# 慢生型大豆根瘤菌(*Bradyrhizobium japonicum*) USDA110 菌株 3-羟丁酸脱氢酶基因(*bdhA*) 的克隆、序列及特性

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摘要:通过功能互补试验,从慢生型大豆根瘤菌 USDA110 菌株基因文库中筛选到能互补广宿主根瘤菌 NGR234 的 bdhA 突变体菌株 NGRPA2 和苜蓿根瘤菌的 bdhA 突变体菌株 Rm11107、使之恢复 Hbu<sup>+</sup> 表型的克隆; 经酶活测定和 Southern 杂交证明该克隆含有 bdhA 基因。测定了 bdhA 基因全序列,并在 GenBank 登试 登记号为: AY077581 )。该基因由 789 个碱基对组成 编码分子量为 27.59 ku、含 262 个氨基酸残基的 3-羟基丁酸脱氢酶。在 该基因的开放阅读框内插入 interposon  $\Omega$ Km,并通过同源重组构建了 Bradyrhizobium japonicum bdhA 突变体(bdh A:  $\Omega$ Km)。植株试验未显示 bdhA 基因的突变对结瘤、固氮有明显影响。

关键词:慢生型大豆根瘤菌 3-羟丁酸脱氢酶 3-羟丁酸脱氢酶基因 聚羟丁酸

# Cloning , Sequence and Characteristics of 3-Hydroxybutyrate Dehydrogenase Encoding Gene ( *bdh*A ) in *Bradyrhizobium japonicum* USDA110 Strain

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Abstract : A clone which can restore the ability of bdh A mutant strains NGRPA2 and Rm11107 to utilize 3hydroxybutyrate as sole carbon source ( Hbu<sup>+</sup> ) was screened out by complementation experiment from *Bradyrhizobium japonicum* USDA110 genomic library. It was confirmed by Bdh assay and Southern blot that this clone contains bdhA gene. The entire sequence of bdhA gene was sequenced and the sequence was deposited in GenBank at the accession number of AY077581. bdhA gene consists of 789 base pairs and encodes Bdh with 262 amino acid and MW 27.59 ku. Interposon  $\Omega$ Km was inserted into the bdhA ORF at EcoR I site and the bdhA mutant was constructed in *B. japonicum* by homologous recombination. Plant test result did not show obvious effects of mutation of bdhA gene on nodulation and nitrogen-fixation.

Key words : Bradyrhizobium japonicum ; Bdh ; bdhA gene ; PHB

很多细菌在非碳素营养(如N、P、O<sub>2</sub>受限)而碳 源过剩时,在细胞内积累聚羟丁酸(poly-3-hydroxybutyrate, PHB),而当碳源不足时,动用这种内部储 存的 PHB 作为碳源和能源<sup>1]</sup>。

根瘤菌普遍具有积累 PHB 的能力,但在不同种属间存在一定差异。如 Rhizobium meliloti 只在自

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由生长阶段及侵染线内的菌体中积累 PHB,而不能 在已分化的类菌体内形成 PHB<sup>2,3</sup>;Bradyrhizobium japonicum 则不同,不仅可在自由生长的细胞内 积累 PHB,而且在共生阶段的类菌体内也可积累高 达细胞干重 50%的 PHB<sup>4</sup>]。有人推测,类菌体中积 累的 PHB 的作用是在植物光合作用降低时为固氮 过程提供能量,或在根瘤衰老后延续固氮作 用<sup>[5~7]</sup>,但迄今没有发现直接证据。

关于细菌 PHB 代谢的生化和分子生物学的研究已有许多报道,其在 Rhizobium meliloti 中的代谢途径已较为明确<sup>8]</sup>。3-羟丁酸脱氢酶(3-hydroxybutyrate dehydrogenase, Bdh; EC1.1.1.30)是 PHB 分解代谢过程中的一个关键酶,催化 3-羟丁酸(3hydroxybutyrate; HB)单体氧化为乙酰乙酸(acetoacetate; AA)<sup>9]</sup>。对 Bradyrhizobium japonicum 的 Bdh 已有一些生化方面的研究<sup>[10]</sup>,但其分子生物学 方面的工作还未见报道。

笔者成功地从 Bradyrhizobium japonicum 基因文库中克隆到 bdhA 基因,完成了全序列测定,构建了突变体并研究了该基因突变对结瘤固氮的影响。

1 材料与方法

1.1 菌株和质粒

见表 1。

1.2 培养基和培养条件

所用培养基有 LB、TY、YMB(g·L<sup>-1</sup>:K<sub>2</sub>HPO<sub>4</sub> 0.5;MgSO<sub>4</sub>·7H<sub>2</sub>O,0.2;NaCl,0.1;Mannitol,10;酵 母提取物 0.5),M9基本培养基<sup>19]</sup>,M9-R-HB(M9 基本培养基中加入终浓度为 10 mmol·L<sup>-1</sup>的 R-HB 作为惟一碳源)。

根瘤菌培养于 30℃,大肠杆菌培养于 30℃或 37℃。如需要使用抗生素 除文中特别说明外,使用 浓度为:Amp,100 µg·ml<sup>-1</sup>;Tc 20 µg·ml<sup>-1</sup>;Km ,20 µg·ml<sup>-1</sup>;Rf 50 µg·ml<sup>-1</sup>。

1.3 酶和试剂

限制性内切酶、连接酶为 Promega 或 MBI 产品 抗生素购自 Sigma 和 Boehringer Mannheim。

### 1.4 DNA 制备

质粒 DNA 的制备按文献 20 的方法进行。根 瘤菌总 DNA 的制备按文献 21 方法进行。

1.5 基因组文库的构建

EcoR I 部分酶切 B. japonicum USDA110 总 DNA,回收10~25 kb片段,与经去磷酸化处理的广 宿主粘粒载体 <sub>p</sub>LAFR1 连接后 ,用 Stratagene 包装 试剂盒进行包装 ,于 DH5α中增殖。

### 1.6 杂交试验

Southern blot 杂交根据 Roche 推荐的方法进行 ;DNA 探针采用 DIG High Prime Kit 进行标记。 1.7 DNA 序列测定

将要测序的片段亚克隆到载体 pUC19,采用 Amersham pharmacia biotech 的 Thermo Sequenase<sup>TM</sup> Cy<sup>TM</sup>5/Cy5.5 末端标记的测序试剂盒在 Visible Genetics OpenGene<sup>TM</sup> Long-Read Tower<sup>TM</sup>测序 仪上进行。所用引物 M13 Forward:5' gtaaaacgacggccagt 3'(17mer);M13 Reverse:5' caggaaacagctatgac 3'(17mer);T7 Forward:5' taatacgactcactataggg 3'(20mer)以及根据需要自行设计的引物 (见文中)。数据采用 Macvector version6.0.1(Oxford Molecular Group)处理。

## 1.8 Bdh(3-hydroxybutyrate dehydrogenase)酶活 测定

收集菌体,用 20 mmol·L<sup>-1</sup> Tris-HCl pH 7.8,1 mmol·L<sup>-1</sup> MgCl<sub>2</sub> 缓冲液洗两遍,然后将菌体悬于 2~4 ml 20 mmol·L<sup>-1</sup> Tris-HCl pH 7.8,1 mmol· L<sup>-1</sup> MgCl<sub>2</sub>,10%甘油,10 mmol·L<sup>-1</sup>巯基乙醇缓冲 液中,然后在超声波破碎仪 Ultrasonics Sonifier Cell Disruptor Model W185-D上4℃处理2 min。15 000 ×g 离心 20 min 去除细胞碎片。无细胞提取液的 总蛋白采用文献 22 防法测定。Bdh(E.C1.1.1. 30)酶活测定根据文献 23 防法进行。

1.9 植株试验

植株试验所用容器是根据 Magenta 缸的原理自 行设计组装的。由上下两容器组成,上面的容器为 上口直径 15 cm 的塑料盆,内装栽培基质,中心部位 穿一条粗棉絮条以使上下两容器相连;下面为1 000 ml 的玻璃烧杯,内盛营养液。栽培基质为 50% 细沙 和 50% 蛭石。每盆加入 400 ml Jensen 氏无氮营养 液,锡箔覆盖,121℃灭菌 1 h,冷却后移去锡箔,植入 3 日龄大豆芽苗(供试大豆品种为 Bayfield)。植苗 后第 2 天,接入 10 ml 浓度为 1 × 10<sup>7</sup> 个 · ml<sup>-1</sup>的菌 液稀释液(YMB 30℃,200 r/min 培养 4 ~ 5 d);空白 对照组接入同样体积的灭菌 ddH<sub>2</sub>O。

供试植株培养于自动调温、调湿、调光的中型培 养室内; $16 h 25^{\circ}$ ,光照  $8 h 20^{\circ}$ ,黑暗;光照强度  $420 \mu E \cdot m^{-2} \cdot Sm^{-1}$ 。根据需要向培养容器中补加 灭菌的  $ddH_2O$ ,植株试验周期为 6 周。

#### 表1 菌株和质粒

Table 1 Strains and plasmids

XBH is DHSs $\Gamma$ and $M$ hold R1X $\eta_1 m_k - 1$ sup E44 thi-1 rec A1X $argF - lacZYA$ ) U169 $\Phi$ S0 dlacZAM159A*GIBCO BRL S0 dlacZAM159A*S17-1 $E$ cold 294 $\Gamma$ thi pro hold R1 rec A derivative[11]MT016MT040 $\Gamma$ [MK000 ) anohiber[12]Rain Dissibility multiplicationmultiplicationMT016MT040 $\Gamma$ [MK000 ) anohiber[13]Rain Dissibility multiplication[13]Rain Dissibility multiplication[14]NGR234 $\Gamma$ ff $\Xi_1$ Bhroad-host , R1 <sup>d</sup> NGR244 $\Gamma$ ff $\Xi_1$ Bhroad-host , R1 <sup>d</sup> NGR245NCR234 hold A1 : S2m5pBidSDA110 <b>B245</b> Wild-type strainBjuSDA110 <b>B245</b> Wild-type strainBjuSDA110 <b>B245</b> Wild-type strainDjuC04bdA1 : 32KmDjuC19CollEl cloning vector , Tc'LAFR1IncP cosmid cloning vector , Tc'DjuC200 mpl N $B_2R92$ ( $m_1$ case Stacide planidDjuC201 mpl N $B_2R92$ ( $m_1$ cases Stacide planid	菌株和质粒 Strains and plasmids	相关特征 Relevant characteristics	来源或参考文献 Sources and references		
DiSaF = adA1 kdR1 η rm, - ) sap E44 thi-1 recA1 Ld arg F - lacZYA ) U169 ΦGBECO BRL SBC 0 BRLS17-1E. col229 (F thi pro hold R) recA derivative[11]MT06MT60X pRtK600 ) mobilizer[12]MT06MT60X pRtK600 ) mobilizer[13]Rain 201Sinorhizobian molilot  [13]Rain 201SU47 str-21[13]Rain 1107Rm1021 bdA1 : Tn 5[14]NGR234C************************************	大肠杆菌 E.coli				
S17-1     E. oil294 (1 <sup>-</sup> this pro had R) rec.A derivative     [11]       MT016     MT060X pRK600) mobilizer     [12]       Rafizebium melilot ( Stnorbizobium melilot )     [13]       Rn1021     Stl47 ur-21     [13]       Rn11070     Rn1021 bdhA1 : Tn5     [8]       Rhizobium spp.     [14]     [14]       NCR242     NCR24 bdhA1 : ΩSmSp     [14]       NCR243     Cfact_, Bhroad-host, Rl <sup>fl</sup> [14]       NCR244     Cfact_, Bhroad-host, Rl <sup>fl</sup> [14]       NCR245     NCR24 bdhA1 : ΩSmSp     [9]       BitS050410     BEtk wild-type strain     USDA Reltsville ADD       Bj0064     bdhA1 : ΩKm     This work       By2522     Ind <sup>2</sup> coming vector , Tc <sup>2</sup> [15]       pLATRI     Ind <sup>2</sup> coming vector , Tc <sup>2</sup> [16]       pDC19     CaEI cloning vector , Tc <sup>2</sup> [17]       plQ200C mpl8)     BKpt fight Gm ( and B Societ de plasmid     [18]       pLAFRI     Ind <sup>2</sup> coming vector , Tc <sup>2</sup> [16]       pDC19     CaEI cloning vector , Ap <sup>2</sup> [16]       plQ200C mpl8)     BKpt fight GM ( add Societ for L 2 bK pn I -Hindll Ftg)     [18]       pDC19     pSP329 carrying the 14 bBamH1 fragment of pDC16     [18]       pDC20     pSP329 carrying the 14 bBamH1 Ftfg     This work       pSP329 car	DH5a	$F^-$ end A1 hsd R17( $r_k^- m_k - $ ) sup E44 thi-1 rec A1Δ arg $F - lac$ ZYA) U169 Φ 80 dlacZΔM15 $\lambda$ A <sup>-</sup>	GIBCO BRL		
MTo16MTO37 μRK000 janobilizer[12]Refield Khizobiar meliloi ( Sinorhizobiar meliloi )Rn1021SL47 str.21Rn1107Ru1021 bdA1 : Tn.5Rn1107Ru1021 bdA1 : Tn.5Rhizobiar msp.NGR234「Gat_, Bbraad-host, Rl <sup>6</sup> NGR244「Gat_, Bbraad-host, Rl <sup>6</sup> NGR25NGR24 bdA1 : SSmSpBrdSDA110野ts with dype strainBjUSDA110野ts with dype strainBjUSDA110Bt example strainBjUSDA110Bt example strainBjUSDA110Ind? cosmid cloning vector . Tc'DLAFRIInd? cosmid cloning vector . Tc'pLAFRIInd? cosmid cloning vector . Tc'pLAFRIInd? cosmid cloning vector . Tc'pLAFRIInd? cosmid cloning vector . Tc'pLAFRISp329Ind? cosmic strain of . source of the GKn interposon[17]plQ200 mpl80plS232 graving Rm1021 BdAA SegX 1.2 kb Kpn 1. Hindlll FdS)pDC16Ap', Km' ; ori GalE 1, source of the GKn interposon[18]pDC16N.B. ; appointant XFGPA3 Zarhön BarbaThis workpDC19pS232 graving Rm1021 BdAA gem(1.2 kb Kpn 1. Hindlll FdS)[9]pDC16N.B. ; appointer XFGPA3 ZarhönThis workpDC19pS232 graving the NLD Barba H FdBThis workpDC20pS232 graving the NLD Barba H FdBThis workpDC23pS232 graving the NLB Barba H FdBThis workpDC24pS232 corring the NLB Barba H FdBThis workpDC25pS232 pSERF pDC160 B9 2.4 kb St I fragment of pDC20This work	S17-1	E. coli294( $F^-$ thi pro hsd R) rec A derivative	[11]		
構 着NameRhizobium meliloti ( Sinorhizobium meliloti )Rn1021StJ47 xr-21Rn1021StJ47 xr-21Rn1021StJ47 xr-21Rhizobium spp.NGR234「T着主, Bbraad-host, Rl <sup>fi</sup> NGR234NGR24 bdA1 : 125mSpBitsDohlium japonicumBjtsDohl0野生株 Wild-type strainBjtsDohl0野生株 Wild-type strainBjtsDohl0BjtsmolpLAFR1IncP cosnid cloning vector. Tc'pLAFR1IncP cosnid cloning vector. Tc'pLAFR1IncP cosnid vector. Ap'pLAFR1IncP cosnid vector. Ap'pLO19ColE1 cloning vector. Ap'pLO20InS202 straing vector. Ap'pLO219ColE1 cloning vector. Ap'pLO200Bif XD data appenderpDC16A B, japonicum 2 strainpDC16A B, japonicum 2 strainpDC17pS232 straing Rm1021 ddAA agen(1.2 to Kpn I-HindIII)FQpDC20pS232 straing the 14 bh BanH1 Fragment of pDC16pDC20pS232 straing the 14 bh BanH1 Fragment of pDC20pDC20pS232 straing the 14 bh BanH1 Fragment of pDC20pDC20pS232 straing the 14 bh BanH1 Fragment of pDC20pDC23pS232 straing the 14 bh BanH1 Fragment of pDC20pDC24pDC29 filt sh BanH1 Pragment of pDC20pDC35pS232 straing the 34 bh BanH1 Fragment of pDC20pDC36pS232 straing the 4.8 bh BanH1 Pragment of pDC20pDC37pS232 straing the 4.8 bh BanH1 Pragment of pDC20pDC38pS24 pDC29 big 3.5 bh Ecol I Ps1 Fragment of pDC20pDC39p	MT616	MT607(pRK600);mobilizer	[12]		
Rhizolam mellol () Sinorhizolum mellol :)[13]Rm1021SU47 str-21[13]Rm1107Rm1021 ddA1 : Tn 5[8]Khizolum spp.[14][14]NGR234[7@±, Bbroad-host, Rf <sup>6</sup> [14]NGR42NGR244 ddA1 : GSm5p[9]Bradyhizolum igponicum <b>5</b> ±K Wild-type strain[9]BjUSDA110 <b>5</b> ±K Wild-type strainUSDA Beltsville, ADDBjUSDA110 <b>5</b> ±K Wild-type strain[15]pLAFR1Ind <sup>2</sup> cosnid cloning vector, Tc <sup>2</sup> [15]pS2239Ind <sup>2</sup> cosnid cloning vector, Tc <sup>2</sup> S. Porter, mupublishedpUC19GAIE cloning vector, Tc <sup>2</sup> S. Porter, mupublishedpUC19GAIE cloning vector, Tc <sup>2</sup> S. Porter, mupublishedpUC19GAIE cloning vector, Tc <sup>2</sup> S. Porter, mupublishedpUC19pS1239 j## Rm1021 bdA gend (1.2 kb Kpn I -Hindll fragment)[9]pDC16M. B. Agnoniza XE#rf AgaBhyBis NGKAZ E3th912Habd2This workpDC19pS1239 carrying the 1021 bdA gend (1.2 kb Kpn I -Hindll fragment)[9]pDC19pS1239 carrying the 1021 bdA gend (1.2 kb Kpn I -Hindll fragment)Fisse workpDC19pS1239 carrying the 12 bb field fragment of pDC16This workpDC19pS1239 carrying the 14 kb BanH I Fag I FagThis workpDC20pS239 carrying the 14 kb BanH I FagThis workpDC23pS239 carrying the 3 kb Sa I fagment of pDC20This workpDC24pS239 carrying the 3 kb Sa I fragment of pDC20Fisse vacpDC25pS239 carrying the 3 kb Sa I fragment of pDC20 <t< td=""><td>根瘤菌 Rhizobia</td><td></td><td></td></t<>	根瘤菌 Rhizobia				
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Rm11107     Rm1021 bdhA1 : Tn5     [8]       Rhizekium spp.        NGR234     广電主, Bhroad-host, Rf <sup>6</sup> [14]       NGR24     NGR234 bdhA1 : TSmSp     [9]       Bradyfrizekium japonizum Bjustnikm japonizum Bjustnikm japonizum Bjustnikm     USDA Alehsville ADD Bj00064     USDA Alehsville ADD Bj00064       Bjustnikm japonizum Bjustnikm Bjust	Rm1021	SU47 str-21	[13]		
Rhizakiam spp.     「宿主, Bbroad-host, Rt <sup>6</sup> [14]       NGR234     广宿主, Bbroad-host, Rt <sup>6</sup> [9]       Bradyhizakiam japonicum     BjuSDA110     野生林, Wild-type strain     USDA Addsville AMD       Bj30064     bdhA: TiKm     USDA Addsville AMD       Bj3007     Bj3087     This work       Bj2071     IneP cloning vector, Tc'     [15]       pLAFR1     IneP coming vector, Ap'     [16]       pUC19     Collit cloning vector, Ap'     [16]       pUC19     Golit cloning vector, Ap'     [16]       pUQ20(mp18)     日茶版和 Cm' saces Sucide plasmid     [18]       pPA7     pSP329 acrying the IIO21 bdhA gaq(1.2 kb kpn 1-Hindll Ftq)     [9]       pDC16     AB japonicum XG# Ph3DBibbs 5 NGR PA2 互补的重组和如     This work       pDC19     pSP329 acrying the I kb BamH 1 fragment of pDC16     This work       pDC20     pSP329 acrying the I kb BamH 1 fragment of pDC16     Fils work       pDC25     pSP329 acrying the I kb BamH 1 fragment of pDC20     Fils work       pDC28     pSP329 acrying the I clo R Shamil 1 fragment of pDC20     Fils work       pDC29     pSP329 acrying the 3.2 kb BanH 1 Fragment of pDC20     Fils work       pDC29     pSP329 acrying the 3.2 kb BanH 1 Fragment of pDC20     Fils work       pDC29     pSP329 acrying the 3.5 kb EcoR1 1 -Fill T fragment of pDC20     Fils work </td <td>Rm11107</td> <td>Rm1021 bdhA1 ::Tn5</td> <td>[8]</td>	Rm11107	Rm1021 bdhA1 ::Tn5	[8]		
NGR24     「宿主, Bbroad-host, Rf <sup>6</sup> [14]       NGR24     MGR234     MGR234     Image and the state of the state	Rhizobium spp.				
NGRPA2         NGR234         bdtRA11: ΩSmSp         [9]           Bradyrhisobium japonicum         BytSDA110         Bytex Wild-type strain         USDA. Beltsville ADD           Bj0064         bdtA1: ΩKm         This work         [15]           Bj2DATH         IncP cosmid cloning vector . Tc'         [15]           pLAFR1         IncP cosmid cloning vector . Tc'         [16]           pVC19         Collel cloning vector . Tc'         S. Porter , unpublished           pUC19         Collel cloning vector . Tc'         [16]           pUC200(mp18)         B\$B\$\overlap{B\$\overlap	NGR234	广宿主,Bbroad-host,Rf <sup>R</sup>	[14]		
Bradyrhizobium japonicum         USDA A.Beltsville ,MD         By Stand         USDA A.Beltsville ,MD           By 30064         bd/A: 33 Km         This work           ØR P Dasmid         [15]         [15]           pLAFRI         IncP cosmid cloning vector ,Tc'         [16]           pS2329         IncP cosmid cloning vector ,Ap'         [16]           pUC19         CoIE1 cloning vector ,Ap'         [16]           pUC19         CoIE1 cloning vector ,Ap'         [16]           pVA67         pS7329 JR#R Rm1021 Bb bd/A & EQ 1.2 kb Kpn I-HindIII ft RQ         [18]           pVC16         A B, izpanicum Xpreh (SR RM)         [18]         [18]           pVC16         A B, izpanicum Xpreh (SR RM)         [16]         [18]           pVC19         pS7329 carrying the14 kb BamH I fragment of pVC16         [16]         [16]           pVC20         pS7329 carrying the14 kb BamH I fragment of pVC16         [16]         [16]           pVC22         pS7329 carrying the 14 kb BamH I fragment of pVC16         [16]         [16]           pVC23         pS7329 carrying the 14 kb BamH I fragment of pVC20         [16]         [16]           pVC24         pS7329 carrying the 14 kb BamH I fragment of pVC20         [16]         [16]           pVC25         pS7329 carrying the 3.8 kb	NGRPA2	NGR234 $bdhA1$ : $\Omega$ SmSp	[9]		
Bj USDA110     野生株 Wild-type strain     USDA Jeditsville ADD       Bj 0064     bdA : ΩKm     This work       Bj Hasmid     IncP cosmid cloning vector .Tc'     [15]       pLAFRI     IncP cosmid vector .Tc'     [16]       pUC19     CaEI cloning vector .Tc'     [16]       pUC19     CaEI cloning vector .Tc'     [16]       pHP45ΩKm     Ap' .Km' ; oriCoIEI , source of the ΩKm interposon     [17]       pJQ200 (mp18)     DAK fm : oriCoIEI , source of the ΩKm interposon     [17]       pJC16     M B. japonicum 文库中分离到的能与 NGRPA2 互补的重组轮如     This work       pDC16     M B. japonicum 文库中分离到的能与 NGRPA2 互补的重组轮如     This work       pDC19     pSP329 carrying the 1 kb BamH I fm Tagment of pDC16     This work       pDC20     pSP329 carrying the 1 kb BamH I fm Tagment of pDC16     This work       pDC25     pSP329 carrying the 4 kb BamH I fm Tagment of pDC20     This work       pDC29     pSP329 carrying the 30 uot 5 kb Sac I fm Tagment of pDC20     This work       pDC32     carrying the 9.2 kb Pst I -BamH I fm Tagment of pDC20     This work       pDC29     pSP329 carrying the 4.8 kb BamH I Pst I FAB     This work       pDC29     pSP329 carrying the 4.8 kb BamH I Pst I FAB     This work       pDC29     pSP329 carrying the 4.8 kb BamH I Pst I FAB     This work       pDC29     pSP329 carrying the 4.8 kb	Bradyrhizobium japonicum				
B30064 bdfA:ΩKm This work This work DATE Plasmid pl.AFR1 IncP cosmid cloning vector ,Tc' [15] p.S7329 IncP cloning vector , Tc' [16] p.S7329 IncP cloning vector , Tc' [16] p.S7329 IncP cloning vector , Ap' [16] p.UC19 CoEEI cloning vector , Ap' [16] p.S7329 carrying Rh 1021 bd AA gem(1.2 kb Kpn I-Hind Ш fragment ) p.UC16 从 B. japonicum 文庫中分离到的能与 NGRA2 互补的重组粘粒 This work Recombinant cosmid plasmid isolated from B. japonicum genomic library and can complement with NGRPA2 strain p.DC19 p.S7329 carrying the 1 kb BamH I fragment of pDC16 p.DC20 p.S7329 上克隆有 p.DC16 691 kb BamH I fragment of pDC16 p.DC20 p.S7329 L克隆有 p.DC20 699 5 kb Sac I 片段 This work p.S7329 carrying the 14 kb BamH I fragment of pDC20 p.DC28 p.S7329 L克隆有 p.DC20 699 5 kb Sac I 片段 This work p.S7329 carrying the 9 kb St I -BamH I fragment of pDC20 p.DC29 p.S7329 L克隆有 p.DC20 694 5 kb Sac I 片段 This work p.DC29 p.S7329 L克隆有 p.DC20 694 5 kb Sac I 片段 This work p.DC29 p.S7329 L克隆有 p.DC20 694 5 kb Sac I 片段 This work p.DC29 p.S7329 L克隆有 p.DC20 694 5 kb Sac I 片段 This work p.DC29 digested with EcoR I then relegated p.MX87 p.UC19 L克隆有 p.DC29 60 6.6 kb EcoR I -Ps I 片段 This work p.DC29 digested with EcoR I then relegated p.MX94 p.UC19 L克隆有 p.DC29 69 0.6 kb EcoR I FAg This work p.UC19 carrying the 0.6 kb EcoR I FAg This work p.UC19 carrying the 0.6 kb EcoR I FAg This work p.UC19 carrying the 0.6 kb EcoR I FAg This work p.UC19 carrying the 0.6 kb EcoR I FAg This work p.UC19 carrying the 0.6 kb EcoR I FAg This work p.UC19 carrying the 0.6 kb EcoR I FAg This work p.UC19 carrying the 0.6 kb EcoR I FAg This work p.UC19 carrying the 0.6 kb EcoR I FAg This work p.UC19 carrying the 0.6 kb EcoR I FAg This work p.UC19 carrying the 0.6 kb EcoR I FAg This work p.UC19 carrying the 0.6 kb EcoR I FAg This work	BjUSDA110	野生株 Wild-type strain	USDA ,Beltsville ,MD		
<table-cell>           原基 Plasmid              pLAFR1</table-cell>	Bj30064	$bdh$ A : $\Omega$ Km	This work		
pLAFRI     IneP cosmid cloning vector ,Te'     [15]       pSP329     IneP cloning vector , Te'     S. Porter , unpublished       pUC19     ColE1 cloning vector , Ap'     [16]       pHP45DKm     Ap', Km'; oriColE1 , source of the ΩKm interposon     [17]       pQ200C mp18)     自着质粒 Gm' saceB Suicide plasmid     [18]       pPA67     pSP329 gdf# Rm1021 bdhA agm(1.2 kb Kpn I -HindIII fragment)     [9]       pDC16     从 B. japonicum 文庫中分高到的能与 NCRPA2 互补的重组移粒     This work       Recombinant cosmid plasmid isolated from B. japonicum genomic     library and can complement with NGRPA2 strain     This work       pDC19     pSP329 carrying the 1k b BamH I 广持段     This work       pSP329 carrying the 1k b BamH I 广持段     This work       pSP329 carrying the 1k b BamH I 广持段     This work       pSP329 carrying the 4b bas Sac I fragment of pDC16     This work       pSP329 carrying the 4b bas Sac I fragment of pDC20     pDC28     pSP329 lzfb@f pDC20 b9 9.2 kb Sac I fragment of pDC20       pDC28     pSP329 lzfb@f pDC20 b9 9.2 kb Pst I -BamH I 广持段     This work       pSP329 carrying the 4.8 kb BamH I -Pst I Fkp     This work       pDC29     pSP329 lzfb@f pDC20 b9 3.5 kb EcoR I -Pst I fragment of pDC20       pDC32     pDC32     pDC39 b0.6 kb EcoR I Fst I fragment of pDC29       pMX87     pUC19 lzfb@f pDC29 b9 0.6 kb EcoR I Fst I fragment of pDC29       pMX87	质粒 Plasmid				
pSP329     IncP cloning vector, Tc'     S. Porter, umpablished       pUC19     CoEI cloning vector, Ap'     [16]       pHP450Km     Ap', Km'; oriCoEI, source of the ΩKm interposon     [17]       pJQ200(mp18)     自杀质粒 Cm', sacB Suicide plasmid     [18]       pPA67     pSP329 d开游 Rm1021 bd/ak 基段 1.2 kb Kpn I-HindIII 片段     [9]       pDC16 <i>M. B. japonicum</i> 文库中分离到的能与 NGRPA2 互补的重组粘粒     This work       Recombinant cosmid plasmid isolated from <i>B. japonicum</i> genomic     library and can complement with NGRPA2 strain       pDC19     pSP329 上克隆有 pDC16 的 14 kb BamH I 并段     This work       pDC20     pSP329 上克隆有 pDC16 的 14 kb BamH I fragment of pDC16     pSP329 carrying the 14 kb BamH I fragment of pDC16       pDC25     pSP329 上克隆有 pDC20 的 59 5 kb Sac I 片段     This work       pSP329 carrying the about 5 kb Sac I 片段     This work       pSP329 carrying the about 5 kb Sac I fragment of pDC20     pSP329 carrying the about 5 kb Sac I fragment of pDC20       pDC29     pSP329 carrying the 3.2 kb Pst I-BamH I Fragment of pDC20     pDC29       pDC29     pSP329 carrying the 3.2 kb Pst I-BamH I Fragment of pDC20     pDC29       pDC29     pSP329 carrying the 3.4 kb BamH I -Pst I Fragment of pDC20     pDC32       pDC32     pDC29 fligested with EcoR I 1 est I fragment of pDC29     pDC39       pDC32     pDC29 fligested with EcoR I 1 est I fragment of pDC29     pDC39     pDC32 bD22 bD3.	pLAFR1	IncP cosmid cloning vector ,Tc <sup>r</sup>	[15]		
pUC19     CoEI cloning vector , Ap'     [16]       pHP45ΩKm     Ap', Km'; oriCoEI , source of the ΩKm intergoon     [17]       pQ200 mp18 )     自杀 质税 Cm', ace S bucide plasmid     [18]       pPA67     pSP329 萬帯 Rm1021 的 bdhA 基区 1.2 kb Kpn I-HindⅢ片段 )     [9]       pDC16     从 B, aponicam Zget 中分离到的能与 NGRPAZ 互补的重组转拉     This work       Recombinant cosmid plasmid isolated from B. japonicum genomic     Ibiary and can complement with NGRPAZ 54xnin     This work       pDC19     pSP329 上克隆有 pDC16 的 1 kb BamH I 片段     This work       pDC29     pSP329 carrying the 1 kb BamH I fragment of pDC16       pDC20     pSP329 carrying the about 5 kb Sac I 片段     This work       pDC25     pSP329 carrying the 9,2 kb Pst I-BamH I 片段     This work       pSP329 carrying the 9,2 kb Pst I-BamH I FAgment of pDC20     pSP329 carrying the 9,2 kb Pst I-BamH I FAgment of pDC20       pDC28     pSP329 carrying the 9,2 kb Pst I-BamH I FAgment of pDC20       pDC32     pSP329 carrying the 3,8 kb BamH I-Pst I FAg     This work       pSP329 carrying the 3,8 kb BamH I-Pst I FAg     This work       pDC32     pDC29 fl coR I jPkfa jDC29 的 3,5 kb EcoR I -Pst I FAg     This work       pDC32     pDC29 fl coR I jPkfa jment of pDC29     pDC39     pDC39 jmg 2,5 kb EcoR I I -Pst I FAgment of pDC29       pMX87     pUC19 上克隆有 pDC29 的 0,6 kb EcoR I FAg     This work       pDC29     pSP329 jmg	pSP329	IncP cloning vector, Tc <sup>r</sup>	S. Porter , unpublished		
pHP45QKm         Ap', Km'; oriCoEl, source of the ΩKm interposon         [17]           pQ200(mp18)         自杀质粒 Gm', scaB Suicide plasmid         [18]           pPA67         pS7329 grar, macB Suicide plasmid         [2]           pB767         pS7329 grar, macB Suicide plasmid         [2]           pDC16 <i>M. B., japonicum 文库中分离到的能与 NGRPA2 互补的重组粘粒</i> This work           Recombinant cosmid plasmid isolated from <i>B. japonicum genomic</i> IIII           ibbrary and can complement with NGRPA2 strain         Fis work           pDC19         pS7329 ± D克健有 pDC16 b1 1 kb BamH I fragment of pDC16         This work           pDC20         pS7329 ± D克健有 pDC16 b1 kb BamH I fragment of pDC16         This work           pDC25         pS7329 carrying the 14 kb BamH I fragment of pDC20         This work           pS7329 carrying the 32 kb Bae I fragment of pDC20         PS7329 ± D克健有 pDC20 b19.5 kb Sae I fragment of pDC20         This work           pD229         pSP329 uzrying the 9.2 kb Pt1 - BamH I fragment of pDC20         This work         PDC29           pDC29         pSP329 uzrying the 4.8 kb BamH I - Pst I fragment of pDC20         This work         PDC29           pDC29         pSP329 uzrying the 4.8 kb BamH I - Pst I fragment of pDC20         This work         PDC29           pDC32         pDC29 fB EcoR I j#t/E 重连接         This work	pUC19	ColE1 cloning vector , Apr	[16]		
pJQ200(mp18.)     自系质粒 Gn', sac8 Suicide plasmid     [18.]       pPA67     pSN329 携带 Rm1021 的 ddA 基因(1.2 kb Kpn I -HindⅢ片段)     [9.]       pDC16     从 B. japonicum 文庫中分离到的能与 NGRPA2 互补的重组粘粒     This work       Recombinant cosmid plasmid isolated from B. japonicum genomic     library and can complement with NGRPA2 strain     This work       pDC19     pSN329 上克曜有 pDC16 的 1 kb BamH J 片段     This work       pSC20     pSN329 上克曜有 pDC16 的 14 kb BamH J 片段     This work       pDC20     pSN329 上克曜有 pDC16 的 14 kb BamH J 片段     This work       pDC25     pSN329 上克曜有 pDC20 的 5 kb Sac J 片段     This work       pS239 carrying the 14 kb BamH J fragment of pDC16     This work       pDC28     pSN329 上克曜有 pDC20 的 5 kb Sac J 片段     This work       pSN329 carrying the about 5 kb Sac I 片段     This work       pSN329 carrying the 9.2 kb Pst I -BamH I 片段     This work       pSN329 carrying the 9.2 kb Pst I -BamH I fragment of pDC20     PDC28       pDC29     pSN329 上克曜有 pDC20 的 4.8 kb BamH I -Pst I 片段     This work       pDC32     pDC29 fl 2.5 Lb fl apDC20 的 5.5 kb EcoR I -Pst I 片段     This work       pDC32     pDC29 fl 2.5 Lb fl apDC20 的 5.6 kb EcoR I -Pst I fl ApD     This work       pDC32     pDC39 fl apDC30 的 5.6 kb EcoR I Fl ApD     This work       pDC39     pDC39 fl apDC30 的 5.6 kb EcoR I fl ApD     This work       pDC32     pDC39 fl	pHP45ΩKm	$\mathrm{Ap}^{\mathrm{r}}$ , $\mathrm{Km}^{\mathrm{r}}$ ; oriColE1 , source of the $\Omega\mathrm{Km}$ interposon	[17]		
pPA67     p5P329 携带 Rm1021 的 bdh 基 基级 1.2 kb Kpn I-Hindll 广段)     [9]       pDC16     从 B. japonicum 文库中分离到的能与 NGRA2 互补的mail and the second plasmid isolated from B. japonicum genomic library and can complement with NGRPA2 strain     This work       pDC19     p5P329 上克隆有 pDC16 的 1 kb BamH I fragment of pDC16     This work       pDC20     p5P329 上克隆有 pDC16 的 1 kb BamH I fragment of pDC16     This work       pDC25     p5P329 urrying the 1 kb BamH I fragment of pDC16     This work       pDC28     p5P329 urrying the about 5 kb Sac I fragment of pDC20     This work       pDC29     p5P329 urrying the 2 kb Fst I-BamH I Fst     This work       pDC28     p5P329 urrying the 2 kb Fst I-BamH I Fst     This work       pDC29     p5P329 urrying the 4.8 kb BamH I-Pst I fragment of pDC20     This work       pDC29     p5P329 urrying the 4.8 kb BamH I-Pst I fragment of pDC20     This work       pDC32     pDC29 digested with EcoRI I then relegated     This work       pDC32     pDC29 fl EcoRI J 消化后 重连接     This work       pMX87     pUC19 上克隆有 pDC20 的 3.5 kb EcoR I -Pst I FtB     This work       pMX94     pUC19 urrying the 0.6 kb EcoRI I fragment of pDC29     This work       pUC19 urrying the 0.65 kb EcoRI I fragment of pDC29     This work     pUC19 urrying the 0.65 kb EcoRI I FtB     This work       pMX100     pUC19 urrying the 4.8 kb BamH I -Pst I FtB     This work     pUC19 urrying the 0.	pJQ200(mp18)	自杀质粒 Gm <sup>r</sup> , <i>sac</i> B Suicide plasmid	[ 18 ]		
pDC16 为 B is provided by the set of the se	pPA67	pSP329 携带 Rm1021 的 bdhA 基因( 1.2 kb Kpn [ -Hind     片段 )	[9]		
Recombinant Cosmid plasmid isolated from B. Japonicum genomic         bilinary and can complement with NGRPA2 strain         pDC19       pSP329 上克隆有 pDC16 的 1 kb BamH I 片段       This work         pDC20       pSP329 carrying the 1 kb BamH I fragment of pDC16       This work         pDC25       pSP329 carrying the 14 kb BamH I fragment of pDC16       This work         pDC25       pSP329 carrying the about 5 kb Sac I fragment of pDC20       This work         pDC28       pSP329 carrying the 9.2 kb Pst I -BamH I fragment of pDC20       This work         pDC29       pSP329 carrying the 9.2 kb Pst I -BamH I fragment of pDC20       This work         pDC29       pSP329 carrying the 9.2 kb Pst I -BamH I fragment of pDC20       This work         pDC29       pSP329 carrying the 4.8 kb BamH I -Pst I fragment of pDC20       This work         pDC32       pDC29 fl EcoR I jit/Lf <b>mither</b> This work         pDC29 digested with EcoR I then relegated       This work         pMX87       pUC19 上克隆有 pDC29 的 0.6 kb EcoR I -Pst I 片段       This work         pUC19 carrying the 0.5 kb EcoR I Fragment of pDC29       This work       pUC19 carrying the 0.6 kb EcoR I fragment of pDC29         pMX95       pUC19 上克隆有 pDC29 的 0.6 kb EcoR I Fragment of pDC29       This work       pUC19 carrying the 0.6 kb EcoR I fragment of pDC29         pMX100       pUC19 carrying the 0.4 kb BamH I -Pst I	pDC16	pSP329 carrying Rm1021 bdhA gene(1.2 kb Kpn I-Hind III fragment) 从 B. japonicum 文库中分离到的能与 NGRPA2 互补的重组粘粒	This work		
pDC20       pSP329 上克隆有 pDC16 的 14 kb BamH I Fragment of pDC16       pSP329 正克隆有 pDC20 的 14 kb BamH I 片段       This work         pDC25       pSP329 上克隆有 pDC20 的 5 kb Sac I 片段       This work         pDC28       pSP329 carrying the about 5 kb Sac I fragment of pDC20       This work         pDC28       pSP329 carrying the 9.2 kb Pst I - BamH I 片段       This work         pDC29       pSP329 L克隆有 pDC20 的 9.2 kb Pst I - BamH I 片段       This work         pDC29       pSP329 carrying the 4.8 kb BamH I -Pst I 片段       This work         pDC32       pDC39 digested with EcoR I then relegated       This work         pMX87       pUC19 上克隆有 pDC20 的 0.6 kb EcoR I -Pst I 片段       This work         pUC19 carrying the 0.6 kb EcoR I Fragment of pDC29       This work       pUC19 L克隆有 pDC29 的 0.6 kb EcoR I Fragment of pDC29         pMX94       pUC19 carrying the 0.6 kb EcoR I Fragment of pDC29       This work       pUC19 L克隆有 pDC29 的 0.6 kb EcoR I Fragment of pDC29         pMX95       pUC19 上克隆有 pDC29 的 0.6 kb EcoR I Fragment of pDC29       This work       pUC19 carrying the 0.65 kb EcoR I Fragment of pDC29         pMX100       pJQ2000 mp18 )上克隆有 pDC29 的 4.8 kb BamH I -Pst I Fragment of pDC29       This work       pUC19 carrying the 4.8 kb BamH I -Pst I Fragment of pDC29         pMX106       pMX100 fiserted ΩKm at the EcoR I fragment of pDC29       This work       pUC19 L克隆有 pDC29 的 4.8 kb BamH I -Pst I Fragment of pDC29<	pDC19	Recombinant cosmid plasmid isolated from <i>B</i> . <i>japonicum</i> genomic library and can complement with NGRPA2 strain pSP329 上克隆有 pDC16 的 1 kb BamH I 片段	This work		
pDC25       pSP329 carrying the 14 kb Bam H I fragment of pDC16         pDC25       pSP329 上克隆有 pDC20 的约 5 kb Sac I 片段       This work         pDC28       pSP329 carrying the about 5 kb Sac I fragment of pDC20       This work         pDC29       pSP329 上克隆有 pDC20 的 9.2 kb Pst I -Bam H I 片段       This work         pDC32       pDC29 hEcoR I 消化后 重连接       This work         pDC32       pDC29 digested with EcoR I then relegated       This work         pMX87       pUC19 上克隆有 pDC29 的 3.5 kb EcoR I -Pst I 片段       This work         pUC19 上克隆有 pDC29 的 0.6 kb EcoR I -Pst I 片段       This work         pWX94       pUC19 上克隆有 pDC29 的 0.6 bb EcoR I F4Q       This work         pUC19 carrying the 0.6 kb EcoR I F4Q       This work       This work         pMX94       pUC19 上克隆有 pDC29 的 0.6 bb EcoR I F4Q       This work         pUC19 carrying the 0.65 kb EcoR I fragment of pDC29       This work       pUC19 carrying the 0.65 kb EcoR I F4Q         pMX100       pJQ200 mp18 上克隆有 pDC29 的 0.6 bb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.65 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.65 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 4.8 kb BamH I -Pst I 片段       This work         pUC19 carrying the 4.8 kb Bam H I -Pst I 片段       This work <t< td=""><td>pDC20</td><td>pSP329 carrying the 1 kb BamH 1 fragment of pDC16 pSP329 上克隆有 pDC16 的 14 kb BamH I 片段</td><td>This work</td></t<>	pDC20	pSP329 carrying the 1 kb BamH 1 fragment of pDC16 pSP329 上克隆有 pDC16 的 14 kb BamH I 片段	This work		
pDC28       pSF329 上克隆有 pDC20 的 9.2 kb Pst I -BamH I 片段       This work         pDC29       pSP329 上克隆有 pDC20 的 9.2 kb Pst I -BamH I fragment of pDC20       This work         pDC29       pSP329 carrying the 9.2 kb Pst I -BamH I fragment of pDC20       This work         pDC32       pDC29 L克隆有 pDC20 的 4.8 kb BamH I -Pst I fragment of pDC20       This work         pDC32       pDC29 用 EcoR I 消化后 重连接       This work         pDC32       pUC19 上克隆有 pDC29 的 3.5 kb EcoR I -Pst I 片段       This work         pUC19 carrying the 3.5 kb EcoR I -Pst I fragment of pDC29       This work         pUC19 carrying the 0.6 kb EcoR I -Pst I fragment of pDC29       This work         pUC19 carrying the 0.6 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.6 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.6 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.6 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.6 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.6 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.65 kb EcoR I fragment of pDC29       This work         pMX100       pJQ200( mp18 ) 上克隆有 pDC29 的 4.8 kb BamH I -Pst I 片段       This work         pJQ200( mp18 ) carrying the 4.8 kb BamH I -Pst I fragment of pDC29       This work	pDC25	pSP329 carrying the 14 kb BamH I fragment of pDC16 pSP329 上克隆有 pDC20 的约 5 kb Sac I 片段	This work		
pSP329 carrying the 9.2 kb Pst I -BamH I fragment of pDC20       This work         pDC29       pSP329 上克隆有 pDC20 的 4.8 kb BamH I -Pst I 片段       This work         pSP329 carrying the 4.8 kb BamH I -Pst I fragment of pDC20       pDC32       pDC29 用 EcoR I 消化后 重连接       This work         pDC32       pDC29 用 EcoR I 消化后 重连接       This work       pDC29 digested with EcoR I then relegated       This work         pMX87       pUC19 上克隆有 pDC29 的 3.5 kb EcoR I -Pst I 片段       This work         pUC19 上克隆有 pDC29 的 0.6 kb EcoR I -Pst I 片段       This work         pUC19 上克隆有 pDC29 的 0.6 kb EcoR I /Fst I fragment of pDC29       This work         pMX94       pUC19 上克隆有 pDC29 的 0.6 kb EcoR I /Fst I       This work         pUC19 上克隆有 pDC29 的 0.6 kb EcoR I /Fst I       This work       This work         pMX95       pUC19 上克隆有 pDC29 的 0.65 kb EcoR I /Fst I       This work         pUC19 carrying the 0.65 kb EcoR I /Fst I fragment of pDC29       This work       This work         pMX100       pJQ200 (mp18 )上克隆有 pDC29 的 4.8 kb BamH I -Pst I /Fst I /Fst I fragment of pDC29       This work         pMX106       pMX100 fa bdhA 基因 ORF 内的 EcoR I 位点插入了 ΩKm       This work         pMX107       pUC19 上克隆有 pDC29 的 1.0 kb Sal I /Fst       This work         pUC19 上克隆有 pDC29 的 1.0 kb Sal I /Fst       This work	pDC28	pSP329 carrying the about 5 kb Sac I fragment of pDC20 pSP329 上克隆有 pDC20 的 9.2 kb Pst I -BamH I 片段	This work		
pDC32       pDC29 用 EcoR I 消化后 重连接       This work         pDC29 digested with EcoR I then relegated       pUC19 上克隆有 pDC29 的 3.5 kb EcoR I -Pst I 片段       This work         pMX87       pUC19 上克隆有 pDC29 的 3.5 kb EcoR I -Pst I 片段       This work         pUC19 carrying the 3.5 kb EcoR I -Pst I fragment of pDC29       This work         pMX94       pUC19 上克隆有 pDC29 的 0.6 kb EcoR I 片段       This work         pUC19 carrying the 0.6 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.65 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.65 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.65 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.65 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.65 kb EcoR I fragment of pDC29       This work         pUQ200 mp18 )上克隆有 pDC29 的 4.8 kb BamH I -Pst I 片段       This work         pJQ200 mp18 )carrying the 4.8 kb BamH I -Pst I fragment of pDC29       This work         pMX106       pMX100 ta bdhA 基因 ORF 内的 EcoR I data入了 ΩKm       This work         pMX107       pUC19 上克隆有 pDC29 的 1.0 kb Sal I 片段       This work         pUC19 carrying the 1.0 kb Sal I fragment of pDC29       This work	pDC29	pSP329 carrying the 9.2 kb Pst 1 -BamH 1 fragment of pDC20 pSP329 上克隆有 pDC20 的 4.8 kb BamH 1 -Pst 1 片段	This work		
pMX87       pUC19 上克隆有 pDC29 的 3.5 kb EcoR I -Pst I 片段       This work         pUC19 carrying the 3.5 kb EcoR I -Pst I fragment of pDC29       This work         pMX94       pUC19 上克隆有 pDC29 的 0.6 kb EcoR I 片段       This work         pUC19 carrying the 0.6 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.6 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.6 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.65 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.65 kb EcoR I fragment of pDC29       This work         pUC19 carrying the 0.65 kb EcoR I fragment of pDC29       This work         pMX100       pJQ200(mp18) 上克隆有 pDC29 的 4.8 kb BamH I -Pst I 片段       This work         pJQ200(mp18) carrying the 4.8 kb BamH I -Pst I fragment of pDC29       This work         pMX106       pMX100 在 bdhA 基因 ORF 内的 EcoR I data入了 ΩKm       This work         pMX107       pUC19 上克隆有 pDC29 的 1.0 kb Sal I 片段       This work         pUC19 carrying the 1.0 kb Sal I fragment of pDC29       This work       This work	pDC32	pDC29 用 EcoR I 消化后 重连接	This work		
pMX94 pUC19 上克隆有 pDC29 的 0.6 kb EcoR I 片段 This mork pUC19 上克隆有 pDC29 的 0.6 kb EcoR I 片段 This work pUC19 carrying the 0.6 kb EcoR I 片段 This work pUC19 上克隆有 pDC29 的 0.65 kb EcoR I 片段 This work pUC19 carrying the 0.65 kb EcoR I fragment of pDC29 pMX100 pJQ200 mp18 上克隆有 pDC29 的 4.8 kb BamH I -Pst I 片段 This work pJQ200 mp18 ) carrying the 4.8 kb BamH I -Pst I 片段 This work pJQ200 mp18 ) carrying the 4.8 kb BamH I -Pst I fragment of pDC29 pMX106 pMX100 在 bdhA 基因 ORF 内的 EcoR I 位点插入了 ΩKm This work pMX107 pUC19 上克隆有 pDC29 的 1.0 kb Sal I 片段 This work pUC19 carrying the 1.0 kb Sal I fragment of pDC29	pMX87	pUC19 由gested with Ecold I then relegated pUC19 上克隆有 pDC29 的 3.5 kb Ecold I -Pst I 片段 pUC19 correction the 3.5 kb Ecold I -Pst I fragment of pDC29	This work		
pMX95 pUC19 上克隆有 pDC29 的 0.65 kb EcoR I 片段 This work pUC19 carrying the 0.65 kb EcoR I 片段 This work pUC19 carrying the 0.65 kb EcoR I fragment of pDC29 pMX100 pJQ200 mp18 )上克隆有 pDC29 的 4.8 kb BamH I -Pst I 片段 This work pJQ200 mp18 )carrying the 4.8 kb BamH I -Pst I fragment of pDC29 pMX106 pMX100 在 bdhA 基因 ORF 内的 EcoR I 位点插入了 ΩKm This work pMX100 inserted ΩKm at the EcoR I site within bdhA ORF pUC19 上克隆有 pDC29 的 1.0 kb Sal I 片段 This work pUC19 carrying the 1.0 kb Sal I fragment of pDC29	рМХ94	pUC19 L 在Fying the 9.5 kb EcoR I -1 st I Hagment of pDC29 pUC19 上克隆有 pDC29 的 0.6 kb EcoR I 片段 pUC19 carrying the 0.6 kb EcoR I fragment of pDC29	This work		
pMX100 pJQ20Q mp18 )上克隆有 pDC29 的 4.8 kb BamH I -Pst I 片段 This work pJQ20Q mp18 )上克隆有 pDC29 的 4.8 kb BamH I -Pst I 片段 This work pJQ20Q mp18 ) carrying the 4.8 kb BamH I -Pst I fragment of pDC29 pMX106 pMX100 在 bdhA 基因 ORF 内的 EcoR I 位点插入了 ΩKm This work pMX100 inserted ΩKm at the EcoR I site within bdhA ORF pUC19 上克隆有 pDC29 的 1.0 kb Sal I 片段 This work pUC19 carrying the 1.0 kb Sal I fragment of pDC29	рМХ95	pUC19 上克隆有 pDC29 的 0.65 kb EcoR I 片段	This work		
pMX106 pMX100 在 bdhA 基因 ORF 内的 EcoR I 位点插入了 ΩKm This work pMX100 inserted ΩKm at the EcoR I site within bdhA ORF pUC19 上克隆有 pDC29 的 1.0 kb Sal I 片段 This work pUC19 carrying the 1.0 kb Sal I fragment of pDC29	pMX100	pJQ200(mp18)上克隆有 pDC29的4.8 kb BamHI-PstI片段 pJQ200(mp18)carrying the 4.8 kb BamHI-PstIfragment of pDC29	This work		
pMX107 pUC19 上克隆有 pDC29 的 1.0 kb Sal [ 片段 This work pUC19 carrying the 1.0 kb Sal [ 片段 This work	pMX106	pMX100 在 $bdhA$ 基因 ORF 内的 EcoR I 位点插入了 $\Omega$ Km pMX100 inserted $\Omega$ Km at the EcoR I site within $bdhA$ ORF	This work		
	pMX107	pUC19 上克隆有 pDC29 的 1.0 kb Sal I 片段 pUC19 carrying the 1.0 kb Sal I fragment of pDC29	This work		

# 2 结果与分析

# 2.1 基因组文库的构建及 bdhA 基因的获得

**2.1.1** *B. japonicum* USDA110 基因组文库的构 建 在含 T<sub>c</sub> 的选择平板上共获得 3 162 个带有重

组粘粒的菌落;随机挑取 10 个菌落,提取质粒 DNA,EcoR [ 酶切检查,各重组粘粒所带外源片段 的平均大小为 12.5 kb。

2.1.2 能与 *bdh*A 突变体功能互补的克隆的获得 以 MT616 为辅助,通过三亲接合将 BjUSDA110 基

因组文库分别引入 Rm11107(*bdh*A1::Tn5),根瘤 菌 NGRPA2(*bdh*A1::ΩSmSp)。将菌液涂布于以 R-HB 为唯一碳源的 M9 培养基(M9-R-HB)上, 30℃培养5d。结果从 NGRPA2 接合组平板上获得 12 个菌落;而从 Rm11107 接合组平板上未得到菌 落。

从 NGRPA2 接合组中提取质粒, BamHI 酶切、 电泳后证实含有一重组质粒 pDC16。随后将 pDC16 引入 Rm11107 后证实能与 Rm11107 互补,使 Rm11107 恢复利用 R-HB 作为唯一碳源的能力(即 表型为 Hbu<sup>+</sup>)。

用 BamHI 酶切 pDC16 后,获得 3 个片段,大小 分别为 23 kb、14 kb 和 1 kb。 23 kb 片段经分析是 载体 pLAFR1 携带部分外源片段(pLAFR1 缺少 BamH I 切点 )。1 kb 和 14 kb 片段分别被克隆到 IncP 克隆载体 pSP329 上,获得质粒 pDC19 和 pDC20。

通过接合分别将 pDC19、pDC20 引入 Rm11107 和 NGRPA2 受体菌,只有携带 pDC20 的菌株 (Rm11107/pDC20,NGRPA2/pDC20)可以利用 R-HB作为唯一碳源而生长,这表明 bdhA 基因位于质 粒 pDC20 的 14 kb BamH [ 片段上。

为缩小 bdhA 基因所在的范围(互补片段的大小),试用多种限制性内切酶消化 pDC20(图1)。将 有关片段克隆到载体 pSP329,所得重组质粒通过接 合引入 Rm11107,然后测定在 M9-R-HB 上的生长 能力。结果,5 kb Sac I 片段(pDC25)和4.8 kb BamHI-Pst I 片段(pDC29)仍然保留与 Rm11107 互补的能力(图2)。4.8 kb BamHI-Pst I 片段是所 得到的最小互补片段。pDC25 和 pDC29 作为进一 步研究的材料。

2.2 bdhA 基因存在的证实

2.2.1 Bdh 酶活测定 pDC25、pDC29 经接合引入 bdhA 突变体菌株 Rm11107 后分别测定其 Bdh 活 性;结果表明,Rm11107/pDC25、Rm11107/pDC29 具有明显的 Bdh 活性(表 2),而阴性对照 Rm11107 不具 Bdh 酶活力。

2.2.2 Southern Blot 以 DIG 标记的 pPA67 的 1.2 kb Kpn I -Hind III 片段(携带 Rm1021 bdhA 基 因的完整序列)<sup>9</sup>作为探针进行杂交,结果显示, pDC29 的 4.8 kb BamH I -Pst I 带呈阳性(图 3 )。 此结果进一步证明,pDC29 的 4.8 kb BamH I -Pst I 片段上存在 bdhA 基因。

2.3 bdhA 基因序列及 Bdh 的氨基酸序列



M:标准(1 kb ladder); A-I:pDC20分别用 BamH [、Pst [、BamH ] + Pst [、BamH I + Pst I + Sac [、Sac I + BamH ]、Sac [、BamH I I + EcoR I、BamH I + Pst I + EcoR I、EcoR I 酶切 M:Market(1 kb ladder); A-I:pDC20 digested with BamH[, Pst], BamH I+ Pst[, BamH[+ Pst] + Sac], BamH[+ Sac], Sac], BamH[+ EcoR], BamH[+ Pst] + EcoR], EcoR], respectively

#### 图 1 pDC20 限制性酶切

Fig.1 Restricted digestion of pDC20



B, P, S 分别代表 BamHI, PstI, SacI

B, P, S represents BamHI, PstI, SacI respectively

图 2 pDC20 的不同片段及其互补能力

Fig. 2 pDC20 fragments and their complementing abilities

#### 表 2 Bdh 酶活测定结果

Table 2 Bdh activity assay result

菌株 Strain	Bdh 活力 Bdh activity nmol·min <sup>-1</sup> ·mg <sup>-1</sup> protein
Rm11107/pDC25	$32.12 \pm 3.2$
Rm11107/pDC29	$35.05 \pm 2.9$
Rm11107	-
Rm1021	$41.32 \pm 4.0$

2.3.1 *bdh*A 基因序列的测定 为减少测序的工作 量和费用,需进一步确定 *bdh*A 基因所在的位置;为 此对 pDC29 4.8 kb BamH [-Pst ] 片段试用不同的 限制性内切酶消化处理并进一步亚克隆。如图 4 所 示 pDC29 的 BamH [-Pst ] 片段上有 3 个 EcoR ] 切点和 2 个 Sal I 切点。pDC29 经 EcoR I 消化后重 新连接,获得了含 3.5 kb EcoR I-Pst I 片段的质粒 pDC32。



M: λ/ HindⅢ; A: pPA67 用 Kpn I 和 HindⅢ 酶切; B: pDC29 用 BamH I 和 Pst I 酶切

 $M:\lambda/ Hind [] ; A:pPA67 \ digested \ with \ Kpn \ I \ and \ Hind []] ; B:pDC29 \ digested \ with \ BamH \ I \ and \ Pst \ I$ 

#### 图 3 pDC29 DNA Southern 杂交图

Fig. 3 pDC29 DNA Southern blot



B, E, S, P分别代表 BamHⅠ, EcoRⅠ, SalⅠ, PstⅠ

B , E , S , P stand for  $BamH\,I\,$  ,  $EcoR\,I\,$  ,  $Sal\,I\,$  ,  $Pst\,I\,$  respectively

图 4 4.8 kb BamH I-Pst I 片段的物理图及其亚克隆

Fig. 4 Physical map and subclones of 4.8 kb BamH I -Pst I fragment

通过接合将 pDC32 引入 Rm-11107 得 Rm-11107/pDC32 划线接种于 M9-R-HB 平板上测定其 Hbu表型。结果 Rm11107/pDC32 不能利用 R-HB 作为唯一碳源生长,即 Hbu<sup>-</sup>。酶活测定证明 Rm11107/pDC32 无 Bdh 活性。这说明 3.5 kb EcoR I-Pst I 片段中的切点 EcoR I,要么位于 bdhA 基因之外,要么破坏了 bdhA 基因的阅读框架。

根据上述结果,分别将 pDC29 上的 0.6 kb

EcoR I 片段、0.65 kb EcoR I 片段、3.5 kb EcoR I-Pst I 片段以及 1.0 kb Sal I-Sal I 片段克隆到 pUC19 载体上,分别获得 pMX94、pMX95、pMX87 和 pMX107 然后分别进行测序。

pMX95、pMX87 序列经 BLASTx 检索,分别具 有 bdhA 部分序列 将两部分序列结合后,得到完整 bdhA 序列。但序列的后半部分还未得到互补链序 列,为此,用软件" Primer3 "设计一引物 5' cgccattegaagtectacte 3',再以 pMX107 DNA 为模板进行测 序,如此获得 bdhA 全部双链序列,见图 5。 B. japonicum USDA110 bdhA 基因开放阅读框架由 789 bp 组成,GC 含量 63.6%, AT 含量 36.4%;该 基因序列在 GenBank 的登记号为 AY077581。

2.3.2 推导出的 BdhA 氨基酸序列 推导出的 Bd-hA 氨基酸序列见图 5,由 262 个氨基酸残基组成, 分子量 27.59 ku。比较发现其与 *Mesorhizobium loti* 的 Bdh 的相似性最高,二者都由 262 个氨基酸 组成,两者间完全相同和相似的氨基酸分别达 72% 和 81%。

2.4 B. japonicum bdhA 突变体的构建与鉴定

2.4.1 *B. japonicum bdh*A 突变体的构建 首先将 pDC29 的 BamH []-Pst [] 片段克隆到自杀质粒 pJQ200(mp18)<sup>18]</sup> 获得 pMX100 ;然后用 EcoR [] 对 pMX100 质粒 DNA 进行部分酶切。再与 pHP-45 $\Omega$ Km 的 EcoR [] 片段 interposon )进行连接 ,在 LB + Gm10Km20 平板上筛选 ,获得 162 个克隆 ;随机 挑取部分克隆 ,提取质粒 DNA ,酶切、电泳检查 ,获得 pMX106 经多种酶切证实系在 *bdh*A 基因开放阅 读框内的 EcoR [] 位点正确插入了 interposon  $\Omega$ Km ;  $\Omega$ Km 的插入破坏了 *bdh*A 基因的阅读框。

将 pMX106 质粒 DNA 转化感受态 S17-1 细胞, 获得 S17-1/pMX106, 然后与 B. japonicum US-DA110 进行双亲接合,将接合后的菌悬液涂布于 YMA+Cm10Km40 平板上培养 6~7 d。

结果在接合组 8 个平板上共得到 626 个菌落, 对照组 S17-1/pMX106 和 B.jUSDA110 平板上均无 菌落出现。

将上述菌落接于 TY + Cm10Km20 + 5% 蔗糖 平板上,测定菌落在含 5% 蔗糖的培养基上的生长 情况。结果约 4.8% 的被试菌落可在含 5% 蔗糖的 TY 培养基上生长。这些菌落再分别划线接种于成 对的 TY + Gm30 和 TY 平板上测定其对 Gm 的敏 感性;结果表明,大部分(约 76.5%)能在 TY + Cm10Km20 + 5% 蔗糖培养基上生长的菌落对Gm

1	tct	acc	gcaį	g ca	aga	attac	ga	atec	tcg	t cca	aagg	gact	a tga	attte	ctcg	cac	gtg	gcc	a				60
61	tg	gtcg	age	a ttg	ggg	aage	c g	gcgt	gcg	cga	acgt	gca	tct g	tcg	atge	gc	aca	ag	gac	a			120
121	gg	ctcs	gate	a gc	cgc	aatc	c g	gcg	agao	ca	tggt	gac	cta c	gat	ctca	icg	ggg	gac	gtc	t			180
181	cc	gcg	ccc	cc g	gca	aaaa	gg a	age	gaat	aga	ATG	kar	AGT	ста	TCA	30C /	AG.	AAC	GCC	GT	с		240
1											м	G	s	L	s	G	ĸ	N		N	6.3	0	
241	GT	GAC	CGG	A TC	GAC	C AGO	001	ATC	GGG	CTC	GCC	TAT	306 0	GT C	юст	TCO	000	cad	XCC (	GT			300
11	v	т	G	s	т	-5	0	1	G	L	Α	Y	۸	R	A	F	A		р	G		0	
301	GC	CAA	C GT	CGT	C AT	C AAG	GGG	: 110	GGG	TCC	GCC	GA	GAG	ATO	GAG	AA	GA	ACC	T O	CG.	AAG		360
31	٨	N	٧	v	1	N	G	F	G	5	A	E	D	1	E	κ	1	£	R	٨	к	50	
361	ATC	GAG	000	GAG	: TT	GOC	GGG		5 GC	G AT	C TAC	тсо	ccc	GCC	GAC	ATG	ACC	-	o co	GG	CC		420
51	1	E	A	D	F	G	G	K	۸	1	Y	s	P	A	D	M	т	K		Р	A	70	
421	GA	ATC	COC	GOO	ATC	ATC	GCG	CTC	GGC	GAG	-	ACC	TTC	000	TCG	GTC	GAG	OT	c ct	c d	TC		480
71	Е	1	۸	G	м	1	٨	L	G	E	ĸ	т	F	G	s	v	D	V	L		v	90	
481	AA	C AAT	GCC	GGC	ATC	CAG	TIC	GTC	TCG	CCG	ATC	GAG	GAA	ттс	CCG	CCG	GAC		A TO	ig (	AC		540
91	N	N	۸	G	1	Q	F	۷	s	P	τ	ŧ.	E	F	p	P	Е	к	1	W	D	110	
541	CA	ATC	ATC	GCG	ATC	AAC	CTG	TCC	TCG	GCC	TIC	CAT	GCC /	TT C	GC	CC C	CG (	ото	ccc	GG	с		600
111	Q	1	1	A	1	N	L.	s	s	٨	F	н	A	t	R	A	A	v	P	G		130	
601	ATC	AAC		G AAC	00	C TGO	001	r co	C ATC	ATC	AAC	ACC	GCG	TCC	GCC	CAG	TCO	CT	G G1	ic o	юc		660
131	м	ĸ	ĸ	ĸ	G	W	/ G	R	1	1	N	т		. S		H		s	L	٧	۸	150	1
661	TCC	CCC	TTC	AAG	TCG	OCC	TAC	GTC	TCG	GCC	AAG	CAC	GGC	ATC	OCC	GGT	CTT	ACC	440	A	C		720
151	5	P	E	к	s	Α	Y	v	s	٨	ĸ	н	G	1	A	0	L	т	ĸ		т	170	
721	GTC	GCC	CTC	GAA	OTO	C OCO	ACC	CAC	AAC	ATC	ACC	TOC	AAC	TGC	ATC	AGC	ccc	GGG	C TA	r G	rc	1	78D
171	٧	A	L	E	٧	۸	т	н	к	1	т	с	N	С	υ	s	P.	G	Y	٧		190	
781	TGG	ACG	CCG	CTG	GTC	GAG	AAG	CAG	ATC	CCC	GAC	ACG	ATG	AAG	GCG	CGC	AAT	CTC	AC	G CO	т		840
191	w	т	P	L.	۷	Ε	ĸ	Q	1	P	D	τ	м	к	۸	R	N	L	. 1		R	210	1
841	GAC	GAO	GTC	ATC	AAC	GAC	GTG	CTG	CIC	GAC	GCC	CAG	CCG	ACC	AAG	GAG	TTC	GT	C AC	CT	CC	2	900
211	D	E	V	1	N	D	٧	L	L	D	A	Q	p	т	κ	E	F	1	1	r.	s	230	)
901	GAC	CAO	GTC	OCC	OCA	CTO	GCG	CIG	TTC	CTG	TOC	AOC	GAC	GAT	OCC	oco	CAG	ATC	: 40	C G	OC		960
231	E	Q	V	Α	A	ι.	۸	L.	F	L	с	s	D	D	Α	Α	Q	1	т		G	250	D
961	ACC	AAC	стс	TCG	ATC	GAC	GGC	GGC	TGG	ACO	oco	GAC	TAG		gac	ttcg	ants	gc	gtaj	gg	gtg	1	020
251	т	N	L	S	1	D	G	G	w	т	۸	8	•									26	2
021	gg	caaa	iggo	gcg	tccį	gtgg	cccc	cgco	cata	cttte	ctcc	gtat	gtcg	gaa	ggc	ggt	ggc	gcg	£			1	080

#### 方框 :起始密码子 :黑体 :EcoR ] 酶切位点 ;方框带阴影 终止密码子 ,阴影 :引物匹配区域

Square : start code ; blacken square : stop code ; boldtype : EcoR I cut site ; blacken part : sequence complementing with primer

图 5 B. japonicum USDA110 bdhA 基因序列及推导的 BdhA 氨基酸序列

Fig. 5 Nucleotide and deduced amino acid sequences of bdhA gene of Bradyrhizobium japonicum

敏感。如此筛选到的对 Gm 敏感的菌株被认为是 bdhA 突变体,命名为菌株 Bj30064。

3 期

2.4.2 Southern Blot 证实 *B*. *japonicum bdh*A 突变体 分别制备 Bj30064 和野生菌株 BjUSDA110 的 总 DNA 经 BamH I 酶切、电泳、转膜后 ,用 DIG 标 记的 pDC29 的 4.8 kb BamH I -Pst I 片段作探针进 行 Southern blot。结果 野生型 BjUSDA110 在约 14 kb 处出现 1 条阳性带,而 Bj30064 则分别于约 12.5 kb 和 1.5 kb 处出现 2 条阳性带(图 6) 这可解释为 14 kb BamH I 片段被 interposon  $\Omega$ Km 于 EcoR I 处 打断(interposon  $\Omega$ Km 也可被 BamH I 从两端切 开),这与预计的结果完全相符。

2.4.3 突变体 Bdh 酶活测定 野生型菌株 BjUS-DA110 和突变体菌株 Bj30064 分别于 YMB 中 30℃, 200 r/min 摇瓶培养 5 d,收集菌体,超声波破碎制备 无细胞提取液,测定 Bdh 酶活性。结果 Bj30064 培养 物的无细胞提取液未测得 Bdh 活性,而野生型菌株 BjUSDA110 测得明显的 Bdh 活性(表 3)。

2.4.4 植株试验 植株试验共设3个重复试验组, 每试验组3个处理,分别为接种野生型菌株 BjUS-DA110、接种突变体菌株 Bj30064 和不接种(以等体 积 ddH<sub>2</sub>O 代替菌液);每处理3盆,每盆3株。结 果,未接菌的阴性对照组的植株培养至第18天开始 明显变黄,生长缓慢;根部无根瘤。接种 BjUS-DA110和 Bj30064的试验组植株外观健壮,生长较 快,根部明显结瘤。突变体接种组和野生型菌株接 种组各测定指标统计分析未显示显著区别;而二者 与阴性对照组之间差别显著(表4)。



36卷

0.6kb — ())

M:\/ HindⅢ;A:BjUSDA110 总 DNA,BamHⅠ酶切;B:Bj30064 总 DNA,BamHⅠ酶切

 $\label{eq:main_state} \begin{array}{l} M: \mathcal{W}Hind \end{array} ; A: Genomic DNA of BjUSDA110 digested with BamH \\ I ; B: Genomic DNA of Bj30064 digested with BamH I \end{array}$ 

#### 图 6 bdhA 突变体和野生型菌株总 DNA Southern blot

Fig. 6 Southern blot of *bdh*A mutant and wild-type strain genomic DNA

#### 表4 植株试验结果

Table 4 Plant test result

#### 表 3 Bdh 酶活性测定结果

Table 3 Bdh activity assay result

菌株 Strain	相关特征 Relevant characteristic	Bdh 活性 Bdh activity (nmolN・min <sup>-1</sup> ・ mg <sup>-1</sup> protein)
BjUSDA110	野生株 Wild strain	41.12 ± 3.6
Bj30064	bdhA 突变株 Mutant	-

# 3 讨论

NGRPA2 菌株系快生型根瘤菌(*Rhizobium* spp.)NGR234 的 *bdh*A 突变体(*bdh*A::ΩSmSp), *bdh*A 基因突变后,菌株不能合成 3-羟基丁酸脱氢 酶,不能将 3-羟基丁酸分解成乙酰乙酸进而进入三 羧酸循环代谢产能,因此不能以 R-HB 为唯一碳源 而生长。利用该特性,以其为受体菌,笔者成功地从 *B.japonicum* USDA110 菌株的基因文库中克隆到 *bdh*A 基因,证明采用快生型根瘤菌突变体菌株筛选

菌株 Strain	每株瘤数 Nodule number per plant	每株瘤重 Average weight per plant (mg)	平均单瘤重 Average weight per nodule ( mg )	植株平均干重 Average shoot dry weight per plant(g)
Bj USDA110	38.80	396.70	10.22	3.625
Bj30064	34.89	379.98	10.89	3.426
CK( - )	-	-	-	0.886

克隆 B. japonicum 某些基因是可行的,该方法具有 较好的重复性。

Aneja 和 Charles<sup>[9]</sup>报道在 Rhizobium meliloti 中, bdhA 基因和 xdhA 基因组成 bdhA-xdhA 操纵 子, xdhA 位于 bdhA 的下游,与 bdhA 共用一个启动 子。bdhA 和 xdhA 共存的意义还不清楚,推测可能 与碳饥饿和氮饥饿间的生理关联性有关。笔者分别 测定了 B. japonicum USDA110 bdhA 基因的部分 上游序列(0.9 kb)和下游序列(0.6 kb),均未发现 xdhA 基因序列的存在,说明在 B. japonicum 中 bdhA 基因并不像 Rhizobium meliloti 那样与 xdhA 基因组成操纵子。

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