Potential role of flavobacterial Por secretion system (PorSS) in root colonization and plant defense system stimulation

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Abstract

Plants are the key primary producers in most terrestrial ecosystems and generally exploit soils for resources using complex root systems. The rhizosphere is the soil environment that is directly influenced by the presence and activities of roots, and defined as a nutrient-rich area surrounding roots with decreased microbial diversity and increased biomass, activity, biological interactions and genetic exchange relative to the bulk soil. Moreover, plant–microbe interactions in the rhizosphere have both direct and indirect effects on plant health and nutrition, and therefore bacteria are an essential determinant of plant health and productivity [1-3]. Decades of culture-dependent and independent analyses of root-associated bacterial community composition, have determined that members of the *Bacteroidetes* phylum, especially those belonging to the *Flavobacterium* genus, are often highly enriched in the rhizosphere. Additionally, it was establish that under fertilized controlled conditions, *Flavobacterium* abundance could reach up to 30% of the total defined bacterial genera in the rhizosphere of a wide array of plants including lettuce (*Lactuca sativa*), potato (*Solanum tuberosum*), onion (*Allium cepa*), broccoli (*Brassica oleracea* var. *botrytis*), tall fescue pasture grass (*Festuca arundinacea*), barley (*Hordeum vulgare*), tomato (*Solanum lycopersicum*), sweet pepper (*Capsicum annuum*), cucumber (*Cucumis sativus*) and *Arabidopsis* [4-9]. Recently it was also proposed that this *Flavobacterium* enrichment is associated with their strong copitrophic properties and positively correlated with increases in nitrogen (N) availability [10]. Moreover it was shown that some *Flavobacterium* isolates have biocontrol capabilities. For instance, selected *Flavobacterium* isolates were highly antagonistic toward the soilborne fungal pathogens *Sclerotium rolfsii*, *Lasiodiplodia theobromae*, *Colletotrichum musae*, *Phytophthora cactorum* and *Phytophthora capsiki* which can infect a range of agricultural and horticultural crops [11-14]. Flavobacteria commonly possess an arsenal of extracellular enzymes such as proteinases and chitinases which enable them to degrade bacteria, fungi, insects and nematode constituents [15]. They often produce and secrete secondary metabolites, including a wide range of antibiotics [16], which may be beneficial in the highly competitive root environment. Additionally, many flavobacteria can move rapidly over solid surfaces by gliding motility. This movement is closely associated with the unique *Bacteroidetes*-affiliated Por protein secretion system (PorSS) [17–19]. Generally disruption of

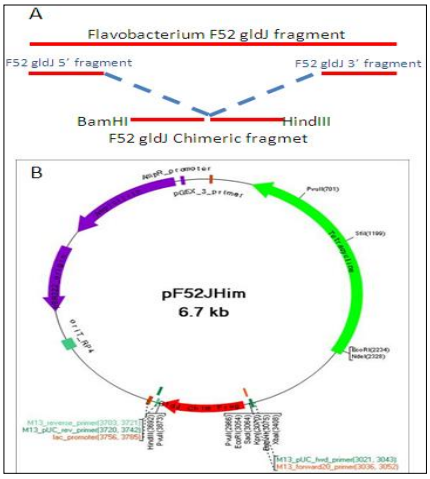

Figure 1. Schematic representation of pF52JHim plasmid. (A) Construction of the chimeric PCR fragment of gldJ. (B) Insertion of the PCR fragment into suicide plasmid pLYL001 to generate pF52JHim.

gliding motility genes lead to loss of both gliding and Por secretion [20]. Although flavobacteria are ubiquitous in root environments and preliminary data indicates that they are positively correlated to plant disease suppression, the appropriate explanation for their high abundance in rhizosphere is still missing. Many studies have revealed that effective colonization of antagonistic bacteria on roots through competition for limited nutrients and/or niches against plant pathogens leads to successful disease suppression by protecting infection courts from plant pathogens [2,21]. Effective root colonization and root exudate utilization are two potential explanations for the *Flavobacterium* abundance in the rhizosphere. The high abundance of flavobacteria in the rhizosphere and indications that it induces plant resistance, led us to hypothesize that gliding motility may give flavobacteria a competitive advantage in the rhizosphere, and that extracellular enzymes excreted by the PorSS may stimulate plant protection against phytopathogens.

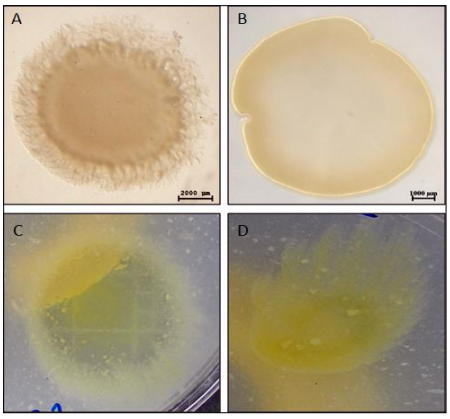

Figure 2. Phenotypic characterization of *Flavobacterium* F52 *gldJ*⁻ mutant. Plasmid pF52JHim was inserted into *Flavobacterium* F52 by triparental conjugation. Generated mutants were observed by their lack of gliding motility and chitin utilization. Left panel (A, C) represents *Flavobacterium* F52 wild type, right panel (B, D) represents *Flavobacterium* F52 *gldJ*⁻ mutant. Upper panel (A, B) represents gliding motility, bottom panel (C, D) represents chitin utilization.

To explore this hypothesis, we isolated a collection of root-associated *Flavobacterium* spp and screened them for biochemical and physiological properties that are attributed to plant growth promotion and protection. Based on integrative evaluation of these traits, *Flavobacterium* strain F52 was chosen as a model strain for studying *Flavobacterium*-root interactions. We recently sequenced the *Flavobacterium* strain F52 genome (accession number: NZ_AKZQ00000000) in order to detect genes linked to rhizosphere fitness, and to compare genomic characteristics of root-associated flavobacteria to previously sequenced flavobacterial genomes from other environments [22]. Interestingly, phylogenetic analyses of the sequenced genomes revealed two environmentally-distinct clusters: a soil/rhizosphere clade and an aquatic-associated clade. The genomes of the soil/rhizosphere flavobacterial clade were nearly twice the size of the marine flavobacterial strains, and were characterized by a wide array of genes involved in the metabolism of complex plant related sugars, proteins and fatty acids. The large genomes of the soil and root-associated flavobacteria may be dictated by the need for higher metabolic flexibility in terrestrial environments compared to aquatic ones.

Previous comprehensive genetic and biochemical analysis of the gliding motility/PorSS apparatus in the model strain *Flavobacterium johnsoniae* indicated that lipoprotein GldJ is obligatory for both gliding motility and chitin utilization [23]. To assess the role of gliding motility/PorSS apparatus in rhizosphere competence we constructed a *Flavobacterium* F52 *gldJ*⁻ mutant strain that lacks gliding motility and extracellular chitinase activity (Fig. 1 and Fig. 2).

Inoculation of one month old tomato (*Solanum lycopersicum*) roots with *Flavobacterium* F52 and its *gldJ*⁻ mutant strain (approx. 10^8-10^9 cells/ml) revealed that over a three-week period persistence of wild type flavobacterial strains was significantly higher than their corresponding *gldJ*⁻ mutants (Fig. 3). A similar experiment that applied the bacterial gram-positive phytopathogen *Clavibacter michiganens* to the tomato canopy (approx. 10^8-10^9 cells/ml) following inoculation of tomato roots with *Flavobacterium* F52 wild type or *gldJ*⁻ mutants, showed that the disease severity in plants inoculated with the wild type strain was lower than in those inoculated with the *gldJ*⁻ mutant (Fig. 4). These preliminary results suggest a central role of gliding motility/PorSS in flavobacterial rhizosphere competence and stimulation of plant defense systems.

The data presented here provides a functional connection between flavobacterial physiology (the unique flavobacterial gliding motility/secretion system) and the high abundance of flavobacteria in the rhizosphere of fertilized crops.

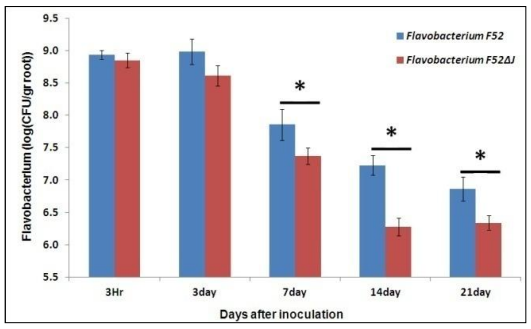

Figure 3. *Flavobacterium* F52 survival on plant roots. Tomato plant (*Solanum lycopersicum*) roots was inoculate with *Flavobacterium* F52 wild type or its *gldJ*⁻ mutant strain (F52ΔJ, approx. 10^8-10^9 cells/ml). Survival of flavobacterium, was inspected based on antibiotic enrich CYE agar plates and normalized to root mass. Statistical analyses were done with probabilities of 0.05.

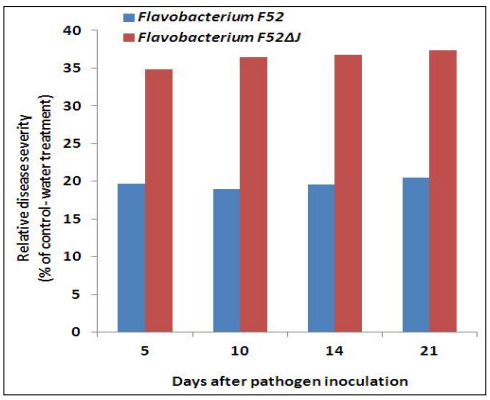

Figure 4. Role of gliding motility/PorSS system in stimulation of plant defense. Tomato (*Solanum lycopersicum*) roots were inoculated with *Flavobacterium* F52 wild type or its *gldJ*⁻ mutant strain (F52ΔJ, approx. 10^8-10^9 cells/ml) 3 days prior to the application of the bacterial gram-positive phytopathogen *Clavibacter michiganens* to the tomato canopy (approx. 10^8-10^9 cells/ml). Plant disease propagation was estimated during 3 weeks. Effect of *Flavobacterium* strains presents in the rhizosphere was calculated as a ratio of plant disease severity in *Flavobacterium* strains treatments to water (control) treatments.

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