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cDNA-AFLP analysis of differential gene expression related to cell chemotactic and encystment of *Azospirillum brasilense*

Huamin Li[a], Yanhua Cui[a,b], Lixian Wu[a], Ran Tu[a], Sanfeng Chen[a,*]

[a]*State Key Laboratory of Agrobiotechnology and College of Biological Sciences, China Agricultural University, Beijing 100193, PR China*
[b]*School of Food Science and Engineering, Harbin Institute of Technology, Harbin 150090, PR China*

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Summary

Our previous study indicated *org35* was involved in chemotaxis and interacted with nitrogen fixation transcriptional activator NifA via PAS domain. In order to reveal the role of *org35* in nitrogen regulation, the downstream target genes of *org35* were identified. We here report differentially expressed genes in *org35* mutants comparing with wild type Sp7 by means of cDNA-AFLP. Four up-regulated transcript-derived fragments (TDFs) homologues of chemotaxis transduction proteins were found, including CheW, methyl-accepting chemotaxis protein and response regulator CheY-like receiver. Three distinct TDFs (AB46, AB58 and AB63) were similar to PHB de-polymerase C-terminus, cell shape-determining protein and flagellin domain protein. And 11 TDFs showed similarities with signal transduction proteins, including homologous protein of the nitrogen regulation protein NtrY and nitrate/nitrite response regulator protein NarL. These data suggested that the *Azospirillum brasilense org35* was a multi-effecter and involved in chemotaxis, cyst development and regulation of nitrogen fixation.

1. Introduction

Azospirillum brasilense is a nitrogen-fixing α-proteobacterium which mainly colonizes the rhizosphere of many important agricultural grasses and crops, promoting plant growth and cereals yield (Steenhoudt and Vanderleyden 2000). In most diazothrophs, the expression of the nitrogen fixation (*nif*) genes is dependent on the transcriptional activator NifA. It was reported that NifA played a key role in regulating the synthesis and activity of nitrogenase in response to ammonia

*Corresponding author.
E-mail address: chensf@cau.edu.cn (S. Chen).

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and oxygen available in *A. brasilense* (Arsène et al. 1996). In previous study we reported a chemotaxis-related gene *org35* of *A. brasilense* Sp7, which was originally identified to be able to interact with NifA in yeast-two-hybrid system (Cui et al. 2010; Chen et al. 2005a). Experiments demonstrated that interaction between Org35 and NifA was mediated via PAS domain of Org35 and the N-terminal GAF domain of NifA (Tu et al. 2006). It was also found that the *org35* mutant Sp7353 has a 1.5-fold elevation in nitrogenase activities than the wild type. We suspected that Org35 may negatively regulate the NifA activities and was a repressor of NifA. All these results suggested that Org35 is involved in chemotaxis and that can also participate in nitrogen regulation through the PAS domains. It is possible that the phosphorylated Org35 outputs the signal to NifA or other proteins.

Genome-wide expression analysis is a valuable tool for determining the function of genes and elucidating the genetic networks in which they participate (Valverde et al. 2006). cDNA-amplified fragment length polymorphism (cDNA-AFLP) is a robust, high-throughput, genome-wide expression tool for gene discovery and analysis of genetic network (Gabriëls et al. 2006; Polesani et al. 2008). cDNA-AFLP does not require any prior knowledge of gene sequences and is more stringent and reproducible than many other methods because it can amplify low-abundance transcripts (Lievens et al. 2001; Valverde et al. 2006). cDNA-AFLP is widely used in the analysis of transcription profiles in plants and to a lesser extent in bacteria, including *A. brasilense* (Dellagi et al. 2000; Breyne and Zabeau 2001; Valverde et al. 2006; Wang et al. 2009).

In present study, to gain insights into signal transduction mechanisms of Org35 in *A. brasilense*, differentially expressed genes were screened in *org35* mutants comparing with wild type Sp7 by means of cDNA-AFLP approach. The results provide a clue to the elucidation of signal transduction mechanisms of nitrogen fixation in *A. brasilense*.

2. Materials and methods

2.1. Bacterial strain and growth conditions

The strains and plasmids used in this study are listed in Table 1. *A. brasilense* strains were routinely grown at 30 °C in LD medium (Chen et al. 2005b). *Escherichia coli* strains were grown in Luria—Bertani (LB) medium at 37 °C. Antibiotics ampicillin (Amp), kanamycin (Km) and tetracycline (Tc) were used for *E. coli* at 100 μg/ml, 50 μg/ml and 12.5 μg/ml, respectively. Ampicillin and nalidixic acid (Nx) were used for *A. brasilense* at 25 μg/ml and 5 μg/ml, respectively.

2.2. Construction of mutants

To construct mutant with in-frame deletion of *org35*, two fragments corresponding to upstream and downstream of *org35* were PCR amplified. A 1.9 kb fragment upstream of *org35* was firstly PCR amplified with primers 35PAS1 (5′-CGGGATCCACCCTCATGGTGGAGTC-3′) [an *Eco*RI site underlined] and 35PAS2 (5′-CCCAAGCTTGAGATGTCCAGCAGGCAGT-3′) [a *Hin*d III site underlined]. The fragment was cut with *Pst* I and *Hin*d III, and got a 980 bp fragment

Table 1. The bacterial strains used in this work.

Strains or plasmids	Relevant characteristics	Source or references
Strains		
A. brasilense Sp7	Wild type, ATCC29145, Amp^r Nx^r	ATCC 29145
A. brasilense Sp70351	*A. brasilense* Sp7 derivative, *org35::kan* mutant, Amp^r Nx^r Km^r	This work
A. brasilense Sp70352	*A. brasilense* Sp7 derivative, *org35::kan* mutant, Amp^r Nx^r Km^r	This work
Plasmids		
pBlueScript KS+	Cloning vector, Amp^r	Stratagene
pPHU281	Suicide vector, lacZ' mob (RP4), Tc^r	Hübner et al. (1993)
pMD18-T	T-vector	Takara
pBS-PAS	pBlueScript derivative carrying 980bp 5′-terminal region of *org35*	This work
pBS-PD	pBlueScript derivative carrying 980bp 5′-terminal region and 840bp 3′-terminal region of *org35*	This work
pBS-PDK	pBlueScript derivative carrying 5′-terminal and region of *org35*, between them *kan* gene was insert	This work
pPHU-PDK	pPHU281 derivative carrying *Eco*R I to *Xho* I fragments from pBS-PDK, Tc^r	This work