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阴沟肠杆菌B8拮抗活性基因‘*admA*’及上游调控序列的克隆与功能鉴定

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摘要 为了阐明水稻白叶枯病拮抗菌阴沟肠杆菌B8的作用机理, 文章采用转座子标签法和染色体步移技术克隆到突变株B8B中Tn5插入位点周边拮抗活性相关片段, 并通过基因敲除验证了获得的拮抗相关片段?*admA*’上游调控序列的功能。以转座子中Kan抗性基因为标签, 克隆了B8B菌株中Tn5插入位点左侧2 608 bp序列, 经两次染色体步移得到Tn5插入位点右侧的2 354 bp序列。序列拼接后获得B8菌株拮抗相关序列4 611 bp 的B contig。生物信息学分析显示该序列含有7个ORF, 分别对应于3-磷酸甘油醛脱氢酶(GADPH)基因的部分编码区、2个LysR家族转录调控因子、弧菌假设蛋白VSWAT3-20465及成团泛菌(*Pantoea agglomerans*) andrimid生物合成基因簇的*admA*、*admB*和部分*admC*基因序列。B8B菌株Tn5插入分别位于同源于弧菌假设蛋白的*anrP* ORF及‘*admA*’基因上游200 bp和894 bp处。通过同源重组技术, 借助敲除质粒pMB-BG, 获得拮抗活性消失的突变株B-1和B-3。结果表明B8B突变株中Tn5的插入可能影响了anrP蛋白的转录和表达, 进而调控拮抗物质编码基因簇的生物合成。B8菌株中拮抗物质相关基因是类似于andrimid 生物合成基因簇的基因家族, 其上游调控区对该抗生素的生物合成具有重要的作用。

关键词： 阴沟肠杆菌B8 拮抗机理 Andrimid 染色体步移

Abstract： To reveal the antagonistic mechanism of B8 strain to *Xanthomonas oryzae* pv. *oryzae*, transposon tagging method and chromosome walking were deployed to clone antagonistic related fragments around Tn5 insertion site in the mutant strain B8B. The function of up-stream regulatory sequence of gene ‘*admA*’ involved in the antagonistic activity was further identified by gene knocking out technique. An antagonistic related left fragment of Tn5 insertion site, 2 608 bp in length, was obtained by tagging with *Kan* resistance gene of Tn5. A 2 354 bp right fragment of Tn5 insertion site was amplified with 2 rounds of chromosome walking. The length of the B contig around the Tn5 insertion site was 4 611 bp, containing 7 open reading frames (ORFs). Bioinformatic analysis revealed that these ORFs corresponded to the partial coding regions of glyceraldehyde-3-phosphate dehydrogenase, two LysR family transcriptional regulators, hypothetical protein VSWAT3-20465 of *Vibrionales* and *admA, admB*, and partial sequence of *admC* gene of *Pantoea agglomerans* biosynthetic gene cluster, respectively. Tn5 was inserted in the up-stream of 200 bp or 894 bp of the sequence corresponding to *anrP* ORF or *admA* gene on B8B, respec-tively. The B-1 and B-2 mutants that lost antagonistic activity were selected by homeologuous recombination technology in association with knocking out plasmid pMB-BG. These results suggested that the transcription and expression of *anrP* gene might be disrupted as a result of the knocking out of up-stream regulatory sequence by Tn5 in B8B strain, further causing biosythesis regulation of the antagonistic related gene cluster. Thus, the antagonistic related genes in B8 strain is a gene family similar as andrimid biosynthetic gene cluster, and the upstream regulatory region appears to be criti-cal for the antibiotics biosynthesis.

Keywords： *Enterobactor cloacae* B8, antagonistic mechanism, andrimid, chromosome walking

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