

Supplementary Materials

Table S1 Mycolic acid-containing bacteria (MACB) and streptomycetes used in this study.

| No. | Strain number | Genus | Most closely related type strain | 16S rRNA gene similarity |
|-----|---------------|----------------------|---|--------------------------|
| 1. | HX09-1 | <i>Mycobacterium</i> | <i>Mycobacterium hodleri</i> JCM 12141 ^T | 99.32 % |
| 2. | HX09-8 | <i>Rhodococcus</i> | <i>Rhodococcus qingshengii</i> djl-6 ^T | 100.00 % |
| 3. | HX09-20 | <i>Nocardia</i> | <i>Nocardia cummidelens</i> R89 ^T | 99.78 % |
| 4. | HX10-42 | <i>Mycobacterium</i> | <i>Mycobacterium madagascariense</i> P2 ^T | 98.64 % |
| 5. | HX10-55 | <i>Rhodococcus</i> | <i>Rhodococcus opacus</i> DSM 43205 ^T | 99.92 % |
| 6. | HX11-30 | <i>Rhodococcus</i> | <i>Rhodococcus yunnanensis</i> YIM 70056 ^T | 99.70 % |
| 7. | HX11-35 | <i>Rhodococcus</i> | <i>Rhodococcus cercidiphylli</i> YIM 65003 ^T | 99.77 % |
| 8. | HX14-21 | <i>Nocardia</i> | <i>Nocardia fluminea</i> DSM 44489 ^T | 98.64 % |
| 9. | WS03-62 | <i>Nocardia</i> | <i>Nocardia abscessus</i> NBRC 100374 ^T | 98.94 % |
| 10. | WS04-9 | <i>Rhodococcus</i> | <i>Rhodococcus qingshengii</i> JCM 15477 ^T | 100.00 % |
| 11. | FXJ1.050 | <i>Streptomyces</i> | <i>Streptomyces hygroscopicus</i> subsp. CGMCC 4.1527 ^T | 100.00 % |
| 12. | FXJ1.068 | <i>Streptomyces</i> | <i>Streptomyces hygroscopicus</i> subsp. CGMCC 4.1252 ^T | 99.85 % |
| 13. | FXJ1.069 | <i>Streptomyces</i> | <i>Streptomyces yeochonensis</i> NBRC 100782 ^T | 98.08 % |
| 14. | FXJ1.076 | <i>Streptomyces</i> | <i>Streptomyces hygroscopicus</i> subsp. NBRC 16551 ^T | 100.00 % |
| 15. | FXJ1.172 | <i>Streptomyces</i> | <i>Streptomyces naganishii</i> NRRL B-1816 ^T | 99.21 % |
| 16. | FXJ1.235 | <i>Streptomyces</i> | <i>Streptomyces lasalocidi</i> ATCC 31180 ^T | 99.06 % |
| 17. | FXJ1.253 | <i>Streptomyces</i> | <i>Streptomyces hygroscopicus</i> subsp. NBRC 16551 ^T | 100.00 % |
| 18. | FXJ1.264 | <i>Streptomyces</i> | <i>Streptomyces cuspidosporus</i> NBRC 12378 ^T | 99.78 % |
| 19. | FXJ1.4008 | <i>Streptomyces</i> | <i>Streptomyces anandii</i> JCM 4720 ^T | 99.24 % |
| 20. | FXJ1.4012 | <i>Streptomyces</i> | <i>Streptomyces diastaticus</i> subsp. <i>ardesiacus</i> NRRL B-1773 ^T | 99.77 % |
| 21. | FXJ1.4013 | <i>Streptomyces</i> | <i>Streptomyces misionensis</i> NBRC 13063 ^T | 100.00 % |
| 22. | FXJ1.4014 | <i>Streptomyces</i> | <i>Streptomyces naganishii</i> NBRC 12892 ^T | 100.00 % |
| 23. | FXJ1.4033 | <i>Streptomyces</i> | <i>Streptomyces achromogenes</i> subsp. <i>rubradiris</i> NBRC 14000 ^T | 99.25 % |
| 24. | FXJ1.4034 | <i>Streptomyces</i> | <i>Streptomyces aldersoniae</i> NRRL 18513 ^T | 99.71 % |
| 25. | FXJ1.4035 | <i>Streptomyces</i> | <i>Streptomyces aurantiogriseus</i> NBRC 12842 ^T | 98.43 % |
| 26. | FXJ1.4037 | <i>Streptomyces</i> | <i>Streptomyces avellaneus</i> NBRC 13451 ^T | 99.63 % |
| 27. | FXJ1.4038 | <i>Streptomyces</i> | <i>Streptomyces chromofuscus</i> NBRC 12851 ^T | 98.13 % |
| 28. | FXJ1.4041 | <i>Streptomyces</i> | <i>Streptomyces cuspidosporus</i> NBRC 12378 ^T | 99.78 % |
| 29. | FXJ1.4044 | <i>Streptomyces</i> | <i>Streptomyces durhamensis</i> NRRL B-3309 ^T | 98.83 % |
| 30. | FXJ1.4054 | <i>Streptomyces</i> | <i>Streptomyces rameus</i> LMG 20326 ^T | 99.33 % |
| 31. | FXJ1.4056 | <i>Streptomyces</i> | <i>Streptomyces somaliensis</i> NBRC 12916 ^T | 97.84 % |
| 32. | FXJ1.4059 | <i>Streptomyces</i> | <i>Streptomyces spiralis</i> NBRC 14215 ^T | 100.00 % |
| 33. | FXJ1.4061 | <i>Streptomyces</i> | <i>Streptomyces thermoviolaceus</i> subsp. <i>apingens</i> DSM 41392 ^T | 99.18 % |
| 34. | FXJ1.4075 | <i>Streptomyces</i> | <i>Streptomyces corchorusii</i> NBRC 13032 ^T | 99.48 % |
| 35. | FXJ1.4082 | <i>Streptomyces</i> | <i>Streptomyces malachitofuscus</i> NBRC 13059 ^T | 99.18 % |

Supplementary Materials

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| 36. | FXJ1.4087 | <i>Streptomyces</i> | <i>Streptomyces rameus</i> LMG 20326 ^T | 99.26 % |
| 37. | FXJ1.4088 | <i>Streptomyces</i> | <i>Streptomyces spinoverrucosus</i> NBRC 14228 ^T | 98.44 % |
| 38. | FXJ1.4089 | <i>Streptomyces</i> | <i>Streptomyces thermoviolaceus</i> subsp. <i>apingens</i> DSM 41392 ^T | 99.18 % |
| 39. | FXJ1.4097 | <i>Streptomyces</i> | <i>Streptomyces azureus</i> NBRC 12744 ^T | 99.93 % |
| 40. | FXJ1.4098 | <i>Streptomyces</i> | <i>Streptomyces bingchenggensis</i> BCW-1 ^T | 99.70 % |
| 41. | FXJ1.4099 | <i>Streptomyces</i> | <i>Streptomyces caeruleatus</i> GIMN4 ^T | 99.41 % |
| 42. | FXJ1.4101 | <i>Streptomyces</i> | <i>Streptomyces europaeiscabiei</i> KACC 20186 ^T | 99.85 % |
| 43. | FXJ1.4104 | <i>Streptomyces</i> | <i>Streptomyces hygrosopicus</i> subsp. <i>ossamyceticus</i> NBRC 13983 ^T | 99.93 % |
| 44. | FXJ1.4106 | <i>Streptomyces</i> | <i>Streptomyces cinereoruber</i> subsp. <i>cinereoruber</i> NBRC 12756 ^T | 99.78 % |
| 45. | FXJ1.4107 | <i>Streptomyces</i> | <i>Streptomyces glauciniger</i> NBRC 100913 ^T | 99.20 % |
| 46. | FXJ1.4108 | <i>Streptomyces</i> | <i>Streptomyces hygrosopicus</i> subsp. <i>ossamyceticus</i> NBRC 13983 ^T | 100.00 % |
| 47. | FXJ1.4109 | <i>Streptomyces</i> | <i>Streptomyces panaciradicis</i> 1MR-8 ^T | 99.26 % |
| 48. | FXJ1.4110 | <i>Streptomyces</i> | <i>Streptomyces pseudovenezuelae</i> NBRC 12904 ^T | 99.18 % |
| 49. | FXJ1.4111 | <i>Streptomyces</i> | <i>Streptomyces thermoviolaceus</i> subsp. <i>apingens</i> DSM 41392 ^T | 99.26 % |
| 50. | FXJ1.4112 | <i>Streptomyces</i> | <i>Streptomyces yaanensis</i> Z4 ^T | 99.93 % |
| 51. | FXJ1.532 | <i>Streptomyces</i> | <i>Streptomyces ferralitis</i> CGMCC4.1985 ^T | 99.19 % |
| 52. | FXJ1.535 | <i>Streptomyces</i> | <i>Streptomyces ferralitis</i> CGMCC4.1985 ^T | 98.53 % |
| 53. | FXJ1.907 | <i>Streptomyces</i> | <i>Streptomyces spinoverrucosus</i> NBRC 14228 ^T | 98.38 % |
| 54. | FXJ23y | <i>Streptomyces</i> | <i>Streptomyces achromogenes</i> subsp. <i>rubradiris</i> NBRC 14000 ^T | 99.19 % |

Table S2 Linear elution ratio of methanol and water in HPLC-PDA analysis.

| Time (min) | 0 | 15 | 20.5 | 20.51 | 26.51 |
|------------------------|----|-----|------|-------|-------|
| ddH ₂ O (%) | 80 | 0 | 0 | 80 | 80 |
| Methanol (%) | 20 | 100 | 100 | 20 | 20 |

Table S3 HPLC-PDA/HRMS data of 1.264HX-1-6.

| Chemical data | 1.264HX-1 | 1.264HX-2 | 1.264HX-3 | 1.264HX-4 | 1.264HX-5 | 1.264HX-6 |
|----------------------|--|-----------------------|--|--|--|--|
| HRMS (positive) | [M+H] ⁺ 373.1432 | — | [M+H] ⁺ 387.1599 | — | — | — |
| HRMS (negative) | [M-H] ⁻ 371.1292 | — | [M-H] ⁻ 385.1433 | [M-H] ⁻ 743.2671 | [M-H] ⁻ 743.2645 | [M-H] ⁻ 743.2634 |
| Molecular formula | C ₂₄ H ₂₀ O ₄ | — | C ₂₅ H ₂₂ O ₄ | C ₄₈ H ₄₀ O ₈ | C ₄₈ H ₄₀ O ₈ | C ₄₈ H ₄₀ O ₈ |
| Molecular weight | 372 | — | 386 | 744 | 744 | 744 |
| UV absorptivity (nm) | 195, 251, 285, 376 | 195, 251, 285, 376 | 195, 251, 285, 376 | 195, 251, 285, 376 | 195, 251, 285, 376 | 195, 251, 285, 376 |

Note: '—', no corresponding signal detected in mass spectrum.

Supplementary Materials

Table S4 NMR data of 1.264HX-3 in $CDCl_3$ (500 MHz).

| Number | Chemical shift of ^{13}C (ppm) | Chemical shift of 1H (ppm, J in Hz) | DEPT135 |
|--------|-------------------------------------|--|-----------------|
| 1 | 13.3 | 1.53 (d) | CH ₃ |
| 2 | 28.7 | 1.18 (s) | CH ₂ |
| 3 | 32.49 | 3.6 (d) | CH ₂ |
| 3-1? | 32.51 | 3.6 (d) | CH ₂ |
| 4 | 54.7 | 3.8 (s) | CH ₃ |
| 5 | 85.72 | 5.00 (m) | CH |
| 5-1? | 85.69 | 5.00 (m) | CH |
| 6 | 88.54 | 5.12 (m) | CH |
| 6-1? | 88.57 | 5.12 (m) | CH |
| A | 99.14 | | C |
| A-1? | 98.79 | | C |
| 7 | 99.69 | 6.40 (d, 2.5) | CH |
| 7-1? | 99.99 | 6.415 (d, 2.5) | CH |
| 8 | 101.01 | 6.30 (d, 2.5) | CH |
| 8-1? | 101.0 | 6.307 (d, 2.) | CH |
| 9 | 105.22 | 6.24 (s) | CH |
| 9-1? | 106.71 | 6.29 (s) | CH |
| 10 | 121.23 | 6.15 (d, 15.5) | CH |
| 10-1? | 117.49 | 5.80 (d, 12) | CH |
| 11 | 124.4 | 7.5 (d, 4) | CH |
| 11-1? | 125.08 | 7.5 (d, 4) | CH |
| 12 | 125.67 | 7.14 (m) | CH |
| 12-1? | 125.75 | 7.14 (m) | CH |
| 13 | 125.99 | 7.14 (m) | CH |
| 14 | 127.46 | 6.78, 6.75 (dd, 11.2, 15.5) | CH |
| 14-1? | 127.39 | 7.91, 7.88 (dd, 11.5, 15.5) | CH |
| 15 | 128.98 | 7.14 (m) | CH |
| 15-1? | 128.81 | 7.14 (m) | CH |
| 16 | 133.38 | 7.2 (d, 11) | CH |
| 17 | 133.67 | 7.04 (d, 15.5) | CH |
| 18 | 133.95 | 6.97 (d, 15.5) | CH |
| 19 | 134.35 | | C |
| 19-1? | 134.38 | | C |
| 19-2? | 135.13 | | C |
| 20 | 137.58 | | C |
| 20-1? | 137.41 | | C |
| 21 | 138.29 | | C |
| 21-1? | 138.19 | | C |
| 22 | 151.4 | | C |
| 22-1? | 152.9 | | C |
| 23 | 162.75 | | C |
| 23-1? | 162.65 | | C |
| 24 | 164.58 | | C |
| 24-1? | 164.43 | | C |

Supplementary Materials

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|-------|--------|-----|
| 25 | 165.73 | C |
| 25-1? | 165.68 | C |
| 26 | 204.23 | C=O |
| 26-1? | 204.20 | C=O |

Note: '?', the chemical shifts of ^{13}C have not yet been determined.



Figure S1 The device for non-contact co-culture. Diffusion of blue ink through a 0.22- μm polyether sulfone membrane from the right compartment to the left was observed within 2 hours.

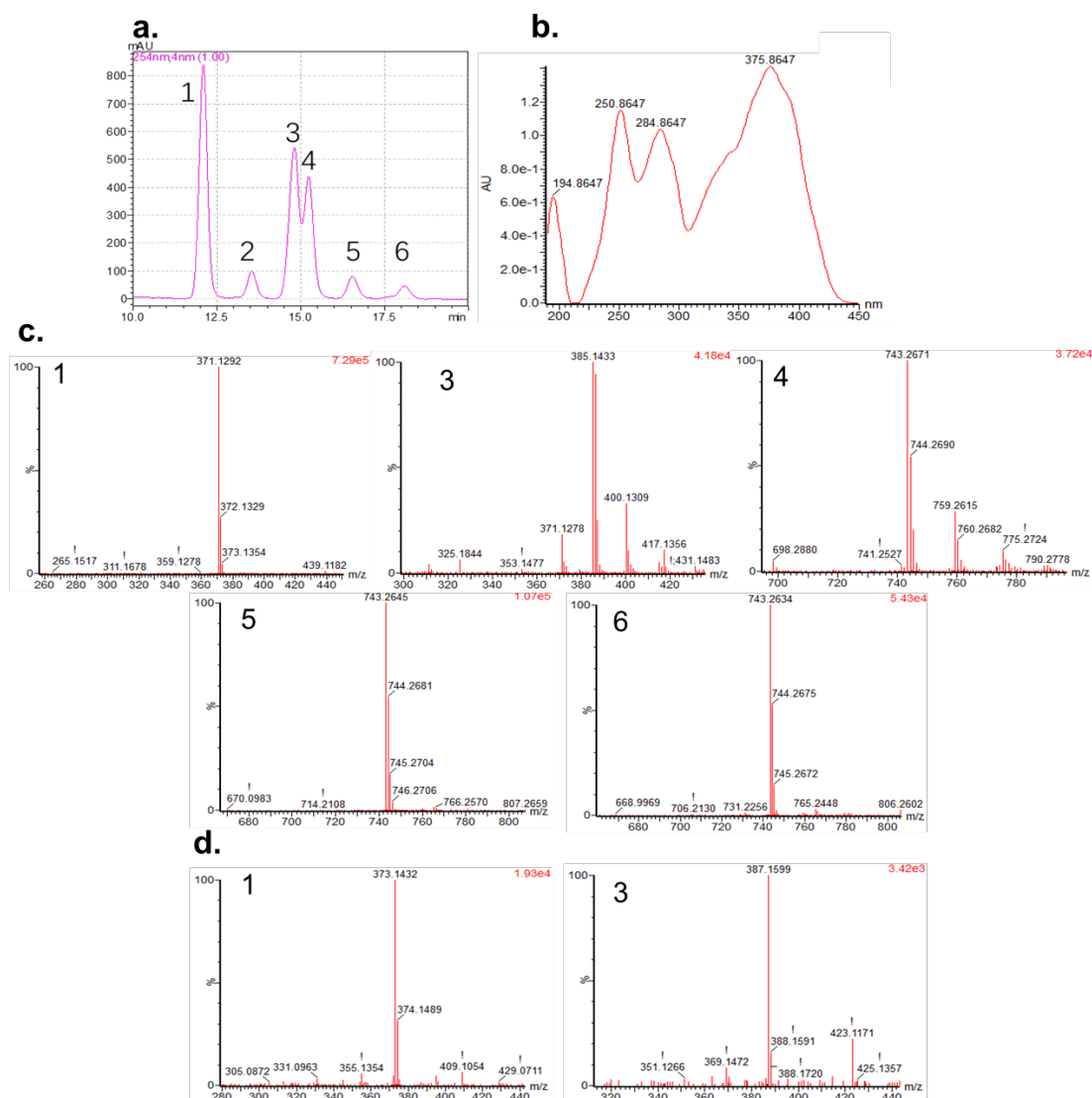


Figure S2 HPLC and HRMS chromatograms of compounds 1.264HX-1-6; (a) HPLC chromatogram of 1.264HX-1-6; (b) Ultraviolet absorption data of 1.264HX-1-6 at 254 nm; (c) HRMS data (negative ion mode) of 1.264HX-1-6 corresponding to the absorption peaks in (a); (d) HRMS data (positive ion mode) of 1.264HX-1 and 1.264HX-3.

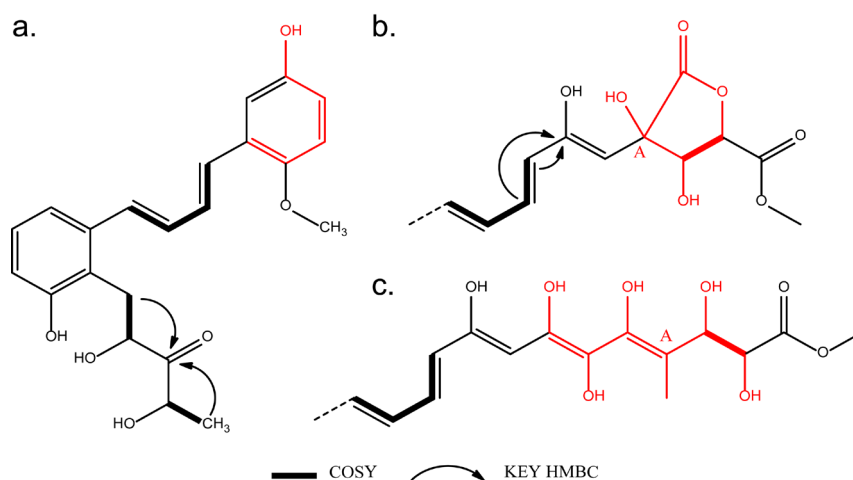


Figure S3 Three putative structures of 1.264HX-3; the black and red parts in the diagram represent the determined and undetermined structure elucidation, respectively.

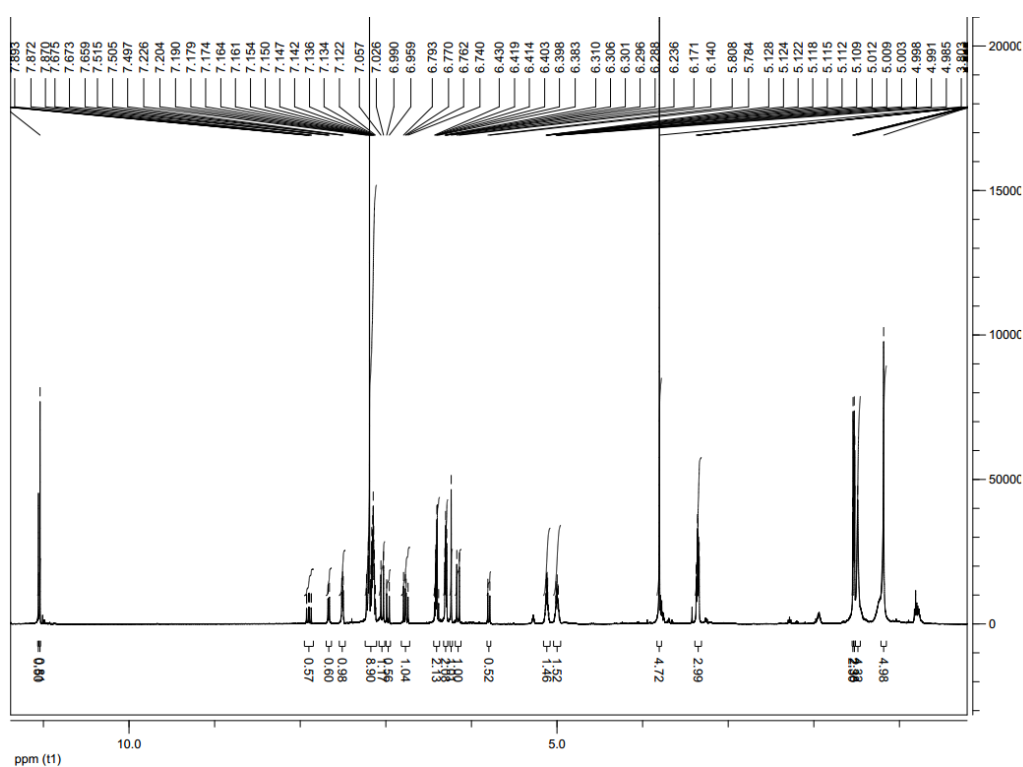


Figure S4 ^1H spectrum of 1.264HX-3.

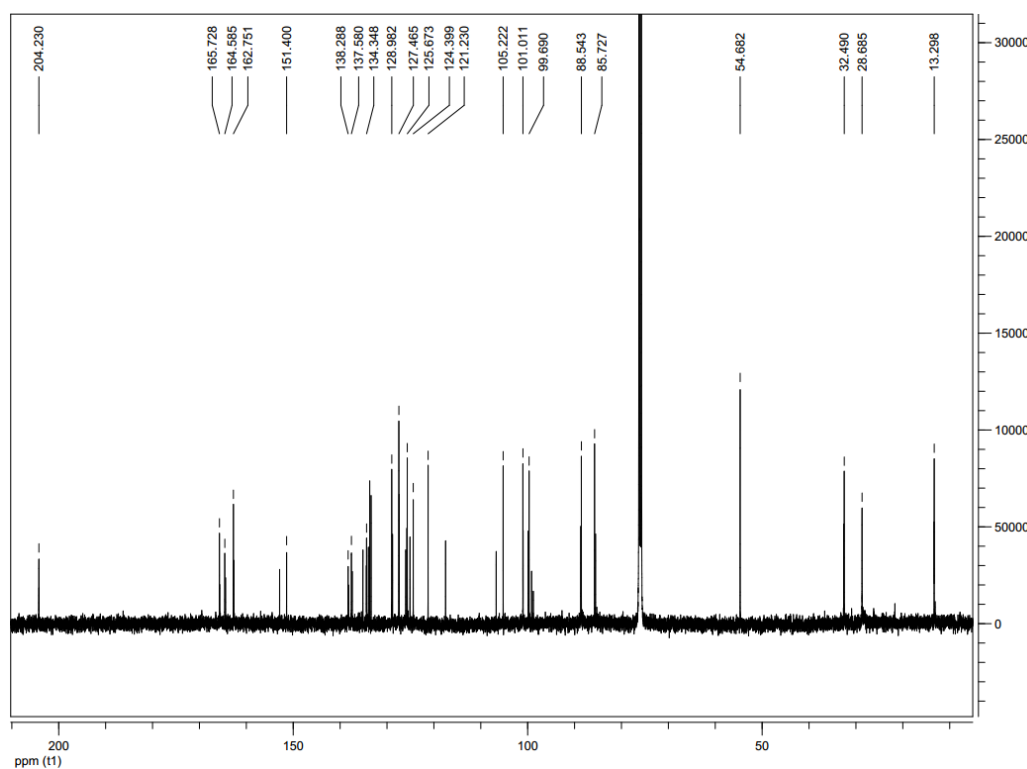


Figure S5 ^{13}C spectrum of 1.264HX-3.

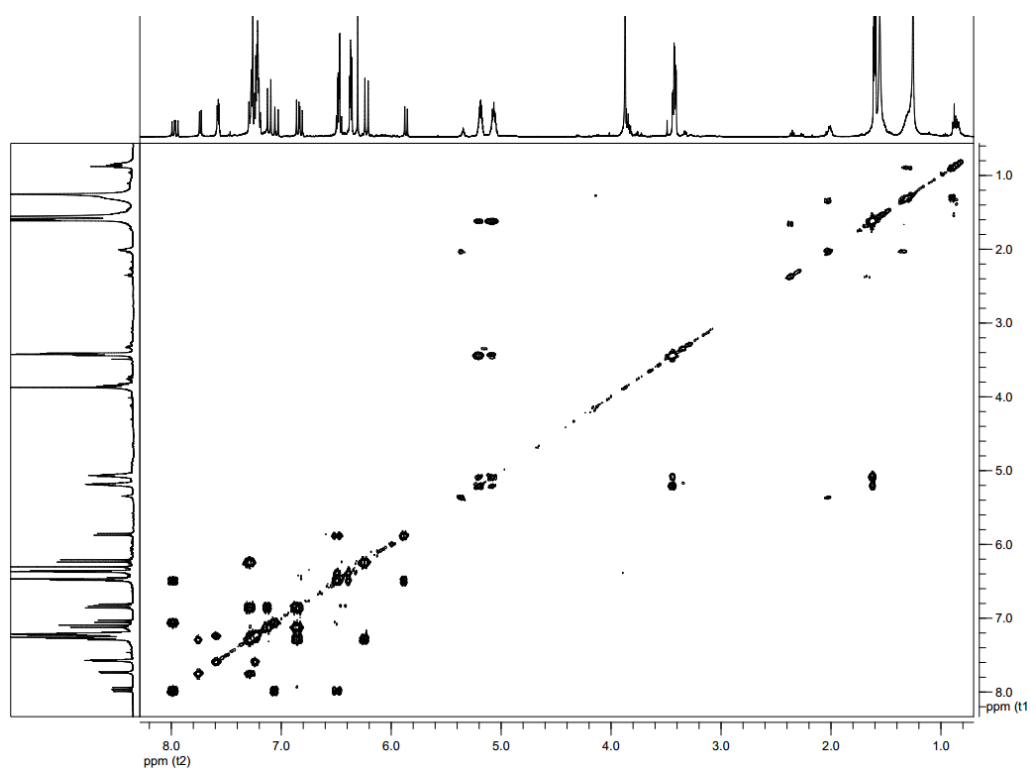


Figure S6 H-H COSY spectrum of 1.264HX-3.

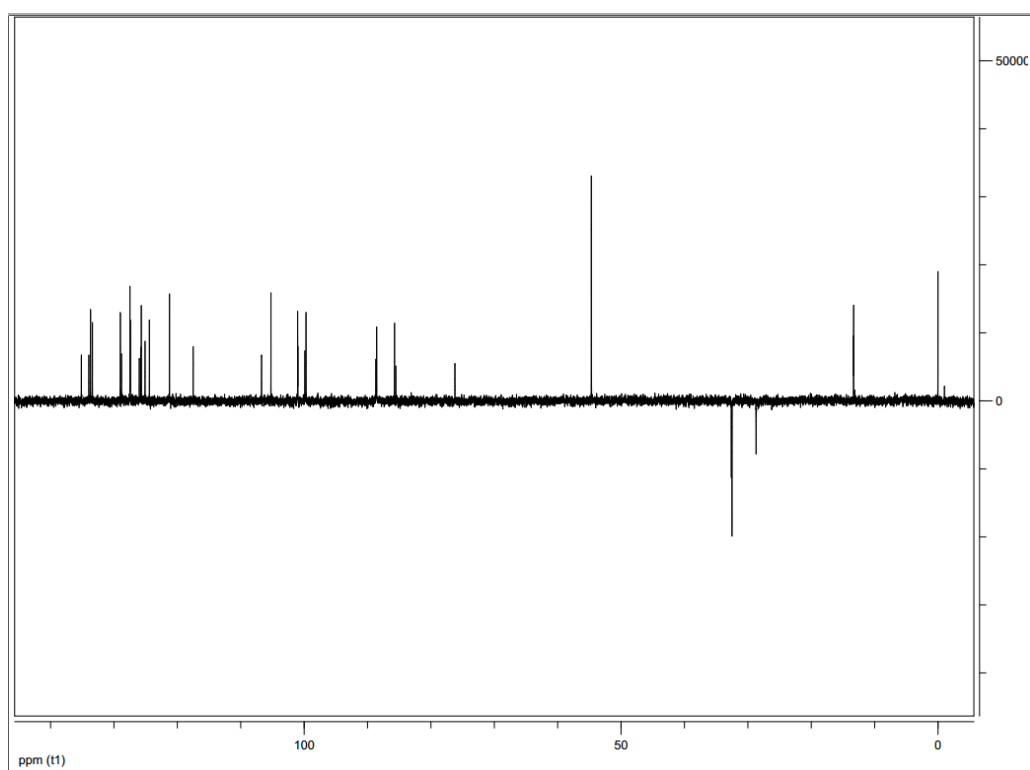


Figure S7 DEPT135 spectrum of 1.264HX-3.

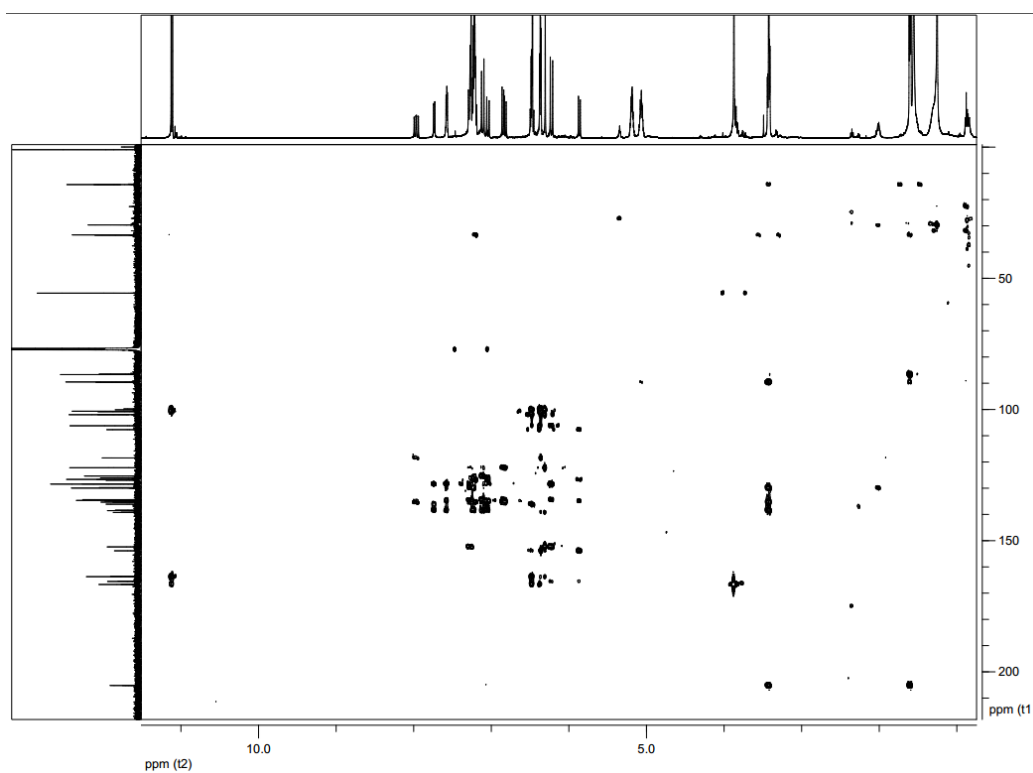


Figure S8 HMBC spectrum of 1.264HX-3.

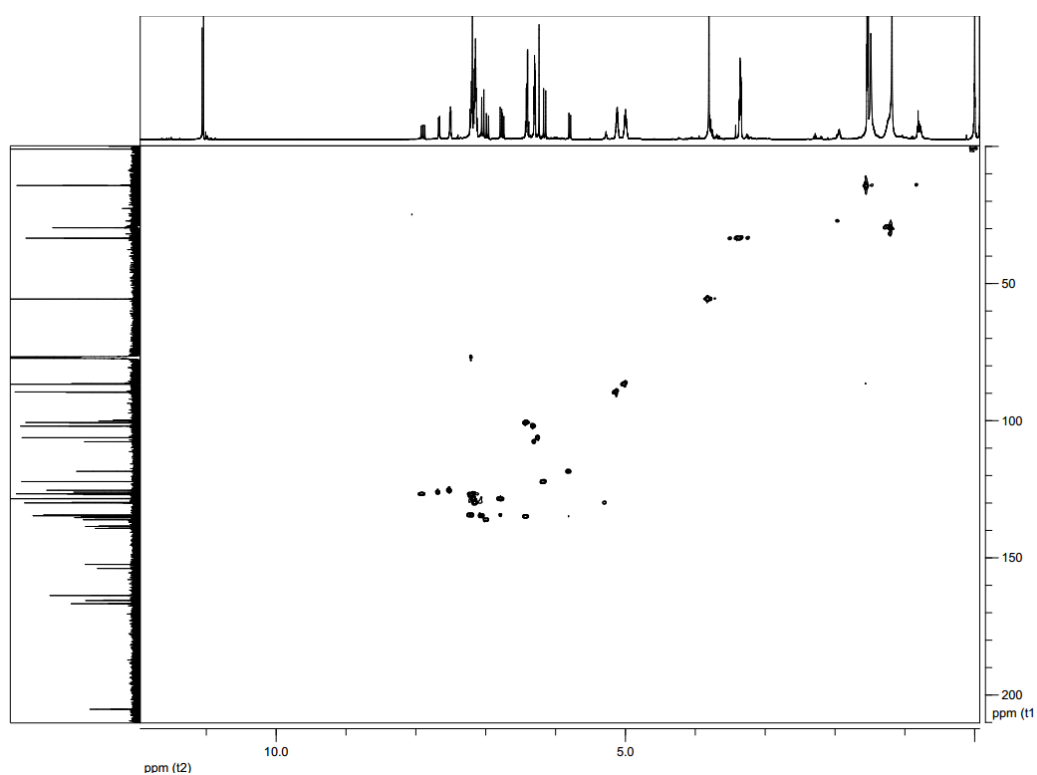


Figure S9 HSQC spectrum of 1.264HX-3.

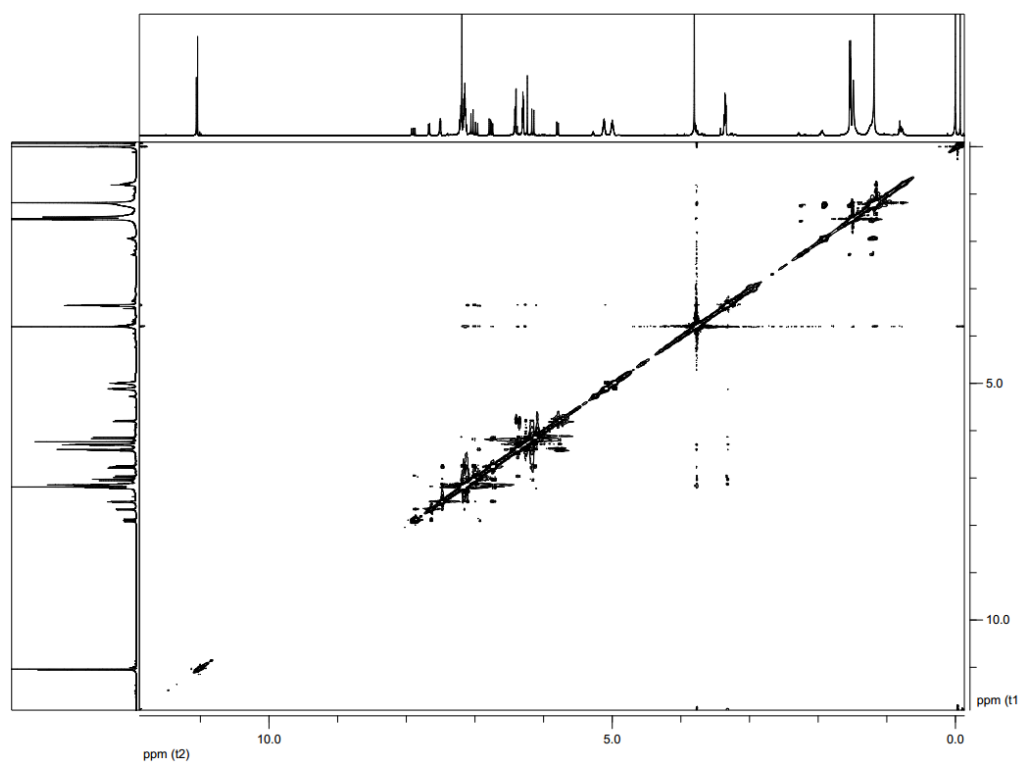


Figure S10 NOESY spectrum of 1.264HX-3.

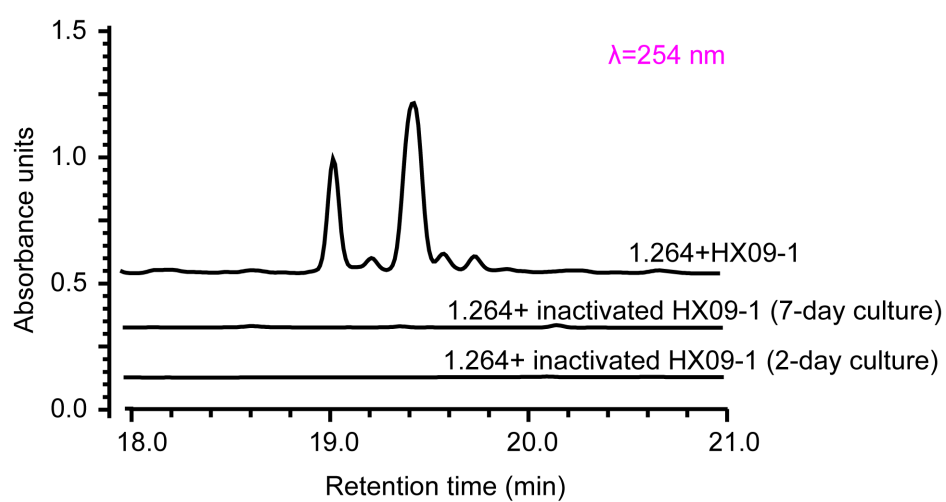


Figure S11 HPLC analysis of fermentation extracts of co-cultures of *S. sp.* FXJ1.264 and heat-killed *M. sp.* HX09-1.

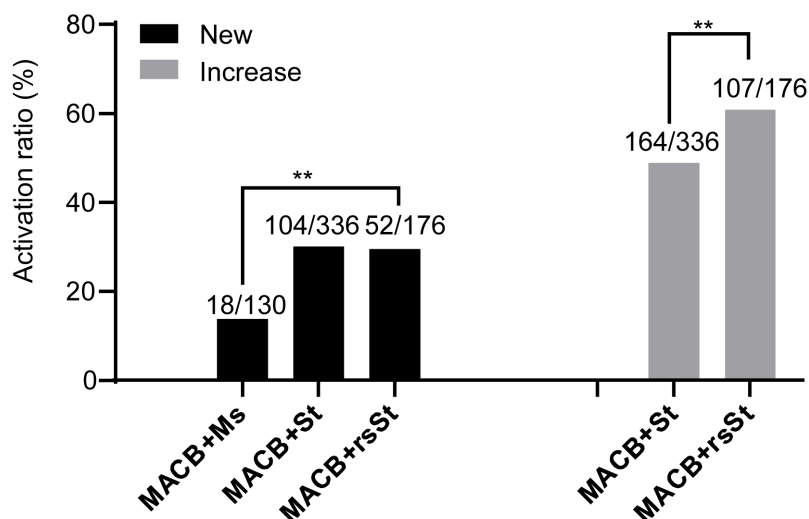


Figure S12 Comparison of activation effects of MACB on different actinobacteria.

Ms, marine *Micromonosporaceae*; St, *Streptomyces*; rsSt, red soil-derived *Streptomyces*; New, 'new' products detected in co-cultures; Increase, the increase in the yield of original metabolites; MACB+Ms, co-cultures of MACB and *Micromonosporaceae* [1]; MACB+St, co-cultures of MACB and *Streptomyces* [2]; MACB+ rsSt, co-cultures of MACB and red soil-derived *Streptomyces* (this study); **, $p < 0.01$; the increase in the yield of original metabolites of MACB+Ms is unavailable.

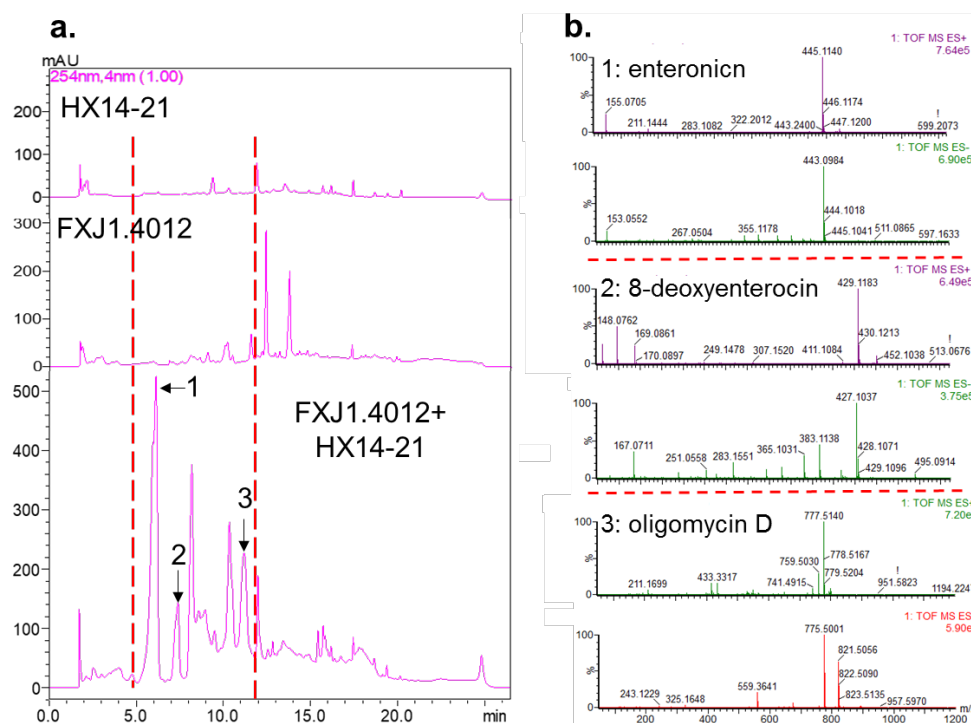


Figure S13 HPLC and HRMS analysis of fermentation extracts of *S. sp.* FXJ1.4012, *N. sp.* HX14-21, and their co-culture. (a) HPLC analysis of fermentation extracts of *S. sp.* FXJ1.4012, *N. sp.* HX14-21, and their co-culture; (b) HRMS data corresponding to peaks in a. $[M+H]^+ = 445.1140$ and $[M+H]^+ = 443.0984$ for enterocin; $[M+H]^+ = 429.1183$ and $[M+H]^+ = 427.1037$ for 8-deoxyenterocin; $[M+H]^+ = 777.5140$ and $[M+H]^+ = 775.5001$ for oligomycin D.

References

Supplementary Materials

1. Adnani, N.; Vazquez-Rivera, E.; Adibhatla, S.N.; Ellis, G.A.; Braun, D.R.; Bugni, T.S. Investigation of interspecies interactions within marine *Micromonosporaceae* using an improved co-culture approach. *Mar. Drugs* **2015**, *13*, 6082-6098.
2. Onaka, H.; Mori, Y.; Igarashi, Y.; Furumai, T. Mycolic acid-containing bacteria induce natural-product biosynthesis in *Streptomyces* species. *Appl. Environ. Microbiol.* **2011**, *77*, 400-406.