**Supporting data**

# New tyrosinase inhibitors from *Paecilomyces gunnii*

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**Supplementary Figure 1.** The inhibiton of the methanol extracts of the strain RCEF0199 on tyrosinase diphenolase. (A) Half inhibition concentration (IC50) (B) Reversible inhibitory mechanism at different concentration: the concentration of A-E is 25、50、100、150、200 μg/mL, respectively (C) Competitive inhibitory type at different concentration of the extracts: the concentration of A-F is 0、25、50、100、150、200 μg/mL, respectively (D) Slope versus the concentration of the extract for determining the inhibition constants $K_I$.

**Supplementary Figure 2.** Preparative HSCCC separation of the crude sample from *P. gunnii* using two-step elution with solvent systems. Solvent system: *n*-hexane: ethyl acetate: methanol: acetic acid: water = 3.5:5:3.5:0.15:5 (v/v/ v/v/v); rotary speed, 850 rpm; column temperature, 25 °C; flow rate, 2.0 mL/min; detection, 254 nm; sample size, 100 mg of crude sample dissolved in 4 mL upper phase and 4 mL lower phase; retention of the stationary phase, 72%. Peaks: Paecilomycone A (**1**) Paecilomycone B (**2**) and Paecilomycone C (**3**).

**Supplementary Figure 3.** $^{1}H$ (A), $^{13}C$ NMR (B), and HMBC (C) of compound **1**

**Supplementary Figure 4.** $^{1}H$ (A-B) , and $^{13}C$ NMR (C) of compound **2**

**Supplementary Figure 5.** $^{1}H$ (A), $^{13}C$ NMR (B) of 3

**Supplementary Figure 6**. Ninhydrin reaction with the three compounds showed only compound **3** reacted positively with a purple color: 0.2% Ninhydrin solution. TLC developing solvent: $CHCl_3$: Acetone = 3:8). E: Methanol extract, **1**: Paecilomycone A; **2**: Paecilomycone B; **3**: Paecilomycone C.

**Supplementary Table 1.** Inhibition Rates of Mycelia Extracts from Different Strains

against Diphenolase of Tyrosinase (25 μg/mL)

**Table S2**. Pared *t*-tests on Inhibibition Rates of Mycelia Extracts and Fermentation Broth Extracts

**Fig. S1**

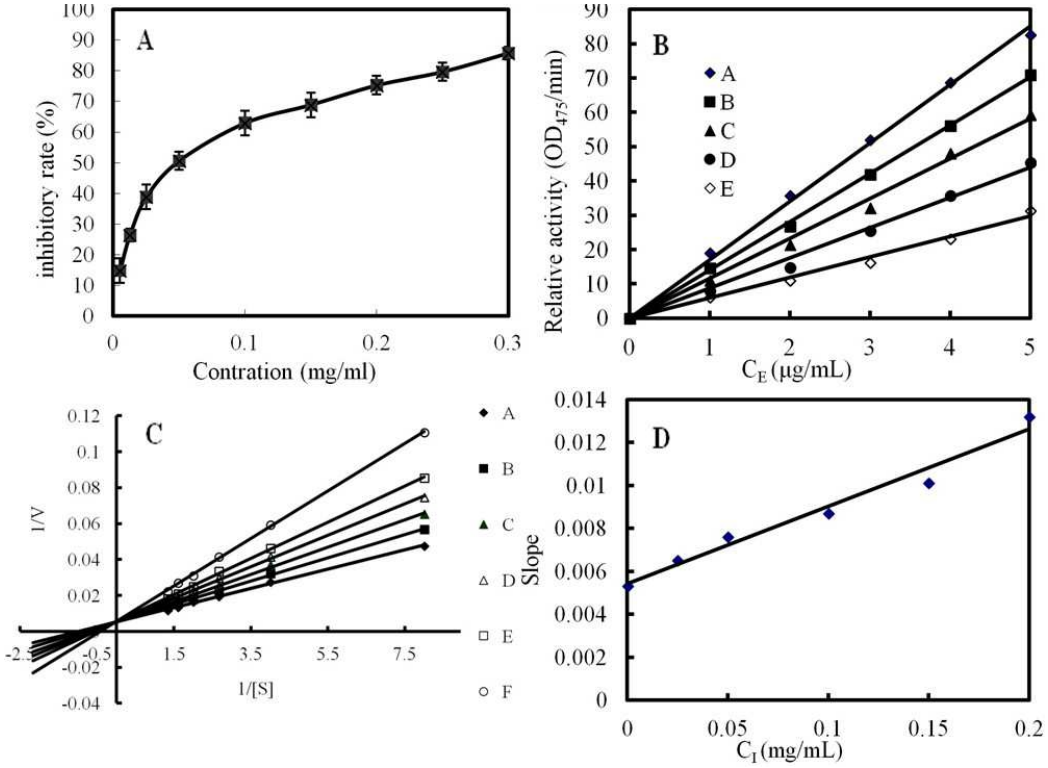

A
inhibitory rate (%)
Contration (mg/ml)
B
Relative activity (OD475/min)
A
B
C
D
E
CE (μg/mL)
C
1/V
1/[S]
A
B
C
D
E
F
D
Slope
CI (mg/mL)

**Fig.S2**

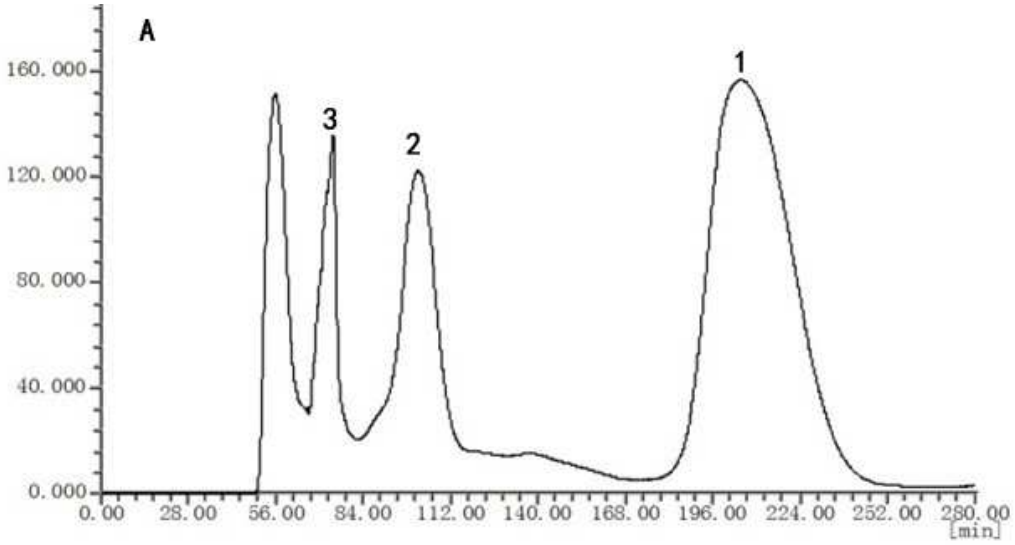
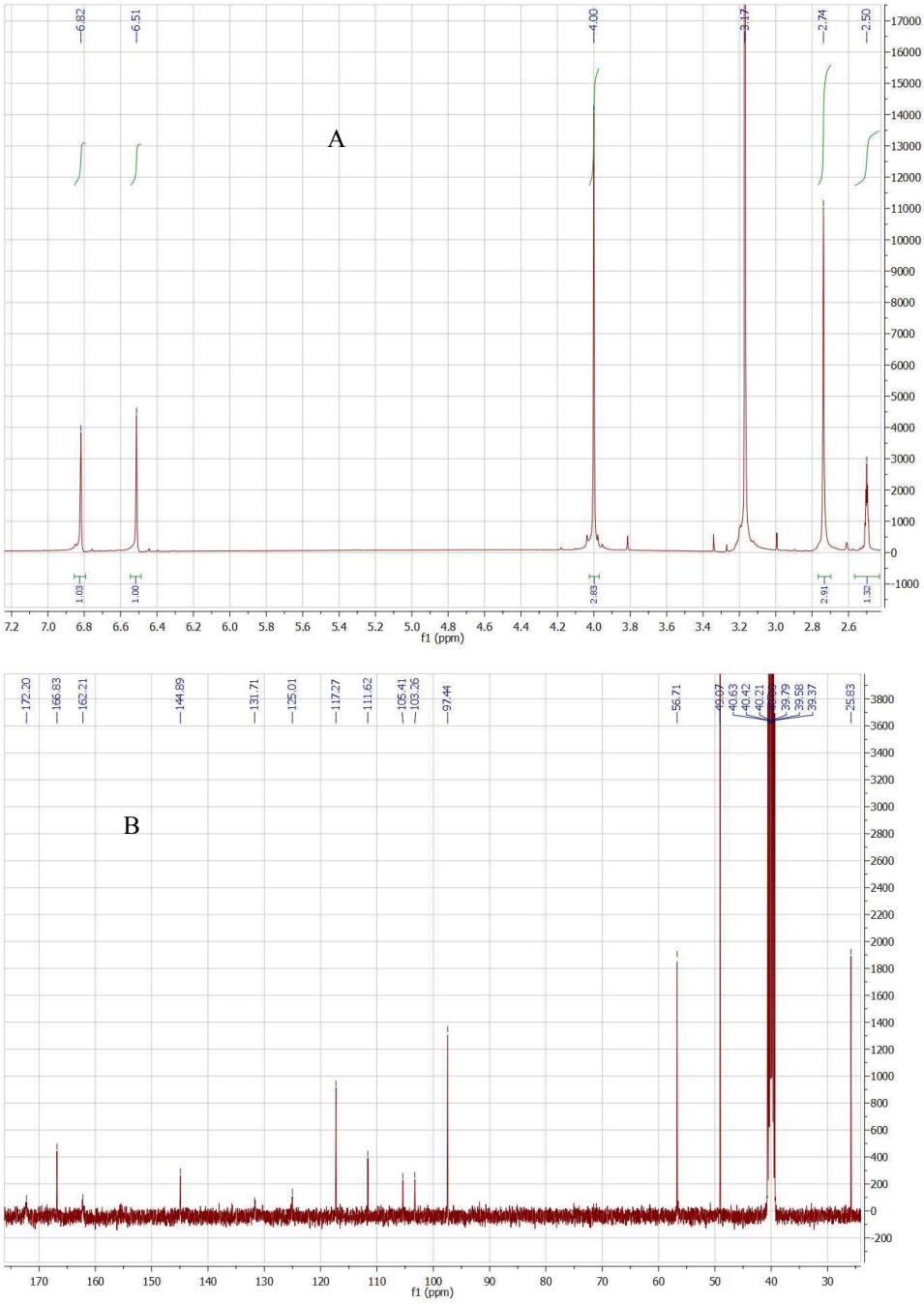

A
160.000
120.000
80.000
40.000
0.000
0.00
28.00
56.00
84.00
112.00
140.00
168.00
196.00
224.00
252.00
280.00
[min]
1
2
3

**Fig. S3.**

$^{1}H$ (A), $^{13}C$ NMR (B), and HMBC (C) of 1



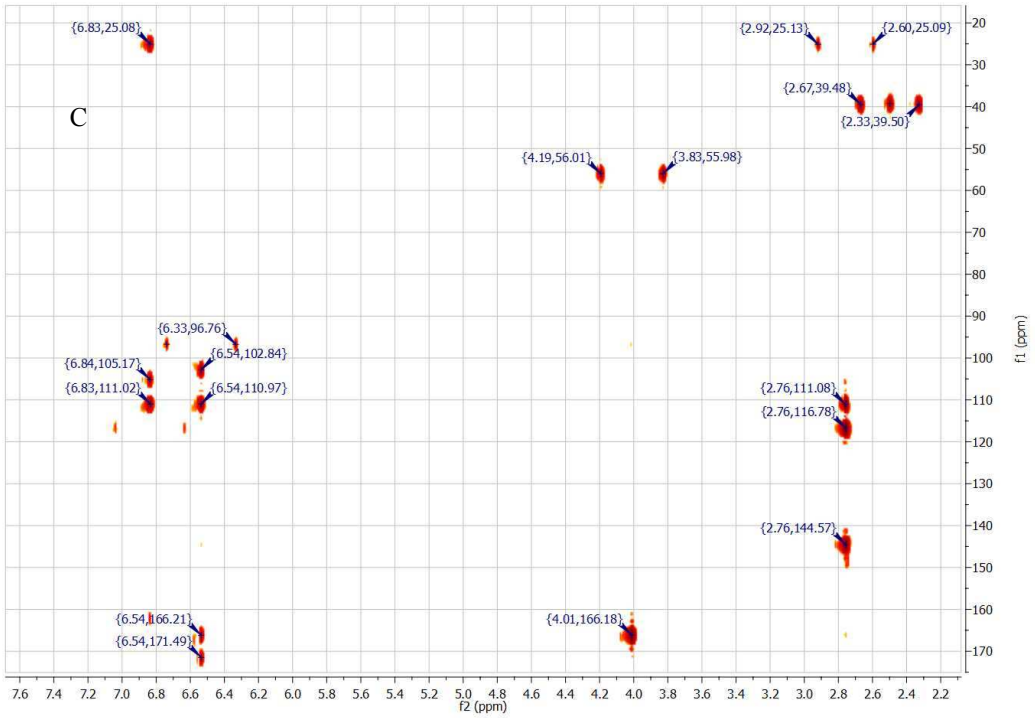
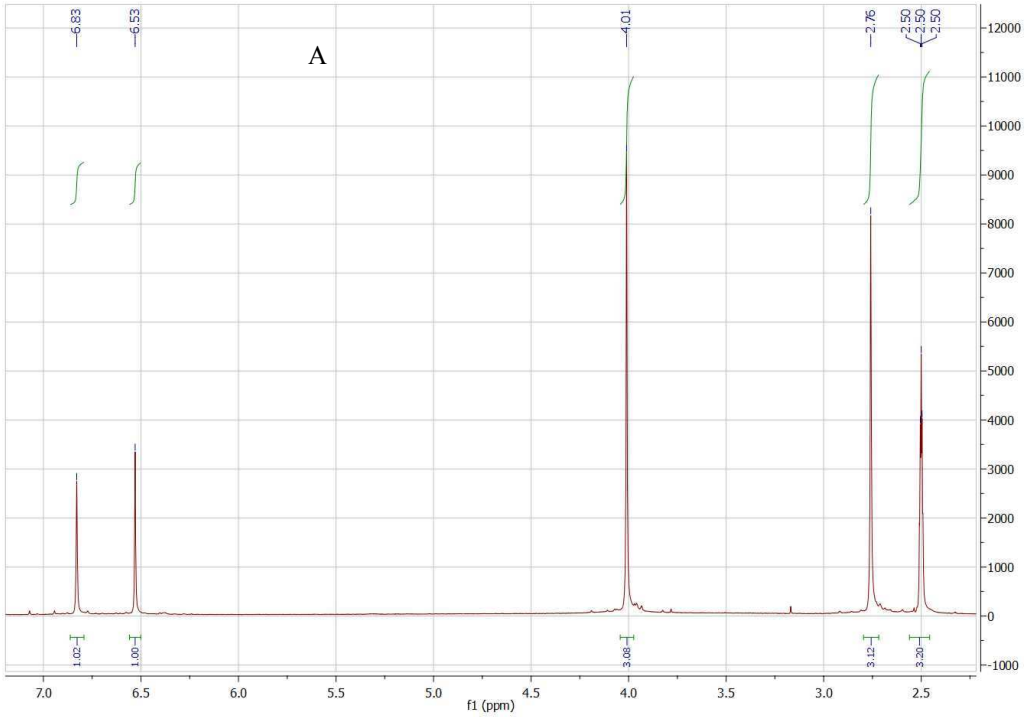
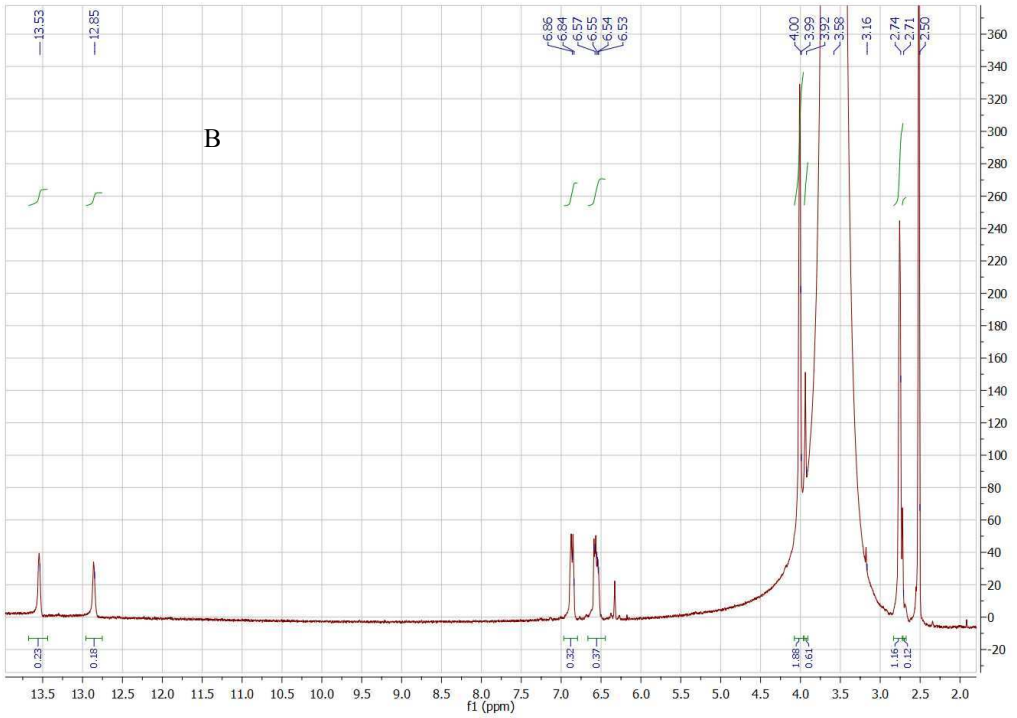



**Fig. S4**

$^{1}$H (A-B) , and $^{13}$C NMR (C) of 2




B
13.53
12.85
6.86
6.84
6.57
6.55
6.54
6.53
4.00
3.99
3.92
3.58
3.16
2.74
2.71
2.50
f1 (ppm)

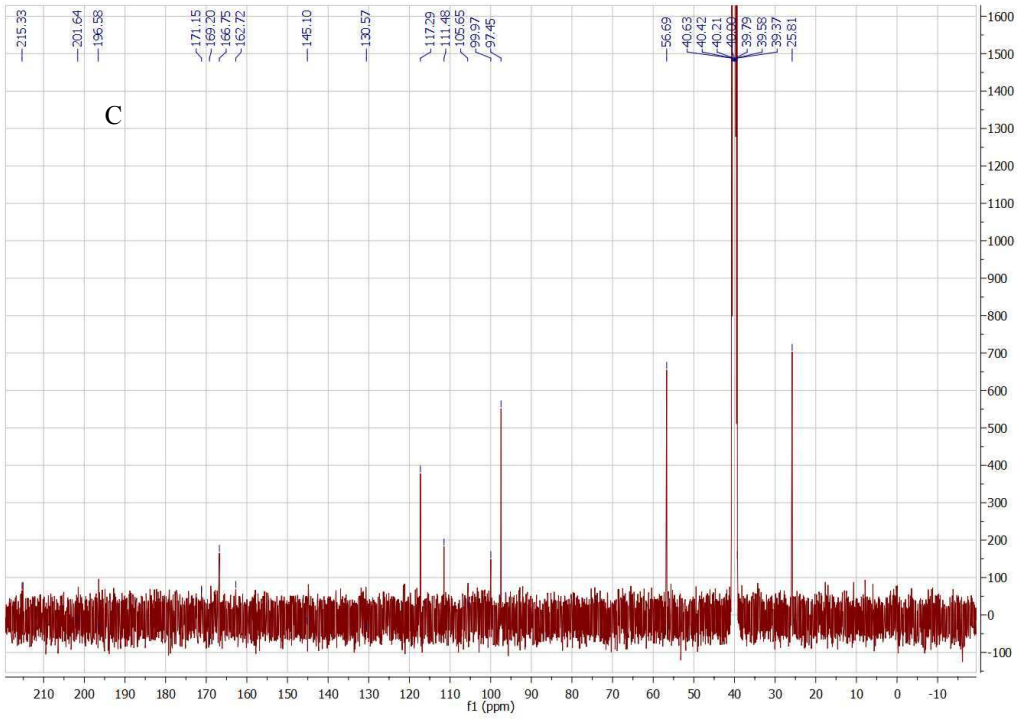

C
215.33
201.64
196.58
171.15
169.20
166.75
162.72
145.10
130.57
117.29
111.48
105.65
99.97
97.45
56.69
40.63
40.42
40.21
40.00
39.79
39.58
39.37
25.81
f1 (ppm)

**Fig. S5**

$^{1}H$ (A), $^{13}C$ NMR (B) of 3

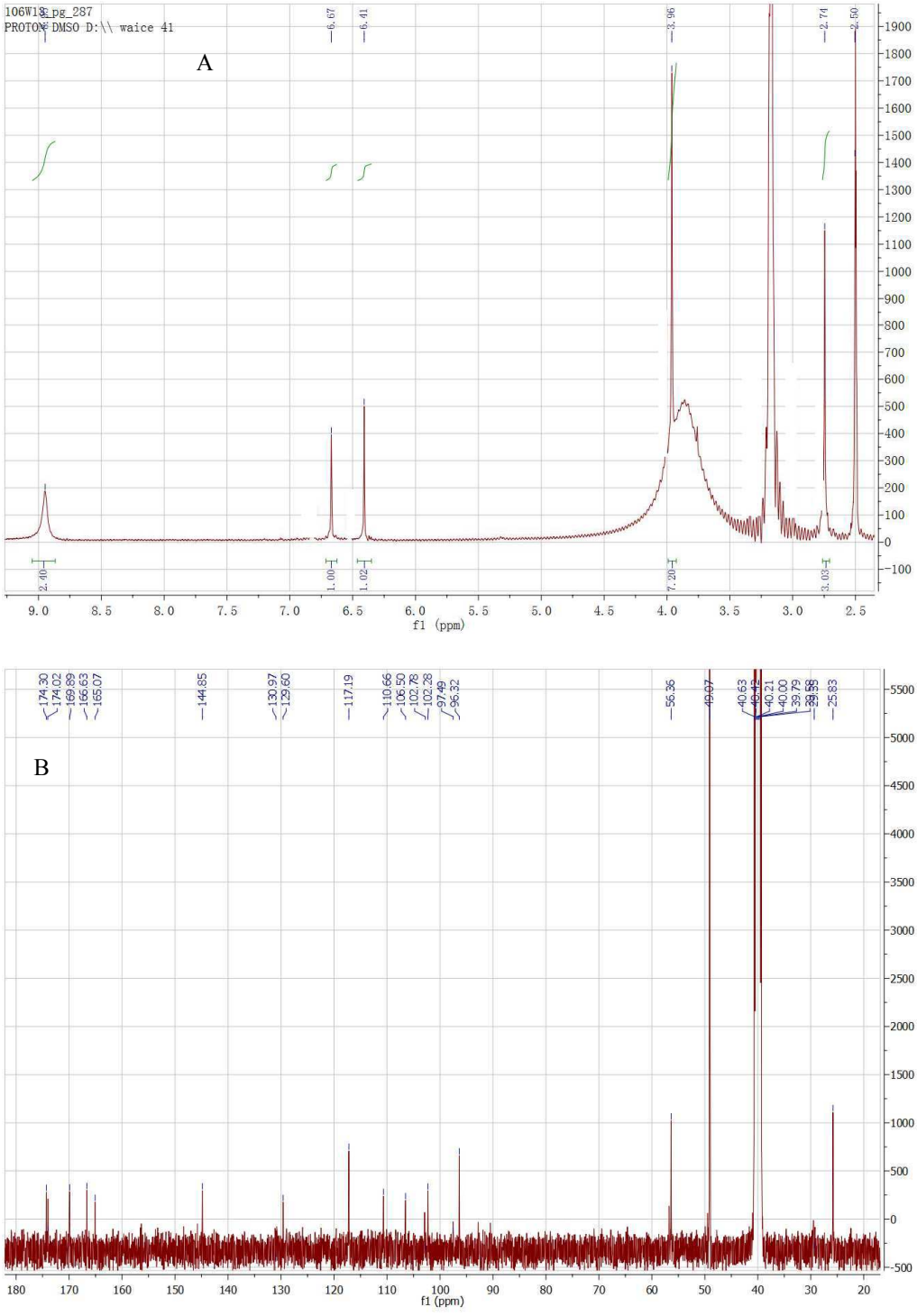


Fig.S6

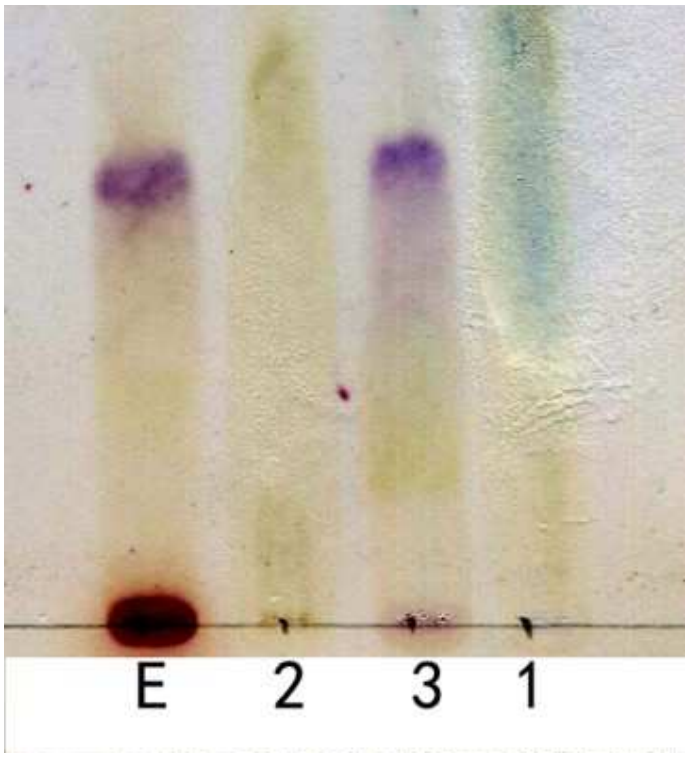



Table S1

| Strains number | Strains name | Host | Geographic origin | Inhibition rates (%) |
|---|---|---|---|---|
| RCEF0882 | *Hirsutella sp.* | *Cicadae* adult | Guniujian in Anhui | 21.38±2.35 |
| RCEF0883 | *Hirsutella sp.* | *Cicadae* adult | Guniujian in Anhui | 18.16±2.05 |
| RCEF0795 | *Isaria cateni* | *Formicidae* | Daweishan in Yunnan | 10.21±1.24 |
| RCEF0952 | *Isaria cateni* | *Lepodoptera* pupa | Xuanzhou in Anhui | 13.32±1.51 |
| RCEF5104 | *Isaria cateni* | *Noctuidae* pupa | Chengde in Hebei | 15.38±1.55 |
| RCEF4220 | *Isaria cicadae* | *Cicdae* larva | Guniujian in Anhui | 5.13±1.02 |
| RCEF4736 | *Isaria cicadae* | *Cicdae* larva | Xuancheng in Anhui | 8.94±1.17 |
| RCEF4744 | *Isaria cicadae* | *Cicdae* larva | Xuancheng in Anhui | 10.12±1.30 |
| RCEF4785 | *Isaria cicadae* | *Cicadae* adult | Xuancheng in Anhui | 6.23±0.95 |
| RCEF4953 | *Isaria cicadae* | *Cicadae* adult | Mingxi in Fujian | 4.37±0.82 |
| RCEF4957 | *Isaria cicadae* | *Cicadae* adult | Mingxi in Fujian | 6.78±0.96 |

| | | | | |
|---|---|---|---|---|
| RCEF0778 | *Isaria fumosoroseus* | *Lepodoptera* pupa | Tiantangzhai in Anhui | 11.43±1.29 |
| RCEF1004 | *Isaria fumosoroseus* | *Monochamus alternatus* | Huangshan in Anhui | 5.29±0.73 |
| RCEF4228 | *Isaria fumosoroseus* | *Lepodoptera* pupa | Jinzhai in Anhui | 14.48±1.38 |
| RCEF4256 | *Isaria tenuip* | *Lepodoptera* larva | Jinzhai in Anhui | 22.31±2.26 |
| RCEF4278 | *Isaria tenuip* | *Lepodoptera* pupa | Jinzhai in Anhui | 27.08±2.88 |
| RCEF4342 | *Isaria tenuip* | *Lepodoptera* pupa | Jinzhai in Anhui | 26.93±3.12 |
| RCEF4344 | *Isaria tenuip* | *Lepodoptera* pupa | Jinzhai in Anhui | 17.39±1.85 |
| RCEF4381 | *Isaria tenuip* | *Lepodoptera* pupa | Shucheng in Anhui | 15.97±1.79 |
| RCEF0673 | *Isaria tenuipes* | *Formicidae* | Tiantangzhai in Anhui | 19.02±2.24 |
| RCEF0961 | *Isaria tenuipes* | *Monochamus alternatus* | Huangshan in Anhui | 23.77±2.43 |
| RCEF5108 | *Isaria tenuipes* | *Diptera* pupa | Jinyuan in Ningxia | 13.29±1.30 |
| RCEF4691 | *Mariannaea puinosa* | *Lepodoptera* pupa | Guniujiang in Anhui | 28.08±3.08 |
| RCEF4694 | *Mariannaea puinosa* | *Lepodoptera* pupa | Guniujiang in Anhui | 39.66±3.95 |
| RCEF4730 | *Mariannaea puinosa* | *Lepodoptera* pupa | Huangshan in Anhui | 30.82±2.87 |
| RCEF0772 | *Metarhizikum taii* | *Noctuidae* larva | Huoshan in Anhui | 23.92±2.41 |
| RCEF0842 | *Metarhizikum taii* | *Lepodoptera* larva | Huangshan in Anhui | 19.39±2.22 |
| RCEF4126 | *Metarhizikum taii* | *Noctuidae* larva | Jinzhai in Anhui | 24.49±2.56 |
| RCEF4127 | *Metarhizikum taii* | *Noctuidae* larva | Jinzhai in Anhui | 21.68±2.34 |
| RCEF4734 | *Metarhizikum taii* | Unknown | Huangshan in Anhui | 17.59±1.78 |
| RCEF0577 | *Metarhizium anisopliae* | *Phasmatodea* | Tiantangzhai in Anhui | 11.71±1.35 |
| RCEF1404 | *Metarhizium anisopliae* | *Monochamus alternatus* larva | Xuancheng in Anhui | 8.31±1.04 |
| RCEF0292 | *Nomuraea rilreyi* | *Lepodoptera* larva | Huoshan in Anhui | 12.97±1.36 |
| RCEF5069 | *Nomuraea rilreyi* | Silkworm | Tianshui in Gansu | 4.34±0.65 |
| RCEF5084 | *Nomuraea rilreyi* | *Lepodoptera* larva | Chengde in Hebei | 6.22±0.72 |
| RCEF0199 | *Paecilomyces gunnii* | *Hepialus* larva | Guniujiang in Anhui | 78.57±5.61 |
| RCEF4118 | *Paecilomyces gunnii* | *Hepialus* larva | Jinzhai in Anhui | 51.03±4.28 |
| RCEF4119 | *Paecilomyces gunnii* | *Hepialus* larva | Jinzhai in Anhui | 65.60±5.30 |
| RCEF4121 | *Paecilomyces gunnii* | *Hepialus* larva | Mazongling in Anhui | 31.27±3.19 |
| RCEF0720 | *Paecilomyces militari* | *Lepodoptera* larva | Jinzhai in Anhui | 11.25±1.23 |

| | | | | |
|---|---|---|---|---|
| RCEF3298 | *Paecilomyces militari* | *Lepodoptera* pupa | Tiantangzhai in Anhui | 16.07±1.72 |
| RCEF3895 | *Paecilomyces militari* | *Sphingida* pupa | Shucheng in Anhui | 22.81±2.35 |
| RCEF4330 | *Paecilomyces militari* | *Lepodoptera* pupa | Jinzhai in Anhui | 27.94±2.84 |
| RCEF4334 | *Paecilomyces militari* | *Lepodoptera* pupa | Shucheng in Ahui | 16.29±1.80 |
| RCEF4731 | *Paecilomyces militari* | *Lepodoptera* pupa | Huangshan in Anhui | 9.81±0.96 |
| RCEF0724 | *Septafusidium bifusisporum* | *Lepodoptera* cocoon | Tiantangzhai in Anhui | 19.78±2.01 |
| RCEF4548 | *Septafusidium bifusisporum* | *Limacodidae* pupa | Shucheng in Anhui | 17.69±1.85 |
| RCEF1006 | *Verticillium* | *Lepodoptera* adult | Yuexi in Anhui | 0.85±0.43 |
| RCEF1007 | *Verticillium* | Unknown | Huoshan in Anhui | 5.81±0.68 |
| RCEF4597 | *Verticillium* | *Gryllidae* | Guniujiang in Anhui | 0.93±0.49 |

Table S2

| Strains number | Solid mycelia | Liquid mycelia | Culture broth | t-test results |
|---|---|---|---|---|
| RCEF 0259 | 30.54±2.74 | 32.67±2.17 | 13.89±3.11 | Difference between solid and liquid mycelia t=3.4362 p=0.0139 < 0.05 |
| RCEF 0199 | 78.57±5.61 | 80.41±6.12 | 43.72±2.42 | |
| RCEF 4121 | 31.27±3.19 | 33.70±3.03 | 11.95±3.37 | |
| RCEF 4118 | 51.03±4.28 | 58.24±4.21 | 22.28±2.55 | Difference between solid mycelia and culture broth t=6.6242 p=0.0006 < 0.01 |
| RCEF 4119 | 65.60±5.30 | 74.08±3.52 | 29.07±2.12 | |
| RCEF 0860 | 28.46±2.35 | 28.93±2.98 | 13.27±3.63 | |
| RCEF 0260 | 32.35±2.53 | 40.07±3.15 | 17.71±3.31 | |