

基因工程菌 *Streptomyces bingchenggensis* BCJ60 的次级代谢产物研究

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摘要:对基因工程菌 *Streptomyces bingchenggensis* BCJ60 的次级代谢产物化学成分及杀虫活性进行研究, 采用正相硅胶柱层析、反向硅胶柱层析和 Sephadex LH-20 分离纯化, 结合核磁共振、质谱等波谱学方法鉴定化合物结构, 从中分离获得 11 个化合物, 分别为 3,4-dihydro-3-hydroxy-5-oxomilbemycin A3 (**1**)、3,4-dihydro-3-hydroxy-5-oxomilbemycin A4 (**2**)、seco-milbemycin C (**3**)、seco-milbemycin A (**4**)、米尔贝霉素 H (**5**)、米尔贝霉素 β_{13} (**6**)、米尔贝霉素 ST906 (**7**)、米尔贝霉素 β_3 (**8**)、25-ethylmilbemycin β_3 (**9**)、米尔贝霉素 β_6 (**10**) 和米尔贝霉素 β_7 (**11**)。其中化合物 **1** 和 **2** 为新化合物, 杀虫活性结果显示, 化合物 **1** 和 **2** 对朱砂叶螨 ($LC_{50} = 0.121 \pm 0.010$ mg/L 和 0.116 ± 0.015 mg/L) 和松材线虫 ($LC_{50} = 5.217 \pm 0.064$ mg/L 和 5.581 ± 0.059 mg/L) 具有显著的杀虫活性, 与商品化的杀虫剂米尔贝霉素 A3/A4 相比没有显著性差异。

关键词:基因工程菌; *Streptomyces bingchenggensis* BCJ60; 次级代谢产物; 杀虫杀螨活性

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Secondary metabolites from the genetically engineered strain *Streptomyces bingchenggensis* BCJ60

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Abstract: To study the chemical constituents from a culture of the genetically engineered strain *Streptomyces bingchenggensis* BCJ60. All compounds were isolated and purified by various column chromatographies, including silica gel, Rp-18 and Sephadex LH-20, and their structures were elucidated through the interpretation of NMR and MS spectroscopic data. Eleven milbemycin derivatives were isolated from methanol extract of *Streptomyces bingchenggensis* BCJ60, which were identified as 3,4-dihydro-3-hydroxy-5-oxomilbemycin A3 (**1**), 3,4-dihydro-3-hydroxy-5-oxomilbemycin A4 (**2**), seco-milbemycin C (**3**), seco-milbemycin A (**4**), milbemycin H (**5**), milbemycin β_{13} (**6**), milbemycin ST906 (**7**), milbemycin β_3 (**8**), 25-ethylmilbemycin β_3 (**9**), milbemycin β_6 (**10**) and milbemycin β_7 (**11**). Compounds **1** and **2** were new compounds. Compounds **1** and **2** possessed potent activities against *Tetranychus cinnabarinus* ($LC_{50} = 0.121 \pm 0.010$ mg/L and 0.116 ± 0.015 mg/L) and *Bursaphelenchus xylophilus* ($LC_{50} = 5.217 \pm 0.064$ mg/L and 5.581 ± 0.059 mg/L), which were comparable to those of commercial pesticide milbemycins A3/A4.

Key words: genetically engineered strain; *Streptomyces bingchenggensis* BCJ60; secondary metabolites; acaricidal and nematocidal activities

聚酮类化合物主要是由聚酮合酶催化将低级羧酸通过连续的缩合反应产生的一类天然产物,包括大环内酯、四环素、蒽醌和聚醚等^[1,2]。从药理学的角度来看,聚酮类化合物是新型药物的重要来源,主要用作抗生素、免疫抑制剂、抗寄生虫、降胆固醇和抗肿瘤药物^[3]。聚酮合酶每个结构域的功能都与最终产物的结构呈现对应关系,因而,可以利用这种对应关系来编辑生物合成基因,从而设计合成产物,由此产生出一个崭新的研究领域——聚酮合酶的组合生物学^[4]。学者们主要通过减少、增加或者替换聚酮合酶的模块及模块中的结构域来组合产生新的产物,如通过对链霉菌 *Streptomyces avermitilis* 中聚酮合酶基因簇 *aveLAT-ACP* 进行改造获得一株基因工程菌,从该工程菌次级代谢产物中分离获得两个新的阿维菌素衍生物 25-甲基伊维菌素和 25-乙基伊维菌素^[5]。

本课题组在前期的研究中,对冰城链霉菌 *Streptomyces bingchenggensis* BC-120-4 中编码聚酮合酶的 *milF* 基因进行改造得到基因工程菌株 *Streptomyces bingchenggensis* BCJ60,该菌株不能编码 C-5 酮基还原酶,从而阻断米尔贝霉素 A3/A4/B2/B3 的合成^[6]。通过基因定向改造,生物合成基因簇发生改变,有可能激活了某些生物合成途径得到新的代谢产物。因此,本研究主要对基因工程菌株 *Streptomyces bingchenggensis* BCJ60 的次级代谢产物进行研究,以期获得具有应用价值的高效杀虫抗生素。

1 材料与方法

1.1 仪器与试剂

高效液相色谱仪 Agilent 1260(美国安捷伦科技有限公司);超导核磁共振仪 Bruker AVANCE-400(德国 Bruker 公司);旋转蒸发仪 RE2000(上海亚荣仪器有限公司);发酵罐 100 L(上海保兴生物设备有限公司);凝胶 Sephadex LH-20(美国 Pharmacia 公司);正相硅胶(100~200目)(青岛海洋化工厂);薄层层析(TLC)用硅胶板(烟台新诺化工有限公司);甲醇、乙醇、二氯甲烷等试剂均为分析纯,购于台州市椒江中西化工有限公司;酵母膏、蛋白胨、葡萄糖、大豆饼粉等培养基购于中国新兴化工试剂研究所;NaCl、KNO₃ 等无机盐购于浙江临安化工试剂厂。

1.2 菌株来源

基因工程菌株 *Streptomyces bingchenggensis* BCJ60,由本实验室保存,该菌株是由冰城链霉菌

Streptomyces bingchenggensis 经诱变和基因改造获得^[6]。

1.3 菌株培养

1.3.1 斜面培养

培养基组成:可溶性淀粉 20 g, NaCl 0.5 g, KNO₃ 1 g, K₂HPO₄ · 3H₂O 0.5 g, MgSO₄ · 7H₂O 0.5 g, 琼脂 15 g, 蒸馏水 1 000 mL。于 121 °C 下高压蒸汽灭菌 20 min,接种后在 28 °C 下培养 7 天。

1.3.2 种子培养

培养基组成:蔗糖 10 g, 脱脂奶粉 1 g, 酵母提取物 5 g, 蛋白胨 3.5 g, K₂HPO₄ 0.5 g, 蒸馏水 1 000 mL, pH 7.0。用 1 000 mL 的三角瓶每瓶分装 250 mL 的上述培养基。向长有孢子的斜面上,加入 9 mL 无菌水,用无菌的接种铲刮下培养基表面的孢子制成孢子悬浮液接于种子培养基中培养,28 °C, 250 rpm 震荡培养 24 h。

1.3.3 发酵培养

培养基组成:黄豆饼粉 20 g, 蔗糖 80 g, 脱脂奶粉 1 g, FeSO₄ · 7H₂O 0.1 g, K₂HPO₄ · 3H₂O 1 g, CaCO₃ 3 g, 蒸馏水 1 000 mL, pH 7.0~7.2。采用 100 L 发酵罐,按 8% 接种量,将种子液接入到 100 L 发酵罐中(60 L 培养基),28 °C, 100 r/min 震荡培养 6~7 天。

1.4 提取、分离与鉴定

发酵液 60 L 经离心得到菌丝体 12 L,菌丝体用去离子水洗涤后再用 40 L 甲醇浸提两次,每次搅拌 4 h,过滤合并甲醇浸提液,50 °C 减压浓缩至干,得到 52.0 g 油状物质。将所得的油状物质上硅胶(100~200目)层析,石油醚:丙酮 = 100:0→60:40 (V/V) 梯度洗脱,流速 30 mL/min,收集洗脱液,每份 250 mL,薄层层析(TLC)检测(石油醚:丙酮 = 2:1),得到四个组分 Fr. 1~4。Fr. 1(12.5 g)上凝胶 Sephadex LH-20(二氯甲烷:甲醇 = 1:1)层析获得化合物 **3**(7.5 mg)、**4**(6.8 mg)、**5**(8.3 mg)和 **6**(7.9 mg)。Fr. 2(9.7 g)上凝胶 Sephadex LH-20(二氯甲烷:甲醇 = 1:1)得到含有化合物 **1** 和 **2** 的混合物,进一步上 Agilent 1260 高压液相色谱层析,色谱柱 Agilent Zorbax SB C3 column(5 μm, 250 mm × 9.4 mm),洗脱剂甲醇:乙腈:水 = 42:42:16 (V/V/V),流速 1.5 mL/min,检测波长 λ = 240 nm,得到化合物 **1**(9.5 mg, *t_R* = 19.4 min)和化合物 **2**(8.3 mg, *t_R* = 23.2 min)。Fr. 3(8.3 g)上凝胶 Sephadex LH-20(二氯甲烷:甲醇 = 1:1)层析获得化合物 **7**(6.3

mg)、**8**(7.3 mg)和**9**(6.7 mg)。Fr.4(5.6 g)经反向硅胶柱色谱梯度洗脱(甲醇:水 = 30% →90%)得到化合物**10**(6.5 mg)和**11**(8.4 mg)。化合物结构采用一维、二维核磁共振、质谱、高分辨质谱等波谱学方法以及对比文献数据进行鉴定。

1.5 杀虫活性测试

1.5.1 杀螨虫活性测试

将化合物用甲醇溶解,用含0.01%表面活性剂烷基酚聚氧乙烯醚的水稀释10倍,制成100 mg/mL的溶液,再依次稀释得到1、0.5、0.25、0.1、0.05和0.025 mg/mL的溶液作为样品溶液。将对有机磷类杀虫药敏感的朱砂叶螨接种到豇豆的初生叶上。接种一天后,将豇豆的叶子浸泡在样品溶液中1~2 s。将叶子在25℃放置3天后,用显微镜观察存活成虫的个数,计算死亡率(%)。设置对照组,以米尔贝霉素A3和A4(30:70, V/V)作为阳性对照。

1.5.2 杀线虫活性测试

将化合物用甲醇溶解,再用水稀释成100 mg/mL的溶液,再依次稀释得到10、5、2、1、0.5、0.25、0.1和0.05 mg/mL的溶液。分别取不同浓度溶液10 μL加入到90 μL含活松材线虫水悬液中。混合液震荡后在25℃放置15 h。在显微镜下计数不动线虫的数量和测试线虫的总数,计算测试线虫的死亡率(%)。设置对照组,以米尔贝霉素A3和A4(30:70, V/V)作为阳性对照。

1.5.3 数据处理

以上每组实验设置三个平行实验组,结果取平均值。用统计软件SPSS 13.0计算半数致死量 LC_{50} (mg/L), Student's *t*-test 检验 *P* 值,观察与阳性对照米尔贝霉素A3/A4的活性差异,如果 $P > 0.05$ 则认为无显著性差异。

2 结果与分析

2.1 化合物结构鉴定

化合物**1** 白色粉末状固体;易溶于氯仿、丙酮、甲醇,不溶于水;比旋度 $[\alpha]_D^{25} + 101$ (c 0.38, EtOH);紫外吸收UV(EtOH) λ_{max} nm (log ϵ): 240 (4.22);红外吸收IR(KBr): 3337、2925、2869、1711、1453、1379、1245、1056、993 cm^{-1} ;HR-ESI-MS: m/z 545.3080 $[M + H]^+$, 567.2903 $[M + Na]^+$ (calcd for $C_{31}H_{44}NaO_8$, 567.2870), 分子式为 $C_{31}H_{44}O_8$ 。¹H NMR 显示五个甲基氢信号 δ_H 0.82(3H, d, $J = 6.5$ Hz, H-30), 1.02(3H, d, $J = 6.5$ Hz, H-28), 1.12(3H, d, $J = 6.5$ Hz, H-31), 1.34(3H, d, $J =$

6.7 Hz, H-26)和1.55(3H, s, H-29);一对反式双键上的氢信号 δ_H 5.43(1H, dd, $J = 9.9, 14.3$ Hz, H-11), 5.93(1H, dd, $J = 14.3, 11.0$ Hz, H-10)。分析核磁数据显示31个碳信号,包括5个甲基(δ_C 10.2、16.1、17.8、19.6和22.0),7个亚甲基(δ_C 27.8、34.7、35.7、36.0、40.6、48.4和60.5),9个脂肪族次甲基(δ_C 36.0、36.5、46.2、51.8、67.3、70.3、71.2、75.0和81.1),1个连氧季碳(δ_C 77.6),1个缩酮碳信号(δ_C 97.5),6个 sp^2 杂化碳信号(δ_C 120.9、122.6、128.6、130.6、135.9和144.3)和2个羰基(δ_C 171.4、209.2)。氢氢相关二维谱(¹H-¹H COSY)显示四个自旋体系 H-2/H-3/H-4/H₃-26, H-9/H-10/H-11/H-12/H₂-13, H-15/H₂-16/H-17/H₂-18/H-19/H₂-20 和 H₂-22/H₂-23/H-24/H-25/H₃-31,从而确认了四个结构单位连接方式分别是 C-2-C-26, C-9-C-13, C-15-C-20 和 C-22-C-31。碳氢远程相关谱(HMBC)中观察到一系列远程相关信号:H₃-26(δ_H 1.34)与 C-3(δ_C 75.0)、C-4(δ_C 46.2)、C-5(δ_C 209.2), H₃-29(δ_H 1.55)与 C-13(δ_C 48.4)、C-14(δ_C 135.9)、C-15(δ_C 120.9), H-19(δ_H 5.42)与 C-1(δ_C 171.4)、C-17(δ_C 67.3)、C-21(δ_C 97.5)。通过分析1D(¹H NMR、¹³C NMR)和2D NMR(¹H-¹H COSY、HSQC、HMBC)归属核磁数据见表1,并与已知化合物5-oxomilbemycin A3^[6]对比核磁数据发现二者结构相似,在碳氢远程相关谱(HMBC)中 H₃-26 与 C-3、C-4、C-5 存在相关信号,说明 C-3、C-4 之间以单键相连,氢氢相关二维谱(¹H-¹H COSY)中 H-2/H-3/H-4/H₃-26 相关信号进一步证明了 C-3/C-4 的连接方式,从 C-3 位化学位移(δ_H 4.61, δ_C 75.0)推断 C-3 位连有一个羟基,与高分辨质谱 HR-ESI-MS: m/z 545.3080 $[M + H]^+$, 567.2903 $[M + Na]^+$ (calcd for $C_{31}H_{44}NaO_8$, 567.2870)得到的分子式 $C_{31}H_{44}O_8$ 相吻合。从而确认了化合物**1**的平面结构(见图1),经SciFinder检索确定其为新化合物并命名为3,4-dihydro-3-hydroxy-5-oxomilbemycin A3。

在NOESY谱中H-2/H-4存在氢氢远程相关信号,说明H-2和H-4空间朝向一致;H-3/H₃-26存在氢氢远程相关信号,说明H-3和H₃-26空间朝向一致;H-10和H-11之间较大的耦合常数(δ_H 14.3 Hz)以及H₃-27/H-10, H₃-29/H₂-16之间NOESY信号表明C-8/C-9, C-10/C-11, C-14/C-15是三个反式双键;H-17/H-25, H₃-30/H-25, H-17/H-19之间的NOESY信号表明H-17、H-19、H-25和H-30有相同

的空间朝向。C-6、C-7、C-12 和 C-21 位手性中心的相对构型通过与 5-oxomilbemycin A3 对比确认。

酮、甲醇, 不溶于水; 比旋度 $[\alpha]_D^{25} + 81$ (c 0.40, EtOH); 紫外吸收 UV (EtOH) λ_{\max} nm ($\log \epsilon$): 236 (4.28); 红外吸收 IR (KBr): 3441、2951、2923、1707、

化合物 2 白色粉末状固体; 易溶于氯仿、丙

表 1 化合物 1 和 2 的 ^1H 和 ^{13}C NMR 数据

Table 1 NMR data of compounds 1 and 2

Position	δ_{H} (mult., J in Hz)		δ_{C}	
	1	2	1	2
1			171.4	171.4
2	3.62(1H, br s)	3.62(1H, br s)	51.8	51.8
3	4.61(1H, br s)	4.61(1H, d, 3.7)	75.0	75.0
4	2.63(1H, m)	2.64(1H, m)	46.2	46.2
5			209.2	208.8
6	4.37(1H, s)	4.36(1H, s)	81.1	81.1
7			77.6	77.7
8			130.6	130.0
9	6.38(1H, d, 11.0)	6.39(1H, d, 11.1)	128.6	128.7
10	5.93(1H, dd, 14.3, 11.0)	5.94(1H, dd, 14.3, 11.1)	122.6	122.7
11	5.43(1H, dd, 14.3, 9.9)	5.45(1H, dd, 14.3, 10.0)	144.3	144.0
12	2.39(1H, m)	2.40(1H, m)	36.0	36.0
13	2.14(1H, m) 1.82(1H, m)	2.16(1H, m) 1.83(1H, m)	48.4	48.4
14			135.9	135.8
15	4.85(1H, d, 10.0)	4.82(1H, br d, 9.9)	120.9	120.8
16	2.27(2H, m)	2.26(2H, m)	34.7	34.5
17	3.59(1H, m)	3.56(1H, m)	67.3	67.3
18	0.66(1H, m) 1.70(1H, m)	0.67(1H, m) 1.71(1H, m)	36.0	36.0
19	5.42(1H, m)	5.41(1H, m)	70.3	70.3
20	1.52(1H, t, 12.0) 1.90(1H, m)	1.52(1H, t, 12.0) 1.91(1H, m)	40.6	40.5
21			97.5	97.3
22	1.55(1H, m) 1.70(1H, m)	1.53(1H, m) 1.72(1H, m)	35.7	35.7
23	1.55(2H, m)	1.51(2H, m)	27.8	27.9
24	1.23(1H, m)	1.31(1H, m)	36.5	34.5
25	3.26(1H, m)	3.01(1H, m)	71.2	76.1
26	1.34(3H, d, 6.7)	1.34(3H, d, 6.8)	10.2	10.2
27	4.42(1H, d, 14.5) 3.54(1H, d, 14.5)	4.38(1H, d, 14.7) 3.55(1H, d, 14.7)	60.5	60.6
28	1.02(3H, d, 6.5)	1.01(3H, d, 8.1)	22.0	21.8
29	1.55(3H, s)	1.57(3H, s)	16.1	16.1
30	0.82(3H, d, 6.5)	0.82(3H, d, 6.3)	17.8	17.7
31	1.12(3H, d, 6.5)	1.32(1H, m) 1.67(1H, m)	19.6	25.7
32		0.96(3H, t, 7.3)		10.2

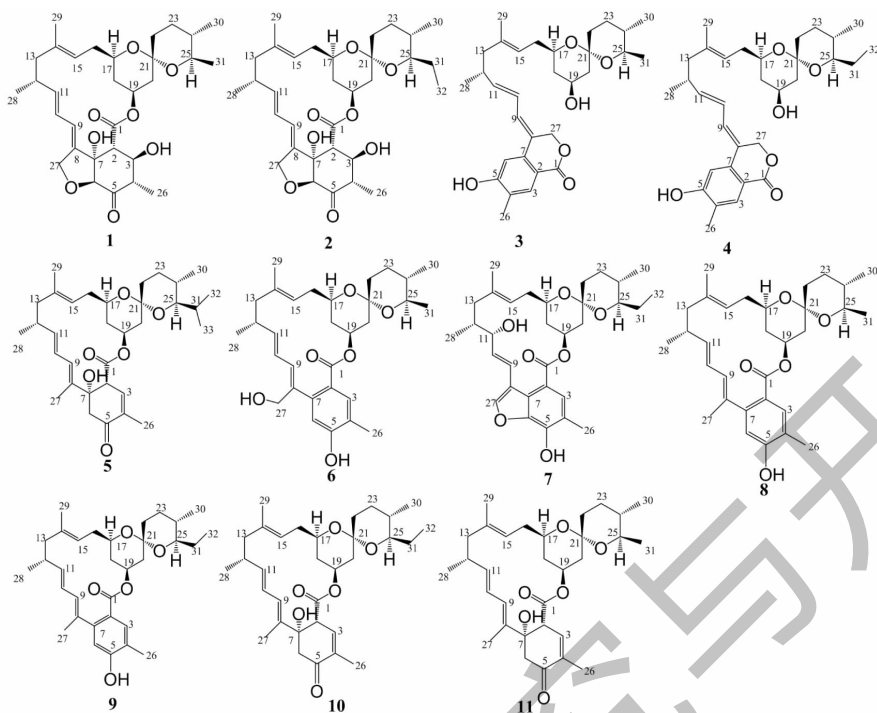


图1 化合物1~11的化学结构

Fig. 1 Chemical structures of compounds 1-11

1456, 1370, 1269, 1059, 985 cm^{-1} ; 高分辨质谱 HR-ESI-MS: m/z 581.3064 [$M + Na$] $^+$ (calcd for $C_{32}H_{46}NaO_8$, 581.3085), 分子式为 $C_{32}H_{46}O_8$ 。通过与化合物 1 对比核磁数据发现二者结构相似, 在氢氢相关二维谱 (1H - 1H COSY) 中 H-25/H₂-31/H₃-32 存在相关信号, 说明 C-25 位连接有一个乙基基团。因此, 确认了化合物 2 的结构, 经 SciFinder 检索确定其为新化合物并命名为 3,4-dihydro-3-hydroxy-5-oxomilbemycin A4。化合物 2 手性中心的相对构型通过与化合物 1 对比确认。化合物 1 和 2 的核磁数据见表 1, 二维相关信号见图 2 和图 3。

化合物 3 白色粉末状固体; 易溶于氯仿、甲醇、丙酮, 难溶于水; ESI-MS: m/z 509.1 [$M - H$] $^-$,

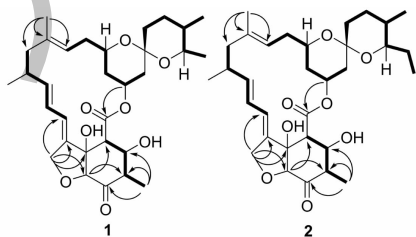
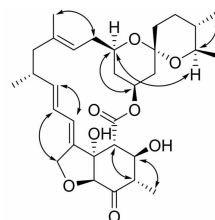
图2 化合物1和2的主要 1H - 1H COSY和HMBC相关信号Fig. 2 Key 1H - 1H COSY and HMBC correlations of 1 and 2

图3 化合物1的主要NOESY相关信号

Fig. 3 Key NOESY correlations of compound 1

分子式为 $C_{31}H_{42}O_6$ 。 1H NMR (400 MHz, $CDCl_3$) δ : 7.77 (1H, s, H-3), 7.01 (1H, s, H-6), 6.48 (1H, d, $J = 11.0$ Hz, H-9), 6.19 (1H, dd, $J = 14.8, 11.0$ Hz, H-10), 5.96 (1H, dd, $J = 14.8, 6.6$ Hz, H-11), 5.05 (2H, s, H-27), 4.24 (1H, m, H-19), 3.60 (1H, m, H-17), 3.25 (1H, m, H-25), 2.53 (1H, m, H-12), 2.28 (2H, m, H-16), 2.20 (2H, m, H-13), 2.20 (3H, s, H-26), 2.11 (1H, m, H-18), 2.08 (1H, m, H-20), 1.65 (1H, m, H-22), 1.62 (3H, br s, H-29), 1.54 (1H, m, H-22), 1.50 (2H, m, H-23), 1.34 (1H, br t, $J = 12.0$ Hz, H-20), 1.24 (1H, m, H-24), 1.20 (1H, m, H-18), 1.10 (3H, d, $J = 5.8$ Hz, H-31), 1.08 (3H, d, $J = 6.2$ Hz, H-28), 0.82 (3H, d, $J = 6.5$ Hz, H-30); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 165.8 (C-1),

114.5 (C-2), 133.2 (C-3), 126.1 (C-4), 160.5 (C-5), 107.5 (C-6), 138.2 (C-7), 123.6 (C-8), 128.0 (C-9), 121.7 (C-10), 146.4 (C-11), 33.8 (C-12), 48.2 (C-13), 135.4 (C-14), 122.3 (C-15), 34.3 (C-16), 68.1 (C-17), 40.3 (C-18), 65.8 (C-19), 44.7 (C-20), 97.6 (C-21), 35.8 (C-22), 27.8 (C-23), 36.6 (C-24), 71.3 (C-25), 15.6 (C-26), 66.7 (C-27), 19.4 (C-28), 16.1 (C-29), 17.9 (C-30), 19.4 (C-31)。以上数据与文献^[7]报道基本一致,因此鉴定化合物 **3** 为 *seco-milbemycin C*。

化合物 4 白色粉末状固体;易溶于氯仿、甲醇、丙酮,难溶于水;ESI-MS: m/z 523.3 [M - H]⁻, 分子式为 C₃₂H₄₄O₆。¹H NMR (400 MHz, CDCl₃) δ : 7.78 (1H, s, H-3), 7.02 (1H, s, H-6), 6.51 (1H, d, J = 11.0 Hz, H-9), 6.17 (1H, dd, J = 14.8, 11.0 Hz, H-10), 5.97 (1H, dd, J = 14.8, 6.6 Hz, H-11), 5.11 (1H, t, J = 7.0 Hz, H-15), 5.05 (2H, s, H-27), 4.24 (1H, m, H-19), 3.63 (1H, m, H-17), 3.25 (1H, m, H-25), 2.53 (1H, m, H-12), 2.28 (2H, m, H-16), 2.20 (2H, m, H-13), 2.20 (3H, s, H-26), 2.11 (1H, m, H-18), 2.08 (1H, m, H-20), 1.65 (1H, m, H-22), 1.62 (1H, m, H-31), 1.62 (3H, br s, H-29), 1.54 (1H, m, H-22), 1.50 (2H, m, H-23), 1.34 (1H, br t, J = 12.0 Hz, H-20), 1.24 (1H, m, H-24), 1.25 (1H, m, H-31), 1.20 (1H, m, H-18), 1.08 (3H, d, J = 6.2 Hz, H-28), 0.92 (3H, t, J = 6.2 Hz, H-32), 0.82 (3H, d, J = 6.5 Hz, H-30); ¹³C NMR (100 MHz, CDCl₃) δ : 165.6 (C-1), 114.5 (C-2), 133.1 (C-3), 126.2 (C-4), 160.6 (C-5), 107.4 (C-6), 138.3 (C-7), 123.5 (C-8), 128.1 (C-9), 121.8 (C-10), 146.6 (C-11), 33.7 (C-12), 48.3 (C-13), 135.5 (C-14), 122.5 (C-15), 34.2 (C-16), 68.0 (C-17), 40.1 (C-18), 65.7 (C-19), 44.8 (C-20), 97.5 (C-21), 35.7 (C-22), 27.8 (C-23), 36.5 (C-24), 71.4 (C-25), 15.6 (C-26), 66.8 (C-27), 19.5 (C-28), 16.1 (C-29), 17.9 (C-30), 25.6 (C-31), 10.3 (C-32)。以上数据与文献^[8]报道基本一致,因此鉴定化合物 **4** 为 *seco-milbemycin A*。

化合物 5 白色粉末状固体;易溶于氯仿、甲醇、丙酮,难溶于水;ESI-MS: m/z 541.1 [M + H]⁺, 分子式为 C₃₃H₄₈O₆。¹H NMR (400 MHz, CDCl₃) δ : 6.42 (1H, br s, H-3), 6.25 (1H, d, J = 11.8 Hz, H-9), 6.03 (1H, dd, J = 14.8, 11.8 Hz, H-

10), 5.38 (1H, d, J = 14.8, 9.1 Hz, H-11), 5.36 (1H, m, H-19), 4.83 (1H, br d, J = 10.0 Hz, H-15), 3.75 (1H, m, H-17), 3.61 (1H, br d, H-2), 3.38 (1H, s, H-6), 3.06 (1H, dd, J = 9.6, 2.0 Hz, H-25), 2.50 (1H, m, H-12), 2.43 (1H, m, H-13), 2.42 (1H, s, H-6), 2.31 (1H, m, H-16), 2.23 (1H, m, H-16), 2.01 (1H, m, H-18), 1.98 (1H, m, H-20), 1.94 (3H, s, H-27), 1.87 (1H, m, H-13), 1.84 (3H, s, H-26), 1.65 (1H, m, H-22), 1.64 (3H, s, H-29), 1.53 (1H, m, H-22), 1.52 (2H, m, H-23), 1.50 (1H, m, H-31), 1.43 (1H, m, H-20), 1.32 (1H, m, H-24), 1.03 (3H, d, J = 6.7 Hz, H-28), 0.89 (3H, d, J = 6.2 Hz, H-32), 0.84 (3H, d, J = 6.2 Hz, H-33), 0.79 (1H, q, J = 11.8 Hz, H-18), 0.79 (3H, d, J = 6.5 Hz, H-30); ¹³C NMR (100 MHz, CDCl₃) δ : 173.5 (C-1), 49.2 (C-2), 118.1 (C-3), 139.6 (C-4), 198.1 (C-5), 41.6 (C-6), 75.9 (C-7), 124.0 (C-8), 123.8 (C-9), 126.4 (C-10), 144.6 (C-11), 36.1 (C-12), 48.3 (C-13), 137.5 (C-14), 122.3 (C-15), 34.2 (C-16), 67.0 (C-17), 36.6 (C-18), 70.0 (C-19), 41.3 (C-20), 97.5 (C-21), 35.7 (C-22), 27.8 (C-23), 31.5 (C-24), 78.2 (C-25), 18.8 (C-26), 19.8 (C-27), 14.2 (C-28), 16.3 (C-29), 17.9 (C-30), 28.6 (C-31), 14.2 (C-32), 20.9 (C-33)。以上数据与文献^[9]报道基本一致,因此鉴定化合物 **5** 为米尔贝霉素 H。

化合物 6 白色粉末状固体;易溶于氯仿、甲醇、丙酮,难溶于水;ESI-MS: m/z 509.3 [M - H]⁻, 分子式为 C₃₁H₄₂O₆。¹H NMR (400 MHz, CDCl₃) δ : 7.38 (1H, s, H-3), 6.70 (1H, s, H-6), 6.24 (1H, dd, J = 14.9, 11.0 Hz, H-10), 5.82 (1H, d, J = 11.0 Hz, H-9), 5.50 (1H, m, H-19), 5.42 (1H, dd, J = 14.9, 6.6 Hz, H-11), 4.91 (1H, t, J = 9.7 Hz, H-15), 4.56 (1H, d, J = 10.0 Hz, H-27), 4.53 (1H, d, J = 10.0 Hz, H-27), 3.71 (1H, m, H-17), 3.30 (1H, m, H-25), 2.53 (1H, m, H-12), 2.34 (1H, m, H-16), 2.25 (1H, m, H-13), 2.25 (1H, m, H-16), 2.22 (3H, s, H-26), 2.14 (1H, m, H-18), 2.02 (1H, m, H-20), 1.86 (1H, br t, J = 12.4 Hz, H-13), 1.65 (1H, m, H-22), 1.64 (3H, br s, H-29), 1.54 (1H, m, H-22), 1.54 (2H, m, H-23), 1.44 (1H, br t, J = 12.0 Hz, H-20), 1.24 (1H, m, H-24), 1.15 (3H, d, J = 6.2 Hz, H-31), 1.07 (3H, d, J = 6.2 Hz, H-28), 0.87 (1H, m, H-18), 0.85 (3H, d, J = 6.5 Hz, H-30); ¹³C NMR

(100 MHz, CDCl_3) δ : 169.2 (C-1), 123.7 (C-2), 132.1 (C-3), 123.2 (C-4), 156.6 (C-5), 114.4 (C-6), 140.6 (C-7), 136.1 (C-8), 131.8 (C-9), 123.8 (C-10), 143.1 (C-11), 35.7 (C-12), 48.7 (C-13), 135.5 (C-14), 121.5 (C-15), 33.8 (C-16), 67.6 (C-17), 36.6 (C-18), 68.7 (C-19), 41.3 (C-20), 97.8 (C-21), 35.8 (C-22), 27.8 (C-23), 36.5 (C-24), 71.3 (C-25), 15.5 (C-26), 61.5 (C-27), 20.9 (C-28), 16.1 (C-29), 17.9 (C-30), 19.3 (C-31)。以上数据与文献^[10]报道基本一致,因此鉴定化合物 **6** 为米尔贝霉素 β_{13} 。

化合物 7 白色粉末状固体;易溶于氯仿、甲醇、丙酮,难溶于水;ESI-MS: m/z 539.3 [$\text{M} + \text{H}$]⁺, 分子式为 $\text{C}_{32}\text{H}_{42}\text{O}_7$ 。¹H NMR (400 MHz, CDCl_3) δ : 7.74 (3H, s, H-27), 7.57 (1H, s, H-3), 6.83 (1H, d, $J = 15.6$ Hz, H-9), 6.15 (1H, dd, $J = 15.6, 8.9$ Hz, H-10), 5.63 (1H, m, H-19), 5.33 (1H, t, $J = 7.7$ Hz, H-15), 4.39 (1H, d, $J = 8.9$ Hz, H-11), 3.93 (1H, m, H-17), 3.12 (1H, m, H-25), 2.42 (1H, m, H-16), 2.31 (3H, s, H-26), 2.30 (1H, m, H-16), 2.16 (1H, m, H-20), 2.14 (1H, m, H-18), 2.10 (2H, m, H-13), 1.84 (1H, m, H-12), 1.73 (3H, br s, H-29), 1.67 (1H, m, H-31), 1.65 (1H, m, H-22), 1.54 (1H, m, H-22), 1.50 (2H, m, H-23), 1.40 (1H, m, H-18), 1.35 (1H, m, H-31), 1.34 (1H, m, H-20), 1.34 (1H, m, H-24), 1.03 (3H, d, $J = 6.9$ Hz, H-28), 1.01 (3H, t, $J = 6.2$ Hz, H-32), 0.85 (3H, d, $J = 6.5$ Hz, H-30); ¹³C NMR (100 MHz, CDCl_3) δ : 167.3 (C-1), 117.2 (C-2), 129.6 (C-3), 119.6 (C-4), 142.4 (C-5), 142.3 (C-6), 123.5 (C-7), 120.0 (C-8), 126.8 (C-9), 126.4 (C-10), 86.6 (C-11), 40.1 (C-12), 43.3 (C-13), 137.5 (C-14), 122.3 (C-15), 33.2 (C-16), 67.0 (C-17), 35.6 (C-18), 70.0 (C-19), 41.3 (C-20), 97.5 (C-21), 35.7 (C-22), 27.8 (C-23), 34.5 (C-24), 76.2 (C-25), 14.8 (C-26), 144.4 (C-27), 14.2 (C-28), 18.3 (C-29), 17.9 (C-30), 25.6 (C-31), 10.2 (C-32)。以上数据与文献^[11]报道基本一致,因此鉴定化合物 **7** 为米尔贝霉素 ST906。

化合物 8 白色粉末状固体;易溶于氯仿、甲醇、丙酮,难溶于水;ESI-MS: m/z 495.1 [$\text{M} + \text{H}$]⁺, 分子式为 $\text{C}_{31}\text{H}_{42}\text{O}_5$ 。¹H NMR (400 MHz, CDCl_3) δ : 7.37 (1H, s, H-3), 6.61 (1H, s, H-6), 6.15

(1H, dd, $J = 15.0, 10.9$ Hz, H-10), 5.71 (1H, d, $J = 10.9$ Hz, H-9), 5.49 (1H, m, H-19), 5.29 (1H, d, $J = 15.0, 9.1$ Hz, H-11), 4.90 (1H, br d, $J = 10.1$ Hz, H-15), 3.63 (1H, m, H-17), 3.10 (1H, m, H-25), 2.60 (3H, s, H-26), 2.50 (1H, m, H-12), 2.31 (1H, m, H-16), 2.23 (1H, m, H-16), 2.21 (1H, m, H-13), 2.07 (3H, s, H-27), 2.01 (1H, m, H-18), 1.98 (1H, m, H-20), 1.87 (1H, m, H-13), 1.65 (1H, m, H-22), 1.64 (3H, s, H-29), 1.53 (1H, m, H-22), 1.52 (2H, m, H-23), 1.43 (1H, m, H-20), 1.32 (1H, m, H-24), 1.10 (3H, d, $J = 5.8$ Hz, H-31), 1.03 (3H, d, $J = 6.7$ Hz, H-28), 0.85 (3H, d, $J = 6.5$ Hz, H-30), 0.79 (1H, q, $J = 11.8$ Hz, H-18); ¹³C NMR (100 MHz, CDCl_3) δ : 169.1 (C-1), 123.5 (C-2), 132.0 (C-3), 123.2 (C-4), 156.4 (C-5), 114.3 (C-6), 140.7 (C-7), 136.2 (C-8), 131.7 (C-9), 123.6 (C-10), 143.2 (C-11), 35.7 (C-12), 48.7 (C-13), 135.6 (C-14), 121.4 (C-15), 33.8 (C-16), 67.5 (C-17), 36.6 (C-18), 68.8 (C-19), 41.2 (C-20), 97.9 (C-21), 35.8 (C-22), 27.7 (C-23), 36.6 (C-24), 71.2 (C-25), 15.5 (C-26), 16.8 (C-27), 20.8 (C-28), 16.2 (C-29), 17.9 (C-30), 19.3 (C-31)。以上数据与文献^[12]报道基本一致,因此鉴定化合物 **8** 为米尔贝霉素 β_3 。

化合物 9 白色粉末状固体;易溶于氯仿、甲醇、丙酮,难溶于水;ESI-MS: m/z 507.4 [$\text{M} - \text{H}$]⁻, 分子式为 $\text{C}_{32}\text{H}_{44}\text{O}_5$ 。¹H NMR (400 MHz, CDCl_3) δ : 7.37 (1H, s, H-3), 6.62 (1H, s, H-6), 6.14 (1H, dd, $J = 15.0, 10.9$ Hz, H-10), 5.72 (1H, d, $J = 10.9$ Hz, H-9), 5.49 (1H, m, H-19), 5.28 (1H, d, $J = 15.0, 9.1$ Hz, H-11), 4.90 (1H, br d, $J = 10.1$ Hz, H-15), 3.63 (1H, m, H-17), 3.10 (1H, m, H-25), 2.60 (3H, s, H-26), 2.50 (1H, m, H-12), 2.31 (1H, m, H-16), 2.23 (1H, m, H-16), 2.21 (1H, m, H-13), 2.07 (3H, s, H-27), 2.01 (1H, m, H-18), 1.98 (1H, m, H-20), 1.87 (1H, m, H-13), 1.67 (3H, m, H-31), 1.65 (1H, m, H-22), 1.64 (3H, s, H-29), 1.53 (1H, m, H-22), 1.52 (2H, m, H-23), 1.43 (1H, m, H-20), 1.35 (2H, m, H-31), 1.32 (1H, m, H-24), 1.03 (3H, d, $J = 6.7$ Hz, H-28), 1.01 (3H, t, $J = 6.2$ Hz, H-32), 0.85 (3H, d, $J = 6.5$ Hz, H-30), 0.79 (1H, q, $J = 11.8$ Hz, H-18); ¹³C NMR (100 MHz, CDCl_3) δ : 168.9 (C-1), 123.9 (C-2), 132.1 (C-3), 123.4 (C-4), 156.7

(C-5), 114.4 (C-6), 140.8 (C-7), 136.3 (C-8), 131.8 (C-9), 123.9 (C-10), 143.0 (C-11), 35.7 (C-12), 48.8 (C-13), 135.6 (C-14), 121.6 (C-15), 33.8 (C-16), 67.6 (C-17), 36.7 (C-18), 68.8 (C-19), 41.4 (C-20), 97.6 (C-21), 35.7 (C-22), 27.9 (C-23), 34.4 (C-24), 76.3 (C-25), 15.5 (C-26), 18.5 (C-27), 20.9 (C-28), 16.1 (C-29), 17.9 (C-30), 25.8 (C-31), 10.1 (C-32)。以上数据与文献^[10]报道基本一致,因此鉴定化合物 **9** 为 25-ethylmilibmycin β_3 。

化合物 10 白色粉末状固体;易溶于氯仿、甲醇、丙酮,难溶于水;ESI-MS: m/z 525.3 [M - H]⁻, 分子式为 C₃₂H₄₆O₆。¹H NMR (400 MHz, CDCl₃) δ : 6.38 (1H, s, H-3), 6.28 (1H, d, J = 11.9 Hz, H-9), 6.01 (1H, dd, J = 15.0, 11.9 Hz, H-10), 5.38 (1H, d, J = 15.0, 9.1 Hz, H-11), 5.34 (1H, m, H-19), 4.84 (1H, br d, J = 10.1 Hz, H-15), 3.63 (1H, m, H-17), 3.59 (br d, H-2), 3.38 (1H, s, H-6), 3.10 (1H, m, H-25), 2.50 (1H, m, H-12), 2.40 (1H, s, H-6), 2.31 (1H, m, H-16), 2.23 (1H, m, H-16), 2.21 (1H, m, H-13), 2.01 (1H, m, H-18), 1.98 (1H, m, H-20), 1.94 (3H, s, H-27), 1.87 (1H, m, H-13), 1.84 (3H, s, H-26), 1.67 (1H, m, H-31), 1.65 (1H, m, H-22), 1.64 (3H, s, H-29), 1.53 (1H, m, H-22), 1.52 (2H, m, H-23), 1.43 (1H, m, H-20), 1.35 (1H, m, H-31), 1.32 (1H, m, H-24), 1.03 (3H, d, J = 6.7 Hz, H-28), 1.01 (3H, t, J = 6.2 Hz, H-32), 0.85 (3H, d, J = 6.5 Hz, H-30), 0.79 (1H, q, J = 11.8 Hz, H-18); ¹³C NMR (100 MHz, CDCl₃) δ : 173.1 (C-1), 48.6 (C-2), 136.1 (C-3), 136.4 (C-4), 196.6 (C-5), 47.6 (C-6), 75.6 (C-7), 135.1 (C-8), 148.6 (C-9), 121.6 (C-10), 153.1 (C-11), 36.7 (C-12), 48.6 (C-13), 136.5 (C-14), 121.4 (C-15), 34.4 (C-16), 67.5 (C-17), 36.6 (C-18), 68.8 (C-19), 40.7 (C-20), 97.8 (C-21), 35.7 (C-22), 27.6 (C-23), 36.6 (C-24), 71.2 (C-25), 15.5 (C-26), 16.6 (C-27), 21.1 (C-28), 16.1 (C-29), 25.7 (C-31), 10.2 (C-32)。以上数据与文献^[10]报道基本一致,因此鉴定化合物 **10** 为米尔贝霉素 β_6 。

化合物 11 白色粉末状固体;易溶于氯仿、甲醇、丙酮,难溶于水;HR-ESI-MS: 535.3061 [M + Na]⁺ (calcd for C₃₁H₄₄NaO₆, 535.3074), 分子式为

C₃₁H₄₄O₆。¹H NMR (400 MHz, CDCl₃) δ : 6.38 (1H, s, H-3), 6.28 (1H, d, J = 11.9 Hz, H-9), 6.01 (1H, dd, J = 15.0, 11.9 Hz, H-10), 5.38 (1H, d, J = 15.0, 9.1 Hz, H-11), 5.34 (1H, m, H-19), 4.84 (1H, br d, J = 10.1 Hz, H-15), 3.63 (1H, m, H-17), 3.59 (1H, br d, H-2), 3.38 (1H, s, H-6), 3.25 (1H, m, H-25), 2.50 (1H, m, H-12), 2.40 (1H, s, H-6), 2.31 (1H, m, H-16), 2.23 (1H, m, H-16), 2.21 (1H, m, H-13), 2.01 (1H, m, H-18), 1.98 (1H, m, H-20), 1.94 (3H, s, H-27), 1.87 (1H, m, H-13), 1.84 (3H, s, H-26), 1.65 (1H, m, H-22), 1.64 (1H, s, H-29), 1.53 (1H, m, H-22), 1.52 (2H, m, H-23), 1.43 (1H, m, H-20), 1.23 (1H, m, H-24), 1.11 (3H, m, H-31), 1.03 (3H, d, J = 6.7 Hz, H-28), 0.85 (3H, d, J = 6.5 Hz, H-30), 0.79 (1H, q, J = 11.8 Hz, H-18); ¹³C NMR (100 MHz, CDCl₃) δ : 173.0 (C-1), 48.5 (C-2), 136.0 (C-3), 136.2 (C-4), 196.4 (C-5), 47.6 (C-6), 75.7 (C-7), 135.2 (C-8), 148.7 (C-9), 121.6 (C-10), 153.2 (C-11), 36.7 (C-12), 48.7 (C-13), 136.6 (C-14), 121.4 (C-15), 34.4 (C-16), 67.5 (C-17), 36.6 (C-18), 68.8 (C-19), 40.7 (C-20), 97.9 (C-21), 35.8 (C-22), 27.6 (C-23), 36.6 (C-24), 71.2 (C-25), 15.5 (C-26), 16.6 (C-27), 21.1 (C-28), 16.1 (C-29), 17.9 (C-30), 19.2 (C-31)。以上数据与文献^[10]报道基本一致,因此鉴定化合物 **11** 为米尔贝霉素 β_7 。

2.2 化合物杀虫活性测试

对化合物 **1** 和 **2** 杀虫活性测试结果(见表2)显示该两个化合物对朱砂叶螨和松材线虫都具有显著的杀灭活性,与阳性对照组米尔贝霉素 A3/A4 活性相比无显著性差异($P > 0.05$)。

3 讨论与结论

本文通过对基因工程菌 *Streptomyces bingchengensis* BCJ60 次级代谢产物进行了系统的研究,得到 11 个化合物,其中化合物 **1** 和 **2** 是两个新化合物。在之前的研究中,本课题组已对基因工程菌 *Streptomyces bingchengensis* BCJ60 次级代谢产物进行了初步的研究获得了 12 个新化合物,并且都是米尔贝霉素类化合物^[13-18]。这说明通过基因定向改造,生物合成基因簇发生改变,激活了某些生物合成途径,得到新的代谢产物,有助于进一步了解米尔贝类化合物生物合成机制。对新化合物杀虫活性实验表明,化

表 2 化合物 1 和 2 的杀螨和杀线虫活性

Table 2 The acaricidal and nematocidal activities of compounds 1 and 2

化合物 Compound	半数致死量 LC ₅₀ (mg/L)	
	朱砂叶螨 <i>Tetranychus cinnabarinus</i>	松材线虫 <i>Bursaphelenchus xylophilus</i>
1	0.121 ± 0.010 *	5.217 ± 0.064 *
2	0.116 ± 0.015 *	5.581 ± 0.059 *
米尔贝霉素 A3/A4 Milbemycins A3/A4	0.107 ± 0.007	4.900 ± 0.068

注:与阳性对照相比, * $P > 0.05$ 。

Note: Compared with positive control, * $P > 0.05$.

合物 1 和 2 对朱砂叶螨和松材线虫都表现了显著的杀灭活性,并且与商品化的米尔贝霉素 A3/A4 无显著性差异,说明化合物 1 和 2 不仅有望作为米尔贝霉素 A3/A4 的替代杀虫剂,还有可能通过对其结构修饰得到活性更高的新一代农用或兽用抗生素。

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