Anti-phytopathogenic Bacterial Metabolites From the Seaweed-Derived Fungus Aspergillus sp. D40

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In order to search for new lead compounds with anti-phytopathogenic bacterial activity, three pairs of new furanone derivatives, sclerotiorumins D–F (1–3), and eight known compounds (4–11) were isolated from the seaweed-derived fungus, Aspergillus sp. D40, fermented with potato dextrose seawater (PDW) medium. Their structures were determined using comprehensive spectroscopic analyzes including HRESIMS, 1D and 2D NMR data. Compounds 1–4 and 9 existed as inseparable mixtures of a pair of epimers. Penicillic acid (7) exhibited clear antibacterial activity against Ralstonia solanacearum and several other plant pathogenic bacteria with IC50 values ranging from 11.6 to 58.2 µg/mL.

Keywords: furanones, penicillic acid, Ralstonia solanacearum, phytopathogenic bacteria, antibacterial activity

INTRODUCTION

Phytopathogenic bacteria cause many serious diseases in plants and limit the quality and production of crops all over the world, and as a result pose a significant threat to global food safety (Sundin et al., 2016). For instance, Ralstonia solanacearum – the causative agent of bacterial wilt and Moko disease – is ranked second on the top ten bacterial plant pathogen list (Manfield et al., 2012). R. solanacearum affects more than 200 plant species belonging to over 50 different botanical families, including Solanaceae – tomato and potato – and many weeds, crops, shrubs, and trees (dicot as well as monocot). This pathogen invades the xylem conduit from the roots of the plant, and spreads to the aerial parts of the plant through the vascular system. The extensive colonization results in vascular dysfunction, thereby causing the wilting symptoms (Genin and Denny, 2012).

Current integrated management strategies for R. solanacearum and other phytopathogenic bacteria include the use of resistant cultivars, pathogen-free transplants, and crop rotation with non-host cover crops, all of which have had limited effects (Pradhanang et al., 2005). A few – mainly chemical – pesticides are used to control phytopathogenic bacteria, however, this leads to environmental pollution, pesticide residues, food safety issues, and pathogen resistance (Fujiiwa et al., 2011). It is well known that marine fungi can produce secondary metabolites with novel structures and potential antibacterial activities, as part of their repertoire of survival strategies and metabolic mechanisms endowed by the unique marine environment. Thus, they have been a hotspot for study of new antibacterial agents (Carroll et al., 2020). However, there have been few
reports focused on their potential for applications in agriculture, making this a promising new field for identification and study of antibacterial biopesticides.

In our ongoing search for new bioactive secondary metabolites from marine-derived fungi (Huang et al., 2018; Zhao et al., 2018; Zhao et al., 2019), our attention was drawn to Aspergillus sp. D40, isolated from a red seaweed Grateloupia filicina, because an ethyl acetate (EtOAc) extract of a culture grown in potato dextrose seawater (PDW) medium exhibited obvious antibacterial activity against R. solanacearum because an ethyl acetate (EtOAc) extract of a culture grown for 30 days. The mycelia were mechanically broken and then ultrasonically disrupted for 10 min, and then extracted twice with CH$_2$Cl$_2$:MeOH (1:1, v/v). The solutions were concentrated under reduced pressure to yield a residue, which was extracted three times with EtOAc. The culture media was also extracted three times with EtOAc, and the combined EtOAc extracts from both the mycelia and culture media were concentrated under reduced pressure to yield the total EtOAc extract (22.3 g). The extract was subjected to silica gel vacuum liquid chromatography (VLC), eluted using a linear gradient of petroleum ether (PE)–EtOAc (0–100%) and subsequently EtOAc–MeOH (0–100%) to obtain seven fractions (Fr.1–Fr.7). Fraction 2 was initially fractionated using reverse silica gel column chromatography (CC) with a step gradient elution of MeOH–H$_2$O (50–90%), followed by separation on Sephadex LH-20 CC (CH$_2$Cl$_2$/MeOH, v/v, 1/1) to afford Fr. 2-1 and Fr. 2-2. Fraction 2-1 was then purified by reversed phase (RP)-HPLC and eluted with 30% MeOH–H$_2$O to obtain 5 (168.0 mg) and 6 (30.6 mg). Fraction 2 was run on a reverse silica gel column and separated on silica gel CC (CH$_2$Cl$_2$/MeOH, v/v, 200/1–0/100) to obtain Fr. 3-1–Fr. 3-6. Fraction 3–5 was further separated by HPLC using 15% MeCN–H$_2$O (0.1% TFA) to yield 7 (271.1 mg) and 8 (5.8 mg). Fraction 4 was subjected to octadecylsilyl silica gel (ODS) CC separation using a gradient elution of 30–90% MeOH–H$_2$O to afford subfractions Fr.4-1–Fr.4-2. Fraction 4-1 and Fraction 4-2 were separated by silica gel CC (CH$_2$Cl$_2$/MeOH, v/v, 500/1–0/100) to obtain 9 (72.0 mg) and 10 (5.5 mg). Fraction 4-2-2 was purified by HPLC utilizing 5% MeOH–H$_2$O to afford 11 (16.0 mg), 12 (12.0 mg), 13 (42.5 mg), and 14 (80.7 mg). Fraction 1 was run on a reverse silica gel CC, eluted with 30–90% MeOH in H$_2$O, and further separated on silica gel CC (CH$_2$Cl$_2$/MeOH, v/v, 200/1–0/100) to obtain Fr. 2-1. Fraction 2-1 was further fractionated by HPLC utilizing 30% MeCN–H$_2$O (0.1% TFA) to afford 12 (16.0 mg), 13 (12.0 mg), 14 (42.5 mg), and 15 (80.7 mg). Fraction 6 was run on a reverse silica gel CC, eluted with 30–90% MeOH in H$_2$O, and further separated on silica gel CC (CH$_2$Cl$_2$/MeOH, v/v, 200/1–0/100) to obtain Fr. 2-1. Fraction 2-1 was further fractionated by HPLC utilizing 30% MeCN–H$_2$O (0.1% TFA) to afford 11 (25.0 mg). The purities of all the isolated compounds were >95% based on the peak area normalization method.

**Fungal Material**

The fungal strain *Aspergillus* sp. D40 was isolated from a red seaweed *Grateloupia filicina*, which was collected from coastal habitats in Qingdao, China (120°20’18.18”E, 36°03’15.90’’N), in July 2016. The fungus was identified based on its morphological characteristics and by a molecular protocol based on the amplification of the ITS region of the rDNA gene followed by sequence determination (Zhao et al., 2018). The strain was deposited in the Marine Agriculture Research Center, Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao, China, with the GenBank (NCBI) accession number MK968521.

**Extraction and Isolation**

The fungal strain *Aspergillus* sp. D40 was fermented in 80 L of potato dextrose seawater (PDW) medium at 28°C for 30 days. The mycelia were mechanically broken and then ultrasonically disrupted for 10 min, and then extracted twice with CH$_2$Cl$_2$:MeOH (1:1, v/v). The solutions were concentrated under reduced pressure to yield a residue, which was extracted three times with EtOAc. The culture media was also extracted three times with EtOAc, and the combined EtOAc extracts from both the mycelia and culture media were concentrated under reduced pressure to yield the total EtOAc extract (22.3 g). The extract was subjected to silica gel vacuum liquid chromatography (VLC), eluted using a linear gradient of petroleum ether (PE)–EtOAc (0–100%) and subsequently EtOAc–MeOH (0–100%) to obtain seven fractions (Fr.1–Fr.7). Fraction 2 was initially fractionated using reverse silica gel column chromatography (CC) with a step gradient elution of MeOH–H$_2$O (50–90%), followed by separation on Sephadex LH-20 CC (CH$_2$Cl$_2$/MeOH, v/v, 1/1) to afford Fr. 2-1 and Fr. 2-2. Fraction 2-1 was then purified by reversed phase (RP)-HPLC and eluted with 30% MeOH–H$_2$O to obtain 5 (168.0 mg) and 6 (30.6 mg). Fraction 3 was run on a reverse silica gel column and separated on silica gel CC (CH$_2$Cl$_2$/MeOH, v/v, 200/1–0/100) to obtain Fr. 3-1–Fr. 3-6. Fraction 3–5 was further separated by HPLC using 15% MeCN–H$_2$O (0.1% TFA) to yield 7 (271.1 mg) and 8 (5.8 mg). Fraction 4 was subjected to octadecylsilyl silica gel (ODS) CC separation using a gradient elution of 30–90% MeOH–H$_2$O to afford subfractions Fr.4-1–Fr.4-2. Fraction 4-1 and Fraction 4-2 were separated by silica gel CC (CH$_2$Cl$_2$/MeOH, v/v, 500/1–0/100) to obtain 9 (72.0 mg) and 10 (5.5 mg). Fraction 4-2-2 was purified by HPLC utilizing 5% MeOH–H$_2$O to afford 11 (16.0 mg), 12 (12.0 mg), 13 (42.5 mg), and 14 (80.7 mg). Fraction 1 was run on a reverse silica gel CC, eluted with 30–90% MeOH in H$_2$O, and further separated on silica gel CC (CH$_2$Cl$_2$/MeOH, v/v, 200/1–0/100) to obtain Fr. 2-1. Fraction 2-1 was further fractionated by HPLC utilizing 30% MeCN–H$_2$O (0.1% TFA) to afford 12 (16.0 mg), 13 (12.0 mg), 14 (42.5 mg), and 15 (80.7 mg). Fraction 6 was run on a reverse silica gel CC, eluted with 30–90% MeOH in H$_2$O, and further separated on silica gel CC (CH$_2$Cl$_2$/MeOH, v/v, 200/1–0/100) to obtain Fr. 2-1. Fraction 2-1 was further fractionated by HPLC utilizing 30% MeCN–H$_2$O (0.1% TFA) to afford 11 (25.0 mg). The purities of all the isolated compounds were >95% based on the peak area normalization method.

**Sclerotiorumin D (1):** pale yellow powder; [α]$_{20}^{D}$ +1.9 (c 0.34, MeOH); UV (MeOH) λ$_{max}$ (log ε) 228 (3.51), 260 (3.47) nm; $^1$H
TABLE 1 | $^1$H NMR Data (500 MHz, DMSO-d$_6$, $\delta$ in ppm, J in Hz) for 1 and 2.

| Position | 1a | 1b | 2a | 2b |
|----------|----|----|----|----|
| 5        | 6.62, s | 6.66, s | 6.62, s | 6.66, s |
| 6        | 2.11, dd | 1.62, brd | 2.13, dd | 1.67, brd |
|          | (14.0, 3.0) | (14.5) | (14.4, 1.8) | (14.4) |
| 7        | 1.38, m | 1.35, m | 1.36, m | 1.33, m |
|          | 1.72–1.75, | 1.64–1.66, | 1.76–1.82, | 1.70–1.74, |
|          | m | m | m | m |
| 10       | 5.32, s | 5.37, s | 5.34, s | 5.37, s |
| 12       | 3.94, s | 3.95, s | 3.94, s | 3.95, s |
| 13       | 1.06, s | 1.01, s | 1.07, s | 1.02, s |
| 14       | 3.82, s | 3.83, s | 3.82, s | 3.84, s |
| 15       | 0.58, d | 0.77, d | 0.53, d | 0.71, d |
|          | (7.0) | (6.6) | (6.6) | (7.2) |

and $^{13}$C NMR data, Tables 1, 2; HRESIMS m/z 333.0940 [M + Na]$^+$ (calcd for C$_{15}$H$_{18}$O$_2$Na, 333.0945).

Sclerotiorum E (2): pale yellow powder; [α]$_D^{20}$ -0.82 (c 0.38, MeOH); UV (MeOH) $\lambda_{max}$ (log e) 225 (3.84), 260 (3.71) nm; $^1$H and $^{13}$C NMR data, Tables 1, 2; HRESIMS m/z 328.1392 [M + NH$_4$]$^+$ (calcd for C$_{15}$H$_{12}$O$_2$N, 328.1391).

Sclerotiorum F (3): pale yellow oil; [α]$_D^{20}$ -0.37 (c 0.48, MeOH); UV (MeOH) $\lambda_{max}$ (log e) 223 (3.80), 269 (3.67) nm; $^1$H and $^{13}$C NMR data, Tables 1, 2; HRESIMS m/z 283.1181 [M + H]$^+$ (calcd for C$_{14}$H$_{19}$O$_6$, 283.1176).

Sclerotiorum B (4): pale yellow oil; [α]$_D^{20}$ -0.41 (c 0.42, MeOH); UV (MeOH) $\lambda_{max}$ (log e) 226 (3.71), 269 (3.57) nm; $^1$H and $^{13}$C NMR data, Tables 1, 2; HRESIMS m/z 283.1176 [M + H]$^+$ (calcd for C$_{14}$H$_{19}$O$_6$, 283.1176).

Antibacterial Assay for the Isolated Compounds

The antibacterial activity of the isolated compounds was determined using a conventional broth dilution assay (Oppong-Danquah et al., 2020). Apart from R. solanacearum (bacterial wilt of tobacco), another five phytopathogenic bacterial strains, including Acidovorax avenae (bacterial fruit blotch), Clavibacter michiganensis (bacterial wilt and canker of tomato), Erwinia carafavara (tobacco hollow stalk), Xanthomonas campestris (cotton angular leaf spot), and Xanthomonas citri (bacterial canker of citrus) were used. Streptomycin sulfate was used as the positive control. Dimethyl sulfoxide was used as the solvent and the concentration was 1%. The MIC was determined as the lowest concentration at which no growth was observed. The inhibition rate was calculated according to the following formula, and the IC$_{50}$ was calculated from the regression equation.

\[
\text{Inhibition rate} = \frac{(\text{OD value}_\text{bs} - \text{OD value}_\text{t})}{(\text{OD value}_\text{bs} - \text{OD value}_\text{ck})} \times 100\%
\]

bs, bacterial suspension; t, tested compounds; ck, blank control.

RESULTS AND DISCUSSION

Structural Elucidation of Compounds 1–11

Sclerotiorum D (1) was obtained as a pale-yellow powder, and had a molecular formula of C$_{15}$H$_{12}$O$_2$ based on HRESIMS data (m/z 333.0942 [M + Na]$^+$) (Supplementary Figure S7), accounting for seven degrees of unsaturation. Although it was isolated as a pure compound by HPLC, its $^1$H- and $^{13}$C-NMR signals (Supplementary Figures S1–S5) appeared as a mixture of two geometric isomers (1a and 1b) with a ratio of 1:1.3 (Supplementary Figures S1, S2). Further attempts to separate the two isomers using a chiral column failed due to spontaneous and immediate isomerization between the two forms.

The $^1$H NMR spectrum of 1a (Table 1) displayed signals for two olefinic protons at $\delta_H$ 6.62 (s) and 5.32 (s), two methoxy groups at $\delta_H$ 3.94 (s) and 3.82 (s), one set of non-equivalent

TABLE 2 | $^{13}$C NMR Data (125 MHz, DMSO-d$_6$, $\delta$ in ppm) for 1–4.

| Position | 1a | 1b | 2a | 2b | 3a | 3b | 4a | 4b |
|----------|----|----|----|----|----|----|----|----|
| 1        | 202.8, C | 202.3, C | 201.7, C | 201.6, C |
| 2        | 50.1, C | 50.0, C | 49.9, C | 49.9, C |
| 3        | 200.6, C | 200.7, C | 201.5, C | 201.3, C |
| 4        | 170.9, C | 171.1, C | 170.9, C | 171.1, C |
| 5        | 171.7, C | 171.1, C | 171.7, C | 171.1, C |
| 6        | 173.6, C | 173.6, C | 173.6, C | 173.7, C |
| 7        | 173.6, C | 173.6, C | 173.6, C | 173.7, C |
| 8        | 173.6, C | 173.6, C | 173.6, C | 173.7, C |
| 9        | 173.6, C | 173.6, C | 173.6, C | 173.7, C |
| 10       | 173.6, C | 173.6, C | 173.6, C | 173.7, C |
| 11       | 173.6, C | 173.6, C | 173.6, C | 173.7, C |
| 12       | 173.6, C | 173.6, C | 173.6, C | 173.7, C |
| 13       | 173.6, C | 173.6, C | 173.6, C | 173.7, C |
| 14       | 173.6, C | 173.6, C | 173.6, C | 173.7, C |
| 15       | 173.6, C | 173.6, C | 173.6, C | 173.7, C |
methylenic protons at $\delta_H$ 2.11 (dd, $J = 14.0, 3.0$ Hz) and $\delta_H$ 1.38 (m), one methine proton at $\delta_H$ 1.72–1.75 (m), and two methyl groups at $\delta_H$ 1.06 (s) and 0.58 (d, $J = 7.0$ Hz). The $^{13}$C NMR and DEPT spectra of 1a (Table 2) showed resonances for 15 carbon signals which could be classified as one methylene, three methines (one aliphatic and two olefinic), seven quaternary carbons including two $\alpha,\beta$-unsaturated ketones ($\delta_C$ 202.6, 200.7), two methoxy groups ($\delta_C$ 59.8, 59.3), and two methyl groups ($\delta_C$ 20.8, 15.1). These spectroscopic features suggested that 1a belongs to the family of furanones and that ring B in 1a is very similar to sclerotiorum in B (4), isolated from a co-culture of Aspergillus sclerotiorum and Penicillium citrinum (Bao et al., 2017). Analysis of their $^1$H and $^{13}$C NMR spectra indicated that the main differences were present in the ring A. The additional methoxy group ($\delta_H$ 3.94, $\delta_C$ 59.3) in 1a, and the disappearance of one methyl group ($\delta_H$ 1.61, $\delta_C$ 5.1) compared to 4a, suggested that there was a methoxy rather than a methyl group anchored at C-4 in 1a. The additional $\alpha,\beta$-unsaturated ketone ($\delta_C$ 202.6) and the upfield shifts of C-2 ($\delta_C$ 49.9) and C-5 ($\delta_C$ 117.5) in 1a, revealed that C-1 in 4a was replaced by a carbonyl. The observed HMBC correlations (Figure 2 and Supplementary Figure S5) from H-5 to C-1, C-2, and C-3, from H-6 to C-1, from H-12 to C-4, and from H-13 to C-1, C-2, and C-3 confirmed the above deduction. Thus, the planar structure of 1a was determined.

Comparison of the 1D and 2D NMR data for 1a and 1b indicated that they had the same planar structure. There were also no differences between the two compounds in the NOESY spectrum of 1a and 1b (Figure 2 and Supplementary Figure S6). The correlations of H-6a with H-13, H-6b with H-15, and of H-15 with H-13 were unstable, and resulted in the R/S configurations of C-8.

Sclerotiorum in F (3) was isolated as pale-yellow oil and also presented as an inseparable mixture of two geometric isomers (3a and 3b). Its HRESIMS data ($m/z$ 283.1176 [M + H]$^+$) revealed a molecular formula of C$_{14}$H$_{18}$O$_6$, requiring six degrees of unsaturation (Supplementary Figure S21). Detailed analysis of the 1D (Table 3 and Supplementary Figures S15, S16) and 2D NMR data showed that 3a and 3b have the same planar structure as 4, indicating that they were stereoisomers of 4 (Supplementary Figures S17–S19). In the NOESY spectrum (Figure 2 and Supplementary Figure S20), the correlation signals were the same between 3a and 3b, but different from those of 4. The correlations of H-6b with H-13 and H-15, and of H-15 with H-13 indicated that the two groups are positioned in the same plane. It may be impossible to determine the absolute configurations of 1–4 due to the unstable hemiacetal group. Methylation was as 1 (Supplementary Figure S14). Similar to 1, compound 2 also existed as geometric isomers in the same ratio of 1:1.3. Comparison of the 1D (Tables 2 and Supplementary Figures S8, S9) and 2D NMR data of 2 with those of 1 revealed that these compounds have the same planar structures (Supplementary Figures S10–S12). The correlation of H-13 with H-15 in the NOESY spectrum (Figure 2 and Supplementary Figure S13), which was different from that of 1, indicated that these two groups have a syn-relationship. These data indicated that 2a and 2b were also epimers at C-8.

Sclerotiorum in F (3) was isolated as pale-yellow oil and also presented as an inseparable mixture of two geometric isomers (3a and 3b). Its HRESIMS data ($m/z$ 283.1176 [M + H]$^+$) revealed a molecular formula of C$_{14}$H$_{18}$O$_6$, requiring six degrees of unsaturation (Supplementary Figure S21). Detailed analysis of the 1D (Table 3 and Supplementary Figures S15, S16) and 2D NMR data showed that 3a and 3b have the same planar structure as 4, indicating that they were stereoisomers of 4 (Supplementary Figures S17–S19). In the NOESY spectrum (Figure 2 and Supplementary Figure S20), the correlation signals were the same between 3a and 3b, but different from those of 4. The correlations of H-6b with H-13 and H-15, and of H-15 with H-13 indicated that the two groups are positioned in the same plane. It may be impossible to determine the absolute configurations of 1–4 due to the unstable hemiacetal group. Methylation was

| Position | 3a | 3b | 4a | 4b |
|----------|----|----|----|----|
| 5        | 8.43, s | 8.42, s | 8.41, s | 8.44, s |
| 6        | 2.19, brd | 1.79, brd | 2.25, brd | 1.68, brd |
| 7        | 1.92–1.97, m | 1.97–2.01, m | 1.71–1.74, m | 1.63–1.66, m |
| 10       | 5.35, s | 5.37, s | 5.34, s | 5.36, s |
| 12       | 1.60, s | 1.60, s | 1.61, s | 1.61, s |
| 13       | 1.22, s | 1.19, s | 1.25, s | 1.20, s |
| 14       | 3.85, s | 3.85, s | 3.83, s | 3.81, s |
| 15       | 0.68, d | 0.77, d | 0.53, d | 0.71, d |

| Phytopathogenic bacterial strains | IC$_{50}$ (µg/mL) | MIC (µg/mL) |
|----------------------------------|-----------------|-------------|
|                                  | Streptomycin sulfate | 7 | Streptomycin sulfate | 7 |
|                                  |                  |             |                  |             |
| $R$. solanacearum               | 58.2             | 7.63         | 200              | 16.4         |
| $A$.avenae                      | 11.6             | 17.6         | 51.3             | 56.9         |
| $C$.mitchigianensis             | 37.2             | 8.74         | 118              | 50.2         |
| $E$.carafata                    | 57.8             | 50.7         | 218              | 200          |
| $X$.campestris                  | 58.2             | 11.2         | 200              | 50.0         |
| $X$.citri                       | 14.8             | 6.94         | 100              | 25.0         |
performed to fix the hydroxyl group at C-8, but failed, possibly because the lactone was disrupted under alkaline conditions.

The known compounds 4–11 were identified on the basis of their spectroscopic data by comparison with those in the literature. These compounds were identified as sclerotiorumins B (4) (Supplementary Figures S22–S28) (Bao et al., 2017), orcinol (5) (Witjak et al., 1967), vanillic acid (6) (Lee et al., 1992), penicillic acid (7) (Suzuki et al., 1971), dihydropenicillic acid (8) (Phainuphong et al., 2017), 4-hydroxy-2-methoxy-5-methylcyclopent-2-enone (9) (Wang et al., 2015), 4-hydroxy-3-methoxy-3-methylcyclopent-2-enone (10) (Wang et al., 2015), and oxaline (11) (Konda et al., 1980). Compound 9 was previously reported as a pure compound (Wang et al., 2015), however, in the present study, 9 existed as a pair of epimers with a trans relationship of H-4/H-5 (9a, J4,5 = 1.0 Hz), while H-4/H-5 of 9b had a cis relationship (J4,5 = 5.5 Hz). Therefore, 9b was identified as a new compound with a relative configuration different from that of 9a.

**Antibacterial Activity of 1–11 From D40 in PDW Medium**

All the isolated compounds were evaluated for their antibacterial activity against *R. solanacearum* (bacterial wilt of tobacco), and another five phytopathogenic bacterial strains – *Acidovorax avenue* (bacterial fruit blotch), *Clavibacter michiganensis* (bacterial wilt and canker of tomato), *Erutima carafavora* (tobacco hollow stalk), *Xanthomonas campestris* (cotton a angular leaf spot), and *Xanthomonas citri* (bacterial canker of citrus) – and only penicillic acid (7) showed potent antibacterial activity. Penicillic acid has been reported to have anti-plant pathogenic bacterial activity (Nguyen et al., 2016). In the present study, its antibacterial activity toward other phytopathogens or those from different plants were studied. The IC50 values of 7 against six plant pathogens are shown in Table 4 and Supplementary Figure S29. As indicated, 7 exhibited obvious antibacterial effects against all 6 tested strains. Although the positive control streptomycin sulfate showed a stronger effect than 7 (Supplementary Figure S30), its application has been prohibited in agriculture in China since 2016. Penicillic acid was first reported in an examination of fungal growth in maize and the potential for fungal involvement in pellagra, and was found to be more common on stored cereals, as the best producers are mostly cereal-borne (Frisvad, 2018). Its toxicity to poultry, mice, rats, and rabbits, as well as human beings was soon discovered (Barkai-Golan, 2008). However, hormesis, the phenomena of low-dose stimulation/signaling and high-dose toxicity by the same molecule, is very common for microbial natural products (Schmidt et al., 2019).

**CONCLUSION**

In summary, we successfully isolated and identified 11 compounds from the seaweed-derived *Aspergillus* sp. D40 cultured with PDW medium, including three pairs of new furanone derivatives, sclerotiorumins D–F (1–3), which existed as inseparable mixtures of epimers. Among these compounds, penicillic acid exhibited potent anti-bacterial activity toward different plant pathogens that showing the potential to develop into an anti-bacterial biopesticide.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

**AUTHOR CONTRIBUTIONS**

C-SZ and D-LZ conceived and designed the experiments. R-HH, WLI, J-YL, and DW performed the experiments. PZ, Y-QL, and X-QW analyzed the data. D-LZ wrote the manuscript. WLI provided the fungal material and performed fungal strain screening. D-LZ and WLI revised the manuscript. All authors reviewed the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00313/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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