

Virulence Factors of Plant Growth-Promoting Bacteria: Analysis of *Pseudomonas protegens* Genomes

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Abstract. *Pseudomonas protegens* is the species of plant growth-promoting bacteria, which is widely used in agriculture. In article, previously unknown virulence factors of this microorganism are revealed. When researching the three bacterial genomes *P. protegens*, genes encoding adherence, antiphagocytosis, iron uptake, biofilm formation, immune evasion, serum resistance, and other virulence traits have been found. More research is needed to learn the role of predicted virulence factors in phytopathology and medicine.

1 Introduction

Plant growth-promoting bacteria (PGPB) are a group of beneficial microorganisms that include 60 bacterial genera, such as *Bacillus*, *Pseudomonas*, and *Burkholderia*, which widely colonize plant leaves and soil, promote plant growth, and/or inhibit pathogen infection [1]. One of them is the species *Pseudomonas protegens*, which was first described by Alban Ramette and coworkers in 2011 as widespread plant-protecting bacteria producing the antimicrobial compounds 2,4-diacetylphloroglucinol and pyoluteorin [2].

Known as biocontrol agents, these pseudomonads exhibit antibacterial and antifungal activity. It found a practical application in various fields of agriculture. For example, volatile organic compounds produced by acid-tolerant *P. protegens* CLP-6 had excellent inhibitory effects on *Ralstonia solanacearum*, which is the causative agent of tobacco bacterial wilt [3]. The endophytic bacterium *P. protegens* NSJ-2101 inhibited the apple ring rot on postharvest fruits by activating the defense system of apple fruit and repressing the pathogenic factor of *Botryosphaeria dothidea* [4]. Cell-free secretions from *P. protegens* PBL3 inhibited the growth of bacterium *Burkholderia glumae* in vitro and also prevented *B. glumae* from causing bacterial panicle blight of rice [5]. Bacterial consortium of three Chilean strains of *P. protegens* inhibited wheat crown and root rot pathogens *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia cerealis*, and *Fusarium culmorum* [6]. The strain of *P. protegens* Pf-5 strongly inhibited the oomycete pathogen *Aphanomyces euteiches* (pea *Aphanomyces* root rot causative agent) [7]. Application of the bacterial

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strain *P. protegens* DA1.2 increased the amount of harvested bread wheat (*Triticum aestivum* L.) grain from 2.0-2.2 t/ha to 3.2-3.6 t/ha [8].

As per current information, infection caused by *P. protegens* in plant or animal/human host has not been described. However, virulence factors of *P. protegens* have not been analyzed. In this article the present author analyzes the genomes of different *P. protegens* strains to search for a bacterial potential pathogenic attributes.

2 Materials and methods

The complete genomes of three *P. protegens* strains were analyzed (Table 1). The type strain CHA0 was isolated from the roots of tobacco in Swiss soil, which was naturally suppressive to black root rot in tobacco caused by *Thielaviopsis basicola* [9]. *P. protegens* SN15-2 was isolated from the rhizosphere of tomato roots in Shanghai, China [10]. The biocontrol strain *P. protegens* Cab57 was isolated from the rhizosphere of shepherd's purse growing in a field in Hokkaido, Japan, by screening the antibiotic producers [11]. For comparison, complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen, was used [12].

Table 1. Analyzed sequences of *P. protegens* strains in this work.

Genome	NCBI Reference Sequence	GenInfo Identifier (GI)
<i>P. protegens</i> CHA0 chromosome 1, complete sequence 6,868,303 bp circular DNA	NZ_LS999205.1	1604088553
<i>P. protegens</i> strain SN15-2 chromosome, complete genome 7,075,587 bp circular DNA	NZ_CP043179.1	1783957276
<i>P. protegens</i> Cab57 chromosome, complete genome 6,827,892 bp circular DNA	NZ_AP014522.1	751653884

The characterization of genomes was carried out using the Virulence Factor Database (VFDB, <http://www.mgc.ac.cn/VFs/>), which is an integrated and comprehensive online resource for curating information about virulence factors of bacterial pathogens. Since its inception in 2004, VFDB has been dedicated to providing up-to-date knowledge of VFs from various medically significant bacterial pathogens. Instead of using simple BLAST searches, VFAnalyzer first constructs orthologous groups within the query genome and pre-analyzed reference genomes from VFDB to avoid potential false positives due to paralogs. Then, it conducts iterative and exhaustive sequence similarity searches among the hierarchical pre-build datasets of VFDB to accurately identify potential untypical/strain-specific VFs. Finally, via a context-based data refinement process for VFs encoded by gene clusters, VFAnalyzer can achieve relatively high specificity and sensitivity without manual curation [13].

3 Results and Discussion

The results of investigation showed, that although highlighted strains were isolated in different parts of the world (two in Asia, and one in Europe), and from the rhizosphere of miscellaneous plants, all of them found virulence factors. These factors are grouped into several categories (Table 2).

Table 2. Predicted virulence factors of *P. protegens* strains in comparison with *P. aeruginosa* PAO1.

Category	Virulence factor(s)	Related genes	Presence in strains of <i>P. protegens</i> (<i>P. p.</i>) and <i>P. aeruginosa</i> (<i>P. a.</i>)			
			<i>P. p.</i> CHA0	<i>P. p.</i> SN15-2	<i>P. p.</i> Cab57	<i>P. a.</i> PAO1
Adherence	Type IV pili biosynthesis	24	18*	18	18	+
	Type IV pili twitching motility related proteins	10	6	6	6	+
	LPS O-antigen (<i>P. aeruginosa</i>)	1	+	+	+	+
	Flagella	46	+	45	+	+
Antimicrobial activity	Polar flagella (<i>Aeromonas</i>)	62	2	3	3	-
	Phenazines biosynthesis	17	-	-	-	+
Antiphagocytosis	Alginate biosynthesis	13	+	+	+	+
	Alginate regulation	12	8	9	8	+
	Capsular polysaccharide (<i>Vibrio</i>)	29	1	1	1	-
	Capsule (<i>Klebsiella</i>)	1	2		2	-
Biosurfactant	Capsule I (<i>Burkholderia</i>)	26	-	1		-
	Rhamnolipid biosynthesis	3	-	-	-	+
Enzyme	Hemolytic phospholipase C	1	-	-	-	+
	Non-hemolytic phospholipase C	1	+	+	+	+
	Phospholipase C	1	-	-	-	+
	Phospholipase D	1	-	-	-	+
Iron uptake	Pyoverdine	16	+	+	+	+
	Pyoverdine receptors	1	+	+	+	+
	Pyochelin	10	9	9	9	+
	Pyochelin receptor	1	+	+	+	+
	Achromobactin biosynthesis and transport	8	-	-	-	-
	Yersiniabactin	9	-	-	-	-
Protease	Elastase	2	-	-	-	+
	Alkaline protease	1	+	+	+	+
	Protease IV	1	-	-	-	+
Quorum sensing	N-(butanoyl)-L-homoserine lactone QS system	2	-	-	-	+
	N-(3-oxo-dodecanoyl)-L-homoserine lactone QS system	2	-	-	-	+
	N-(3-oxo-hexanoyl)-L-homoserine lactone QS system	2	-	-	-	-
	Acylhomoserine lactone synthase	1	+	+	+	+
Regulation	GacS/GacA two-component system	2	+	+	+	+
	Carbon storage regulator A (<i>Legionella</i>)	1	+	+	+	-
	Two-component system (<i>Bordetella</i>)	2	+	+	+	-
	Two-component system (<i>Acinetobacter</i>)	2	-	+		-
Secretion system	<i>P. aeruginosa</i> TTSS	36	-	-	-	+
	<i>P. aeruginosa</i> TTSS translocated effectors	4	-	-	-	3
	<i>P. syringae</i> TTSS	32	-	-	-	-
	Harpins, pilus-associated proteins and other candidate TTSS helpers	6	-	-	-	-
	<i>P. syringae</i> TTSS effectors	81	3	3	3	-
	Hcp secretion island-1 encoded type VI secretion system (H-T6SS)	21	20	20	20	+
	Mxi-Spa TTSS effectors controlled by MxiE (<i>Shigella</i>)	10	1	1	1	-
	T4SS effectors (<i>Coxiella</i>)	130	1	1	1	-
Toxin	Exolysin	2	+	+	+	-
	Exotoxin A (ETA)	1	-	-	-	+
	Phytotoxin coronatine	20	-	-	-	-
	Phytotoxin phaseolotoxin	21	1	1	1	-
	Phytotoxin syringopeptin	3	-	-	-	-
	Phytotoxin syringomycin	7	1	1	1	-
	Hydrogen cyanide production	3	+	+	+	+
	TccC-type insecticidal toxins	1	-	-	-	-
	RTX toxin (<i>Vibrio</i>)	4	1	1	1	-
	The repeat in toxin (RTX) (<i>Aeromonas</i>)	6	1	1	1	-
Biofilm formation	PNAG (Polysaccharide poly-N-acetylglucosamine) (<i>Acinetobacter</i>)	4	1	1	1	-
Efflux pump	AcrAB (<i>Klebsiella</i>)	2	1	+	1	-
Glycosylation system	O-linked flagellar glycosylation (<i>Campylobacter</i>)	18	1		1	-
Immune evasion	LPS (<i>Brucella</i>)	31	2		2	-
	Capsule (<i>Acinetobacter</i>)	1		2		-
	Exopolysaccharide (<i>Haemophilus</i>)	6		1	1	-
Invasion	Invasion of brain endothelial cells (Ibes) (<i>Escherichia</i>)	3	1	1	1	-
Lipid and fatty acid metabolism	Isocitrate lyase (<i>Mycobacterium</i>)	1	+	+	+	-
Magnesium uptake	Mg2+ transport (<i>Salmonella</i>)	2	1	1	1	-
Others	O-antigen (<i>Yersinia</i>)	1	+	+	+	-
	Virulence-associated proteins (<i>Bartonella</i>)	5	1			-
Serum resistance	LPS rfb locus (<i>Klebsiella</i>)	1	+	+	+	-
Stress adaptation	Catalase (<i>Neisseria</i>)	1	+	+	+	-
	Catalase-peroxidase (<i>Mycobacterium</i>)	1	+	+	+	-
	Manganese transport system (<i>Neisseria</i>)	3	1	1	1	-

* the number of related genes present; + all related genes present; - all related genes absent or inactive; TTSS Type III secretion system

First step in bacterial pathogenesis is usually adherence to tissue. Bacterial motility is an important capability for a successful pathogen to avoid hostile environments and discover useful resources for survival. Flagella contributes to swimming motility, play a role in biofilm formation, which largely improves their resistance to antimicrobials and host immunities to contribute to the survival, dispersion and colonization of the bacteria. By type IV pili bacterial cells attach to host cells, causing a twitching motility that allows the bacteria to move along the cell surface [13-15]. Mucoïd exopolysaccharide alginate also affects *P. aeruginosa* biofilm development and architecture [16].

Iron is an essential nutrient for the proliferation and pathogenicity of bacterial pathogens. The well-characterized host defense strategy of iron sequestration highlights the crucial role of iron acquisition systems in bacterial pathogenesis [13]. Pyoverdine is effective at acquiring iron from transferrin and lactoferrin [17]. Pyochelin, a structurally unique siderophore possessing phenolate, but neither a hydroxamate nor a catecholate moiety, is effective at promoting iron uptake in *P. aeruginosa*. Also, pyochelin also binds other transition metals (e.g. Mo(IV), Ni(II) and Co(II)) with appreciable affinity and is, in fact, implicated in the delivery of both Co(II) and Mo(IV) to *P. aeruginosa* cells [18].

Pore-forming toxin exolysin (*exlA*, *exlB*) responsible for host cell membrane disruption [19]. The 77-kilodalton non-hemolytic phospholipase C (*plcN*) hydrolyzes phosphatidylcholine as well as phosphatidylserine [20]. Alkaline protease (*aprA*) inhibits the function of the cells of the immune system (phagocytes, NK cells, T cells), inactivates several cytokines (IL-1, IL-2, IFN- γ , TNF), cleaves immunoglobulins and inactivate complement [21].

Catalase (*katA*) detoxifies H₂O₂, protects against reactive oxygen species, a family of chemical that are oxidized version of molecular oxygen (hydrogen peroxide, superoxide, and hydroxyl radicals). Catalase:peroxidase (*katG*) degrades H₂O₂ and organic peroxides, the major role is to catabolize the peroxides generated by phagocyte.

4 Conclusion

The obtained results indicate that the beneficial saprophytic rhizosphere bacteria have virulence genes in their genome. Under certain conditions (for example, climate change, exposure to pesticides, or introduction into the immunodeficient organism) this circumstance is bound to be of great importance.

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