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ONE STRAIN-MANY COMPOUNDS METHOD FOR PRODUCTION OF DIVERSE METABOLIC PROFILES USING *Chaetomium* sp. FROM *Astragalus membranaceus*

Hongchi Zhang,[1,2] Rui Liu,[1,2*] Yang Yang,[1,2] Hui Li,[1,2] and Feng Zhou[1,2]

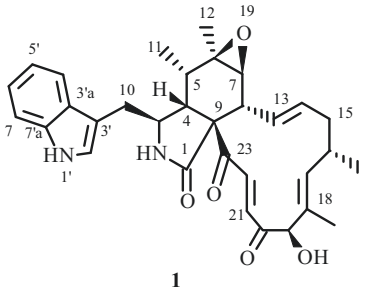
Previously, we found that the endophytic fungus AR-17-7 from the root of *Astragalus membranaceus* (Fisch.) Bunge associated with potato dextrose agar (PDA) media can produce structurally diverse chaetoglobosins. This encouraged further study on the use of the endophytic fungus AR-17-7 for the production of diverse metabolic profiles by the OSMAC technique. The OSMAC (one strain of many compounds) strategy, developed by Zeeck in the early 2000s, has been demonstrated to be a simple and effective method [1]. In this strategy, the silent gene sequence was activated by changing culture conditions, including media composition, medium phase, and temperature [2]. The OSMAC approach has been successfully applied to produce new secondary metabolites from single microbial strains during the last 10 years [3].

Since metabolite production of endophytic fungi was influenced by culture media as mentioned earlier, the impact of growth media (PDB, Czapek′s liquid culture, Sabouraud′s medium, and rice medium) was therefore studied on the metabolite production by *Chaetomium* sp. AR-17-7, with the aim of generating different structural types metabolites. Herein, four chaetomugilins, seven chaetoglobosins, and two malforms produced by the endophytic fungus AR-17-7 after changing growth media for fungal cultivation were reported.

The endophytic *Chaetomium* sp. AR-17-7 was grown on PDA at 28°C for 7 days. Three pieces (diameter 0.5 cm) of mycelial agar plugs were inoculated into Erlenmeyer flasks (250 mL) containing 100 mL potato dextrose broth (PDB). The flasks were incubated at 28°C on a rotary shaker (150 rpm) for 7 days to obtain the fungus seed. The seed liquids were inoculated separately in four different media, modified PDB (potato 40 g/L, glucose 20 g/L, $MgSO_4 \cdot 7H_2O$ 1.5 g/L, K_2HPO_4 3 g/L, yeast extract 1 g/L), Czapek′s medium (Sucrose 30 g/L, KH_2PO_4 1.0 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $NaNO_3$ 3.0 g/L, KCl 0.5 g/L and $FeSO_4$ 0.01 g/L), Sabouraud′s medium (peptone 10 g/L, glucose 40 g/L) and rice medium (100 g rice and 100 mL modified liquid medium contains NaCl 30 g/L, $NaNO_3$ 2 g/L, sodium glutamate 3.5 g/L). Fermentation was performed in 500 mL Erlenmeyer flasks, each Erlenmeyer flask containing 200 mL of culture medium at 28°C. The modified PDB fermentation (60 L) was maintained on a rotary shaker at 140 rpm for 15 days, Czapek′s medium fermentation (80 L) for 14 days, Sabouraud′s medium fermentation (40 L) for 15 days, and rice medium fermentation (20 L) for 30 days. The extracts of the four fermentations were analyzed by HPLC, and the chromatograms were detected by a UV detector.

The PDB culture was extracted with ethyl acetate three times. The culture afforded a dark brown crude extract, which was subjected to silica gel column chromatography and eluted successively with ethyl acetate–methanol gradient to yield six fractions (A–F). Fraction B containing chaetoglobosins and Fr. C containing chaetomugilins were detected and chromatographed on silica gel column with a gradient of ethyl acetate in petroleum ether separately. Subfraction B2-2 was by further subjected to repeated silica gel column chromatography and semipreparative reversed-phase HPLC (H_2O–MeOH, 2:3) to afford compounds **1** and **2**. Subfraction C2 was separated by semipreparative RP-HPLC (H_2O–MeOH, 1:4, 2.5 mL/min) to yield compounds **3**, **4**, and **5**.

1) College of Life Science, Shanxi Datong University, 037009, Datong Shanxi, P. R. China, e-mail: liurlw@163.com; 2) Applied Biotechnology Institute, Shanxi Datong University, 037009, Datong Shanxi, P. R. China. Published in *Khimiya Prirodnykh Soedinenii*, No. 6, November–December, 2021, pp. 1003–1005. Original article submitted October 13, 2020.



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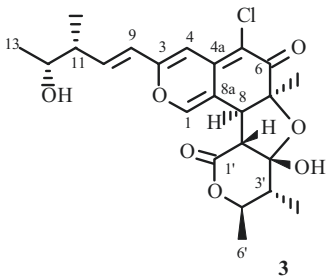

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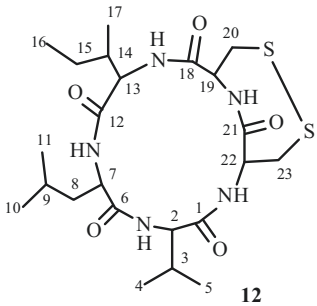

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Czapek′s liquid culture was extracted with ethyl acetate three times. The culture afforded a dark brown crude extract, which was subjected to silica gel column chromatography and eluted successively with dichloromethane–methanol gradient to yield five fractions (A–E). Fraction B was chromatographed on silica gel column with a gradient of ethyl acetate in petroleum ether to give three subfractions (Subfrs. B1–B3). Further Subfr. B2 was purified by MPLC (H_2O–MeOH, 3:2–3:7) to give six subfractions (Subfrs. B2-1–B2-6). Subfraction B2-2 was chromatographed on a Sephadex LH-20 column and eluted with methanol to afford compounds **6** and **7**. Subfraction B2-3 was by further repeated silica gel column chromatography and semipreparative RP-HPLC (H_2O–MeOH, 3:2) to afford compound **1**. Fraction C was separated by Sephadex LH-20 chromatography (MeOH) and MPLC to give five fractions (Subfrs. C1–C5). Subfraction C2 was chromatographed over a silica gel column and eluted with petroleum ether–ethyl acetate, then subjected to PTLC to afford compounds **8** and **2**. Subfraction B5 was also purified by filtration through Sephadex LH-20 (CH_2Cl_2–MeOH, 1:1) and semipreparative RP-HPLC (H_2O–MeOH, 7:13) to give compound **9**.

Sabouraud′s medium was extracted with ethyl acetate three times. The crude extract was then applied to a silica gel column and eluted with a chloroform–methanol gradient to give six fractions (A–F). Fraction B was chromatographed on silica gel column with a gradient of ethyl acetate in petroleum ether to give four subfractions (Subfrs. B1–4). Subfraction B1 was chromatographed on MCI gel eluted with a MeOH–H_2O mixture and further purified on Sephadex LH-20 to afford compounds **10** and **8**. Subfraction B2 was chromatographed over a silica gel column and eluted with petroleum ether–ethyl acetate, then subjected to PTLC to afford compound **11**.

The rice medium was extracted with acetone four times. The extract was then poured into 50% MeOH and further extracted with chloroform five times. The chloroform portion was subjected to silica gel column chromatography and eluted successively with ethyl acetate–methanol gradient to yield eight fractions (A–H). Fraction B containing chaetoglobosins was separated by column chromatography on silica gel with a gradient of ethyl acetate in petroleum ether to give nine subfractions (Subfrs. B1–9). Subfraction B2 was separated by silica gel column chromatography and eluted with petroleum ether–ethyl acetate to give compound **6** and **1**. Subfraction B3 was by further subjected to repeated silica gel column chromatography and semipreparative RP-HPLC (H_2O–MeOH, 3:2) to afford compounds **10**, **8**, and **2**. Fraction C was subjected to repeated chromatographic purifications using ethyl acetate–methanol and Sephadex LH-20 to give compounds **12** and **13**.

The compounds were subjected to characterization using spectroscopic analysis and identified as chaetoglobosin A (**1**) [4], chaetoglobosin C (**2**) [5], chaetomugilin A (**3**) [6], chaetomugilin D (**4**) [7], chaetomugilin S (**5**) [8], chaetoglobosin F (**6**) [5], chaetoglobosin D (**7**) [4], chaetoglobosin G (**8**) [9], chaetoglobosin Y (**9**) [10], chaetoglobosin V (**10**) [11], chaetomugilin O (**11**) [12], malform A (**12**) [13], and malform B (**13**) [14] by comparison of their spectral data with the reported data in the literature.

Chaetomugilin A (3), white powder. HR-ESI-MS *m/z* 451.2449 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.27 (1H, s, H-1), 6.61 (1H, dd, J = 15.0, 6.2, H-10), 6.57 (1H, s, H-4), 6.15 (1H, d, J = 15.0, H-9), 4.30 (1H, q, J = 10.3, 6.5, H-5′), 3.81 (1H, s, H-12), 3.06 (1H, d, J = 10.0, H-2′), 2.98 (1H, d, J = 10.0, H-8), 2.45 (1H, d, J = 6.2, H-11), 1.90 (1H, q, J = 10.3, 6.2, H-4′), 1.41 (3H, d, J = 6.5, H-6′), 1.40 (3H, s, CH_3-7), 1.20 (3H, d, J = 6.2, H-13), 1.13 (3H, d, J = 6.2, CH_3-11), 1.13 (3H, d, J = 6.2, CH_3-4′). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 145.7 (C-1), 157.1 (C-3), 105.5 (C-4), 140.1 (C-4a), 110.4 (C-5), 189.3 (C-6), 84.0 (C-7), 50.6 (C-8), 114.3 (C-8a), 122.1 (C-9), 142.5 (C-10), 44.3 (C-11), 70.9 (C-12), 20.5 (C-13), 23.2 (CH_3-7), 14.9 (CH_3-11), 170.5 (C-1′), 58.2 (C-2′), 104.2 (C-3′), 44.9 (C-4′), 76.9 (C-5′), 18.7 (C-6′), 8.8 (CH_3-4′).

Chaetomugilin S (5), yellow powder. HR-ESI-MS *m/z* 435.1526 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.71 (1H, s, H-1), 6.53 (1H, s, H-4), 6.52 (1H, dd, J = 15.5, 6.5, H-10), 6.06 (1H, d, J = 15.5, H-9), 4.34 (1H, q, J = 10.2, 6.2, H-5′), 3.30 (1H, d, J = 9.0, H-8), 3.04 (1H, d, J = 9.0, H-2′), 2.94 (1H, s, 3′-OH), 2.26 (1H, t, J = 6.5, H-11), 1.79 (1H, q, J = 10.0, 7.0, H-4′), 1.43 (1H, m, H-12), 1.38 (3H, s, CH_3-7), 1.35 (3H, d, J = 6.2, H-6′), 1.11 (3H, d, J = 7.0, CH_3-4′), 1.08 (3H, d, J = 6.5, CH_3-11), 0.90 (3H, t, J = 7.5, H-13).

Chaetoglobosin V (10), white powder. HR-ESI-MS *m/z* 551.2578 $[M + Na]^+$. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.46 (1H, s, NH-1′), 7.44 (1H, d, J = 7.8, H-4′), 7.31 (1H, d, J = 8.1, H-7′), 7.16 (1H, t, J = 7.3, H-6′), 7.09 (1H, t, J = 7.5, H-5′), 6.94 (1H, s, H-2′), 6.38 (1H, s, NH-2), 6.38 (1H, m, H-13), 5.41 (1H, t, J = 13.3, H-14), 4.17 (1H, d, J = 10.0, H-7), 3.67 (1H, m, H-22b), 3.53 (1H, t, J = 7.4, H-3), 3.19 (1H, s), 2.88 (1H, s, H-17), 2.61 (1H, m, H-22a), 2.60 (2H, m, H-10), 2.60 (1H, m, H-15b), 2.43 (1H, m, H-21), 2.35 (1H, t, J = 10.4, H-8), 2.33 (1H, m, H-16), 1.92 (3H, s, CH_3-18), 1.85 (1H, m, H-15a), 1.66 (3H, s, H-12), 1.43 (3H, s, H-11), 0.69 (3H, d, J = 6.5, CH_3-16). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 175.6 (C-1), 58.1 (C-3), 40.2 (C-4), 127.6 (C-5), 133.3 (C-6), 69.8 (C-7), 54.8 (C-8), 63.9 (C-9), 33.3 (C-10), 18.2 (C-11), 14.9 (C-12), 130.2 (C-13), 135.9 (C-14), 39.1 (C-15), 32.7 (C-16), 43.7 (C-17), 148.5 (C-18), 147.4 (C-19), 203.6 (C-20), 40.6 (C-21), 41.8 (C-22), 209.3 (C-23), 124.2(C-2′), 111.7 (C-3′), 127.5 (C-3′a), 119.1 (C-4′), 120.3 (C-5′), 122.9 (C-6′), 112.2 (C-7′), 137.1 (C-7′a), 16.3 (CH_3-16), 12.9 (CH_3-18).

Chaetomugilin O (11), yellow powder, mp 95–97°C. HR-ESI-MS *m/z* 417.1452 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.33 (1H, s, H-1), 6.80 (1H, d, J = 7.0, H-5′), 6.50 (1H, dd, J = 15.7, 7.0, H-10), 6.49 (1H, s, H-4), 6.04 (1H, d, J = 15.7, H-9), 4.37 (1H, d, J = 12.0, H-2′), 4.11 (1H, d, J = 12.0, H-8), 2.25 (1H, t, J = 7.0, H-11), 1.94 (3H, d, J = 7.0, H-6′), 1.84 (3H, s, CH_3-4′), 1.63 (3H, s, CH_3-7), 1.41 (1H, m, H-12), 1.07 (3H, d, J = 7.0, CH_3-11), 0.89 (3H, t, J = 7.0, H-13).

Malform A_1 (12), yellow powder, mp > 300°C. HR-ESI-MS *m/z* 530.2451 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.84 (1H, d, J = 4.2, NH-19), 8.59 (1H, d, J = 6.9, NH-13), 7.95 (1H, d, J = 8.9, NH-2), 7.39 (1H, d, J = 9.4, NH-7), 7.10 (1H, d, J = 10.8, NH-22), 4.70 (1H, d, J = 4.4, 11.0, H-22), 4.46 (1H, dt, J = 6.1, 9.3, H-7), 3.95 (1H, dd, J = 3.2, 7.1, H-19), 3.92 (1H, dd, J = 9.0, 10.3, H-2), 3.86 (1H, dd, J = 6.8, 10.5, H-13), 3.50 (1H, dd, J = 3.7, 14.6, H-20a), 3.11–3.24 (1H, m, H-20b), 3.11–3.24 (2H, m, H-23), 2.03 (1H, d, J = 6.7, 13.3, H-3), 1.65–1.72 (1H, m, H-14), 1.35–1.59 (3H, m, H-8, 9), 1.35–1.59 (1H, m, H-15a), 1.09–1.18 (1H, m, H-15b), 0.88 (3H, d, J = 6.6, H-4), 0.85 (3H, d, J = 6.6, H-5), 0.80–0.82 (6H, m, H-10, 11), 0.80–0.82 (3H, m, H-16), 0.77 (3H, d, J = 6.9, H-17). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 170.5 (C-1), 58.8 (C-2), 26.8 (C-3), 19.7 (C-4), 18.7 (C-5), 173.0 (C-6), 50.3 (C-7), 40.6 (C-8), 24.4 (C-9), 22.7 (C-10), 21.7 (C-11), 172.7 (C-12), 58.0 (C-13), 33.9 (C-14), 24.7 (C-15), 9.9 (C-16), 14.9 (C-17), 173.9 (C-18), 52.3 (C-19), 46.2 (C-20), 169.7 (C-21), 52.9 (C-22), 45.2 (C-23).

Malform B_2 (13), yellow powder, mp > 300°C. HR-ESI-MS *m/z* 516.2326 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.01 (1H, d, J = 4.2, NH-18), 8.61 (1H, d, J = 6.4, NH-12), 8.34 (1H, d, J = 8.5, NH-7), 7.26 (1H, d, J = 9.5, NH-2), 7.00 (1H, d, J = 11.0, NH-21), 4.73 (1H, dt, J = 3.7, 11.0, H-21), 4.23 (1H, dt, J = 6.1, 9.32, H-12), 4.17 (1H, dd, J = 8.1, 8.79, H-2), 3.97 (1H, dd, J = 8.5, 10.5, H-7), 3.97 (1H, m, H-18), 3.53 (1H, m, H-19a), 3.30 (1H, dd, J = 11.5, 14.9, H-22a), 3.16 (1H, m, H-22b), 3.13 (1H, m, H-19b), 2.05 (1H, m, H-8), 1.77 (1H, m, H-3), 1.58 (1H, m, H-14), 1.54 (1H, m, H-13a), 1.25 (1H, m, H-13b), 0.93–0.95 (3H, m, H-15), 0.84–0.92 (6H, m, H-4, 5), 0.84–0.92 (6H, m, H-9, 10), 0.80–0.83 (3H, m, H-16).

The OSMAC strategy has been applied to microbes by changing the ingredients of the culture media. The results in this paper were the first to describe the production of a variety of metabolites using the fungus *Chaetomium* sp. AR-17-7 from *A. membranaceus* as a microbial producer. The metabolite production of *Chaetomium* sp. depended not only on the culture medium but also on the sources of nutrients in PDB, Czapek′s medium, Sabouraud′s medium, and rice medium. In the PDB medium and shaker culture mode, strain AR-17-7 synthesizes mainly chaetoglobosin metabolites. With the use of Czapek′s medium, Sabouraud′s medium, and shaker culture, the metabolism of AR-17-7 undergoes great changes, mainly using tryptophan to synthesize chaetoglobosin metabolites. However, with the use of rice medium and static culture methods, AR-17-7 mainly synthesizes malform cyclic peptides. The present study suggests that endophytic fungi are potentially suitable microorganisms for the OSMAC method, which can enhance the chemical diversity of secondary metabolites. In conclusion, our results demonstrate that *Chaetomium* sp. AR-17-7 secreted a broad spectrum of metabolites and warranted further evaluation for metabolite production. For AR-17-7, the simplest and cheapest medium results in the production of a complex bioactive scaffold.

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