Biological Control 98 (2016) 11–17

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Swarming motility plays the major role in migration during tomato root colonization by *Bacillus subtilis* SWR01

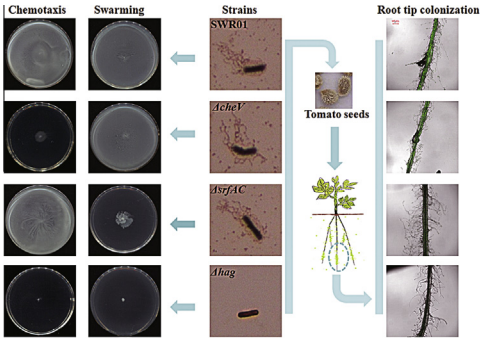
Shengfeng Gao [a,b], Huijun Wu [a], Xinfang Yu [a], Limin Qian [a], Xuewen Gao [a,*]

[a] Key Laboratory of Integrated Management of Crop Diseases and Pests, College of Plant Protection, Ministry of Education, Nanjing Agricultural University, Nanjing, China
[b] Spice and Beverage Research Institute, Chinese Academy of Tropical Agricultural Sciences, Hainan Province, China

HIGHLIGHTS

- Swarming was the major migration method in tomato root colonization by *B. subtilis*.
- Chemotaxis did not contribute as much as believed to root surface migration.
- Flagella motility produced >99% of the root surface migration.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 September 2015
Revised 23 March 2016
Accepted 24 March 2016
Available online 24 March 2016

Keywords:
Root colonization
Bacillus subtilis SWR01
Swarming
Chemotaxis
Flagella motility

ABSTRACT

Root colonization is important for the application of Plant Growth-Promoting Rhizobacteria (PGPR). Previously, chemotaxis was believed to be the major trait for colonization. However, because chemotaxis defective mutants are usually swarming impaired at the same time, in this study we clarified the roles played by swarming motility and chemotaxis in tomato root colonization by *Bacillus subtilis* SWR01. Tomato seeds were treated with *B. subtilis* SWR01 or mutants before being sown in Simons' gnotobiotic system, and the colonization efficacy at root tips were observed after 2 weeks. Both microscopy and plate counts showed that the colonization efficacy of swarming defective and chemotaxis proficient mutants (*ΔswrA*, *ΔminJ* and *ΔsrfAC*) was about 5%–15% that of the parental strain SWR01, while the colonization efficacy of the swarming normal and chemotaxis impaired mutant *ΔcheV* was 78.9% that of the wild-type bacteria. These results demonstrate that while both chemotaxis and swarming motility are important in root colonization, and the role played by swarming is greater than that of chemotaxis. In addition, non-flagellated mutants (*Δhag*) showed 0.47% colonization efficacy relative to that of *B. subtilis* SWR01, suggesting more than 99% root colonization requires the presence of flagella. This study indicates new ways to enhance PGPR rhizoplane colonization, which is useful in agricultural applications.

1. Introduction

Bacillus subtilis is important member of the Plant Growth-Promoting Rhizobacteria (PGPR) with beneficial capabilities in stimulating plant growth and controlling of plant diseases (Stein, 2005; Ongena and Jacques, 2008; Chen et al., 2013). *B. subtilis* cells could form dormant spores to survive in the extreme conditions, which means that *B. subtilis* products can be easily formulated and stored (Piggot and Hilbert, 2004). However, their beneficial effects are believed to be limited by poor root colonization (Weller, 1988). In recent years it has been proven that rhizosphere

* Corresponding author.
E-mail address: gaoxw@njau.edu.cn (X. Gao).

http://dx.doi.org/10.1016/j.biocontrol.2016.03.011
1049-9644/

colonization is required for plant growth-promotion (Gao et al., 2013) and also for some biocontrol mechanisms such as antibiosis (Chin-A-Woeng et al., 2000) and competition for nutrients and niches (Kamilova et al., 2005).

Bacteria move by a range of mechanisms, generally including swarming, swimming, twitching, gliding and sliding (Kearns, 2010). Swarming motility is defined as a rapid multicellular movement of bacteria across a surface, powered by rotating flagella (Henrichsen, 1972). Unlike swarming motility, swimming motility takes place as individual cells rapidly moving in liquid environments, which is oriented by chemotaxis and also powered by rotating flagella. Twitching, gliding and sliding motilities are also kinds of bacteria surface movements like swarming, which do not need the presence of flagella and move in much lower speeds than the first two mechanisms. Scientists have made great efforts to identify the traits and genes involved in root colonization (Lugtenberg and Kamilova, 2009). The trait of flagella motility has received special attention.

Flagella-less mutants of *Pseudomonas fluorescens* WCS374 were reported to inefficiently colonizing tomato root (De Weger et al., 1987). Similar results were obtained for *P. fluorescens* WCS365 (Dekkers et al., 1998) and *Pseudomonas putida* WCS358 (Simons et al., 1996) in colonizing wheat, radish and tomato roots.

Two uncharacterized genes required for swarming were reported to play a role in seedling colonization by *Salmonella enterica* (Barak et al., 2009). In *B. subtilis*, swarming is governed by the *swrA* operon (Kearns et al., 2004) and the flagella and surfactants are also required in this process (Kearns, 2010). The *swrA* operon consists of two genes, *swrA* and *minJ* (previously named *swrAA* and *swrAB* [Calvio et al., 2005]), disruption of either of which could cause the cells elongation and loss of swarming motility. Elongation cells from *swrA* mutant have regular septa, whereas multiple lengths cells from *minJ* mutant do not (Patrick and Kearns, 2008). However, the cell elongation is either a requirement for or an indicator of swarming motility (Kearns, 2010). Flagella encoded by the gene *hag*, is the motor of swarm cell. Surfactants reduce tension between the substrate and the bacterial cell to permit swarming on surfaces. Surfactin, a surfactant encoded by the *srfA-D* operon, has been reported to be important for biofilm formation by *B. subtilis* 6051 on *Arabidopsis* root surfaces (Bais et al., 2004).

cheA mutants of *P. fluorescens* WCS365 that were chemotaxis-defective were found to be deficient in root-tip colonization of tomato (De Weert et al., 2002). The importance of chemotaxis in colonization was also reported for *B. subtilis* FB17 on *Arabidopsis* roots (Rudrappa et al., 2008) and *B. subtilis* N11 on cucumber and banana roots (Zhang et al., 2014). However, measuring the colonization response chemotaxis defective of the mutant *ΔcheA* in *B. subtilis* is not possible because *cheA* mutant is swarming defective besides loss of chemotaxis and the colonization reduced by swarming defective obscures that of the chemotaxis (Kearns and Losick, 2003; Kojima et al., 2007). Mutant *ΔcheV* of *B. subtilis* is chemotaxis defective and swarming normal (Kearns and Losick, 2003), could be a feasible solution of measuring the effect of chemotaxis mutant on root colonization.

In this study, effects of swarming and chemotaxis on root colonization were compared in the background of a lab strain of *B. subtilis* SWR01, which is derivative of an easy transformation strain OKB105 by replacing the mutant *swrAA* gene with the FZB42 *swrA* wild type gene to complement the swarming motility. The swarming proficient and chemotaxis proficient strain SWR01 and various mutants: *ΔcheV* which is swarming proficient but chemotaxis defective; *ΔswrA*, *ΔminJ* and *ΔsrfAC* which are swarming defective but chemotaxis proficient; *ΔcheA* and *ΔcheV*/*ΔsrfAC* which are both swarming and chemotaxis defective; and the flagella-lacking mutant *Δhag* were constructed and their phenotypes of swarming, chemotaxis and colonization were tested. We present evidence that swarming motility plays a greater role than chemotaxis in tomato root colonization by *B. subtilis* SWR01.

2. Materials and methods

2.1. Bacterial strains and growth conditions

B. subtilis SWR01 and its derivatives thereof were grown at 37 °C in Luria-Bertani (LB) broth or on LB plates supplemented with 1.5% agar. When appropriate, antibiotics were included at the following concentrations: 100 μg/ml spectinomycin, 5 μg/ml chloramphenicol, 25 μg/ml kanamycin, 100 μg/ml ampicillin.

2.2. Mutant construction

All primers used in this study are listed in Table 1. All strains and plasmids are listed in Table 2. The "upstream region" + "resistance gene" + "downstream region" cassettes used to produce insertion-deletion mutations of genes *swrA*, *minJ*, *srfAC*, *cheA*, *cheV* and *hag* were generated by overlap extension using primers in Table 1 (Guérout-Fleury et al., 1995). The purified DNA of synthesized cassettes was transformed into strain SWR01 (Kearns and Richard, 2005). Isolates that had excised the "upstream region" + "resistance gene" + "downstream region" cassettes were revealed as light yellow colonies on LB plates with appropriate antibiotic. Six colonies were chosen for each strain, chromosomal DNA was purified, the target gene was PCR amplified with the primer pair

Table 1
DNA primers used in this study.

Name	Sequence (5′–3′)[a]
ΔswrA-1	CCTTTACGAAGAGGATGC
ΔswrA-2	gcgtcagaccccgtagaaGCGGGTTATTGGATGTGG
ΔswrA-3	ccacatccaataacccgcTTCTACGGGGTCTGACGC
ΔswrA-4	tgcctagtctttgtttactCAAGAGGACGCTTTATTC
ΔswrA-5	gaataaagcgtcctcttgAGTAAACAAAGACTAGGCA
ΔswrA-6	GGTTGGAATGGAAGACGG
ΔminJ-1	TTATGACGAACCAATTTG
ΔminJ-2	ggtcaaagccttgtgtatcTGCCTGAAACGAGTATAA
ΔminJ-3	ttatactcgtttcaggcaGATACACAAGGCTTTGACC
ΔminJ-4	tactttgtcggatttggaTTTTCACCGTCATCACCG
ΔminJ-5	cggtgatgacggtgaaaaTCCAAATCCGACAAAGTA
ΔminJ-6	CAAAAGCACTAAGTCTT
ΔsrfAC-1	ACAAGCATCACTGCGTTA
ΔsrfAC-2	ggtgatgacggtgaaaaATACGAGATTCGGTCCTC
ΔsrfAC-3	gaggaccgaatctcgtatTTTTCACCGTCATCACCG
ΔsrfAC-4	tattgccctttggctttTTGATACACAAGGCTTTG
ΔsrfAC-5	caaagccttgtgtatcaaAAAGCCAAAGGGCAATA
ΔsrfAC-6	TTCGGTTCACAAGGTAGG
ΔcheA-1	TGATGAGAGTAAAGAACA
ΔcheA-2	cggtgatgacggtgaaaaAGCTCTTCAAATAAGTTC
ΔcheA-3	gaacttatttgaagagctTTTTCACCGTCATCACCG
ΔcheA-4	gggaccattctcatattTTGATACACAAGGCTTTG
ΔcheA-5	caaagccttgtgtatcaaAATATGAGAATGGTCCC
ΔcheA-6	AATCAGTGCATTACAATC
Δchev-1	ATACGAAATTTTATTGGA
Δchev-2	cggtgatgacggtgaaaaATTTTTTCATAGTCAGGC
Δchev-3	gcctgactatgaaaaaatTTTTCACCGTCATCACCG
Δchev-4	caacacctgaatctgattTTGATACACAAGGCTTTG
Δchev-5	caaagccttgtgtatcaaAATCAGATTCAGGTGTTG
Δchev-6	CACTGATCTCAGGCTTG
Δhag-1	AGAAATTCAGTCATAGCC
Δhag-2	ggtcaaagccttgtgtatcAATGATCTTGACGTAACA
Δhag-3	tgttacgtcaagatcattGATACACAAGGCTTTGACC
Δhag-4	aagaagctgatggttcaTTTTCACCGTCATCACCG
Δhag-5	cggtgatgacggtgaaaaTGAACCATCAGCTTCTT
Δhag-6	AGCGATTCAAATAGGTGC

[a] Parts of primer sequences given in small letters were used for gene splicing by overlap extension PCR.