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脂肽类抗生素fengycin在枯草芽胞杆菌NCD-2菌株抑制番茄灰霉病菌中的功能分析

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The fengycin lipopeptides are major components in the *Bacillus subtilis* strain NCD-2 against the growth of *Botrytis cinerea*

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摘要 枯草芽胞杆菌NCD-2菌株及其无细胞发酵液对多种植物病原真菌具有较强的拮抗活性。为了明确NCD-2菌株抑菌作用机理,本研究利用快速蛋白液相色谱技术(FPLC)结合抑菌活性测定,对NCD-2菌株的脂肽类抑菌物质进行分离、纯化,经质谱鉴定,该抑菌物质为芬荠素(fengycin)。从NCD-2菌株中克隆出fengycin合成酶基因*fenC*及其上游启动子序列,通过同源重组技术对*fenC*基因进行了内缺失突变,同NCD-2野生型菌株相比,*fenC*基因缺失突变子丧失了fengycin的合成能力,同时该突变子显著降低了对番茄灰霉病菌的拮抗活性。进一步在离体叶片上比较了NCD-2野生型菌株和突变子之间脂肽提取物和菌体细胞对番茄灰霉病的保护作用,结果发现,突变子不论是脂肽提取物还是菌体细胞显著降低了对番茄灰霉病的防治作用。

关键词: 枯草芽胞杆菌 番茄灰霉病菌 fengycin 突变

Abstract: The *Bacillus subtilis* strain NCD-2 and its cell-free supernatant showed strong inhibitive activity against the growth of phytopathogens. The NCD-2 lipopeptide extracts showed a strong inhibitive activity against the growth of *Botrytis cinerea*. The major antifungal activity compounds were isolated from the lipopeptides by Fast Protein Liquid Chromatography (FPLC), and identified as fengycin based on the profiles of matrix assisted laser desorption/ionization- time of flight mass spectrometry (MALDI-TOF). The antifungal activity of fengycin was supported by in-frameless deletion mutagenesis and it targeted to suppress the biosynthesis of fengycin production. Compared to the strain NCD-2 wild type, the fengycin-deficient mutant strain decreased the antifungal capability significantly. Further bioassay indicated that the lipopeptides extracted from the fengycin-deficient mutant not only lost the inhibitive activity against the growth of *B. cinerea in vitro*, but also significantly decreased the control effect against tomato grey mold on detached tomato leaves. The results could be confirmed by assays using bacterial suspension between strain NCD-2 wild type and the fengycin-deficient mutant.

Key words: *Bacillus subtilis* *Botrytis cinerea* fengycin mutagenesis

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