毛萼田菁茎瘤菌被种子浸提物诱导表达基因的筛选及其功能

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摘要:【目的】选择毛萼田菁茎瘤菌为研究对象,筛选毛萼田菁茎瘤菌在菌植互作早期被宿主植物种子浸提物诱导表达的基因。【方法】采用新颖的基于抗性的活体表达筛选技术,以种子浸提物为诱导物,筛选获得能够稳定被种子浸提物诱导的突变株。通过中间片断融合报告基因的方法研究信号分子对相关基因的诱导情况。【结果】随机引物产物测序及网上结果表明,被诱导表达的基因为RT=H家族的成员。而且,对另外A个属根瘤菌中*./0基因的研究发现,它们都可以被相应的宿主植物种子浸提物诱导表达。此外,这四株根瘤菌的*./0突变株都表现对刀豆氨酸极为敏感。本文首次报道了*./0家族在根瘤菌中的生理功能。【结论】我们推测:RT=H家族蛋白可能广泛存在于根瘤菌中,并在根瘤菌与宿主植物的早期相互作用中发挥重要作用。
### 1.1.2

- **E. coli**
  - LB
  - Sm
  - Km
  - Ap

- **Km**
  - 50 μg/mL

- **E. coli SM10Ap**
  - pJZ260

#### 1.1.3

- **TaKaRa Fermentas ONPG**
- **IPTG**
- **isopropyl-β-D-thiogalactoside**
- **Sigma Taq DNA**
- **PCR Promega PCR DNA**

### 1.2

- **SE**
- **40 g**
- **70%**
- **100 mL**
- **28 °C**
- **20 °C**

### 1.3

- **Azc0**
- **E. coli SM10Ap**
- **pJZ260**

### 1.4

- **Mariner**
- **Plasmids**
- **Km**
- **Sm100**
- **Km40**
- **SE**
- **YVL**
- **T7**

### 1.5

- **Azc2**
- **50 μg/mL**
- **YVL**
- **SE**
- **8 h**
- **OD**

### 1.6

- **PCR**
- **Arbitrary PCR**
- **137-15''-C-
AGGGACACGGATTTAT-3' 137-2 5'-CTTCCGTC-ACAGGTAGGCCG-3' Arbitrary PCR
137-2 Km-2 DNA
CloneManager Professional Suite DNA sequence
BLAST translated query vs. protein database BlastX

1.7 asiE ssiE msiA rsiE-lacZ

asiE 450 bp EcoRI Xba I PCR pVIK112 Aze0 Aze0 pAze1 asiE Aze0 pAze1 LacZ-out pAze1

1.8 β- 8

1.9 CAN 9

2

2.1

Fig. 1 Growth curve of asiE insertion mutant with seed exudate SE.

2.2 mariner

Aze2 DNA PCR Aze0 5' 3' 800 bp 800 bp DNA

GenBank

Azorhizobium caulinodans ORS571 L- asie 131 bp 132 bp

HMZ0 msiA 56%

2.3 asiE

CZBAU3306 msiA msiA 56% CAN

NCBI BlastX

Sinorhizobium sp. 1128 rsiE asie 55% 54%

lacZ asie CAN

Fig. 2 lysE expression induced by CAN in different Rhizobia species.
three basic amino acids and other amino acids reported to induce gene expression cannot induce it. Moreover, in the four rhizobia, the same homologous genes can be induced to a different extent. By Fig. 2, we can see that the baseline expression level of this class of genes is very low, while the induction ability reaches about 26 times in UW2000.

Discussion

This study adopted a new antibiotic-based live expression screening technology to screen a gene that can be induced by the crude extract in the Caesalpina decapetala nodule bacterium, and compared it with a homologous gene. Through similarity prediction, the protein was found to have six hydrophobic regions, but only five transmembrane regions, which is a typical structure domain of a membrane transport protein. This result is consistent with [4]. Further analysis proved that the crude extract from the seeds can induce the expression of this gene. By analyzing the laboratory existing strains, it was found that they are all induced to a different extent by the canavanine, and the LysE of the canavanine-sensitive mutant is much more sensitive to canavanine. The wild-type V972 is not affected by canavanine. According to [5], canavanine is widely distributed in legumes, especially in the genus Sesbania. It has a similar structure with arginine, and arginine synthesis in rhizobia would also lead to canavanine accumulation. Canavanine synthase can also be used in the synthesis of arginine, and the synthesized canavanine will be used in the protein synthesis. The canavanine in the peptide chain cannot be correctly folded and folded, and the protein becomes denatured, resulting in abnormal morphology and growth of the cell.

Vrijjic et al. (2001) found that the family amino acid transport protein was first discovered in Escherichia coli, with the function of transporting excess lysine in the cell to the extracellular. Later, it was discovered in other bacteria. In E. coli, the L-lactic acid can transport canavanine, and the canavanine-resistant mutant shows sensitivity to canavanine [6]. It was speculated that the Caesalpina decapetala nodule bacterium also exists in the family amino acid transport protein, which can discharge canavanine from the cell to eliminate the toxicity. In many legumes, the seeds can secrete large amounts of canavanine at the time of germination, as a defense factor to control the species and number of microorganisms in the rhizosphere soil, as shown in [7]. Canavanine as a plant defense factor, can inhibit the growth of some rhizosphere bacteria. Since the canavanine-resistant mutant of Caesalpina decapetala nodule bacterium can transport canavanine to the extracellular, it eliminates the toxicity. The results of this experiment show that canavanine can induce the expression of different rhizobia, at the same time, the canavanine-resistant partner can better grow in the surrounding of the legume seeds, which may provide a good basis for the early interaction of the rhizobia with the host plant. This is probably the result of the long-term evolution of the rhizobium and host plant mutual selection and adaptation, and this study provides a theoretical basis and new ideas for the host plant and rhizobium symbiosis.

References

Identification and functional characterization of genes induced by seed exudates in *Azorhizobium caulinodans* ORS571


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**Abstract** To identify genes induced by plant seed exudates in *Azorhizobium caulinodans* ORS571. **Methods** Using promoterless kanamycin resistance gene Km' on transposon as reporter gene and seed exudates as inducers, we screened genes of interest from transposon insertion mutants libraries. We streaked mutants on TY solid medium with Km and another with Km and seed exudates correspondingly. If Km' is inserted into a gene that can be induced by plant signals, Km' will possibly express at the same time. Thus mutants were selected that can grow on medium with Km and exudates rather than on medium with Km. **Results** We identified a lysE family gene named asiE in strain Az50 that can be induced by seed exudates and further analysis indicated that the inducing substance is canavanine CAN. lacZ transcriptional fusion of asiE confirmed that its expression increased by ten-fold or so under the induction of CAN. Besides asiE gene in four different species of Rhizobia can be induced by CAN. lysE mutants are all sensitive to CAN treatment whereas wild type are resistant. **Conclusion** The existence of LysE can make rhizobia better survived in the rhizosphere and may play an important role in early stage of interaction between rhizobia and host plant.

**Keywords** *Azorhizobium caulinodans* ORS571, LysE family, Canavanine, seed exudates