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# Metabolites from a global regulator engineered strain of *Pseudomonas lurida* and their inducement of trap formation in *Arthrobotrys oligospora*

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**Abstract**

**Background** Plant parasitic nematodes (PPNs) cause serious harm to agricultural production. Nematode-trapping fungi (NTF) can produce traps to capture nematodes and are the main resource for controlling nematodes. The number of traps determines the capturing ability of NTF.

**Results** *Pseudomonas lurida* is widely existed in different habitats, which produces active metabolites to induce trap formation of *Arthrobotrys oligospora*, a famous NTF. To further identify the active substances, metabolic regulation was carried out in the strain by molecular biological methods. A mutant strain *P. lurida araC*-PoprL with abundant secondary metabolites was constructed, and 19 metabolites (**1**–**19**) including a new compound, 1,1-dimethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-3-carboxylic acid (**1**), were isolated and identified. The activity assay showed that 1-methylhydantoin (**9**) could effectively induce *A. oligospora* to produce traps.

**Conclusions** *P. lurida* and the metabolite 1-methylhydantoin effectively induced trap formation in *A. oligospora*. Both provide sources for the screening of inducing active materials and show potential use in controlling plant parasitic nematodes.

**Keywords** *Pseudomonas lurida*, Metabolite, Nematode-trapping fungi, Trap, *Arthrobotrys oligospora*

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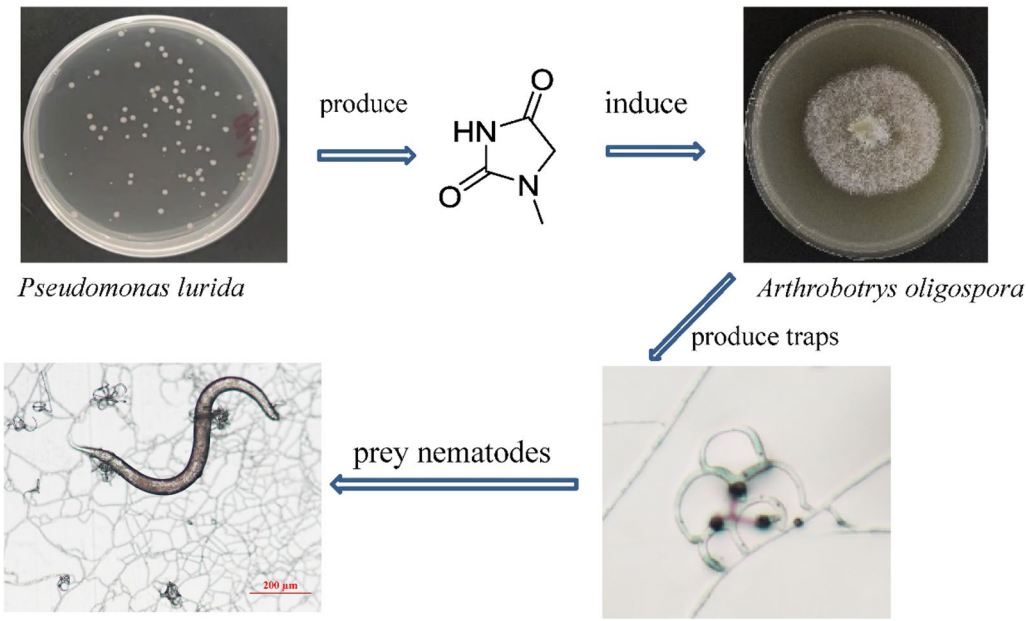
**Graphical Abstract**




## Background

Nematodes are lower multicellular animals that are numerous and widely distributed [1]. Among them, plant parasitic nematodes (PPNs) not only lead to damage to plant tissues, but also promote the infection of plants by pathogenic microorganisms in soil, thus causing or exacerbating other diseases [2]. Almost all crops in the world are infected by PPNs, which is one of the reasons for the enormous economic losses of crops. According to a literature, a global loss of 170 billion dollars annually is caused by PPNs [3].

At present, the main control methods for hazard caused by PPNs can be divided into chemical, physical, agricultural and biological control. The biocontrol of PPNs has been extensively researched because it has few negative environmental impacts [4]. Nematode-trapping fungi (NTF) form diverse special traps to capture nematodes through mycelium specialization, such as three-dimensional networks, adhesive knobs, and constricting rings etc. [4]. It is one of the ideal materials for studying the biocontrol of nematodes. It can kill nematodes directly depending on the number of traps. Many factors can induce the formation of traps, among which natural metabolite is an important factor. Many amino acids and small peptides have been reported to have induced activity [5]. Interestingly, nematodes themselves can induce fungi to produce traps, and their products ascarosides, can also induce traps formation [6, 7].

Recently, bacteria and their metabolites had been found to can induce trap formation in NTF. The research found that the coculture of *Chryseobacterium* sp. and *Arthrobotrys oligospora* could induce traps production [8]. Dipiperazines (DKPs), the metabolites produced by *Chryseobacterium* sp., can enhance the activity of bacteria to promote the formation of traps [9]. Ammonia produced by bacteria can induce the production of traps [10]. The NTF *Arthrobotrys conoides* and *A. oligospora* produced traps after coculture with several bacteria for 48 h [11]. Bacteria and their secondary metabolites are factors that cannot be ignored in the study of trap formation in NTF.

The AraC family of transcriptional regulator is representative of globally regulated genes that have been studied early and are widely distributed in a variety of bacteria [12]. The members of this family can regulate the metabolism of bacteria, for example, the *gapR* gene could regulate glucose metabolism in *Streptomyces aureofaciens* by controlling the expression of glyceraldehyde-3-phosphate dehydrogenase [13]; transcriptional regulator GliR is related to the regulation of glycerolipid metabolism in *Pseudomonas aeruginosa* [14]; the global regulator SAV742 negatively regulates avermectin production in *S. avermitilis* [15]; the regulator MsmR1 is involved in production of polymyxin synthesis in *Paenibacillus polymyxa* SC2 [16]. Promoters

play an important role in gene transcription and expression [17]. In metabolic engineering, the selection of a stably expressed promoter has a great impact on metabolites generation. PoprL, a promoter found in *Pseudomonas putida* [18], has been shown to have a strong enhancement effect on *P. aeruginosa* to increase production of rhamnoolipids [19].

*Pseudomonas lurida* is a gram-negative bacterium that is widely distributed; it was first isolated from strawberry leaves [20], and then obtained from soil in cold regions [21], plant rhizospheres [22], nematode [23], and milk [24]. It has the characteristics of cold tolerance, phosphorus dissolution and plant growth promotion [25]. In the present study, *P. lurida* can induce the formation of traps in *A. oligospora*, but it is not clear which substance plays a role. Up to now, only the compound massetolide E has been reported from the species [26], and further study of its metabolites is needed. Therefore, the study on the secondary metabolites of *P. lurida* aims to tap the active substances and obtain more resources for nematode control.

## Methods

### Equipments

The optical rotation was analyzed by a Jasco DIP-370 digital polarimeter (Tokyo, Japan). Ultraviolet (UV) spectrum was recorded on a Shimadzu UV-2401PC spectrophotometer (Kyoto, Japan). Nuclear magnetic resonance (NMR) spectra were measured on an Avance III-600 spectrometer (Bruker Biospin, Rheinstetten, Germany). Electrospray ionization mass spectrometry (ESI–MS) spectra were recorded on a Thermo high-resolution Q Exactive Focus mass spectrometer (Thermo, Bremen, Germany). Silica gel G (200–300 mesh, Qingdao Ocean Chemical Co., Ltd., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Piscataway, NJ, USA) were used for column chromatography, and silica gel plate GF254 (Qingdao Ocean Chemical Company, Qingdao, China) was used for thin layer chromatography (TLC).

### Strains and nematode cultivation

Stock cultures of *P. lurida* YMF 3.02383, *C. elegans, Escherichia coli* DH5α and *A. oligospora* YMF1.01883 were preserved in Microbial Library of the Germplasm Bank of Wild Species from Southwest China. *P. lurida* was cultivated in liquid media [LB (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl), NB (3 g/L beef extract, 10 g/L peptone, 5 g/L NaCl) or KB (20 g/L tryptone, 0.685 g/L $K_2HPO_4{\cdot}3H_2O$, 1.5 g/L $MgSO_4{\cdot}7H_2O$, 15 mL glycerol)] at 28 °C at 180 rpm for 2 days to prepare prepagula. 1 mL of the prepagula was then inoculated into medium and cultured for 4 days at the above conditions. The fermentation broth was filtered and the broth was collected. The cultured bacterial liquid was used for activity experiments. *A. oligospora* was activated on PDA medium (200 g/L potato, 20 g/L glucose, 15 g/L agar) and inoculated on CMY solid medium (20 g/L corn, 5 g/L yeast extract, 15 g/L agar), and cultured at 28 ℃ for 8–12 d. The proper amount of sterile water and glass beads were added to the triangular flask, shaken to wash all hyphae, and filtered with six layers of Lens paper to obtain a spore suspension. *C. elegans* was cultured with *Escherichia coli* OP50 on NGM (3 g/L NaCl, 2.5 g/L peptone, 1 mL 5 mg/mL cholesterol ethanol solution, 1 mL 1 M $MgSO_4$, 1 mL 1 M $CaCl_2$, 25 mL 1 M $K_2HPO_4{\cdot}3H_2O$, 15 g/L agar) plates at 20 ℃.

### Determination of induced activity of fermentation broth and extract

Spore suspensions of *A. oligospora* at approximately 3000 spores and 100 μL fermentation broth were thoroughly mixed in a 1.5 mL centrifuge tube. The control was 100 μL of culture medium mixing with spore suspensions. The broth was extracted by *n*-butanol and dissolved in methanol to prepare a 30 mg/mL mother liquor. Then, 97 μL of spore liquid and 3 μL of mother liquor were fully mixed in a 1.5 mL centrifuge tube. The mixed solutions (broth and spores, extracts and spores) were coated on a 1.5% water agar plate, and three parallel plates were set up in each experiment. The mixture was cultured at 28 ℃ for 2–3 d. After 24 h of culture, the traps were observed under a microscope. The experiment was repeated three times.

### Overexpression of the *araC* gene in *P. lurida*

The *araC* gene in *P. lurida* was overexpressed in our experiment. The upstream and downstream homologous arm primers of *araC* gene were designed, and the fragments between homologous arms were replaced by strong promoters. The suicide plasmid pK18 mobsacB was used as the carrier, and the plasmid was linearized by enzyme digestion. Four fragments of the upstream homologous arm, PoprL, screening marker fragment gmR and the downstream homologous arm were sequentially connected by overlapping PCR. The fragment was connected to the pK18 mobsacB vector by In-Fusion ligase, transformed into *E. coli* competent DH5α cells and screened to obtain the recombinant plasmid pYUZ180. The recombinant plasmid pYUZ180 was transformed into *P. lurida* for homologous recombination screening to obtain the transformant *P. lurida araC*-PoprL.

### Weight and LC–MS detection of metabolites from *P. lurida* and *P. lurida araC*-PoprL

Two culture media, NB and KB were selected to culture WT and *P. lurida araC*-PoprL. Then, the cultured

fermentation broth was extracted with the same volume of *n*-butanol three times. The solvent was evaporated with a rotary evaporator and the amount of the extract was weighed. The concentration of sample was prepared as 10 mg/mL with chromatography-grade methanol. The sample was filtered and put into a bottle, left overnight at 4 ℃ to ensure that there was no precipitation, and detected by LC–MS. LC–MS was performed on a Dionex UltiMate 3000 LC system coupled with a Q-Exactive Orbitrap mass spectrometer. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in methanol. The 30 min gradient for positive ESI mode was set as follows: 0–3 min, 5% solvent B; 3–22 min, 5–95% solvent B; 22–25 min, 95% solvent B; and 25–30 min, 5% solvent B.

### Extraction and isolation of secondary metabolites from *P. lurida araC*-PoprL

*P. lurida araC*-PoprL was fermented in KB medium and cultured in a shake flask at 28 ℃ and 180 rpm for 4 d, with a total fermentation of 100 L. The fermentation broth was concentrated under reduced pressure and extracted with ethyl acetate to obtain 108.49 g of extract.

The extract was subjected to silica gel G column chromatography (CC) and eluted with petroleum ether/ethyl acetate (100:1, 80:1, 60:1, 40:1, 20:1, 10:1, 5:1, 0:1, v/v), ethyl acetate/methanol (60:1, 40:1, 20:1, 10:1, 4:1, 1:1, 0:1, v/v) and pure methanol in turn to obtain 21 components, E1-E21. Fraction E2 was subjected to Sephadex LH-20 CC with acetone to obtain three fractions, E2-1- to E2-3. Fraction E2-3 was subjected to silica gel G CC eluted with petroleum ether/acetone/formic acid (1000:10:1, 800:10:0.8, v/v) to obtain compound **2** (4.7 mg). Fraction E4 was subjected to Sephadex LH-20 CC with methanol gel to obtain fractions E4-1- to E4-7. Fraction E4-5 was isolated through silica gel G CC eluted with petroleum ether/acetone (100:1, 80:1, v/v) to obtain compound **3** (4.2 mg). Fraction E6 was separated by Sephadex LH-20 CC with methanol to obtain fractions E6-1- to E6-3, and fraction E6-2 was subjected to silica gel G CC eluted with petroleum ether/ethyl acetate (100:1, 90:1, 80:1, 70:1, 60:1, v/v) to afford compound **4** (3.3 mg). Fraction E8 was separated by preparative liquid chromatography [Hypersil BDS C18 (250 mm × 10 mm) semipreparative column was used, mobile phase A was water 5‰ formic acid, and liquid B was methanol containing 5‰ formic acid, and gradient elution (A:B from 90:10 to 0:100) was carried out. The column temperature was normal, the flow rate was 3 mL/min, the injection volume was 0.1 mL], and the detection wavelength was 365 nm to obtain fractions E8-2-1–E8-2-5. Fraction E8-2-4 was subjected to Sephadex LH-20 CC with methanol to obtain fractions E8-2-4-1–E8-2-4-3, among them, E8-2-4-3 was purified by silica gel G CC and eluted with petroleum ether/ethyl acetate/formic acid (80:1:0.08, 60:1:0.06, v/v) to provide compound **5** (7.1 mg). Fraction E9 was separated by Sephadex LH-20 CC with methanol to produce fractions E9-1- to E9-3, and fraction E9-3 was purified by silica gel G CC eluting with petroleum ether/acetone/formic acid (60:1:0.05, 40:1:0.04, 30:1:0.03, v/v) to obtain compound **6** (4.1 mg). Fraction E9-3-2 was subjected to silica gel G CC eluting with petroleum ether/acetone/formic acid (50:1:0.05, 40:1:0.04, 30:1:0.03, 20:1:0.02, 10:1:0.01, v/v) to obtain fractions E9-3-2-1–E9-3-2-3, among them, E9-3-2-2 was separated by preparative liquid chromatography to obtain compound **1** (1.2 mg). Fraction E9-2 was purified by preparative liquid chromatography to obtain compounds **7** (136.0 mg) and **8** (5.5 mg). Fraction E10 was isolated by Sephadex LH-20 CC with methanol to obtain fractions E10-1- to E10-5, in which fraction E10-5 was further purified by silica gel G CC and eluted with chloroform/acetone (80:1, 60:1, 50:1, v/v) to provide compound **9** (16.1 mg). Fraction E11 was subjected to Sephadex LH-20 CC with methanol to obtain fractions E11-1–E11-4. Fraction E11-1 was isolated by silica gel G CC eluting with petroleum ether/acetone (70:1, 60:1, 50:1, 40:1, 30:1, 20:1, 10:1, 5:1, 0:1, v/v) to obtain fractions E11-1-1- to E11-1-7. Fraction E11-1-5 was subjected to Sephadex LH-20 CC with methanol to obtain compound **11** (15.4 mg). Fraction E11-1-6 was separated by preparative liquid chromatography to produce compound **12** (2.0 mg). Fraction E11-1-7 was subjected to Sephadex LH-20 CC with methanol twice to obtain compound **13** (1.5 mg). Fractions E11-3 and E11-4 were further separated with preparative liquid chromatography to afford compounds **10** (2.0 mg) and **14** (6.6 mg), respectively. Fraction E13 was separated by a Sephadex LH-20 CC with methanol to obtain fractions E13-1–E13-10, in which component E13-1 was purified on a silica gel G CC and eluted with chloroform/methanol (100:1, 80:1, 75:1, 70:1, 65:1, 60:1, 56:1, 52:1, 50:1, v/v) to produce compound **15** (3.0 mg). Fraction E13-6 was subjected to silica gel G CC and eluted with chloroform/acetone (25:1, 20:1, 18:1, 16:1, 14:1, v/v) to obtain compound **16** (53.1 mg). Fraction E13-7 was subjected to Sephadex LH-20 CC with methanol and then purified by silica gel G CC eluting with chloroform/acetone (40:1, v/v) to produce compound **17** (1.8 mg). Fraction E15 was subjected to silica gel G CC and eluted with petroleum ether/acetone (70:1, 60:1, 50:1, 45:1, 40:1, 30:1, 20:1, 10:1, 5:1, v/v) to obtain fractions E15-1- to E15-4, and fraction E15-4 was subjected to Sephadex LH-20 CC with methanol to provide compound **18** (12.8 mg). Fraction E16 was purified by silica gel

G CC eluting with chloroform/methanol (60:1, 50:1, 40:1, 30:1, 20:1,10:1, 8:1, 6:1, 4:1, 2:1, v/v) to produce fractions E16-1—E16-2, and E16-1 was sliced by thin-layer chromatography, and then subjected to Sephadex LH-20 CC with methanol gel to obtain compound **19** (13.4 mg).

Compound **1**: Colorless solid; ESI–MS m/z: 244 $[M–H]^-$, 268 $[M+Na]^+$; HR–ESI–MS: 244.0969 ($[M–H]^-$, calc. 244.0968); [α] = − 75.4 (c = 0.25, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) nm: 205 (4.20), 224 (4.44), 278 (3.73); $^1$H- and $^{13}$C- NMR ($CD_3OD$) data are shown in Table 1.

Compound **2**: White solid; the molecular formula is $C_7H_6O_2$; ESI–MS: 121 $[M—H]^-$; $^1$H-NMR (600 MHz, $CDCl_3$) δ: 8.14 (2H, d, *J* = 7.7 Hz, H-6/2), 7.64 (1H, t, *J* = 7.4 Hz, H-4), 7.51 (2H, t, *J* = 7.7 Hz, H-3/5); $^{13}$C-NMR (150 MHz, $CDCl_3$) δ: 172.0 (C-7), 133.8 (C-4), 130.2 (C-6/2), 129.3 (C-1), 128.5 (C-3/5).

Compound **3**: White solid; the molecular formula is $C_{14}H_{11}NO$; ESI–MS: 210 $[M+H]^+$; $^1$H-NMR (600 MHz, $CDCl_3$) δ: 10.3 (1H, brs), 8.55 (1H, d, *J* = 4.9 Hz), 8.16 (2H, d, *J* = 5.1 Hz), 7.62 (2H, m), 7.34 (1H, m) 2.89 (3H, s); $^{13}$C-NMR (150 MHz, $CDCl_3$) δ: 203.3 (s), 141.1 (s), 138.1 (d), 136.0 (s), 135.4 (s), 131.5 (s), 129.3 (d), 129.0 (d), 121.8 (d), 120.7 (d), 120.6 (d), 119.1 (d), 112.0 (d), 25.9 (q).

Compound **4**: Pale yellow oil; the molecular formula is $C_9H_{10}O_3$; ESI–MS: 165 $[M—H]^-$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 7.14 (2H, d, *J* = 8.5 Hz, H-2/6), 6.78 (2H, d, *J* = 8.5 Hz, H-3/5), 3.72 (3H, s, OMe), 3.51 (2H, s, H-7); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 174.6 (C-8), 157.6 (C-4), 131.3 (C-2/6), 126.3 (C-1), 115.5 (C-3/5), 52.3 (OMe), 40.9 (C-7).

Compound **5**: White solid; the molecular formula is $C_9H_9NO_3$; ESI–MS: 180 $[M+H]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 8.65 (1H, d, *J* = 8.0 Hz), 8.08 (1H, d, *J* = 8.0 Hz), 7.56 (1H, t, *J* = 8.0 Hz), 7.15 (1H, t, *J* = 8.0 Hz), 2.15 (3H, s); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 23.6 (q), 116.4 (s), 119.9 (d), 122.6 (d), 131.1 (d), 133.6 (d), 140.9 (s), 170.0 (s), 170.1 (s).

Compound **6**: Yellow powder; the molecular formula is $C_9H_7NO_2$; ESI–MS: 162 $[M+H]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 8.07 (1H, d, *J* = 8.0 Hz, H-4), 7.94 (1H, s, H-2), 7.43 (1H, dd, *J* = 8.0 Hz, H-7), 7.18 (1H, dd, *J* = 8.0, 8.0 Hz, H-6), 7.17 (1H, dd, *J* = 8.0, 8.0 Hz, H-5); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 169.5 (s, COOH), 138.2 (d, C-8), 133.4 (d, C-2), 127.6 (s, C-9), 123.6 (d, C-6), 122.4 (d, C-5), 122.0 (d, C-4), 112.9 (d, C-7), 108.9 (s, C-3).

Compound **7**: White solid; the molecular formula is $C_{14}H_{16}N_2O_2$; ESI–MS: 245 $[M+H]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 1.25 (1H, m), 1.75 (1H, m), 2.08 (1H, m), 3.18 (1H, m), 3.35 (1H, m), 3.52 (1H, m), 4.03 (1H, m), 4.40 (1H, m), 7.21 (5H, m); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 166.8 (s, C-1), 45.9 (t, C-3), 22.7 (t, C-4), 29.2 (t, C-5), 60.0 (d, C-6), 170.9 (s, C-7), 57.5 (d, C-9), 38.0 (t, C-10), 137.4 (s, C-1`), 131.0 (d, C-2`), 130.9 (d, C-3`), 127.9 (d, C-4`).

Compound **8**: Yellow solid; the molecular formula is $C_9H_8O_3$; ESI–MS *m/z*: 187 $[M+Na]^+$; 165 $[M+H]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 7.44 (2H, d, *J* = 8.7 Hz, H-2/6), 6.73 (2H, d, *J* = 8.7 Hz, H-3/5), 6.28 (1H, d, *J* = 15.9 Hz, H-7), 5.77 (1H, d, *J* = 15.9 Hz, H-8); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 127.8 (C-1), 133.3 (C-2/6), 115.8

**Table 1** The NMR data of compound **1** and tryptophan in $CD_3OD$

| Compound 1 | | | | | Tryptophan[a] | |
|---|---|---|---|---|---|---|
| | $^1$H | $^{13}$C | HMBC | COSY | $^1$H | $^{13}$C |
| 1 | – | 140.1, s | – | – | 7.21 (1H, s) | 128.4, d |
| 2 | – | 105.5, s | – | – | – | 108.2, s |
| 3 | – | 127.9, s | – | – | – | 125.6, s |
| 4 | 7.40 (1H, d, *J* = 7.8 Hz) | 118.9, d | C-2, C-3, C-6, C-8 | H-5 | 7.62 (1H, d, *J* = 7.5 Hz) | 112.7, d |
| 5 | 6.97 (1H, t, *J* = 7.5 Hz) | 119.9, d | C-3, C-5 | H-4/H-6 | 7.06 (1H, td, *J* = 7.5, 2.5 Hz) | 120.4, d |
| 6 | 7.05 (1H, t, *J* = 7.5 Hz) | 122.2, d | C-4, C-8 | H-5/H-7 | 7.14 (1H, td, *J* = 7.5, 2.5 Hz) | 123.1, d |
| 7 | 7.28 (1H, d, *J* = 8.1 Hz) | 111.9, d | C-3, C-5 | H-6 | 7.39 (1H, brd, *J* = 7.5 Hz) | 119.1, d |
| 8 | – | 137.9, s | – | – | – | 138.5, s |
| 9 | 2.82 (1H, dd, *J* = 15.0, 11.2 Hz) | 26.9, t | C-1, C-2, C-10, C-11 | H-10 | 3.32 (1H, m) | 27.9, t |
| | 3.08 (1H, dd, *J* = 15.0, 3.5 Hz) | | C-1, C-2 | H-10 | 3.51 (1H, dd, *J* = 5.0, 4.5 Hz) | |
| 10 | 4.52 (1H, dd, *J* = 11.2, 3.5 Hz) | 70.7, d | C-9, C-11, C-12 | H-9 | 4.21 (1H, dd, *J* = 5.0, 4.5 Hz) | 54.9, d |
| 11 | – | 176.3, s | – | – | – | 172.1, s |
| 12 | – | 75.0, s | – | – | – | – |
| 13 | 1.58 (3H, s) | 26.6, q | C-1, C-12, C-14 | – | – | – |
| 14 | 1.61 (3H, s) | 29.3, q | C-1, C-12, C-13 | – | – | – |

[a] The data were cited from reference [28]

(C-3/5), 159.8 (C-4), 146.5 (C-7), 116.89 (C-8), 170.8 (C-9).

Compound **9**: White solid; the molecular formula is $C_6H_6N_2O_2$; ESI–MS: 115 $[M+H]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 2.90 (3H, s), 3.95 (2H, s); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 173.8 (C-4), 159.3 (C-2), 53.9 (C-5), 29.2 (1-$CH_3$).

Compound **10**: White solid; the molecular formula is $C_5H_6N_2O_2$; ESI–MS: 127 $[M+H]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 7.21 (1H, s, H-6), 1.84 (3H, s, H-7); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 167.5 (C-4), 153.7 (C-2), 139.1 (C-6), 110.4 (C-5), 12.1 (C-7).

Compound **11**: White solid; the molecular formula is $C_{11}H_{18}N_2O_2$; ESI–MS: 211 $[M+H]^+$; $^1$H-NMR (600 MHz, $CDCl_3$) δ: 3.59 (2H, m, H-3), 2.03 (1H, m, H-4a), 1.91 (1H, m, H-4b), 2.32 (1H, m, H-5a), 2.10–2.25 (1H, m, H-5b), 4.12 (1H, t, $J$=7.5 Hz, H-6), 4.00 (1H, t, $J$=7.0 Hz), 2.35 (1H, m, H-10), 1.54 (1H, m, H-11), 0.95 (3H, d, $J$=6.5 Hz, H-12), 1.00 (3H, d, $J$=6.6 Hz, H-13); $^{13}$C-NMR (150 MHz, $CDCl_3$) δ: 170.3 (C-1), 45.5 (C-3), 22.7 (C-4), 28.1 (C-5), 59.0 (C-6), 166.2 (C-7), 53.4 (C-9), 38.5 (C-10), 24.6 (C-11), 22.7 (C-12), 21.2 (C-13).

Compound **12**: White solid; the molecular formula is $C_4H_4N_2O_2$; ESI–MS: 113 $[M+H]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 7.39 (1H, d, $J$=7.7 Hz, H-6), 5.60 (1H, d, $J$=7.3 Hz, H-5); $^{13}$C-NMR (150 MHz, $CD_3OD$): 167.4 (C-4), 151.5 (C-2), 143.5 (C-6), 101.7 (C-5).

Compound **13**: White solid; the molecular formula is $C_{10}H_{16}N_2O_2$; ESI–MS: 197 $[M+H]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 4.24 (1H, m, H-6), 3.59 (2H, m, H-3), 3.50 (1H, m, H-9), 2.34 (1H, m, H-10), 2.13 (1H, m, H-5a), 2.00 (1H, m, H-5b), 1.84 (2H, m, H-4), 1.02 (3H, d, $J$=6.8 Hz, H-11), 0.99 (3H, d, $J$=6.8 Hz, H-12); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 171.6 (C-7), 168.0 (C-1), 64.4 (C-9), 59.7 (C-6), 46.7 (C-3), 34.6 (C-10), 30.3 (C-5), 22.9 (C-4), 19.4 (C-11), 18.4 (C-12).

Compound **14**: White solid; the molecular formula is $C_6H_5N_5O$; ESI–MS: 164 $[M+H]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 8.13 (1H, s), 8.10 (1H, s), 7.10 (1H, s); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 163.1 (d, CHO), 155.3 (s), 152.4 (d), 151.2 (s), 139.4 (d), 118.5 (s).

Compound **15**: White solid; the molecular formula is $C_6H_5NO_2$; ESI–MS: 122 $[M—H]^-$; $^1$H-NMR (600 MHz, $CDCl_3$) δ: 9.02 (1H, d, $J$=1.6 Hz, H-2), 8.18 (1H, dd, $J$=1.6, 8.0 Hz, H-4), 7.43 (1H, dd, $J$=4.8, 7.8 Hz, H-5), 8.71 (1H, dd, $J$=1.6, 4.6 Hz, H-6); $^{13}$C-NMR (150 MHz, $CDCl_3$) δ: 148.2 (C-2), 128.5 (C-3), 135.5 (C-4), 129.1 (C-3), 152.6 (C-6), 167.4 (C-7).

Compound **16**: Colorless crystal; the molecular formula is $C_{11}H_{18}N_2O_3$; ESI–MS: 227 $[M+H]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 0.96 (3H, d, $J$=6.4 Hz, H-12), 0.96 (3H, d, $J$=6.4 Hz, H-13), 1.52 (1H, m, H-10), 1.91 (2H, m, H-11/10), 2.10 (1H, ddd, $J$=4.3, 11.1, 13.3 Hz, H-7), 2.28 (1H, dd, $J$=6.5, 13.3 Hz, H-7), 3.44 (1H, d, $J$=12.5 Hz, H-9), 3.66 (1H, dd, $J$=4.3, 12.5 Hz, H-9), 4.17 (1H, m, H-3), 4.45 (1H, m, H-8), 4.53 (1H, m, H-6); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 169.0 (C-2), 55.2 (C-3), 173.1 (C-5), 58.7 (C-6), 38.1 (C-7), 69.1 (C-8), 54.6 (C-9), 39.4 (C-l0), 25.8 (C-11), 22.2 (C-12), 23.3 (C-13).

Compound **17**: White solid; the molecular formula is $C_{11}H_{14}O_3$; ESI–MS: 217 $[M+Na]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 1.00 (6H, d, $J$=6.7 Hz), 2.16 (1H, m), 2.53 (2H, m), 6.23 (1H, s), 7.07 (1H, dd, $J$=6.5, 13.3 Hz, H-7), 3.44 (1H, d, $J$=12.5 Hz, H-9), 3.66 (1H, dd, $J$=4.3, 12.5 Hz, H-9), 4.17 (1H, m, H-3), 4.45 (1H, m, H-8), 4.53 (1H, m, H-6); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 176.0 (s), 160.8 (s), 144.5 (s), 126.3 (d), 125.9 (s), 115.5 (d), 111.0 (d), 47.0 (t), 30.8 (d), 26.7 (q), 22.8 (q).

Compound **18**: White solid; the molecular formula is $C_{11}H_{18}N_2O_3$; ESI–MS: 227 $[M+H]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 3.72 (1H, dd, $J$=12.9, 4.6 Hz), 3.44 (1H, m), 4.47 (1H, m), 2.29 (1H, m), 2.04 (1H, ddd, $J$=13.3, 11.7, 4.3 Hz), 4.48 (1H, m), 4.13 (1H, m), 2.18 (1H, m), 1.51 (1H, m), 1.33 (1H, m), 0.94 (3H, t, $J$=7.4 Hz), 1.08 (3H, d, $J$=7.3 Hz); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 169.0 (C-1), 55.1 (C-3), 69.1 (C-4), 39.4 (C-5), 58.3 (C-6), 173.1 (C-7), 61.2 (C-9), 36.9 (C-10), 25.7 (C-11), 12.6 (C-12), 15.5 (C-13).

Compound **19**: White solid; the molecular formula is $C_7H_{10}N_2O_2$; ESI–MS: 155 $[M+H]^+$; $^1$H-NMR (600 MHz, $CD_3OD$) δ: 3.54 (2H, m, H-3), 2.31 (1H, m, H-5a), 2.01 (3H, m, H-5b/H-4), 4.21 (1H, m, H-6), 4.56 (1H, brs, H-8), 4.08 (1H, d, $J$=16.8 Hz, H-9a), 3.77 (1H, d, $J$=16.8 Hz, H-9b); $^{13}$C-NMR (150 MHz, $CD_3OD$) δ: 166.5 (C-1), 46.3 (C-3), 23.3 (C-4), 29.3 (C-5), 59.8 (C-6), 172.0 (C-7), 46.9 (C-9).

### Determination of induced activity of isolated metabolites

The isolated compounds were dissolved in methanol and diluted with sterile water to different concentrations. The sample solution was mixed with *A. oligospora* spore solution. After fully mixing, it was evenly coated on a 60 mm WA (water agar, 15 g/L agar) plate, and cultured at 28 ℃. Methanol with the same concentration was used as a control. After 24 h, it was observed under a microscope and then observed every 12 h, and the number of traps was counted. The experiment was set up in three parallels and repeated three times. The differences of traps numbers among the different concentrations were compared in order to find statistically significant correlations by using the F (ANOVA) test (significance $p<0.05$).

## Results

### Inducement trap formation in *A. oligospora* by *P. lurida*

The bacterial broth was mixed with spores of *A. oligospora*, and the traps were observed. The result

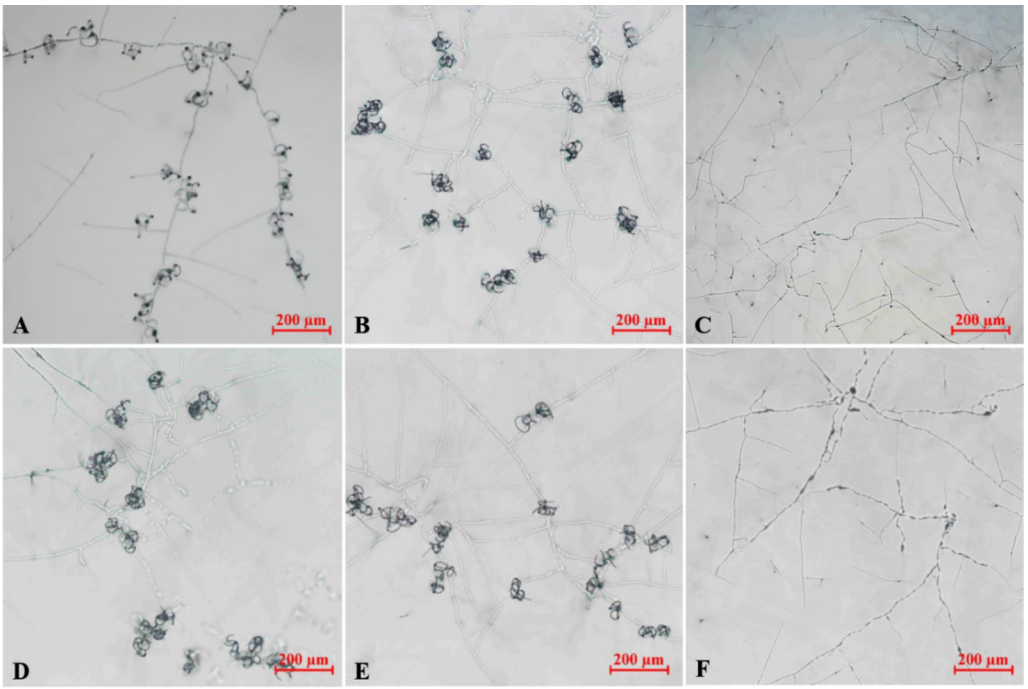



**Fig. 1** Induction activity of fermentation broth and extract of *P. lurida* to *A. oligospora*. **A** and **B** Fermentation broth treatment; **C** Medium control; **D** and **E** Extract treatment; **F** Methanol control

showed that the fermentation broth of *P. lurida* had a good induction effect, traps began to form at 48 h, a large number of traps formed at 60 h (Fig. 1A, B), and the control group treated with medium had no traps (Fig. 1C). To further verify whether the active substances that can induce *A. oligospora* to produce traps are secondary metabolites, the organic compounds in the fermentation broth were extracted with *n*-butanol, and the induced activity of the extracts was also tested. The results showed that the *n*-butanol extract from the fermentation broth of *P. lurida* still had obvious induction activity, and when the concentration was 0.9 mg/mL, it could induce *A. oligospora* to produce a large number of traps (Fig. 1D, E), but the methanol treatment control group had no activity (Fig. 1F).

### Overexpression of the *araC* gene in *P. lurida* and comparison of metabolites of *P. lurida araC*-PoprL with WT

The *araC* gene is a conserved regulatory gene in bacteria [27]. A strong promoter, PoprL, was inserted to overexpress the *araC* gene and the transformant *P. lurida araC*-PoprL was obtained. WT and *P. lurida araC*-PoprL were cultured in 100 mL of NB and KB media, and extracted with *n*-butanol, and the extracts of the two strains were weighed. In the two media, the amount of extracts cultured in KB (141.6 and 236.4 mg for WT and *P. lurida araC*-PoprL) was much higher than that cultured in NB (39.1 and 66.1 mg for WT and *P. lurida araC*-PoprL). Compared with WT, the weight of extract from *P. lurida araC*-PoprL was increased by nearly 2 times, so the transformant was selected for subsequent amplification fermentation and fermented on KB medium. In addition, by analyzing the detection results of *P. lurida araC*-PoprL and WT using LC–MS, metabolites of transformant *P. lurida araC*-PoprL were more abundant. Some distinct peaks were found in the chromatogram of Base Peak, and their retention times were 3.81, 7.36, 12.54, 12.83, 13.07 and 14.95 min (Fig. 2). Therefore, the yield and types of metabolites of *P. lurida araC*-PoprL are more abundant than those of WT.

### Structural identification of compounds

Nineteen metabolites (**1**–**19**) were purified from the extract of *P. lurida araC*-PoprL fermentation broth, and their structures were determined according to NMR and MS data (Fig. 3). Among them, compound **1** is a new metabolite.

Compound **1** was obtained as a colorless solid. According to high-resolution mass spectrometry HR-ESI–MS, its molecular formula is $C_{14}H_{15}O_3N$ (*m/z* 244.0969 $[M-H]^-$, the calculated value is 244.0968), and there are 8 degrees of unsaturation. The NMR spectrum data of compound **1** (Table 1) show that the compound has 14 carbon signals, including 2 methyl groups, 1

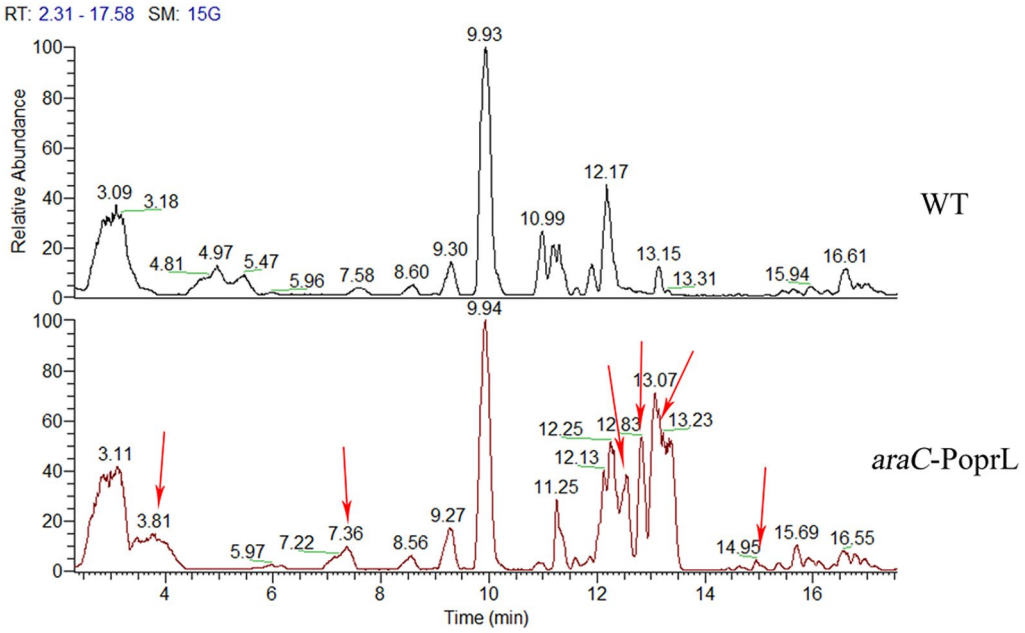



**Fig. 2** LC–MS results of *P. lurida araC*-PoprL and WT

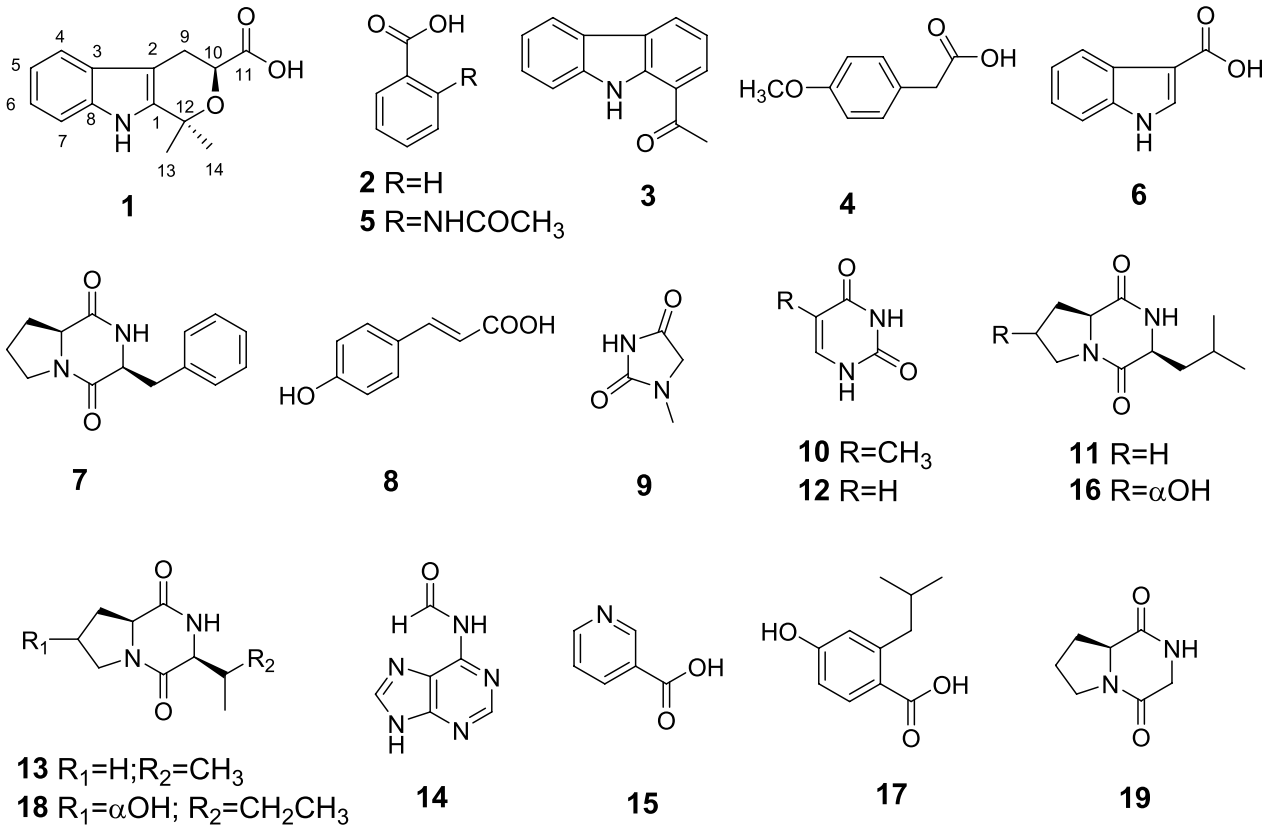



**Fig. 3** Chemical structure of secondary metabolites (**1–19**) from *P. lurida araC*-PoprL

methylene group, 5 methylene groups and 6 quaternary carbons. The NMR data of compound **1** is similar to that of tryptophan (Table 1) [28], and there are three more carbon signals, namely quaternary carbon $\delta_C$ 75.0 and two methyl $\delta_C$ 26.6 and 29.3. In the $^1$H-$^1$H COSY spectrum, the correlations of H-4/H-5/H-6/H-7 and H-9/H-10 provided two structural fragments I and II, which are illustrated in Fig. 4. In the HMBC spectrum, H-4 is correlated with C-2 ($\delta_C$ 105.5), C-3 ($\delta_C$ 127.9), C-6 ($\delta_C$ 122.2) and C-8 ($\delta_C$ 137.9); H-7 is related to C-5 ($\delta_C$ 119.9) and C-3 ($\delta_C$ 127.9); H-9 is related to C-1 ($\delta_C$ 140.1) and C-2 ($\delta_C$ 105.5); H-10 is related to C-12 ($\delta_C$ 75.0) and C-11 ($\delta_C$ 176.3). H-13 and H-14 are related to C-1 ($\delta_C$ 140.1) and C-12 ($\delta_C$ 75.0), respectively. The plane structure of **1** was identified as shown in the figure (Fig. 3). In addition, the relative configuration of compound **1** was determined by the NOE effect between H-10 and H-14 (Fig. 4). The CD curves of compound **1** (Additional file 1: Fig. S1) showed very similar with L-tryptophan positive CE around 216 and 232 nm and negative CE around 202 nm [29, 30], indicating the same absolute configuration as shown in Fig. 4. It is named as 1,1-dimethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-3-carboxylic acid.

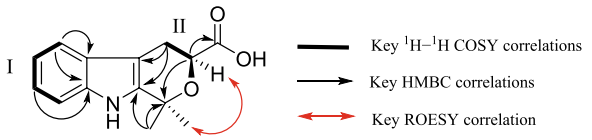



**Fig. 4** The key remote correlations of compound **1**

Compounds **2**–**19** were identified as benzoic acid (**2**) [31], acetyl-9*H*-carbazole (**3**) [32], 4-methoxyphenylacetic acid (**4**) [33], 2-(acetylamino)-benzoic acid (**5**) [34], indole-3-carboxylic acid (**6**) [35], cyclo-(L-phenylalanyl-4R-hydroxy-L-proline) (**7**) [36], *trans*-4-hydroxycinnamic acid (**8**) [37], 1-methylhydantoin (**9**) [38], 5-methyluracil (**10**) [39], cyclo-(Pro-Leu) (**11**) [40], uracil (**12**) [39], cyclo-(L-Pro-L-Val) (**13**) [41], N-9*H*-purin-6-ylformamide (**14**) [42], 3-pyridinecarboxylic acid (**15**) [43], cyclo[L-(4-hydroxyprolinyl)-L-leucine] (**16**) [44], 4-hydroxy-2-(2-methylpropyl)-benzoic acid (**17**), cyclo(L-Hyp-L-Ile) (**18**) [45] and cyclo-(Pro-Gly) (**19**) [41].

### Determination of induced activity of isolated metabolites

All isolated compounds were tested their inducing activity for trap formation in *A. oligospora*. The results showed that compound 1-methylhydantoin (**9**) had good induction activity, but other metabolites had no obvious activity. In a further assay, 1-methylhydantoin (**9**) showed induction activity in a concentration-dependent manner, and the induction effect was the best at 0.1 mg/mL (Fig. 5).

## Discussion

NTF, as natural enemies of nematodes, are ideal materials for controlling of nematodes. Many researchers have focused on inducing NTF to produce traps through external factors, thus enhancing their ability to capture nematodes. Most NTF need to be induced by specific external signals to form traps [7, 46, 47]; among them,

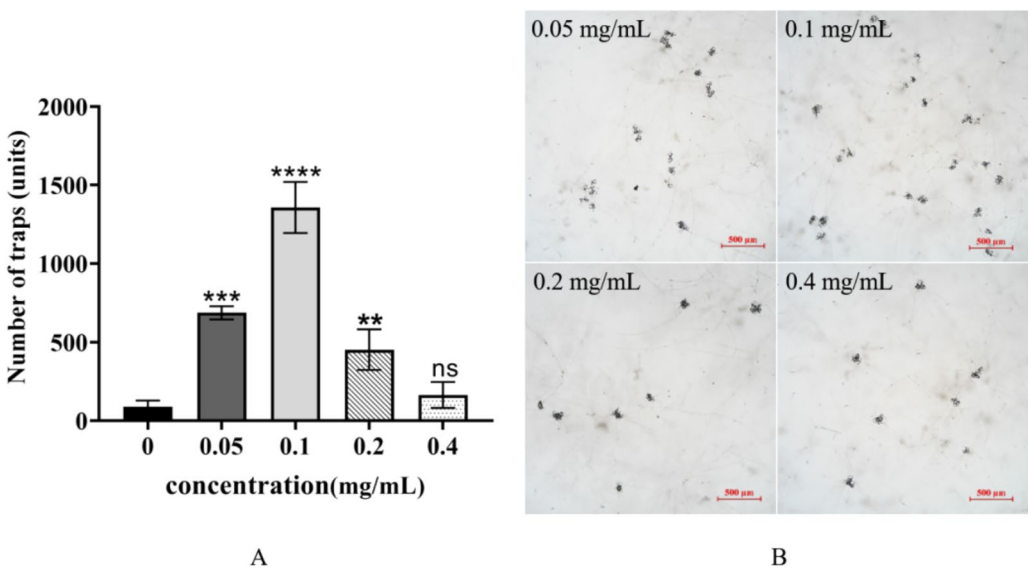



**Fig. 5** Formation of traps induced by compound **9** at different concentrations. **A** The number of traps at different concentrations; **B** Traps induced at different concentrations. **** mean $p < 0.0001$, ***mean $p < 0.001$, ** mean $p < 0.01$

bacteria and their metabolites are one of the potential resources to induce the production of traps. Our previous research reported a new mechanism by which bacteria resist nematodes. Bacteria are food for nematodes, and when facing nematodes, they mobilize the nematode's natural enemies, NTF, to prey on nematodes. That is, bacterium induced NTF produce traps to kill nematodes to maintain their own population [48].

The fermentation broth and its organic extracts of *P. lurida* can effectively induce *A. oligospora* to produce traps. To further explore active substances, we studied the secondary metabolites of *P. lurida*. First, we used molecular biology methods to regulate the metabolism of the bacterium, overexpressed the gene of *araC* global regulation, and obtained *P. lurida araC*-PoprL. The metabolite amount of *P. lurida araC*-PoprL was much higher than that of the WT strain. LC–MS detection showed that the metabolites of *P. lurida araC*-PoprL were significantly abundant compared with those of WT. This result shows that the strong promoter PoprL can enhance the synthesis of secondary metabolites in *P. lurida*, whose *araC* expression is positively regulated. Next, nineteen compounds were identified from the extract of *P. lurida araC*-PoprL, among which, 1,1-dimethyl-1,3,4,9-tetrahydropyrano [3,4-*b*] indole-3-carboxylic acid (**1**) was a new compound.

Benzoic acid (**2**) and its derivatives have biological activities of killing *Meloidogyne incognita* and inhibiting egg hatching [49, 50]. Acetyl-9*H*-carbazole (**3**) was also isolated from sponge *Tedania ignis* [32], and no biological activity has been reported yet. 4-methoxyphenylacetic acid (**4**) was isolated from the fungi *Leptographium qinlingensis* [33] and *Marasmius berteroi* [51], and had nematicidal activity [48]. 2-(Acetylamino)-benzoic acid (**5**) was reported from *Aconitum* spp. [34, 52] and *halophilic actinomycetes* [53], and has antibacterial activity against plant pathogens [53]. Indole-3-carboxylic acid (**6**) is an indole derivative, that is reported to regulate the chemotaxis, oviposition behavior and survival of *C. elegans* [54], and it is also a precursor compound for the synthesis of nematode glycosides [55]. Six known diketopyrazine compounds cyclo-(L-phenylalanyl-4R-hydroxy-L-proline) (**7**), cyclo-(Pro-Leu) (**11**), cyclo-(L-Pro-L-Val) (**13**), cyclo[L-(4-hydroxyprolinyl)-L-leucine] (**16**), cyclo(L-Hyp-L-Ile) (**18**) and cyclo-(Pro-Gly) (**19**), were obtained from fungi, bacteria and animals many times [56–59]. Compounds **7**, **11** and **19** were reported to have cytotoxicity and antibacterial activity [60, 61], and they can be used as signal molecules to inhibit quorum sensing [62–64]. Cyclo-(Pro-Leu) (**11**) has the activity of killing *M. incognita* [65]. Cyclo-(L-Pro-L-Val) (**13**) produced by *Pseudomonas fluorescens* carried by *Bursaphelenchus xylophilus* is cytotoxic and leads to the withering and death of *Pinus thunbergii* seedlings [66]. Compound **16** can promote the adhesion of bacteria to the mycelium surface of nematode-trapping fungi, thus enhancing the production of traps [9]. *Trans*-4-hydroxycinnamic acid (**8**) has nematicidal and ovicidal activities against animal parasitic nematodes [67]. 1-methylhydantoin (**9**) was isolated from microorganisms for the first time. It has anti-inflammatory activity and is an intermediate product of many drug syntheses [38]. 3-pyridinecarboxylic acid (**15**), has been obtained from a variety of biological resources, such as potato leaves, tomatoes and actinomycetes [68, 69], and exhibits antifungal activity [70]. This compound can be used as a pharmaceutical intermediate, which dilates blood vessels and has been widely studied in the medical field [71, 72]. These researches indicated that metabolites from *P. lurida* have diverse bioactivities, and these activities of nematicidal, inhibiting egg hatching of knot-root nematodes, chemotactic activity toward nematode, and inducing trap formation of NTF give the strain the potential to control nematodes.

Soil is a complex ecological environment, and metabolites are important communication tools among organisms living in soil. NTF, as aboriginal organisms in soil, play a significant ecological role in regulating nematode dynamics in soils. With the in-depth research on control of nematodes, integrated control of nematodes is receiving increasing attention. In addition to searching for metabolites that are directly active against nematodes, the factors that can mobilize other organisms in the soil to control nematodes are also worth paying attention to. Therefore, the bacteria and their metabolites with inducing activity in traps formation of NTF provide resources for the development of new biocontrol agents.

## Conclusions

In the present study, *P. lurida* can induce *A. oligospora* to produce traps. The transformant *P. lurida araC*-PoprL was constructed. Nineteen metabolites, including one new compound, were identified from *P. lurida araC*-PoprL. Compound 1-methylhydantoin (**9**) exhibited obvious inducement activity of trap formation in *A. oligospora*. Bacteria-nematodes-fungi share living spaces in the natural environment, and the mutual cooperation and restriction among them is the result of long-term nutrition competition and selection pressure. It was found that *P. lurida* plays an important role between nematodes and nematode-trapping fungi. The study of the interaction among bacteria, nematodes and fungi is beneficial to better control the diseases caused by nematodes.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00547-3.

**Additional file 1: Fig. S1.** CD spectrum of compound **1** in MeOH solution.

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**Author contributions**
SY, ZZ, and LG designed the experiment. SY, ZZ, GX, DJ, and LG. performed the experiments, analysed data and wrote the paper. All authors checked all the details, read and approved the final manuscript.

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**Availability of data and materials**
The data used and/or analyzed during the current study are available from the corresponding author on rational request.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
This research has been confrmed for publication in the journal.

**Competing interests**
The authors have no conficts of interest.

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