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Regulatory roles of *spnT*, a novel gene located within transposon Tn*TIR*

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Abstract

The transposon Tn*TIR* contains *spnIR* quorum-sensing system regulating sliding motility and the production of nuclease, biosurfactant, and prodigiosin in *Serratia marcescens*. Within Tn*TIR*, a gene named *spnT* is upstream of and co-transcribed with *spnI*. SpnT is a cytoplasmic protein and its level peaks during early stationary phase. *spnT* over-expression resulted in inhibition of sliding motility and synthesis of prodigiosin, and biosurfactant similar to *spnR*. *spnT* but not *spnR* over-expression induced cell elongation and aberrant DNA replication in *S. marcescens* and *Escherichia coli* strains. In comparison with wild-type *E. coli* strain, over-expression of *spnT* in an *E. coli priA* and *dnaC* double-mutant strain did not lead to the aberrant cell morphology phenotypes, suggesting SpnT may act through the recombination-dependent DNA replication system. As *spnT* over-expression inhibited swarming but not swimming motility, SpnT may indirectly function as a negative regulator of surface-dependent migration and secondary metabolite production.

Keywords: *Serratia marcescens*; Sliding; Quorum-sensing; SpnT; DNA replication

The bacterial populational surface translocation behavior enables bacterial cells to have better opportunities of access to nutrients, avoidance of toxic substances, and migration to preferred colonization sites, thus enabling bacterial cells to establish a balanced symbiotic, pathological or physiological associations with plant and animal hosts [1–3]. Among the many ways of bacterial surface migrations [4], sliding motility is a flagellum-independent migration behavior by which groups of bacterial cells spread on semi-solid agar surfaces as a biofilm accompanied by the acceleration of biomass production [3–5]. Sliding has been observed in many bacterial species [3,4] including *Serratia marcescens* [5,6], *Mycobacterium smegmatis* and *Mycobacterium avium* [7].

Production of a large amount of biosurfactant under quorum-sensing control is an essential parameter for initiation of sliding [3]. Quorum-sensing is a bacterial cell-to-cell communication system which facilitates the coordination of gene expression in accordance with the bacterial populational cell density [8,9]. Our previous studies in a *S. marcescens* strain SS-1 have shown that sliding motility, biosurfactant, and nuclease production together with prodigiosin synthesis are regulated by acylhomoserine lactone (AHL)-dependent quorum-sensing system *spnIR*, which is unique by their location on a mobile transposon [5,10,11]. In *S. marcescens* SS-1, SpnR represses sliding, prodigiosin synthesis and biosurfactant synthesis. Derepression of SpnR effects via 3-oxo-C6-HSL produced by SpnI leads to increase of prodigiosin production, *nucA* expression and biosurfactant synthesis so reducing surface tension, and initiating sliding [5]. Upstream of *spnI* we located a gene named *spnT* which is co-transcribed with *spnI* to form an operon [5]. Over-expression of *spnT* exerts a profound negative effect on both prodigiosin synthesis and sliding motility [5].

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In this study, we have further explored the function of SpnT. While we failed to specifically delete *spnT* in the *S. marcescens* strain SS-1, due to the natural mobility of Tn*TIR* and duplication of *spnTIR* region in the deletion process [11], *spnT* over-expression did show some other interesting phenotypes. These included inhibition of bio-surfactant production, significant cell elongation, and also an aberrant cell morphology, where the chromosomal DNA was aggregated and centrally localized within the elongated cells in *S. marcescens* SS-1 as well as *E. coli* MG1655. The effect of SpnT with respect to sliding motility in *S. marcescens* SS-1 is subsequently found to be independent of SpnIR/AHL quorum-sensing, and was possibly through interfering PriA, a key protein involved in the recombination-dependent DNA replication system [12,13]. Besides sliding, *spnT* over-expression was further observed to affect swarming, but not swimming, of a *spnT*-negative *S. marcescens* strain CH-1 [14,15]. Thus, *spnT* which together with *spnIR* is carried within a mobile transposon [11] might be horizontally transferred among bacterial species and play an important role in regulation of bacterial populational surface migration behavior.

Materials and methods

Bacterial strains and culture conditions. Bacteria strains and plasmids used in this study are listed in Table 1. *S. marcescens* CH-1 is a quorum-sensing negative clinical isolate [14,15] and *S. marcescens* SS-1 is an environmentally isolated strain with no flagella [16]. *S. marcescens* SS-1ΔI and SS-1ΔR are *spnI* and *spnR* deletion mutants derived from SS-1, respectively [5]. *Escherichia coli* K12 MG1655 is a laboratory-maintained strain and *E. coli* BL21(DE3) is purchased from Stratagene (USA). *E. coli* AG181 was a gift from R.G. Lloyd (Nottingham, UK). *Chromobacterium violaceum* CV026 is an AHL-reporter which responds to exogenous AHLs by producing the purple pigment violacein [17]. *E. coli* was cultured at 37 °C, and *S. marcescens* and *C. violaceum* CV026 at 30 °C in Luria–Bertani (LB) medium [18]. For extraction of AHLs, M9 minimal growth medium (M9 MGM; [18]) was used for overnight bacterial culture. Sliding and swarming assays were performed at 30 °C on LB broth solidified with 0.35% or 0.8% Eiken agar, respectively [5,14].

Enzymes and chemicals. DNA restriction and modification enzymes were purchased from Roche. *Taq* polymerase and PCR-related products were from Takara (Japan) or Perkin-Elmer (USA). Other laboratory grade chemicals were purchased from Sigma (USA), Difco (USA), Merck (Germany) or BDH (Germany). The AHLs used in this study were synthesized and confirmed as described previously [19,20].

Construction of pJR32T and pJR200. Full-length *spnT* gene (GenBank Accession No. AF389912) was amplified by *Pfu* DNA polymerase (Stratagene) from *S. marcescens* SS-1 chromosomal DNA using primers

Table 1
Strains plasmids, and primers used in this study

Strains	Relevant characteristics	Source or reference
Serratia marcescens		
CH-1	Clinical isolate	[14]
SS-1	Environmental isolate	[16]
SS-1ΔI	SS-1 *spnI*::*Sm*, Sm^r	[5]
SS-1ΔR	*SS-1 spnR*::*Sm* Sm^r	[5]
Escherichia coli		
K12MG1655	Wild-type	Hughes C. (University of Cambridge)
BL21(DE3)	*E. coli* B F-*ompT hsdS*(r_B-m_B-)dcm^+ Tet^r gal (DE3) *endA*	Stratagene, USA
CC118 λ *pir*	Δ (*ara-leu*), Δ *lacX*74, *galE*, *galK*, *phoA*20, *vthi*-1, *rpsE*, *rpoB*, *argE*, (*Am*), *recA1*, λ *pir* phage lysogen	[40,41]
S17-1 λ *pir*	Tp^r Sm^r *recA*, *thi*, *pro hsdrR*$^-$ M+RP4:2-Tc:Mu:Km Tn7, λ *pir*, phage lysogen	[40,41]
AG181	*priA2 dnaC212*	[38]
Chromobacterium violaceum CV026	Double mini-Tn5 mutant derived from *C. violaceum.* AHL biosensor Hg^r, *cvil*::Tn5*xyIE*, Km^r, spontaneous Sm^r, non-AHL producer, non-pigmented unless exogenous AHL provided	Williams P. (Nottingham University)
Plasmids		
pUT-Sm	Suicide plasmid contains mini-Tn5 (Smr); requires Pir protein for replication	[40,41]
pBAD18-Kan	*araC* coding region, M13 intergenic region, P_{BAD} promoter,pBR322 origin, kanamycin resistant gene	[21]
pJR200	pBAD18 Km^r contained *spnT* coding region	This study
pJR201	600 bp ORF-less DNA fragment cloned into pBAD18 Km^r, used as a negative control	This study
pET32a	Amp^r, pBR322origin, T7 promoter and terminater, T7 transcription start site, f1 origin, Trx-Tag coding sequence, His-Tag coding sequence, S-Tag coding sequence, *lacI* coding sequence	Novagen, USA
pJR32T	pET32a contained *spnT* coding region	This study
pCR2.1	*lac* promoter, *lacZα fragment*, Amp^r, Km^r, oriCoE1, T7 promoter and primer site, M13 Forward (−20 and −40) and M13 Reverse priming sites, orif1	Invitrogen, USA
pSC200	pCR2.1 contained *spnT* coding region	[5]
Primers		
spnTF1	5′ -ATGGCATCGTTAACAAAAAA-3′	This study
spnTF2	5′-CCCGGTGTTTCATACAGCCAGCCC-3′	This study
spnTF3	5′ -GAGGAACAACAGCAGGCGGTGAT-3′	This study
SpnTR	5′-CATCGTGTATGGTCAGGGGC-3′	This study