Intellectual Contribution

Identification of factors that regulate antibiotic production in a plant-protecting bacterium

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The production of biocontrol factors in a plant-protecting bacterium *Pseudomonas fluorescens* CHA0 depends on the Gac/Rsm signal transduction pathway. In this study, we revealed that the signal transduction pathway is positively regulated by alarmone ppGpp (guanosine 3',5'-bis(diphosphate)) and negatively regulated by the ATP-dependent protease Lon. These findings may facilitate the development of biocontrol strains that show relatively higher performance.

Keywords: biocontrol, plant protection, antibiotic activity, signal transduction pathway

Background

Pseudomonas fluorescens CHA0 is one of the model strains that have been used in studies on the biosynthesis of extracellular enzymes and secondary metabolites with antibiotic activity and thus contribute to plant protection. The expression of these biocontrol factors depends on the Gac/Rsm signal transduction pathway initiated by the GacS/GacA two-component system. In this study, we identified the factors that regulate the Gac/Rsm pathway in strain CHA0.

Results and Discussion

1. In *P. fluorescens* CHA0, the Gac/Rsm signal transduction pathway is instrumental for secondary metabolism via the expression of regulatory small RNAs (sRNAs termed RsmX/Y/Z) (Fig. 1). We performed a metabolome analysis and found a higher level of ppGpp accumulation in a *gacA*-negative mutant as compared to the wild-type strain CHA0.
2. In the CHA0 *relA spoT* double mutant, the ppGpp synthesis is completely inhibited accompanied by significant reduction in expression of sRNAs, antibiotic production (Fig. 2A), root colonization and plant protection. Thus, ppGpp appears to be essential for sustaining epiphytic fitness and biocontrol activity, via positively affecting the Gac/Rsm pathway in the CHA0 strain (Fig. 1).
3. Genetic screening for mutants of strain CHA0 with enhanced Rsm sRNA expression (i.e., the Gac/Rsm pathway is activated) facilitated the isolation of a mutant that has a defect in a gene encoding Lon protease, a member of the ATP-dependent protease family.
4. In the *lon* mutant, the accumulation level of the GacA protein was elevated and antibiotic activity against phytopathogenic fungus *Fusarium oxysporum* was significantly enhanced (Fig. 2B). Thus, Lon protease is an important negative regulator of the Gac/Rsm pathway in strain CHA0 (Fig. 1).

Future prospects

1. Modification of genes involved in the Gac/Rsm signal transduction pathway will help improve bacterial biocontrol ability.
2. Application of chemical compounds that stimulate the Gac/Rsm pathway may also increase the biocontrol efficacy.

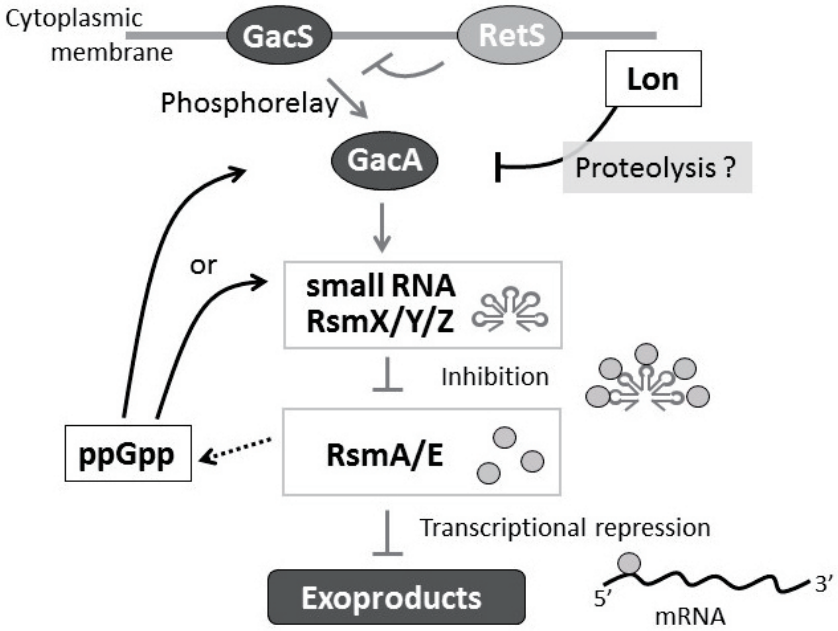

Fig. 1. Model for the Gac/Rsm signal transduction pathway in *Pseudomonas fluorescens* CHA0. In this study, we found that ppGpp has a positive effect on the Gac/Rsm system whereas Lon protease negatively affects GacA protein stability.

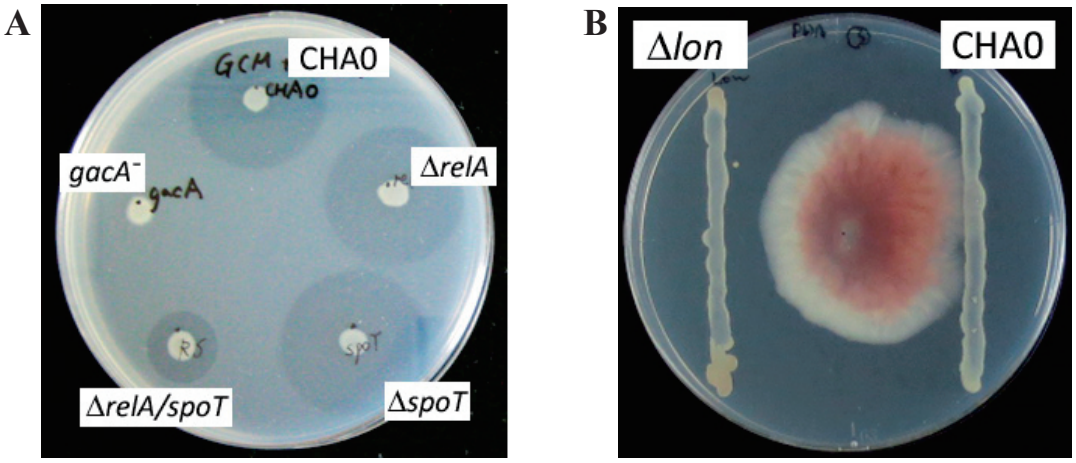

Fig. 2. (A) Effect of *relA* and *spoT* mutations on antibiotic activity toward *Bacillus subtilis*. (B) Effect of a *lon* mutation on antibiotic activity toward *Fusarium oxysporum.* Antibiotic activities of *Pseudomonas fluorescens* CHA0 wild-type strain were compared with those of a *relA* mutant, *spoT* mutant, *relA spoT* double mutant and *gacA* mutant (A) or with a *lon* mutant (B).

References

1. Takeuchi K, Yamada K, Haas D (2012) ppGpp controlled by the Gac/Rsm regulatory pathway sustains biocontrol activity in *Pseudomonas fluorescens* CHA0 ***Molecular Plant-Microbe Interactions*** 25(11):1440-1449
2. Takeuchi K, Tsuchiya W, Noda N, Suzuki R, Yamazaki T, Haas D (2014) Lon protease negatively affects GacA protein stability and expression of the Gac/Rsm signal transduction pathway in *Pseudomonas protegens* ***Environmental Microbiology*** (Published Online DOI: 10.1111/1462-2920.12394)