Research in Microbiology 165 (2014) 300–304

www.elsevier.com/locate/resmic

Brief note

Prevalence of type III secretion system in effective biocontrol pseudomonads

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Available online 12 April 2014

Abstract

Functional type III secretion system (T3SS) genes are needed for effective biocontrol of Pythium damping-off of cucumber by *Pseudomonas fluorescens* KD, but whether biocontrol *Pseudomonas* strains with T3SS genes display overall a higher plant-protecting activity is unknown. The assessment of 198 biocontrol fluorescent pseudomonads originating from 60 soils worldwide indicated that 32% harbour the ATPase-encoding T3SS gene *hrcN*, which was most often found in tomato isolates. The *hrcN*$^+$ biocontrol strains (and especially those also producing 2,4-diacetylphloroglucinol and displaying 1-aminocyclopropane-1-carboxylate deaminase activity) displayed higher plant-protecting ability in comparison with *hrcN*$^-$ biocontrol strains, both in the *Pythium*/cucumber and *Fusarium*/cucumber pathosystems.

Keywords: Type III secretion system; Biocontrol; *Pseudomonas*; 2,4-Diacetylphloroglucinol; ACC deaminase

1. Introduction

Many fluorescent *Pseudomonas* strains can control soil-borne crop diseases, often using antimicrobials e.g. phenazines, hydrogen cyanide, pyrrolnitrin and/or 2,4-diacetylphloroglucinol (Phl) [1–3]. Phl is of particular interest because (i) it inhibits a variety of soil-borne phytopathogens [1,4], (ii) it can trigger induced systemic resistance [3,5], (iii) it induces transcription of phytostimulation-related genes in other plant-beneficial rhizobacteria [6], and (iv) Phl$^+$ biocontrol *Pseudomonas* strains were shown to protect plants better than Phl$^-$ counterparts in two pathosystems [7].

Other properties may also be involved in disease suppression by fluorescent pseudomonads, including the production of extracellular lytic enzymes, surfactants, siderophores, and (so far unknown) effectors secreted by the type III secretion system (T3SS) [1,8]. The implication of T3SS in biological control was shown in plant experiments, where inactivation of T3SS gene *hrcV* strongly diminished the ability of *Pseudomonas* sp. KD to protect cucumber from Pythium damping-off [8], but results with the take-all/wheat pathosystem were not

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http://dx.doi.org/10.1016/j.resmic.2014.03.008
0923-2508/

as clear-cut following T3SS inactivation in *Pseudomonas* sp. Q81r1-96 [9]. In addition to *Pseudomonas* strains KD and Q81r1-96, T3SS genes have also been evidenced in other saprophytic pseudomonads [10–12] including Phl$^+$ strains [9,13,14].

The significance of T3SS genes for biological control by pseudomonads is difficult to determine, because inactivation of T3SS genes has been performed only in two strains, which retained other biocontrol activities [8,9]. Indeed, *Pseudomonas* strains KD and Q8r1-96 can also produce Phl, hydrogen cyanide and siderophore(s). Furthermore, elimination of a particular biocontrol trait may alter the expression of the others in *Pseudomonas* [15].

The objective was to assess at population level whether or not *hrcN*$^+$ biocontrol pseudomonads display higher plant-protecting activity than *hrcN*$^-$ counterparts. This was assessed using 198 biocontrol fluorescent pseudomonads derived from a collection of 230 biocontrol strains selected by screening several thousand *Pseudomonas* isolates [7]. We also compared the occurrence of T3SS gene with that of three other types of plant-beneficial properties, i.e. Phl synthesis [7], 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity [16] and surfactant production [17].

2. Materials and methods

2.1. Isolate origin and biocontrol activity

The 198 biocontrol pseudomonads (Table S1) originated from bulk soil (2 isolates) or macerated surface-disinfected roots of cucumber (98 isolates), tomato (40 isolates), wheat (34 isolates), tobacco (16 isolates), cotton (4 isolates), or bean (4 isolates) grown from surface-disinfected seeds planted in soils obtained from 60 geographic locations in 18 countries [7]. Their biocontrol ability was documented in the *Pythium ultimum*/cucumber pathosystem in steam-pasteurized clay loam soil, and for most of them (i.e. 90%) also in the *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL)/tomato pathosystem in rockwool substrate [7].

2.2. hrcN *screening and trait analyses*

The 198 biocontrol pseudomonads were screened for the presence of the *hrcN* gene by PCR using primers hrcN-5rR/hrcN-4r, as described [13]. The PCR products obtained were purified and both strands sequenced [13]. *hrcN* identity was determined using BlastN and the nr Nucleotide Sequence Database. A phylogenetic analysis was conducted with the 64 partial *hrcN* sequences obtained (250 bp) and others already available. The flagellar ATPase gene *fliI* of *Pseudomonas putida* KT2440 was used as the outgroup. Sequences were aligned using MUSCLE [18], most informative positions were selected using Gblocks [19] and phylogenetic distances were calculated using the GTR model [20]. The maximum likelihood tree was inferred using PhyML [21] and nodal robustness was assessed using 500 bootstrap replicates. All steps were performed using Seaview software [22].

The ability to produce Phl and/or HCN had been determined by Rezzonico et al. [7] and the resulting data were used in this work. Phl had been found (by HPLC analysis, as described [4]) in 71 of the 198 biocontrol fluorescent pseudomonads (i.e. 36%) and HCN (by reaction with copper(II)-ethyl acetoacetate, as described by Castric & Castric [23]) in 144 of the 198 biocontrol fluorescent pseudomonads (i.e. 73%). Here, the capacity to deaminate ACC was scored based on the ability to use ACC as sole nitrogen source on DF salts minimal agar containing 3 mM ACC, as described by Wang et al. [16]. Surfactant production was assessed using the drop-collapse method [24].

Table 1
Prevalence of *hrcN*$^+$ and *hrcN*$^+$ Phl$^+$ AcdS$^+$ traits among the 198 biocontrol pseudomonads based on isolate origin and the capability for HCN production, Phl production, ACC deamination and surfactant production.

	Number of isolates	*hrcN*		*hrcN*$^+$Phl$^+$AcdS	
		hrcN$^+$ isolates[a]	*hrcN*$^-$ isolates	*hrcN*$^+$ Phl$^+$ AcdS$^+$ isolates[b]	Other isolates[d]
	198	64	134	43	155
Origin					
Cucumber	98	30	68	15	83
Tomato	40	21*	19	19‡	21
Wheat	34	7	27	4	30
Tobacco	16	3	13	3	13
Other plants or soil	10	3	7	2	8
Biocontrol traits					
HCN production	145	58*	87	43‡	102
Phl production	71	50*	21	43‡	28
ACC deamination	85	51*	34	43‡	42
Surfactant production[c]	26	0	26	0	26

[a] In each row,* indicates a significantly higher proportion of *hrcN*$^+$ isolates within the different classes for origin (cucumber, tomato, wheat, tobacco or other) or biocontrol traits (HCN production, Phl production, ACC deamination or surfactant production) than expected from the overall proportion (64 of 198 isolates) and a random association (χ^2 tests, $P < 0.05$).

[b] In each row, ‡ indicates a significantly higher proportion of *hrcN*$^+$ Phl$^+$ AcdS$^+$ isolates within the different classes for origin or biocontrol traits than expected from the overall proportion (43 of 198 isolates) and a random association (χ^2 tests, $P < 0.05$).

[c] The association between surfactant production and *hrcN* (or *hrcN* Phl AcdS) could not be tested since *n* was 0.

[d] These other pseudomonads displayed two, one or none of the three traits.