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解淀粉芽孢杆菌赖氨酸-3基因在枯草芽孢杆菌中的克隆

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摘要 限制性核酸内切酶EcoRI酶切解淀粉芽孢杆菌AS1.1099染色体DNA和质粒pUB 110 DNA,T4DNA 连接酶连接，转化枯草芽孢杆菌BR151(trpC2 met B5 lys3 Neo s)感受态细胞。用选择性培 养基两次筛选，得到Neo r和与BR151 kys3营养缺陷突变互补的菌落。用快速测定质粒的方 法检查，其中有13株转化子带有大小相同的重组质粒。分离其中1株转化子BR151-29的重组 策粒pB-L29 DNA，再次转化BR151感受态细胞和原生质体。测定第二次得到的转化子性状， 均与转化子BR151-29相同。琼脂糖凝胶电泳检查这些转化子的质粒，其分子大小也与重组质粒pB-L29相同。以上试验证明，重组质粒pB-L29是由pUB110和解淀粉芽孢杆菌的lys3基因片 段构成的。根据琼脂糖凝胶电泳测定pB-L29的分子量约为5.0kb，推断出lys3基因片段约为0 .5kb。

关键词

分类号

Clonig of Lys3+ Gene of Bacillus amyloliquefaciens in Bacillus subtilis

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Abstract

Chromosomal DNA of Bacillus amyloliquefaciens H AS.1.1099 and plasmid pUB-110 DN A were digested with restriction endonuclease EcoRI and ligated with T4 DNA liga se.Bacillus subtilis BR151 (trpC2 metB5 lys3 Neo s) competent cells were trans-formed by ligated mixture.Cells were plated onto complete medium containing 5μg/ml neomycin.A total of 3,180 Neo r colonies were obtained.These colonies were picked picked onto minimal medium containing 5μg/ml meomycin and lacking the tryptophan,meth-ionine,or lysine separately.74 resistant-neomycin and only complement BR151 lys3 mutation colonies were obtained.Agarose gel electrophoretic analysis of plasmid DNAs from these clonies showed that 13 of them contained inserts of identical size.One of these recombinant plasmid,pB-L29,was studied further.Upon retransformation into BR151 competent cells and protoplasts,pB-L29 was found to confer upon the host resistance to neomycin and complement lys3 mutation indicating that Lys3+ phenotype is associated with the inserted fragment.Electrophoretic mobilities of plasmid DNAs isolated from the secondary transformants were identical to pB-L129.It is a reliable proof that recombinant plasmid pB-L29 consists of plasmid pUB110 with an insert of B.amyloliquefaciens H Lya3+ fragment at its EcoRI site.According to this DNA fragment migration,it was calculated that the MW of pB-L29 is about 5.0 kb,and the MW of Lys3+ fragment is about 0.5 kb.

Key words

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