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Research in Microbiology 159 (2008) 569–578

www.elsevier.com/locate/resmic

A putative $colR_{XC1049}$–$colS_{XC1050}$ two-component signal transduction system in *Xanthomonas campestris* positively regulates *hrpC* and *hrpE* operons and is involved in virulence, the hypersensitive response and tolerance to various stresses

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Received 25 May 2008; accepted 24 June 2008
Available online 24 July 2008

Abstract

The ColR–ColS two-component signal transduction system was originally characterized as a regulatory system involved in the capacity of root-colonizing biocontrol bacterium *Pseudomonas fluorescens* to colonize plant roots. There are three pairs of putative *colR–colS* two-component regulatory systems annotated in the phytopathogen *Xanthomonas campestris* pathovar *campestris*. Mutational studies revealed that one of them, named $colR_{XC1049}$ and $colS_{XC1050}$, is a global regulatory system involved in various cellular processes, including virulence, hypersensitive response and stress tolerance. Growth rate determination showed that, although the $colR_{XC1049}$ and $colS_{XC1050}$ mutants are not auxotrophic, $colR_{XC1049}$ and $colS_{XC1050}$ are required for the pathogen to proliferate well in standard media and host plants. Assays of β-glucuronidase activities of plasmid-driven promoter-*gusA* reporters and/or semi-quantitative RT-PCR demonstrated that $colR_{XC1049}$ and $colS_{XC1050}$ positively regulate expression of *hrpC* and *hrpE* operons, and that expression of $colR_{XC1049}$ and $colS_{XC1050}$ is not controlled by key *hrp* regulators HrpG and HrpX.

Keywords: *Xanthomonas*; Phytopathogen; Pathogenicity; Stress response; ColR–ColS two-component system

1. Introduction

Bacteria have developed two-component signal transduction systems to sense and respond to a wide range of environmental changes. A typical two-component signal transduction system consists of a histidine kinase sensor and a response regulator. The sensor detects an environmental stimulus and activates a cognate response regulator which then controls expression of specific genes [26]. The number of two-component signal transduction systems in a bacterial genome differs from one species to another. For instance, *Escherichia coli* has 64 putative sensors and response regulators [35], while different *Pseudomonas* species have more than 120 putative sensors and regulators [10], and different *Xanthomonas* species or pathovars have 92–121 putative sensors and regulators [20]. The function of most of these

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0923-2508/$ - see front matter © 2008 Published by Elsevier Masson SAS.
doi:10.1016/j.resmic.2008.06.010

putative two-component regulatory systems remains to be clarified.

The ColR–ColS two-component signal transduction system was originally identified from root-colonizing biocontrol bacterium *Pseudomonas fluorescens* as a regulatory system involved in the capacity of the bacterium to colonize plant roots, in which ColS and ColR act as a sensor and a regulator, respectively [9]. Subsequently, the ColR–ColS system has been found to be involved in regulation of different biological responses in *Pseudomonas putida*, such as transposition of transposon Tn4652 [10], phenol tolerance [14] and heavy metal resistance [11]. A survey of NCBI GenBank data (http://www.ncbi.nlm.nih.gov/Genbank/index/html) revealed that *colR–colS* homologous genes were annotated in a large number of bacterial species. Some of them are important phytopathogenic bacteria such as *Pseudomonas syringae* [2], *Ralstonia solanacearum* [22], *Xanthomonas axonopodis* [5], *Xanthomonas campestris* [5,21,27], and *Xanthomonas oryzae* [15,19]. However, the biological functions of the *colR–colS* genes in these bacteria have not been verified.

Xanthomonas campestris pathovar *campestris* (*Xcc*) is the causal agent of black rot disease, one of the most serious diseases of cruciferous crops worldwide [33]. This pathogen infects almost all members of the crucifer family (*Brassicaceae*), including important vegetables such as broccoli, cabbage, cauliflower, mustard and radish, the major oil crop rape, as well as the model plant *Arabidopsis thaliana*, and has been used as a model bacterium for studying plant-pathogen interactions. A total of 106 putative members of two-component signal transduction systems were annotated in the genome of the *Xcc* strain 8004 [21]. Among these, three sensors and three regulators were annotated to be putative ColS and ColR, respectively [21]. The biological functions of these three ColR–ColS-homologous pairs have not been characterized. In this work, we studied their role in the pathogenicity of *Xcc* and demonstrated that one of the *colR–colS* homologous pairs is involved in regulation of various cellular processes, including pathogenesis.

2. Materials and methods

2.1. Bacterial strains, plasmids and culture conditions

Bacteria and plasmids used in this work are listed in Table 1. *E. coli* strains were grown in L medium [18] at 37 °C. *Xcc* strains were grown in rich medium NYG [6], or the minimal medium MMX [7] at 28 °C. Antibiotics were used as described previously [4].

2.2. DNA manipulations

DNA manipulations were performed as described by Sambrook et al. [23]. The conjugation between *Xcc* and *E. coli* strains was performed as described by Turner et al. [28]. Restriction enzymes, DNA ligase and PCR were performed in accordance with the manufacturer's instructions (Promega, Shanghai).

Table 1
Bacterial strains and plasmids used in this work

Strains or plasmids	Relevant characteristics	Reference or source
E. coli		
JM109	*RecA*1, *endA*1, *gyrA*96, *thi*, *supE*44, *relA*1 △(*lac-proAB*)/F′ [*traD*36, *lacI*^{q}, *lacZ* △M15]	[36]
X. campestris* pv. *campestris		
8004	Wild type, Rif^r	[6]
NK1049	As 8004, but XC1049::pK18mob, Rif^r, Kan^r	This work
CNK1049	NK1049 harboring pXC1049, Rif^r, Kan^r, Tc^r	This work
NK1050	As 8004, but XC1050::pK18mob, Rif^r, Kan^r	This work
CNK1050	NK1050 harboring pXC1050, Rif^r, Kan^r, Tc^r	This work
NK3125	As 8004, but XC3125::pK18mob, Rif^r, Kan^r	This work
NK3126	As 8004, but XC3126::pK18mob, Rif^r, Kan^r	This work
NK3451	As 8004, but XC3451::pK18mob, Rif^r, Kan^r	This work
NK3452	As 8004, but XC3452::pK18mob, Rif^r, Kan^r	This work
Δ*hrpG*	*hrpG* deletion mutant of 8004, Rif^r, Kan^r	Our laboratory
Δ*hrpX*	*hrpX* deletion mutant of 8004, Rif^r, Kan^r	Our laboratory
hrcV$^-$	As 8004, but *hrcV*::Tn5*gusA5*, Rif^r, Kan^r, Spc^r, Gm^r	Our laboratory
Plasmids		
pLAFR3	Broad host range cloning vector, Tc^r	[25]
pLAFRJ	Derived from pLAFR3, containing the multiple cloning site of pUC19, Tc^r	Our laboratory
pRK2073	Helper plasmid, Tra$^+$, Mob$^+$, ColE1, Spc^r	[16]
pK18*mob*	Suicide plasmid in *X. campestris* pv. *campestris*, Kan^r	[24]
pK1049	pK18mob containing a 459-bp internal fragment of *XC1049*, Kan^r; for mutant construction	This work
pK1050	pK18mob containing a 488-bp internal fragment of *XC1050*, Kan^r; for mutant construction	This work
pK3125	pK18mob containing a 505-bp internal fragment of *XC3125*, Kan^r; for mutant construction	This work
pK3126	pK18*mob* containing a 473-bp internal fragment of *XC3126*, Kan^r; for mutant construction	This work
pK3451	pK18*mob* containing a 528-bp internal fragment of *XC3451*, Kan^r; for mutant construction	This work
pK3452	pK18*mob* containing a 519-bp internal fragment of *XC3452*, Kan^r; for mutant construction	This work
pXC1049	pLAFR3 containing a 956-bp fragment including intact *XC1049* gene, Tc^r	This work
pXC1050	pLAFRJ containing a 1978-bp fragment including intact *XC1050* gene, Tc^r	This work

Gm^r, gentamicin-resistant; Kan^r, kanamycin-resistant; Rif^r, rifampicin-resistant; Spc^r, spectinomycin-resistant; Tc^r, tetracycline-resistant.

2.3. Construction of mutants

Each *colR* and *colS* homologous gene of the *Xcc* strain 8004 was disrupted by the homologous suicide plasmid integration method as described by Windgassen et al. [34], using pK18*mob* [24], a conjugative suicide plasmid in *Xcc*. A part of the target gene was amplified by PCR from the genomic DNA of strain 8004 using inner primers within the target ORF (Table S1). The amplified DNA fragment was cloned into pK18*mob* and the resulting recombinant plasmid was introduced from the *E. coli* strain JM109 [36] into strain