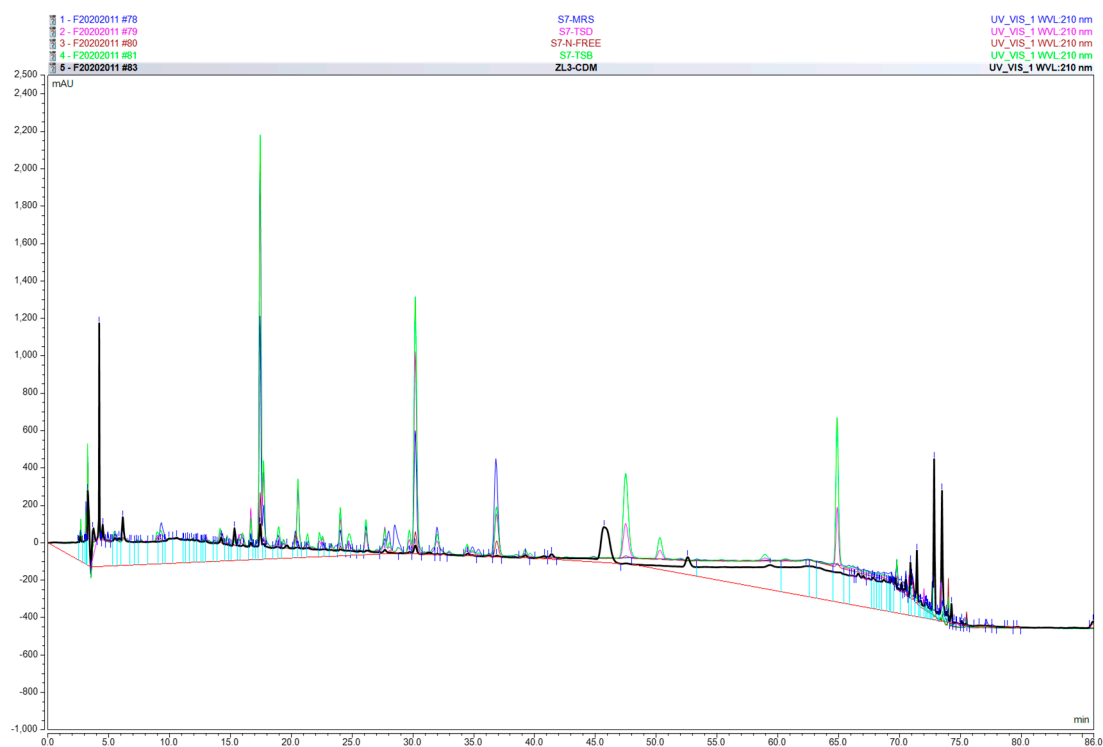


Figure S24. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on different media

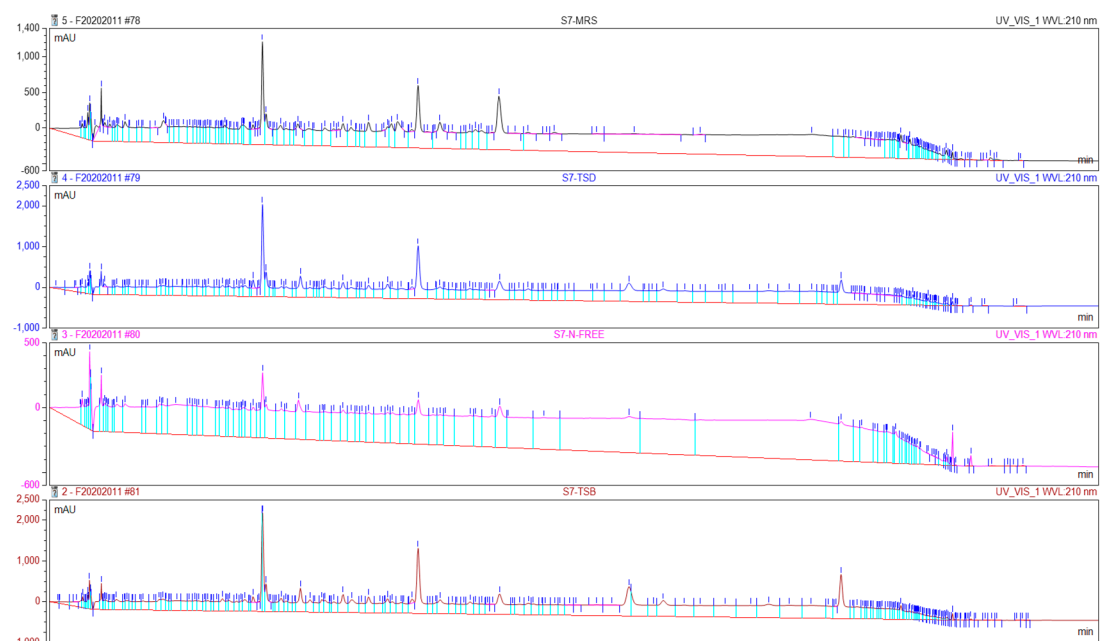


Figure S25. HPLC Analysis of natural products synthesized by most active endophytic bacteria *B. halotolerans* XJB-35 on different media

Chemical composition of most active bacteria strain *B. halotolerans* XJB-35

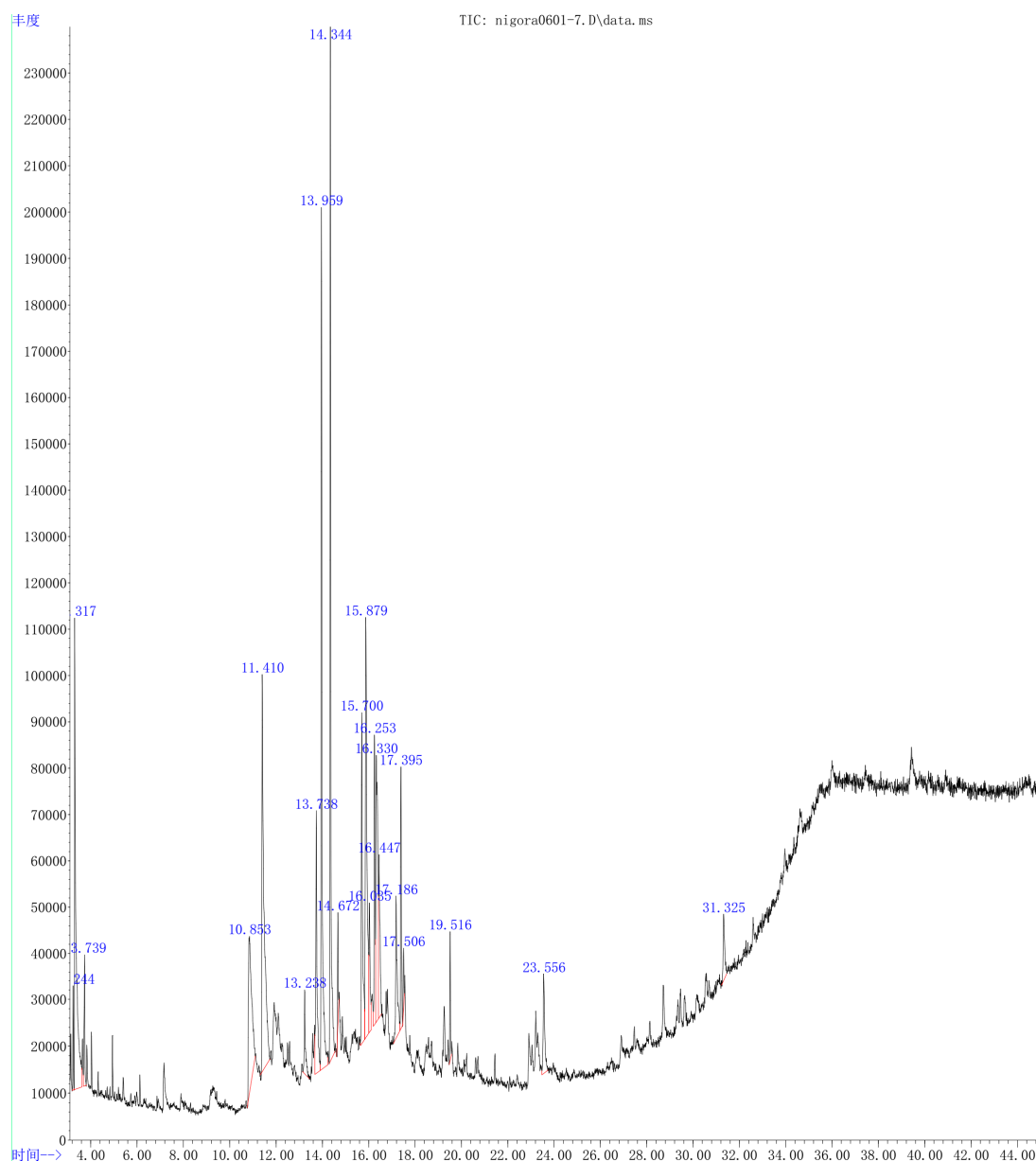


Figure S26. Volatile chemical component of most active endophytic bacteria *B. halotolerans* XJB-35 on different **NB** media

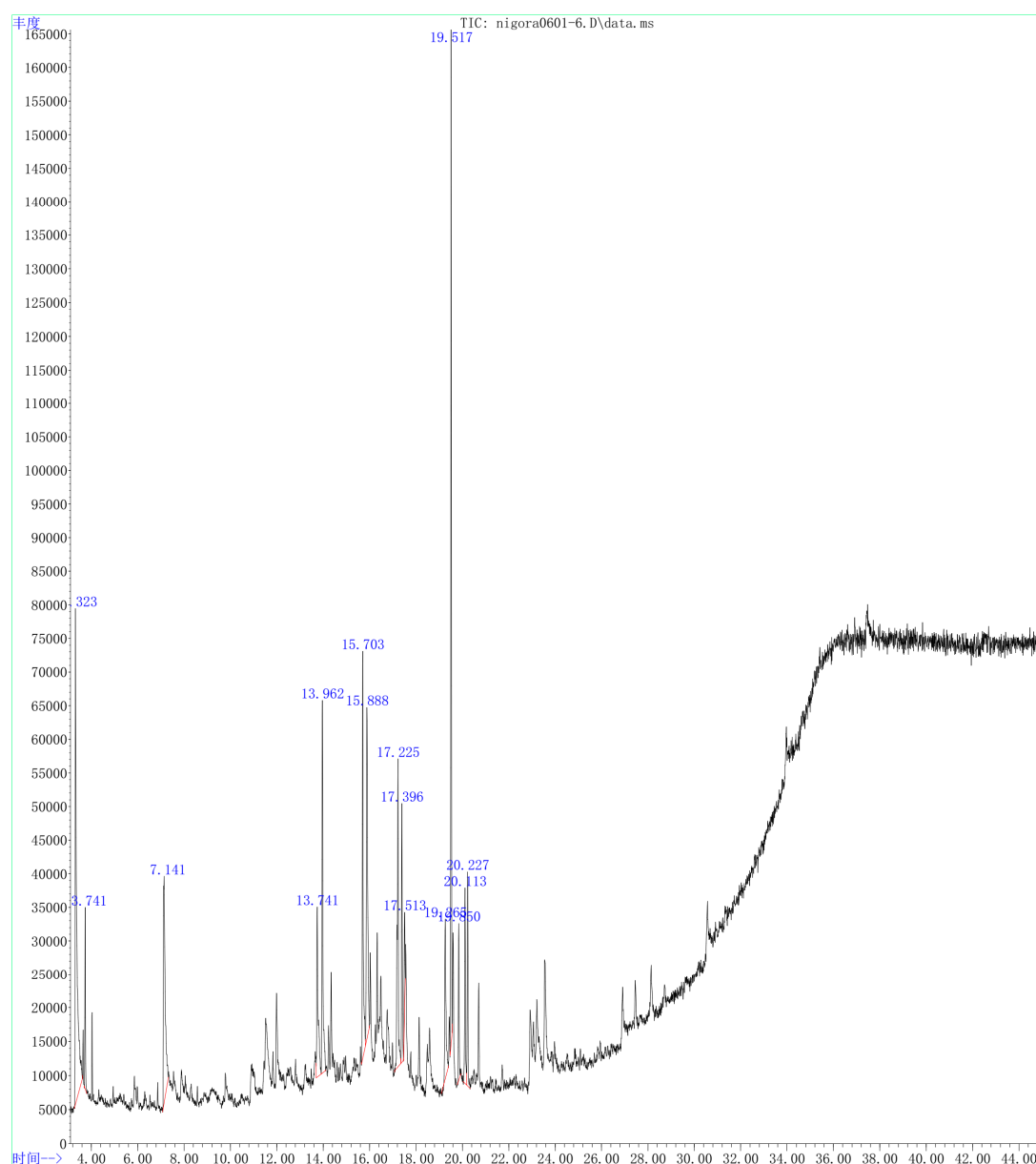


Figure S27. Volatile chemical component of most active endophytic bacteria *B. halotolerans* XJB-35 on different **N-free** media

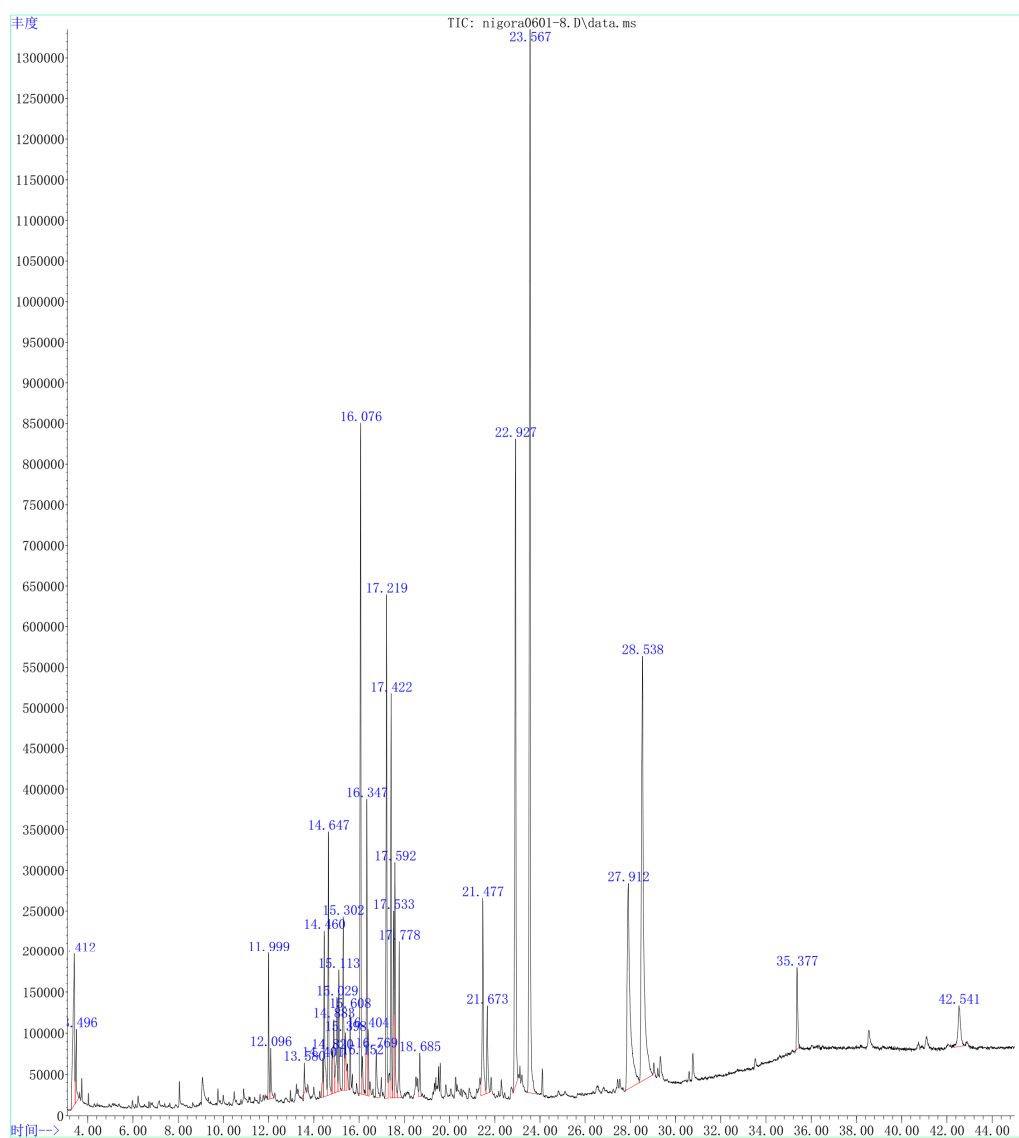


Figure S28. Volatile chemical component of most active endophytic bacteria *B. halotolerans* XJB-35 on different TSD media

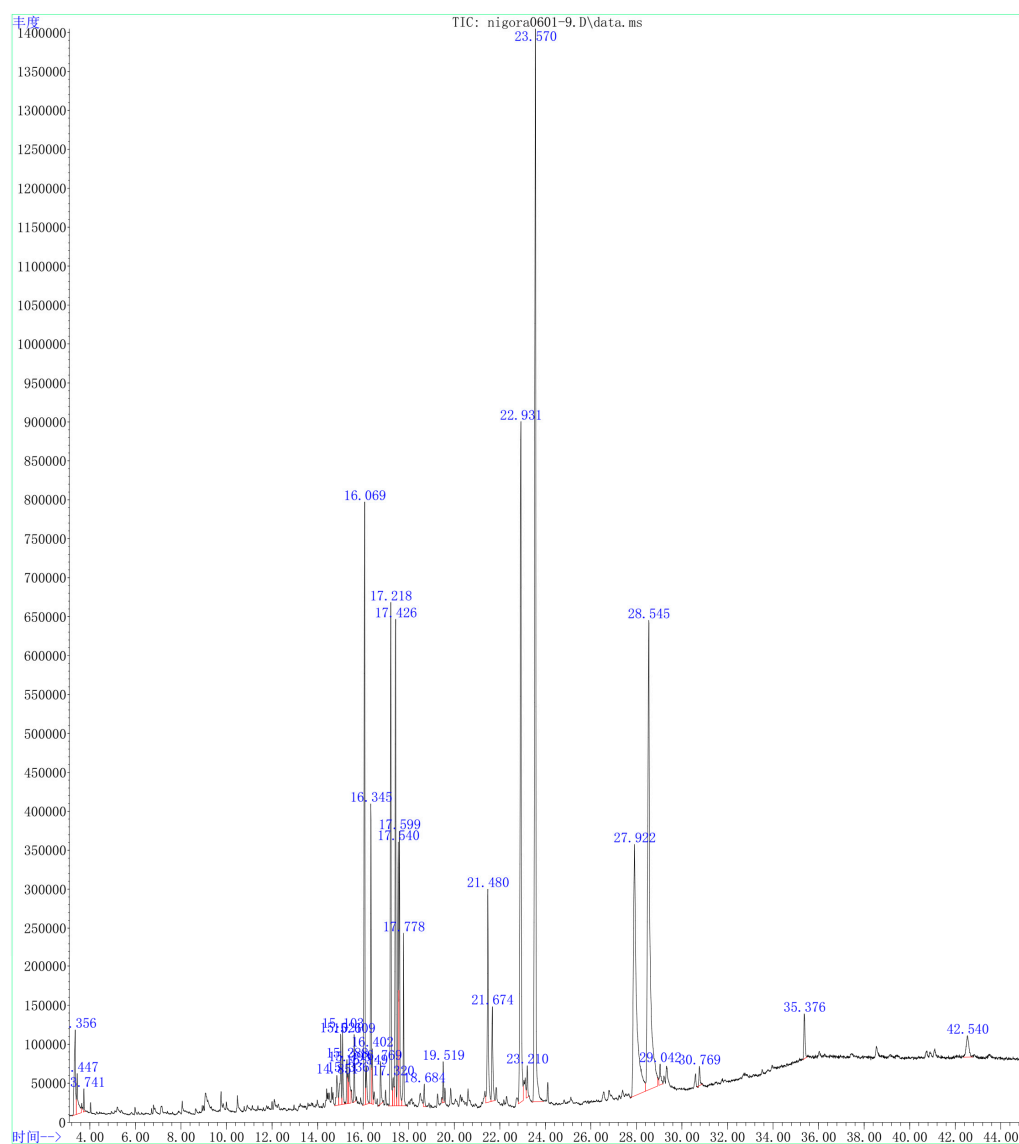


Figure S29. Volatile chemical component of most active endophytic bacteria *B. halotolerans* XJB-35 on different TSB media

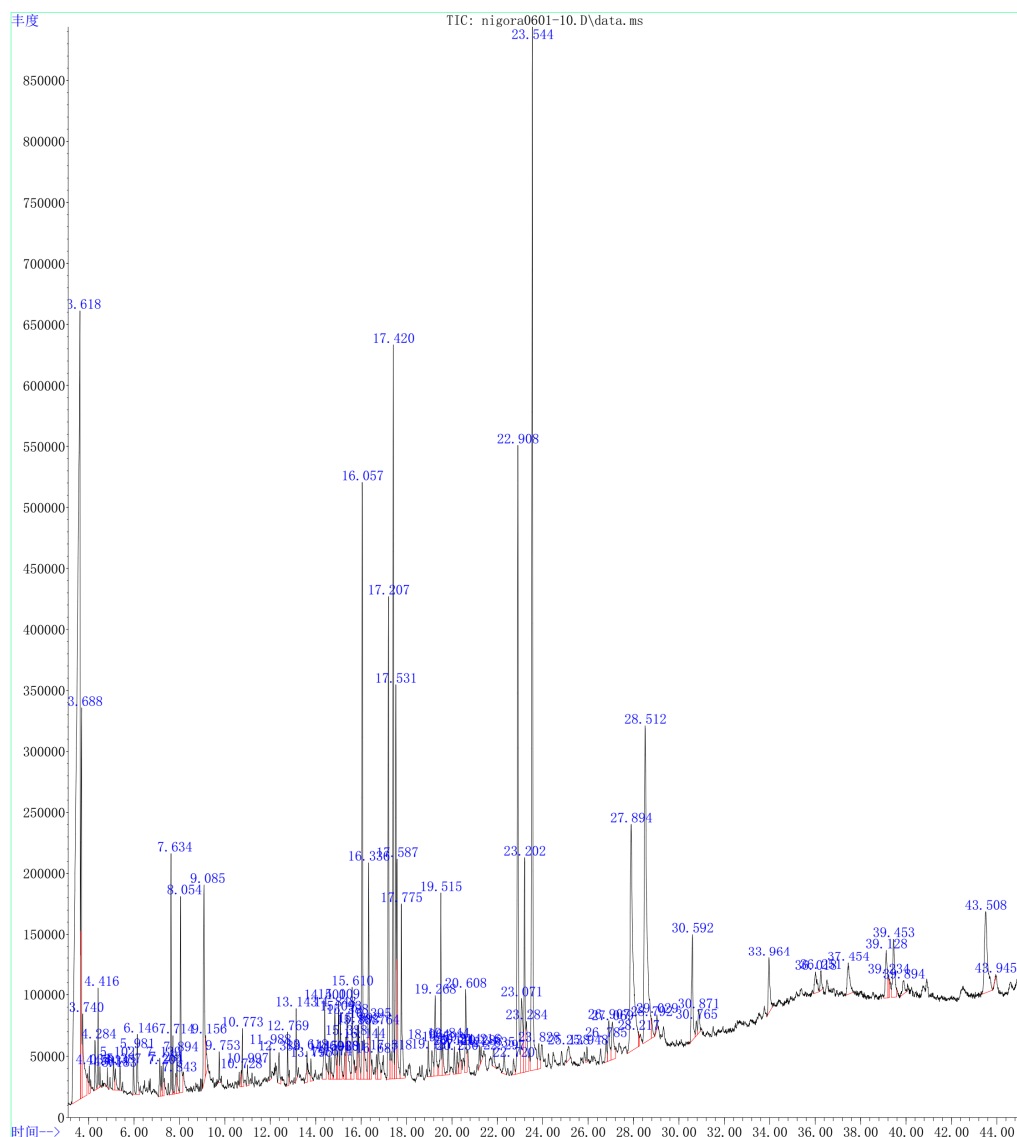


Figure S30. Volatile chemical component of most active endophytic bacteria *B. halotolerans* XJB-35 on different MRS media

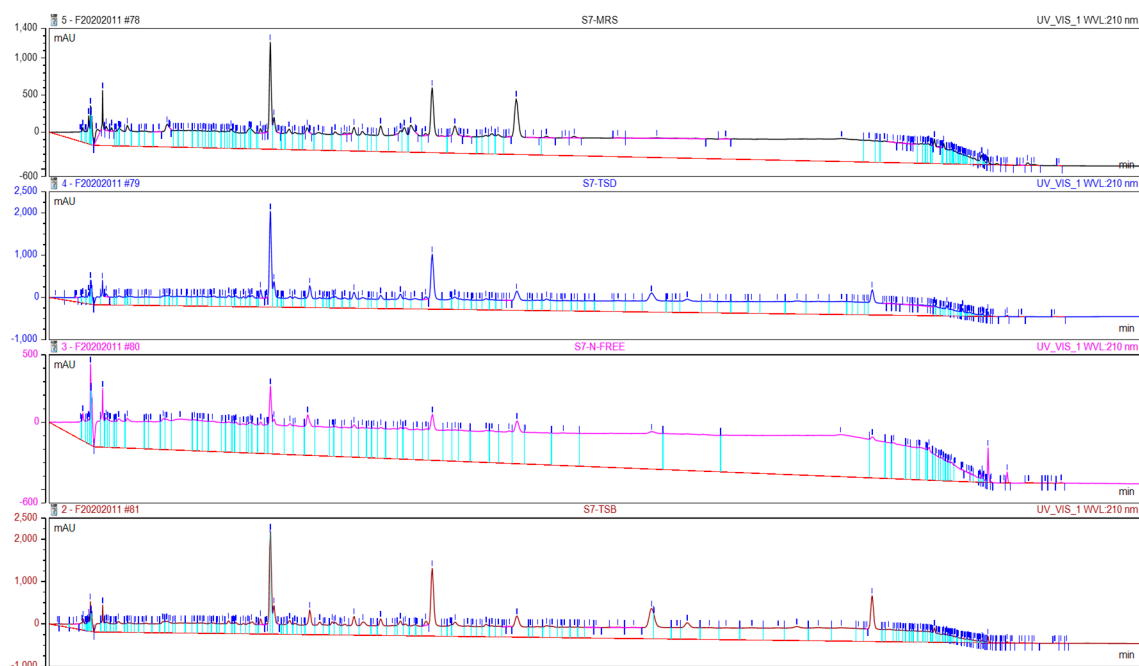


Figure S31. HPLC analysis of secondary metabolites produced by the most-active endophytic bacteria *B. halotolerans* XJB-35 on different culture media