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Research Article

Inhabiting fluorescent *Pseudomonas* on wheat seed promote bacterial leaf streak disease

Aisan Afkhamifar[1], Cobra Moslemkhani[2*], Nader Hasanzadeh[1], Javad Razmi[1] and Leila Sadeghi[2]

1. Department of Plant Protection, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Seed and Plant Certification and Registration Institute, Agricultural Research, education and Extension Organization (AREEO), Tehran, Iran.

Abstract: Wheat seeds harbor different microbial populations, which can be associated with each other in neutral, positive, or negative interactions. The present study investigated the interaction of fluorescent *Pseudomonas* and *Xanthomonas translucens* (*Xt*) as an important wheat seed-borne pathogen. In addition to *P. fluorescens* (A7) and *P. chloroaphis* (A4), which were previously isolated from the rhizosphere of potato as potent biocontrol agents, six more non-pathogenic *Pseudomonas* strains that were isolated from wheat seeds, were studied. According to the general biochemical tests and partial 16S rRNA sequences alignment, the isolated strains were closely related to the species of *P. gessardii, P. orientalis, P. poae, P. koreensis,* and *P. cedrina*. The *Pseudomonas* strains exhibit different antagonistic activities, such as phosphate solubilization, cellulase, protease, and lipase production. Also, they have an apparent inhibition effect under *in vivo* conditions against *X. translucens.* Seed treatment by these strains led to suppressing bacterial leaf streak disease incidence in an early growth stage. However, disease progress enhanced with the seedling growth, resulting in the treated plants' complete death. Only in treated seeds by P. *fluorescens* (A7), P. *chloroaphis(*A4*)*, and *P. orientalis* (Ais119) decrease of AUDPC up to 83%, 74%, and 63% was achieved, respectively, compared with the untreated controls. Our results showed that some fluorescent *Pseudomonas* strains could cause delay at the beginning of the disease appearance due to competition or producing antimicrobial metabolites during that time. In contrast, some may be considered a threat, enhancing disease development through synergistic effects.

Keyword: *Xanthomonas translucens*, *Pseudomonas* spp, disease severity, synergy

Introduction

Diverse communities of bacteria inhabit the outside and inside of different plant tissues (Schlaeppi and Bulgarelli, 2015) associated with the rhizosphere, endosphere, and phyllosphere (Compant *et al*., 2019). Seeds also contain various microbial populations, that are important determinants in seed health, germination, and growth (Chee-Sanford *et al*., 2006; Rodríguez *et*

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*Corresponding author: Moslemkhany@yahoo.com
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al., 2020; Afkhamifar *et al*., 2023a, b) and they can affect the early life stages of a plant (War *et al*., 2023).

Pseudomonads are prominent bacteria found in different habitats. They are associated with plants as pathogens and saprophytes with/without plant-growth-promoting abilities (Peix *et al*., 2009). Some *Pseudomonas* strains are known as successful plant pathogens (Preston, 2004; Compant *et al*., 2005; O'Brien *et al*., 2011), and others may promote plant defense mechanisms and stimulate plant growth (Haas and Keel, 2003; Raaijmakers and Mazzola, 2012).

Beneficial *Pseudomonas* species suppress pathogen activities by producing antibiotics (Haas and Keel, 2003; Raaijmakers and Mazzola, 2012) and other inhibitory compounds (Ramette *et al*., 2003). They compete with pathogens for nutrients or space through the rapid consumption of plant exudates and niche occupation (Weller, 2007; Kamilova *et al*., 2005). They can also induce host plant defense (Preston, 2004; Pieterse *et al*., 2014; Durairaj *et al*., 2017),

Species of Xanthomonas cause more than 350 different plant diseases (Marin *et al*., 2019). Effective chemical control is generally limited and usually unsuccessful. The most viable strategies for controlling bacterial diseases are using healthy seeds, resistant or tolerant cultivars, hygiene practices, and disinfestation of working tools (Marin *et al*., 2019). Using biocontrol agents as a promising strategy for controlling diseases is considered in sustainable agriculture (Xue *et al*., 2013; Pernezny *et al*., 2012; Bale *et al*., 2008). Previous research indicates successful control of some plant diseases by fluorescent *Pseudomonas* spp. (Abo-Elyousr and El-Hendawy, 2008; De Oliveira *et al*., 2011; De Oliveira *et al*., 2016; Khodakaramian *et al*., 2008; Mishra and Arora., 2012; Príncipe *et al*., 2018; Spago *et al*., 2014; da Silva Vasconcellos *et al*., 2014; Bale *et al*., 2008). Some Pseudomonas strains as biocontrol agents can reduce *X. translucens* by decreasing population sizes or disease severity (Stromberg *et al*., 2000). They also can interfere with specific metabolic responses in wheat, known as ecological feedback between wheat and *Pseudomonas* strains (Rieusset *et al*., 2022). Seed treatment with *Pseudomonas* strains as biological control agents has been reported to be effective in controlling several diseases (Amein *et al*., 2008; Khan *et al*., 2006). Wheat seeds carry a complex of *Pseudomona*s population, which may exert beneficial or harmful effects on plant growth and health. Considering the economic importance of bacterial leaf streak (BLS) disease on wheat caused by *X. translucens*, this study aimed to investigate different aspects of seed treatment by fluorescent *Pseudomonas* strains that isolated from wheat seeds and also two other potent biocontrol agents that were previously isolated from potato rhizosphere, on plants that suffer from BLS disease.

Materials and Methods

Seed sampling

Wheat seeds were sampled from six seed lots in Markazi, Azerbaijan Gharbi, Hamedan, and Kurdistan provinces according to the International Seed Testing Association (ISTA) instructions (2020).

Isolation of bacterial strains from wheat seeds

Serial dilutions of wheat seed suspension (50g seed in 0.85% NaCl contains 0.01% Tween 20) were cultured on the N.A. (Nutrient Agar) and K.B. (King's B) media (Asaad *et al*., 2017). Gram-negative colonies suspected to be *Pseudomonas* and the bacterial colonies with blue-green fluorescent pigment on the K.B. medium were isolated. General biochemical assays, including gram reaction, hypersensitivity response (H.R.), color of the colonies on yeast dextrose carbonate agar (YDC) medium, aerobic/anaerobic growth, oxidase, and catalase production, were done for preliminary diagnosis of the *Pseudomonas* isolates (Schaad *et al*., 2001; Klement *et al*., 1964). *X. translucens* isolate (Ais106) was received from the Seed and Plant Certification and Registration Institute.

P. fluorescens (A7) and *P. chloroaphis* (A4) as successful biocontrol agents that previously have been isolated from potato rhizosphere (unpublished data) were received from the Iranian Research Institute of Plant Protection.

Genomic fingerprinting by rep-PCR analysis

The representative strains were selected from among isolates with the same fingerprint pattern by BOX-PCR. DNA was extracted using the method of Rademaker (1997). Amplification reactions were carried out in 25 µl volumes, with 12.5µl Taq 2x Master Mix Red (Ampliqon, Denmark), 2 µM of 22-mer BOXA1R oligonucleotide (5'-CTACGGCAAGGCGACGCTGACG-3') and 5 µl of a bacterial DNA (Versalovic *et al*., 1991). Amplification was performed with the initial denaturation step for 3 min at 94 °C, followed by 35 cycles of 30 seconds at 94 °C, 1 min at 50 °C, 8 min at 72 °C and a final extension at 72 °C for 10 min.

Sequencing of the 16S rRNA gene

Bacterial isolates were identified based on the sequence of the 16S rRNA region (PCR products were sent to Bio Magic Gene Company for sequencing). The DNA sequences were compared with other sequences in the GenBank database using the BLAST search program (http://www.ncbi.nlm.nih.gov/). Obtained sequences were then deposited in the GenBank database for public access. The sequences were aligned (with similar strains downloaded from the EzTaxon-database), and phylogenetic tree was constructed using Neighbour-joining method using MEGA 6 software with bootstrap values calculated from 1000 replicates (Saitou and Nei, 1987).

Traits assessment of Pseudomonas strains

Isolate's ability to inhibit the growth of *X. translucens* was evaluated by measuring the diameter of the zones of *X. translucens* inhibition after 72 hours of incubation at 28 °C. Inhibition zones assay was done by spreading 100 µl of *X. translucens* at 1×10^7 CFU/ml concentration on nutrient agar and spotting selected isolates after drying the surface of the culture plates. Production of indole acetic acid by the Salkowski reagent (Bent *et al*., 2001), production of extracellular compounds, phosphate solubilizing capability (Castagno *et al*., 2011), and enzyme activity including lipase (Tom and Crisan, 1975), proteinase (Majumdar and Chakraborty, 2017), and cellulase (Borkar, 2017) were assessed. The effects of volatile organic compounds produced by *pseudomonas* isolates on *Xt* were also evaluated using a dual-culture assay (Zhang *et al*., 2023).

Biofilm analysis

The qualitative assay for biofilm formation was performed according to the method described by Sorroche *et al*. (2012) with some modifications in the crystal violet staining method. The bacteria were grown in 2 ml N.B. medium for 48 h at 30 °C. The bacterial suspension (150 µl) in fresh medium with OD_{600}: 0.1 was added to each well of ELISA plate and incubated for 24 h at 30 °C. After removing and washing planktonic cells, crystal violet aqueous solution (0.1%, W/V) was added. Crystal violet-stained plate was rinsed after 15 min and then scored for biofilm formation by adding 150 µl, 95% ethanol. The OD_{570} related to dissolved crystal violet was recorded by MicroELISA Reader (Bio-tek ELX 808) device, and then the results were analyzed by Stepanović *et al*. (2000) described method.

Effect of selected *Pseudomonas* strains on BLS disease severity under greenhouse conditions

Effects of eight selected *Pseudomonas* strains on BLS disease severity (on Pishgam cultivar) were investigated under greenhouse conditions. Wheat seeds were treated with 1% CMC and 1×10^7 CFU/ml suspension of each *Pseudomonas* strain for one hour. Treated seeds were sown in soil infected with *Xt* suspension (10 ml of *Xt* suspension with a concentration of 1×10^8 CFU/ml were added to 10 gr soil). Untreated seeds in infected and non-infected soil were used as controls. Disease symptoms were assessed daily from 7 to 30 days after seed sowing. The severity of the disease in the 10th and 30th days was measured as a basis for the area under the disease progress curve (AUDPC) calculation. The severity of bacterial leaf streak was measured by the Duveiller′s method (Duveiller, 1994), and a scale of 0-6 was used for disease severity evaluation (Milus and Mirlohi, 1994). The experiment was conducted in a completely random design with three replicates. Statistical analysis was performed with SPSS (SPSS Inc., Chicago).

Assessment of plant defense responses by Real-Time PCR Analysis

The wheat leaves were harvested from the *Xt*-infected plants 20 days after cultivating the *Pseudomonas*- treated seeds. Leaves were ground in liquid nitrogen, and RNA isolations were performed using a Ribospin plant Kit (GeneAll, South Korea) according to the manufacturer's recommendations. The cDNA was generated by reverse transcription with Hyperscript RT-PCR master mix® according to the manufacturer's instructions (GeneAll, South Korea). The first strand of cDNA was synthesized from 1 µg of total RNA using the Hyperscript RT-PCR master mix® and oligo dT primers according to the manufacturer's instructions.

The qRT-PCR analysis was performed in three biological replicates with the SYBR Premix Ex TaqTM II kit (TaKaRa, Japan). Specific primers CES-F, CES-R (Cellulose synthase), and PR-1F, PR-1R were used to amplify target genes, and primers ActinF and ActinR were used as an internal control for normalizing the transcription. Each qRT-PCR reaction consisted of a mixture containing 15 ng/µL of cDNA, 0.4 µM of each forward and reverse primer, and Master Mix Green. PCR cycling included 95 °C for 10 min (heat activation), 40 cycles at 95 °C for 15 s, 60 °C for 1 min (amplification), and then 72 °C for 10 min. Gene expression was analyzed using the $2^{-\Delta\Delta Ct}$ method. Data were statistically analyzed by conducting the analysis of variance (ANOVA) by SPSS (SPSS Inc., Chicago).

Results

Sixty-four epiphytic bacterial strains were isolated from six wheat seed lots sampled from Markazi, Kurdistan, Hamedan, and Azarbaijan Gharbi provinces. Based on morphological and some biochemical characteristics, isolated bacteria were initially identified at the genus level. Out of 25 non-pathogenic *Pseudomonas* isolates, six isolates were selected as the representative isolate for the following evaluations based on the fingerprint pattern of BOX-PCR (Fig. 1). Some diagnostic tests are summarized in Table 1.

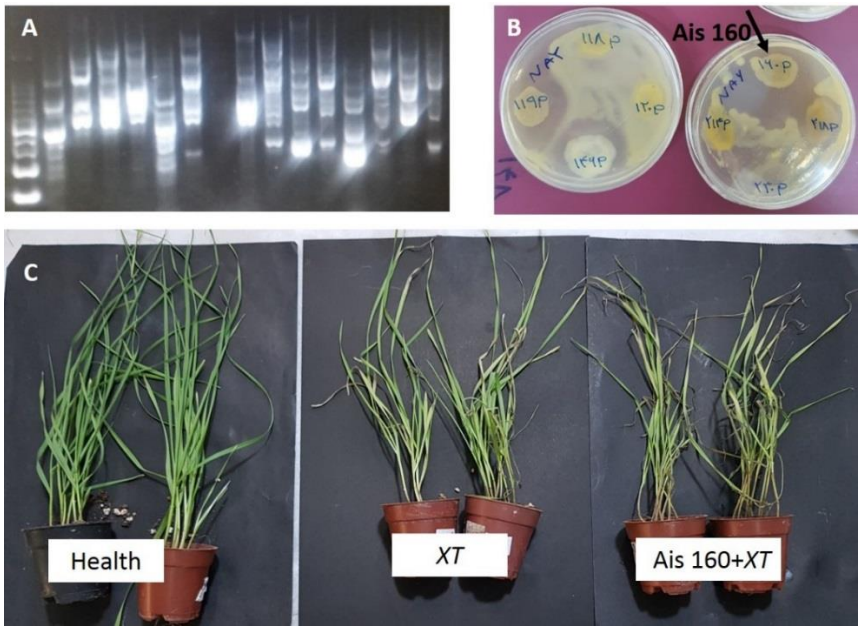

Figure 1 A: BOX-PCR fingerprint patterns of *Pseudomonas* strains, isolated from wheat seeds; B: Antagonistic activity of candidate *Pseudomonas* isolates against *X. translucens*, in dual culture assay; C: Aggravation of the bacterial leaf streak disease severity due to seed treatment with *P. cedrina* (Ais160), 15 Day after sowing wheat seeds (Pishgam cultivar) in *X. translucens* infected soil.

Table 1 Information of bacterial isolates, sampling sites and results of some basic identification tests.

Isolate	Host/Cultivar	Sampling site	Isolation	Colony color onGram test YDC medium		fluorescent onHR King's B medium		Ice nucleation
Ais159	Wheat/ Mihan	Markazi	Seed/epiphyte	Creamy	-	+	-	-
Ais218	Wheat/ Mihan	Kurdistan	Seed/epiphyte	Yellowish cream	-	+	-	-
Ais125	Wheat/ Mihan	Azarbaijan gharbi	Seed/epiphyte	Creamy	-	+	-	-
Ais160	Wheat/ Mihan	Markazi	Seed/epiphyte	Creamy	-	+	-	-
Ais146	Wheat/ Mihan	Markazi	Seed/epiphyte	Whitish cream	-	+	-	-
Ais119	Wheat/ Mihan	Azarbaijan gharbi	Seed/epiphyte	brownish cream	-	+	-	-
A7	Potato	Hamedan	Rhizosphere	Creamy	-	+	-	-
A4	Potato	Hamedan	Rhizosphere	creamy	-	+	-	-
Xt 106	Wheat/ Mihan	Hamedan	Seed/epiphyte	Pale yellow	-	-	+	

+: Positive; -: Negative.

The selected isolates exhibited high similarity with *P. gessardii* (Ais218), *P. orientalis* (Ais119; Ais 125), *P. poe* (Ais146), *P. koreensis* (Ais159) and *P. cedrina* (Ais160) based on the 16S rRNA gene sequencing. These sequences were deposited in NCBI GenBank with the accession number OM095360, OM095357, OM095358, OM095354, OM095359, and OM095362, respectively. Based on the BLAST results and sequences alignment, a phylogenetic tree was constructed by the neighbor-joining method using MEGA 6 software with similar strains downloaded from the EzTaxon-database (Fig. 2).

The severity of BLS disease reduced 10-days post-inoculation (DPI) upon the seed treatment by some *Pseudomonas* strains (Ais159, Ais218, Ais125, Ais160, and Ais146) (Table 2), but gradually, until the 30th day after inoculation the percentage of disease severity and AUDPC were significantly increased up to complete death of plants in comparison to untreated seeds (Table 2). Only *P. fluorescens* (A7) and *P. chloroaphis* (A4), and *P. orientalis* (Ais119) exhibited effective biocontrol activity among other strains under greenhouse conditions. They decreased AUDPC up to 83%, 74%, and 63%, respectively.

P. orientalis (Ais125) and *P. cedrina* (Ais160) inhibited growth of *Xt* with an average inhibition of 21 mm in diameter, more significant than the other strains ($P > 0.01$). Still, they showed a synergistic effect on BLS disease development under greenhouse conditions (Fig. 1C). Meanwhile, *P. chloroaphis* (A4), with a high degree of disease control in greenhouse experiments, showed minimum suppression in the inhibition zones assay.

Production of secondary metabolites such as phosphatase, protease, and cellulase of selected *Pseudomonas* strains were confirmed in this study (Table 3). The cellulolytic activity was positive in *P. cedrina* (Ais160), *P. poae* (Ais146), *P. fluorescens* (A7), and *P. chloroaphis* (A4) strains. The bacterial strains encoded Ais159, Ais218, Ais146, and Ais119 produced proteinase enzymes (Fig. 3). Most of the selected *Pseudomonas* isolates, except Ais218 and Ais125 showed phosphatase activity.

Our study confirmed the antimicrobial activity of *P. orientalis* (strains Ais119 and Ais125) against *Xt* under *in vitro* conditions, but only strain Ais119 exhibited successful and permanent antagonistic activity under *ex vivo*.

We screened *Pseudomonas* strains to determine their ability to induce a defense response in wheat seedlings. The candidate strains induce expression of *PR1* and *Ces* genes in plant defense assessment compared to untreated seeds in pots containing uncontaminated soil. *Xt* as a pathogenic bacterium and *P. fluorescens* (A7) as a beneficial microbe induced most expression of P.R. protein and *Ces* genes. Plants grown in *Xt*-infected soil without any treatment showed a more significant increase in the expression of both defense genes (*PR1* and *Ces*) 10 days after seed sowing (Table 2).

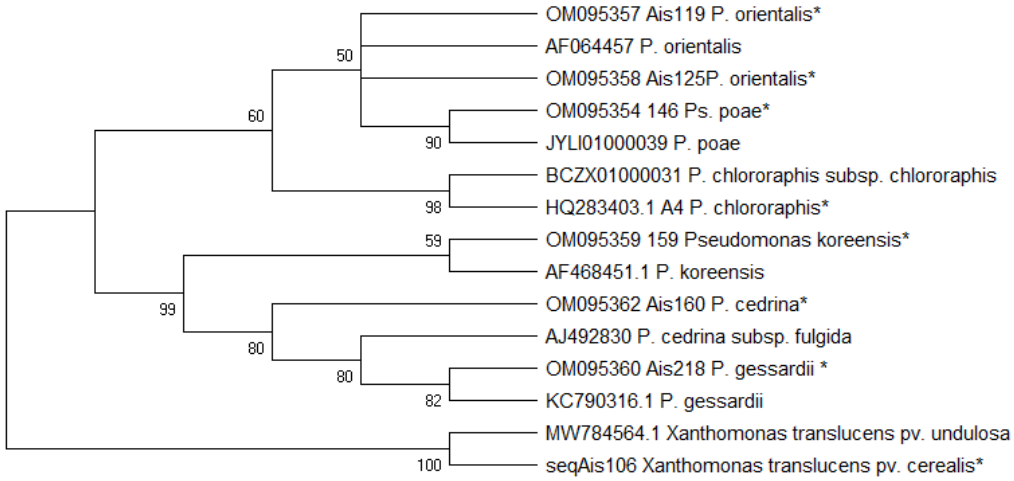

Figure 2 Phylogeny tree of representative fluorescent *Pseudomonas* strains isolated from wheat seeds based on 16S rRNA sequence similarity. *Xanthomonas translucens* was used as an outgroup. Sequences marked with an asterisk correspond to the isolates analyzed in the present study.

Table 2 Mean comparison of bacterial leaf streak disease severity, AUDPC and qRT-PCR expression analysis of *PR1* and *Ces* genes in wheat plants obtained from seeds treated with fluorescent *Pseudomonas* strains.

Seed treatment with bacterial isolates	Infected soil with *Xanthomonas translucens*			Defense gene expression (Real time PCR)	
	Disease severity on 10 DPI	Disease severity on 30 DPI	AUDPC in period of 10 – 30 DPI	*PR1* 10 DPI	*Ces* 10 DPI
P. koreensis (Ais159)	11.465	100	557.32^{a}	0.049^{b}	0.017^{b}
P. gessardii (Ais218)	5.28	100	526.4^{a}	-	-
P. orientalis (Ais125)	13.27	100	566.37^{a}	0.191^{b}	0.034^{b}
P. cedrina (Ais160)	7.97	100	539.87^{a}	0.711^{b}	0.405^{b}
P. poae (Ais146)	12.723	100	563.61^{a}	-	-
P. orientalis (Ais119)	10.79	22.9	168.49^{c}	-	-
P. fluorescens (A7)	2.145	13.69	79.19^{de}	10.230^{a}	0.998^{b}
P. chloroaphis (A4)	2.74	21.35	120.49^{cd}	1.655^{b}	0.483^{b}
Not treated	16.5	76.9	467^{b}	9.881^{a}	7.970^{a}

Means with the same letter are not significantly different (P > 0.01).

Table 3 Some traits of fluorescent Pseudomonas isolates obtained from wheat seeds and potato rhizosphere.

Isolate	Diameter of *Xt* inhibitory halo (mm)	Proteinase	Phosphatase	Cellulase	Lipase	Extracellular substances	Volatile compounds	Biofilm	IAA
P. koreensis (Ais159)	5^{d}	+	+	-	+	L	+	-	+
P. gessardii (Ais218)	5^{d}	+	-	-	+	L	+	-	+
P. orientalis (Ais125)	21^{a}	-	-	-	-	L	-	-	+
P. cedrina (Ais160)	21^{a}	-	+	+	+	L	+	-	+
P. poae (Ais146)	10.33^{b}	+	+	+	-	H	-	-	+
P. orientalis (Ais119)	10.54^{b}	+	+	-	-	H	-	-	+
P. fluorescens (A7)	9.5^{bc}	-	+	+	+	H	-	+	+
P. chloroaphis (A4)	4.5^{d}	-	+	+	+	L	-	+	+

Means with the same letter are not significantly different from each other (P > 0.01).

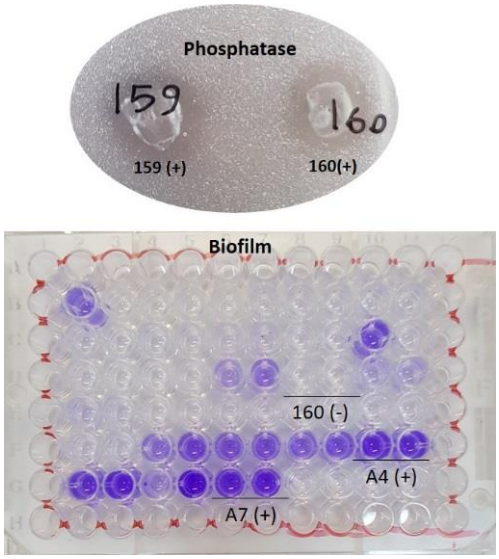

Figure 3 *In vitro* evaluation of selected non-pathogenic *Pseudomona*s (Ais160, A7 and A4) for enzyme activity and assessment of biofilm formation by crystal violet staining method.

Discussion

In this study, the fluorescent pseudomonas strains closely related to *P. gessardii*, *P. orientalis, P. poae, P. koreensis,* and *P. cedrina* were detected from wheat seeds. Garrido-Sanz *et al*.,(2016) declared that the fluorescent *pseudomonas* complex contain eight phylogenomic groups, such as *P. fluorescens* group (containing several species including *P. fluorescens, P. poae, P. orientalis, P. cedrina*), *P. koreensis* group, *P. gessardii* group and *P. chloroaphis* group some of which aredescribed as beneficial microorganism in disease management via induction of plant resistance, competition and production of antibiotics, phytohormones and metabolites (Capdevila *et al*., 2004; Bakker *et al*., 2007; Han *et al*., 2006).

Our study showed, despite strain's antagonistic activities under *invivo* conditions, all of the *Pseudomonas* strains (except for three Ais119, A7 and A4) only caused a relative control of the disease in the early stages under greenhouse condition and over time, they induced the development of disease much more severely than the control. Pliego *et al.* (2011) showed only a few bacteria that had antagonistic properties in laboratory conditions could ultimately play an influential role as a biocontrol agent in greenhouse and field conditions. The antagonistic interactions, due to other biotic interactions in plants may only sometimes lead to successful disease protection (Besset-Manzoni *et al*., 2019). Thus, laboratory and applied practical experiments were recommended for developing a biocontrol product (Besset-Manzoni *et al*., 2019).

Selected *Pseudomonas* strains in this research exhibited different metabolite activity such as phosphatase, protease, and cellulase. These results follow some findings indicating the role of cellulolytic and Proteinases activities of bacteria in plant disease management and plant growth promotion (Szydlowski *et al*., 2015; Majumdar and Chakraborty, 2017; Passari *et al*. 2016). Garrido-Sanz *et al*. (2016) reported that fluorescent *Pseudomonas* produce volatile and non-volatile metabolites in their antagonistic activities. Most of the selected *Pseudomonas* isolates, except Ais218 and Ais125 showed phosphatase activity. Phosphatase enzymes provide available phosphate and suitable conditions for plant growth (Gyaneshwar *et al*. 2002).

Even though the enzymes contribute as control agents in antagonistic bacteria against different plant pathogens, especially fungi (Jadhav *et al*., 2017; Admassie *et al*., 2022; Cho *et al*., 2007), the production of some enzymes can aggravate the bacterial disease. Some enzymes, such as proteinase, involve infection of bacterial pathogens in the host plant via regulatory roles, modulation of virulence factors functioning, avoidance of plant recognition, and suppression of defense response pathways (Figaj *et al*., 2019). Cellulolytic enzymes are required for bacteriapenetrateionand spread in plants through the intercellular space (Yadav *et al*., 2017). Enzymes may play a synergistic role in hydrolyzing the plant host substrates (Carro and Menéndez, 2020), which in turn causes more severe disease damage.

All of investigated strains produced indole-3-acetic acid (IAA). Rafikova *et al*. (2016) reported *that P. koreensis strain IB-4 showed the*

valuable features characteristic of PGPR microorganisms, such as synthesizing indole-3-acetic acid (IAA) and cytokinin-like compounds and couldantagonize plant pathogens. Similar to our results Duman and Soylu. (2019) concluded that *P. gessardii* strain under *in vitro* conditions showed antagonistic activity against *Pseudomonas syringae* pv. *phaseolicola* due to its ability to produce a relatively large amount of extracellular IAA.

Our results showed *P. orientalis* Ais119 and *P. orientalis* Ais125 exhibited different antagonistic and synergistic activities. Hofte and Altier (2010) demonstrated the members of *Pseudomonas* spp. contain effective biocontrol agents, but their biocontrol abilities are strain-dependent. Pathogenic, saprophytic, and plant-growth-promoting strains are often found within the same species (Audenaert *et al*., 2002; Janisiewicz and Marchi, 1992), so risk assessment of the biocontrol agent for prevention of non-target effect (such as synergistic effects on other pathogens) is essential (Winding *et al*., 2004).

In agreement with our studies, Zengerer *et al*. (2018) determined the antagonistic activity of *P. orientalis* (strain F9) against phytopathogenic bacteria, particularly *Erwinia amylovora*. However, they concluded *P. orientalis* revealed phytotoxic traits in the apple flower and produced phenazines, which contributed to the antagonistic activity of bacterial strains against pathogens.

The studied Pseudomonas strains activated defense response in wheat seedlings. This study showed expression of PR1 and Ces genes was positively induced by *Xt* and *P. fluorescens* (A7) more than other strains.

Ces is a crucial gene in plant cellulose synthesis, increasing *Ces* gene expression and augmenting structural barriers via enhancing cell wall thickness (Sun *et al*., 2014; Maleki *et al*., 2020). Cellulose plays a fundamental role in the structure of physical barriers, affecting cell wall strengths and integrity against pathogens during the defense response (Hückelhoven, 2007).

Xanthomonas effectors interacts with host defense-related proteins, which can trigger or suppress plant cell death and defense responses in plants (Han and Hwang, 2017). Garcia-Seco *et al*. (2017) reported the alteration of several enzymes involved in cell wall remodeling after *X. translucens* invasion that caused intracellular signal transduction and the cell defense response. They clarified *X. translucens* attack induces an active interplay between S. A. and J. A. pathways. Buell and Somerville (1995) demonstrated that PR1 accumulation in compatible interaction of Arabidopsis and *Xanthomonas campestris* pv. *campestris*, acts as a sensitive indicator in susceptible plants in response to pathogen invasion. Defense-related proteins are induced through the action of the signaling compounds and increase in response to pathogen attack. The PR-1 is one of the best-characterized P.R. genes used as a SAR marker (Van Loon *et al*., 2006). Different strains of the *Pseudomonas* species can be highlighted as potential biological control agents; they may suppress pathogens through prime and elicit defense responses and induced systemic resistance (Choudhary *et al*., 2009).

Conclusion

Fluorescent Pseudomonas that colonize wheat seeds showed different antagonistic activities and suppressed *Xt* in the early stage. However, over time, the development of the disease in plants from treated seeds (with Ais159, 218, 125, 160, and 146) was more intense than the untreated ones. It seems that the pathogenic bacteria will win in the competition with Pseudomonas strains after some time. Some other Pseudomonas factors, including enzymes, may also act as synergists in the host plant's penetration and development of disease. Our research exhibited that the *Pseudomonas* strains isolated from soil (A4 and A7) were more successful in permanently controlling BLS disease through direct and indirect mechanisms. This study confirmed the different abilities of *Pseudomonas* strains, therefore their potential abilities should be taken into consideration in order to prevent damage to non-target control agents. Since some fluorescent *pseudomonas*

strains can intensify the development of bacterial diseases over time, future research must focus more on this issue for introducing reliable biocontrol agents.

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تشدید توسعه بیماری نواری باکتریایی گندم از طریق فعالیت باکتری‌های سودوموناس فلورسنت مستقر در بذر گندم

آی‌سن افخمی‌فر[۱]، کبری مسلم‌خانی[۲*]، نادر حسن‌زاده[۱]، جواد رزمی[۱] و لیلا صادقی[۲]

۱- گروه بیماری‌شناسی گیاهی، دانشگاه آزاد اسلامی، واحد علوم و تحقیقات، تهران، ایران.
۲- مؤسسه تحقیقات ثبت و گواهی بذر و نهال، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران.
پست الکترونیکي نویسنده مسئول مکاتبه: Moslemkhany@yahoo.com
دریافت: ۱۲ شهریور ۱۴۰۲؛ پذیرش: ۲۹ آبان ۱۴۰۲

چکیده: بذر گندم حامل جمعیت‌های میکروبی متعددی است که این جمعیت‌ها می‌توانند تعاملات خنثی، مثبت یا منفی بر یک‌دیگر داشته باشند. مطالعه حاضر به بررسی اثر سودوموناس‌های فلورسنت بر باکتری *Xanthomonas translucens* (Xt) به‌عنوان بیمارگر بذرزاد مهم گندم پرداخته است. علاوه‌بر جدایه‌های *P. fluorescens* (A7) و *P. chloroaphis* (A4) که قبلاً از ریزوسفر سیب‌زمینی به‌عنوان عامل کنترل زیستی قوی جدا شده بودند، شش جدایه غیربیماری‌زا Pseudomonas فلورسنت براساس الگوی باندی BOX-PCR به‌عنوان جدایه نماینده انتخاب و در تحقیق حاضر مورد بررسی قرار گرفتند. جدایه‌های سودوموناس منتخب با استفاده از نتایج توالی 16S rDNA به‌عنوان *P. gessardii*، *P. orientalis*، *P. poae*، *P. koreensis* و *P. cedrina* شناسایی شدند. جدایه‌های منتخب فعالیت‌های آنتاگونیستی مختلفی نظیر انحلال فسفات، تولید آنزیم‌های سلولاز، پروتئاز و لیپاز را نشان دادند. هم‌چنین در شرایط *in vivo* اثر بازدارندگی معنی‌داری را علیه باکتری *X. translucens* نشان دادند. تیمار بذر توسط تمامی این جدایه‌ها منجر به سرکوب بیماری در مراحل اولیه رشد شد اما در برخی تیمارها با توسعه و رشد گیاهچه‌ها، به‌تدریج شدت بیماری تا حد مرگ کامل گیاه با سرعت بیش‌تری در مقایسه با بذرهای تیمار نشده (در شرایط آلودگی خاک) پیش رفت. تنها تیمار بذر با جدایه‌های *P. fluorescens* (A7) و *P. chloroaphis* (A4) و *P. orientalis* (Ais119) باعث کاهش AUDPC بیماری به‌ترتیب تا ۸۳، ۷۴ درصد و ۶۳ درصد نسبت به بذرهای تیمار نشده (در شرایط آلودگی خاک) شدند. نتایج ما نشان داد که برخی از جدایه‌های سودوموناس فلورسنت به‌دلیل رقابت با عامل بیمارگر یا تولید متابولیت‌های ضدمیکروبی باعث تأخیر در شروع بیماری نواری باکتریایی گندم شدند اما به مرور زمان از طریق اثرات هم‌افزایی با عامل بیمارگر به‌دلیل تولید آنزیم‌های تخریب‌کننده اجزاء سلولی گیاه یا به‌دلیل سرکوب پاسخ‌های دفاعی گیاه توانستند باعث تشدید و افزایش سرعت توسعه بیماری شوند.

واژگان کلیدی: *Xanthomonas translucens*، گونه‌های سودوموناس، شدت بیماری و هم‌افزایی