A histidine kinase sensor protein gene is necessary for induction of low pH tolerance in *Sinorhizobium* sp. strain BL3

Panlada Tittabutr[1], Waraporn Payakapong[1], Neung Teaumroong[1], Nantakorn Boonkerd[1], Paul W. Singleton[2] and Dulal Borthakur[3,*]

[1]*School of Biotechnology, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand;* [2]*Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, Honolulu, HI, 96822, USA;* [3]*Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, HI, 96822, USA; *Author for correspondence (e-mail: dulal@hawaii.edu; phone: +1-808-956-6600; fax: +1-808-956-3542)*

Received 30 May 2005; accepted in revised form 26 September 2005

Key words: actX, Adaptive acid tolerance response, Histidine-kinase, Nodulation, *Rhizobium*–legume symbiosis, *Sinorhizobium*

Abstract

The aim of this investigation was to identify and isolate genes involved in acid tolerance from *Sinorhizobium* sp. strain BL3. It was hypothesized that acid tolerance of strain BL3 could be enhanced by high level expression of certain genes involved in acid tolerance, following insertion of these genes in a multiple copy plasmid. A cosmid clone library of BL3 was introduced into BL3, and the transconjugant colonies were selected at low pH. A single cosmid containing genes for acid tolerance was isolated from 40 different colonies. By transposon–insertion mutagenesis, subcloning and DNA sequencing, a gene involved in acid tolerance, *actX*, was identified in a 4.4-kb fragment of this cosmid. The *actX* mutant of BL3 showed increased acid sensitivity and was complemented by the 4.4-kb subcloned fragment. *Phaseolus lathyroides* seedlings inoculated with recombinant strains containing multiple copies of *actX* showed increased symbiotic performance at low pH. By constructing an *actX::gus* fusion, it was shown that *actX* was induced at low pH. *actX* encodes a putative histidine kinase sensor protein of a two-component regulatory system. The method of gene identification used in this study for isolation of *actX* may be applied for the isolation of other genes involved in tolerance to adverse environmental factors.