The alternative sigma factor RpoH2 is required for salt tolerance in *Sinorhizobium* sp. strain BL3

Panlada Tittabutra, Waraporn Payakaponga, Neung Teaumroonga, Nantakorn Boonkerd a, Paul W. Singleton b, Dulal Borthakur c,∗

a School of Biotechnology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand
b Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, Honolulu, HI 96822, USA
c Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, HI 96822, USA

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Abstract

The objectives of this investigation were to isolate the *rpoH2* gene encoding an alternative sigma factor from *Sinorhizobium* sp. BL3 and to determine its role in exopolysaccharide (EPS) synthesis, salt tolerance and symbiosis with *Phaseolus lathyroides*. The *rpoH2* gene of *Rhizobium* sp. strain TAL1145 is known to be required for EPS synthesis and effective nodulation of *Leucaena leucocephala*. Three overlapping cosmids clones containing the *rpoH2* gene of BL3 were isolated by complementing an *rpoH2* mutant of TAL1145 for EPS production. From one of these cosmid, *rpoH2* of BL3 was identified within a 3.0-kb fragment by subcloning and sequencing. The cloned *rpoH2* gene of BL3 restored both EPS production and nodulation defects of the TAL1145 *rpoH2* mutants. Three *rpoH2* mutants of BL3 were constructed by transposon-insertion mutagenesis. These mutants of BL3 grew normally in complete or minimal medium and were not defective in EPS synthesis, nodulation and nitrogen fixation, but they failed to grow in salt stress conditions. The mutants complemented with cloned *rpoH2* from either BL3 or TAL1145 showed higher levels of salt tolerance than BL3. The expression of *rpoH2* in BL3 started increasing during the exponential phase and reached the highest level in the mid-stationary phase. These results indicate that RpoH2 is required for salt tolerance in *Sinorhizobium* sp. BL3, and it may have additional roles during the stationary phase.

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1. Introduction

TAL1145 is a strain of *Rhizobium* sp. that forms nitrogen-fixing nodules on the tree legume *Leucaena leucocephala* (*leucaena*). It produces large amounts of exopolysaccharide (EPS) in medium containing mannitol. We have shown previously that in this strain, the alternative sigma factor RpoH2 regulates the transcription of several *exo* genes required for EPS synthesis [16]. *rpoH2* mutants of TAL1145 are also defective in nodulation and nitrogen fixation on the tree legume *Leucaena leucocephala*. Conversely, *rpoH2* mutants of *Sinorhizobium meliloti* are not defective in EPS synthesis and symbiosis with the host legume alfalfa [24]. However, cloned *rpoH2* of *S. meliloti* Rm1021 complemented the *rpoH2* mutant of *Rhizobium* TAL1145 for EPS synthesis, suggesting that RpoH2 of TAL1145 and *S. meliloti* may have similar regulatory functions [16]. It is not known if RpoH2 is required for EPS synthesis and symbiosis in other *Rhizobium* and *Sinorhizobium* strains.

Beside EPS synthesis, alternative sigma factors can also be involved in transcriptional regulation of genes for sporulation, flagellum biosynthesis, stationary phase survival, nitrogen fixation and stress response in bacteria [36]. For example, the *rpoS* gene products are responsible for the transcription of many genes expressed during stationary phase and osmotic stress [12]. In *Bacillus subtilis*, an alternative sigma factor, SigB, is expressed during stationary phase in rich medium or after depletion of glucose, phosphate or oxygen in the medium, and also in response to heat and osmotic stresses [11]. In *Mycobac-