

Frankia 基因文库的构建及与根瘤菌结瘤基因区同源克隆的筛选*

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收稿日期 修回日期 网络版发布日期 接受日期

摘要 应用无色肽酶(Achromopeptidase)加溶菌酶系统破壁,提取分别来自白色赤杨、细技术麻黄和沙棘的3株代表性Frankia菌株的总DNA。以可在很多革兰氏阴性细菌中稳定复制和诱动转移的广谱寄主性质粒pLAFR1为载体,构建了其基因组文库。基于经EcoRI酶切后的Frankia总DNA中有与根瘤菌结瘤基因同源性的片段,以豌豆根瘤菌结瘤基因为探针,通过菌落原位杂交对文库进行了筛选,较强杂交克隆经斑点杂交复筛,初步得到了几个阳性克隆,为进一步研究Frankia的结瘤基因及有关共生固氮的其它基因奠定了基础。

关键词 [Frankia,基因文库,结瘤基因](#)

分类号

Construction of Frankia Genomic Libraries and Isolation of Clones Homologous to Nodulation Genes from Rhizobium leguminosarum*

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Abstract

High molecular genomic DNAs were isolated by using the lysozyme plus achromopeptidase system from Frankia strains At4,Ccol and Hr16,the root nodule endophytes of Alnus,Casuarina and Hippophae respectively,and used to construct genomic libraries in pLAFR1,a broad host range cosmid vector within many gram-negative hosts. The genomic libraries were screened by in situ colony hybridization to identify clones homologous to common nodulation genes of Rhizobium leguminosarum,based on the sequence homology of EcoRI-digested Frankia total DNA to nod ABC from Rhizobium meliloti. Several clones showing relatively strong hybridization were found ,the recombinant plasmid was isolated,and their homology with Rhizobium nodulation genes was confirmed by spot hybridization.Further work on these positive clones is now underway.

Key words [Frankia](#) [Genomic libraries](#) [Nodulation genes](#)

DOI:

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