SUPPLEMENTARY MATERIAL

Induced production of cytochalasans in co-culture of marine fungus *Aspergillus flavipes* and actinomycete *Streptomyces* sp.

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Abstract

Secondary metabolites profiles of co-culture of *Aspergillus flavipes* and *Streptomyces* sp. that isolated from the same habitat showed an induced production of a series of cytochalasans (five aspochalasins and rosellichalasin, determined by MS and NMR analysis). These cytochalasans were found to be produced by *A. flavipes* in LC-MS comparison analysis, and biological activity assays revealed that they were able to cause cytotoxic effects against *Streptomyces* sp. within a wide range of concentrations without causing any effect to the producer *A. flavipes*, which favored the producer in competition. Further induction mechanism study applying membrane-separated culture and morphology study with scanning electron microscopy (SEM) suggested that the successful induction of active secondary metabolites required microbial physical contact.

Keywords: co-culture; *Aspergillus flavipes*; *Streptomyces*; cytochalasans; aspochalasin

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1. Structures analysis data of compounds 1-6

Rosellichalasin (1): Colorless gum. HRESIMS m/z 486.2248 $[M+Na]^+$ ($C_{28}H_{33}NO_5$, $[M+Na]$ calculated 486.2256). UV λ_{max} : 202, 247 nm. ^{13}C NMR ($CDCl_3$): δ 205.4, 171.4, 169.2, 143.1, 136.7, 135.3, 131.7, 129.5, 129.5, 129.0, 129.0, 84.6, 60.3, 57.2, 53.9, 49.2, 47.1, 44.3, 39.8, 39.7, 36.6, 36.0, 19.7, 17.4, 12.9, 12.8. 1H NMR ($CDCl_3$): δ 7.35 (2H, t, $J = 7.3$ Hz), 7.28 (1H, t, $J = 7.3$ Hz), 7.7 (2H, d, $J = 7.2$ Hz), 6.34 (1H, br.s), 5.83 (1H, ddd, $J = 15.0, 9.9, 1.5$ Hz), 5.45 (1H, ddd, $J = 15.2, 11.3, 3.9$ Hz), 3.69 (1H, t, $J = 6.0$ Hz), 3.30 (1H, m), 3.26 (1H, dd, $J = 12.0, 6.8$ Hz), 2.99 (1H, dd, $J = 12.0, 6.8$ Hz), 2.88 (1H, dd, $J = 10.0, 5.4$ Hz), 2.84 (2H, d, $J = 6.6$ Hz), 2.71 (1H, dd, $J = 5.3, 1.5$ Hz), 2.67 (1H, d, $J = 6.3$ Hz), 2.23 (1H, m), 2.21 (1H, dd, $J = 12.7, 7.0$ Hz), 2.03 (1H, m), 1.85 (3H, s), 1.22 (3H, s), 1.12 (3H, d, $J = 6.7$ Hz), 1.04 (3H, d, $J = 7.1$ Hz).

Aspochalasin E (2): Colorless gum. HRESIMS m/z 442.2565 $[M+Na]^+$ ($C_{24}H_{37}NO_5$, $[M+Na]$ calculated 442.2569). UV λ_{max} : 204 nm. ^{13}C NMR ($CDCl_3$): δ 212.0, 176.0, 139.6, 136.8, 125.7, 124.6, 77.0, 76.8, 68.8, 68.2, 51.2, 50.4, 48.7, 44.1, 43.8, 37.8, 35.3, 29.3, 24.8, 23.5, 21.5, 19.8, 15.7, 13.4. 1H NMR ($CDCl_3$): δ 7.08 (1H, br.s), 5.98 (1H, d, $J = 10.6$ Hz), 5.35 (1H, br.s), 4.42 (1H, br.s), 4.32 (1H, br.s), 3.99 (1H, br.s), 3.85 (1H, m), 3.50 (1H, m), 3.39 (1H, m), 3.26 (1H, m), 3.16 (1H, m), 3.11 (1H, br.s), 2.77 (1H, m), 2.52 (1H, m), 2.51 (1H, m), 1.95 (1H, m), 1.71 (1H, m), 1.69 (3H, s), 1.58 (1H, m), 1.54 (1H, m), 1.44 (3H, s), 1.19 (1H, m), 1.15 (1H, m), 1.14 (3H, d, $J = 6.6$ Hz), 1.07 (1H, m), 0.84 (6H, d, $J = 6.6$ Hz).

Aspochalasin P (3): Colorless gum. HRESIMS m/z 424.2456 $[M+Na]^+$ ($C_{24}H_{35}NO_4$, $[M+Na]$ calculated 424.2464). UV λ_{max} : 205 nm. ^{13}C NMR ($CDCl_3$): δ 209.8, 209.1, 176.6, 139.5, 136.8, 125.9, 125.0, 74.4, 68.7, 55.1, 51.2, 48.8, 42.2, 37.1, 35.4, 33.8, 32.7, 30.7, 25.0, 23.7, 21.3, 19.9, 18.4, 13.4. 1H NMR ($CDCl_3$): δ 6.95 (1H, s), 6.16 (1H, d, $J = 10.9$ Hz), 5.35 (1H, br.s), 4.34 (1H, dd, $J = 18.9, 11.8$ Hz), 4.22 (1H, d, $J = 6.0$ Hz), 3.25 (1H, d, $J = 10.6$ Hz), 3.14 (1H, m), 2.83 (1H, t, $J = 10.6$ Hz), 2.69 (1H, m), 2.61 (1H, m), 2.43 (1H, m), 2.41 (1H, m), 2.34 (1H, m), 2.21 (1H, m), 2.20 (1H, m), 2.04 (1H, m), 1.74 (3H, s), 1.63 (1H, m), 1.37 (3H, s), 1.27 (1H, m), 1.19 (1H, m), 1.18 (3H, d, $J = 7.3$ Hz), 0.94 (3H, d, $J = 6.5$ Hz), 0.93 (3H, d, $J = 6.5$ Hz).

Aspochalasin H (4): Colorless gum. HRESIMS m/z 440.2406 $[M+Na]^+$ ($C_{24}H_{35}NO_5$, $[M+Na]$ calculated 440.2413). UV λ_{max} : 204 nm. ^{13}C NMR (DMSO): δ 206.3, 173.5, 140.3, 134.8, 125.1, 124.4, 77.3, 71.9, 67.3, 62.5, 53.8, 51.9, 50.8, 48.7, 43.5, 38.6, 35.0, 29.2, 23.9, 23.6, 21.3, 19.7, 15.0, 13.3. 1H NMR (DMSO): δ 8.3 (1H, s), 5.97 (1H, d, $J = 10.8$ Hz), 5.33 (1H, s), 5.18 (1H, d, $J = 4.9$ Hz), 4.08 (1H, d, $J =$

1.6 Hz), 3.72 (1H, d, $J = 2.7$ Hz), 3.05 (1H, t, $J = 6.0$ Hz), 2.99 (1H, m), 2.91 (1H, br.d, $J = 10.8$ Hz), 2.42 (2H, m), 2.35 (1H, dd, $J = 7.7, 1.6$ Hz), 2.04 (1H, t, $J = 11.7$ Hz), 2.00 (1H, dd, $J = 10.3, 4.5$ Hz), 1.88 (1H, t, $J = 14$ Hz), 1.61 (1H, m), 1.32 (3H, s), 1.32 (1H, m), 1.14 (3H, d, $J = 6.7$ Hz), 1.05 (1H, m), 0.84 (6H, d, $J = 6.2$ Hz).

Aspochalasin M (**5**): Colorless gum. HRESIMS m/z 424.2455 $[M+Na]^+$ ($C_{24}H_{35}NO_4$, $[M+Na]$ calculated 424.2464). UV λ_{max} : 206 nm. ^{13}C NMR ($CDCl_3$): δ 215.8, 214.1, 175.5, 140.1, 136.9, 125.5, 124.0, 77.5, 67.2, 51.8, 51.0, 48.6, 43.5, 37.0, 36.8, 35.3, 34.2, 26.3, 25.0, 23.6, 21.5, 19.9, 16.1, 13.5. 1H NMR ($CDCl_3$): δ 7.14 (1H, br.s), 6.17 (1H, d, $J = 10.4$ Hz), 5.37 (1H, br.s), 4.07 (1H, d, $J = 4.2$ Hz), 3.83 (1H, br.s), 3.51 (1H, m), 3.25 (1H, t, $J = 12.4$ Hz), 3.16 (1H, m), 2.99 (1H, br.s), 2.63 (1H, t, $J = 13.0$ Hz), 2.58 (1H, dd, $J = 6.2, 2.7$ Hz), 2.51 (1H, m), 2.22 (1H, m), 2.15 (3H, m), 1.91 (1H, m), 1.73 (3H, s), 1.61 (3H, s), 1.57 (1H, m), 1.19 (1H, m), 1.19 (3H, d, $J = 7.0$ Hz), 1.09 (1H, m), 0.93 (3H, d, $J = 6.6$ Hz), 0.91 (3H, d, $J = 6.6$ Hz).

19,20-dihydro-aspochlasin C (**6**): Colorless gum. HRESIMS m/z 426.2613 $[M+Na]^+$ ($C_{24}H_{37}NO_4$, $[M+Na]$ calculated 426.2620). UV λ_{max} : 200, 280 nm. ^{13}C NMR ($CDCl_3$): δ 208.1, 176.0, 139.5, 136.7, 125.9, 124.9, 74.0, 73.0, 68.3, 53.4, 50.8, 48.8, 43.1, 37.8, 35.3, 34.7, 28.5, 26.6, 25.1, 23.5, 21.5, 19.9, 15.8, 13.4. 1H NMR ($CDCl_3$): δ 6.07 (1H, s), 6.05 (1H, s), 5.42 (1H, s), 4.20 (1H, m), 3.91 (1H, br.s), 3.34 (1H, m), 3.26 (1H, m), 3.14 (1H, m), 2.85 (1H, br.s), 2.60 (1H, m), 2.54 (1H, m), 2.49 (1H, m), 2.15 (1H, m), 2.15 (1H, m), 2.01 (1H, m), 1.81 (1H, m), 1.74 (3H, s), 1.72 (1H, m), 1.61 (1H, m), 1.54 (3H, s), 1.54 (1H, m), 1.38 (1H, m), 1.20 (1H, m), 1.19 (3H, d, $J = 7.1$ Hz), 1.15 (1H, m), 0.91 (3H, d, $J = 6.4$ Hz), 0.89 (3H, d, $J = 6.4$ Hz).

2. Figure S1. LC-MS analysis of *A. flavipes* and *Streptomyces* sp. co-culture and mono-culture