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Original Article

# The phosphotransferase system gene *ptsI* in *Bacillus cereus* regulates expression of *sodA2* and contributes to colonization of wheat roots

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**Abstract**

Plant growth-promoting rhizobacteria effectively enhance plant growth and root colonization by the bacteria is a prerequisite during the process. *Bacillus cereus* 905, a rhizosphere bacterium originally isolated from wheat roots, colonizes the wheat rhizosphere with a large population size. We previously showed that a manganese-containing superoxide dismutase (MnSOD2), encoded by the *sodA2* gene, plays an important role in colonization of the wheat rhizosphere by *B. cereus* 905. In this study, we identified a gene, *ptsI*, which positively regulates transcription of *sodA2*. *ptsI* encodes Enzyme I of the phosphotransferase system (PTS), a major regulator of carbohydrate uptake in bacteria. Assays of β-galactosidase activity and real-time quantitative PCR showed that loss of *ptsI* caused a 70% reduction in *sodA2* expression. The Δ*ptsI* mutant also showed a 1000-fold reduction in colonization of wheat roots, as well as a reduced growth rate in minimal media with either glucose or succinate as the sole carbon source. Artificial induction of *sodA2* in the Δ*ptsI* mutant partially restored root colonizing ability and utilization of succinate, but not glucose. These results suggest that the PTS plays an important role in rhizosphere colonization by both promoting nutrient utilization and regulating *sodA2* expression in *B. cereus* 905.


*Keywords:* *Bacillus cereus*; *ptsI*; Superoxide dismutase; Phosphotransferase system; Root colonization

## 1. Introduction

*Bacillus* species are well known for their activities in plant biological control [1]. A variety of biological control mechanisms have been elucidated in different *Bacillus* species. For example, *Bacillus subtilis* and *Bacillus amyloliquefaciens* showed antagonistic capacities toward fungal and bacterial pathogens by producing several families of well-known cyclic lipopeptides (LPs), including surfactins, bacillomycins (iturin family), fengycins and locillomycins [2–7]. Aside from control of plant diseases, *Bacillus* species also promote plant growth as another important beneficial activity [8–10]. The effective biocontrol activities of *Bacillus* spp. are thought to be principally due to competitive root colonization and strong environmental persistence [11–14]. A number of studies demonstrated that bacteria have evolved a variety of mechanisms to enhance colonization capacity, including biofilm formation, surfactin production and production of metabolic enzymes that allow the bacteria to efficiently utilize plant-derived nutrients [7,15–17].

The bacterial phosphoenolpyruvate phosphotransferase system (PTS) functions to uptake and catalyze phosphorylation of its sugar substrates by using phosphoenolpyruvate (PEP) as the phosphoryl donor [18]. PTS is a complex system that

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contains two general cytoplasmic energy-coupling proteins, enzyme I (EI) and HPr (heat-stable, histidine-phosphorylatable protein) and a specific membranous multicomponent enzyme II [19,20] (Fig. 1A). The enzyme II complex consists of three functional subunits, IIA, IIB and IIC, although those belonging to the mannose family contain an additional subunit, IID [21]. The PTS system is modular, in that there are multiple enzyme II complexes for uptake of different PTS sugars, while enzyme I and HPr are shared. In the genome of *B. subtilis*, there are multiple genes encoding 15 complete PTS permeases, but only one copy of the gene (*ptsI*) encoding enzyme I and one for HPr (*ptsH*) [22]. Thus far, the PTS has been found in many different classes of bacteria, and is a pleiotropic regulator of various essential physiological processes [21]. In addition to its primary function in sugar transport and phosphorylation, the PTS has been shown to play important roles in other aspects of bacterial physiology, including a variety of ramifications for metabolic and transcriptional regulation [19]. Previous studies suggested that the PTS proteins participate in chemotaxis, regulation of carbon metabolism, coordination of carbon and nitrogen metabolism, virulence, biofilm formation, plant colonization, etc. [23,24]. For instance, in *Bacillus anthracis*, the PTS was shown to be involved in virulence by regulating the activity of the AtxA transcription factor, which controls key virulence genes [25]. In *Bacillus cereus*, the enzyme I (PtsI) was shown to affect biofilm formation and wheat root colonization, yet the role of PtsI in wheat root colonization by *B. cereus* has remained elusive [26].

Superoxide dismutases (SODs) are metalloenzymes specifically catalyzing the dismutation of superoxide anions ($O_2^-$) to form hydrogen peroxide ($H_2O_2$) and molecular oxygen ($O_2$) [27]. Hydrogen peroxide is further detoxified to water by catalases. Various SODs are distinguished from each other by their metal cofactors, either iron (Fe), manganese (Mn) or copper-zinc (Cu-Zn) [28]. SODs are key components of the cellular defense mechanism against oxidative stress [27]. SODs also play an important role during plant-microbe interactions. Studies mainly in Gram-negative bacteria have led to identification of signaling pathways and genes involved in regulation of SOD expression [29]. The oxidative stress-responsive transcription factors SoxRS, OxyR and Fur are among the key regulators for control of SOD expression in Gram-negative bacteria [30–32]. In contrast, less is known about MnSOD and regulation of MnSOD expression in the Gram-positive bacteria. In the model bacterium *B. subtilis*, a MnSOD encoded by the *sodA* gene was found to play an important role in

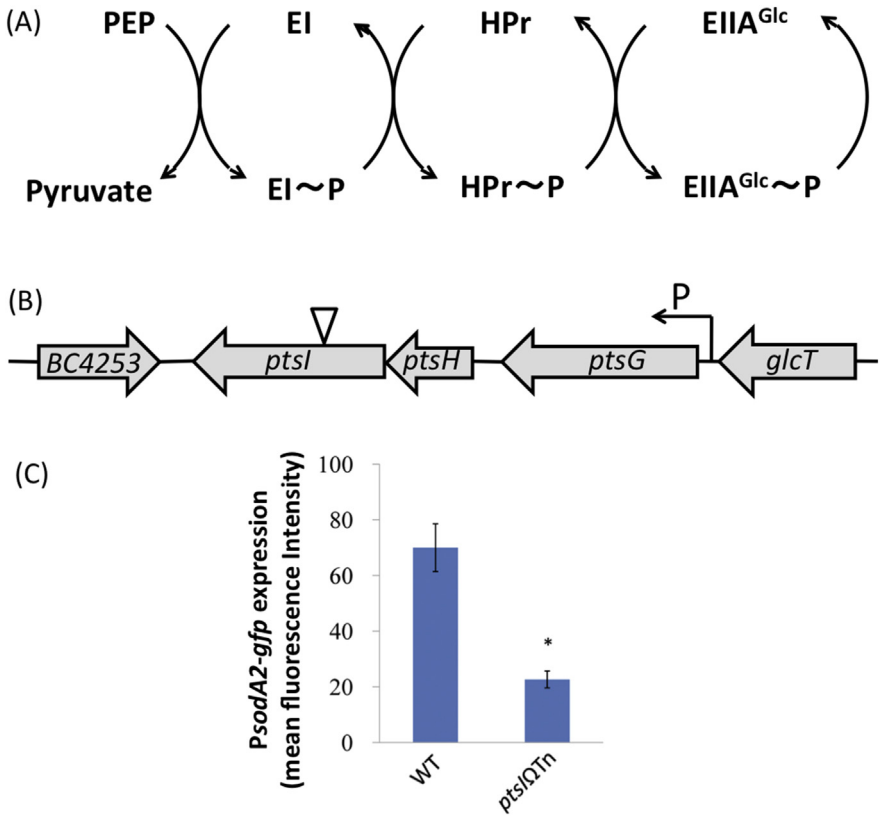


Fig. 1. *ptsI* is important for *sodA2* expression in *B. cereus* 905. (A) An overview of the PTS system in *B. cereus*. In the PTS phosphotransfer cascade, a phosphate group is transferred sequentially from PEP to enzyme I (EI), to HPr and to subunit A of enzyme II (EII). (B) A schematic drawing of the *pts* operon in *B. cereus* 905. The *pts* operon consists of *ptsI*, *ptsH* and *pts*G genes, encoding enzyme I (EI), HPr (heat-stable, histidine-phosphorylatable protein) and specific membranous multicomponent enzymes II, respectively. Promoter of the *pts* operon is indicated by arrow. The position of the Tn*YLB*-1 transposon insertion in the *ptsI* gene on the chromosome of *B. cereus* 905 is indicated by the triangle. (C) Expression of *sodA2* gene in *B. cereus* 905 wild-type (WT) and the Tn*YLB*-1 insertional mutant (*ptsI*ΩTn). The *sodA2* promoter activities at $OD_{600} = 0.5$ of bacterial growth were determined. Green fluorescent protein (GFP) mean fluorescence intensity (MFI) was determined for gated populations of bacterial cells by flow cytometry. Values are representative of three experiments, and three replicates were used for each experiment. Asterisk indicates statistically significant difference in GFP MFI between wild type (WT) and mutant (*ptsI*ΩTn) ($P < 0.05$, Student's *t* test).