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Efficacy of the strains of *Pseudomonas* and *Acinetobacter* as biocontrol agents against bacterial wilt disease in chili

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Abstract Bacterial wilt of chili (*Capsicum* spp.) caused by *Ralstonia solanacearum* is an economically damaging disease of chili production in Thailand and tropical regions worldwide. This study screened bacteria isolated from the rhizosphere soil of healthy chili plants from Surin, Ratchaburi, and Chanthaburi provinces with strong antagonistic activity against *R. solanacearum.* A dual-culture assay revealed the best three isolated strains, D402-5(3), M601-4, and KJB01, which were able to inhibit the growth of the pathogen. D402-5(3) showed the largest inhibition zone diameter of 2.63 cm, followed by M601-4 and KJB01 with diameters of 2.50 cm and 2.33 cm, respectively. By morphological observation and molecular characterization via 16S rDNA gene sequencing analysis, D402-5(3) and KJB01 were identified as *Pseudomonas* sp., and M601-4 was identified as *Acinetobacter* sp. The suppression of bacterial wilt disease in chili by the three antagonistic strains was carried out by pot experiments in the greenhouse. D402-5(3) exhibited the highest biocontrol efficacy of 73.12% on the 12th day post *R. solanacearum* inoculation, followed by M601-4 and KJB01 with biocontrol efficacies of 58.19% and 43.2%, respectively. Plant growth-promoting traits and lytic enzyme production abilities of the three strains were detected. The results showed that D402-5(3) was able to produce cellulase and protease, KJB01 produced only protease, and M601-4 could solubilize phosphate and fix nitrogen.

Keywords: Antagonistic bacteria, Biocontrol, *Capsicum* spp., *Ralstonia solanacearum*

Introduction

Chili (*Capsicum* spp.), belonging to the family Solanaceae, is an important vegetable crop in most areas of the world (Lozada *et al*., 2022). Chili has been extensively used for a long time as a spice for cooking, as a herb, and as an ingredient in industrial food products. An enormous consumption of chili worldwide has driven its cultivation. In Thailand, the export value of chili and its processed products was 4,256 million baht in 2019 and was continuously increasing (Sukhawatthanakun, 2022). Besides, in 2020, Thailand was the

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world's second-biggest producer of dry chili at 322,886 tons. Chili, therefore, has become an economically important crop in Thailand. However, many chili diseases influence the quality and yield of chili crops. One of the most devastating diseases affecting chili production is bacterial wilt.

Ralstonia solanacearum is a soil-borne, Gram-negative, plant pathogenic bacterium that causes lethal bacterial wilt disease in chili and other crops. *R. solanacearum* is ranked as the world's second most damaging bacterial phytopathogen (Wang *et al*., 2023). In Thailand, bacterial wilt harm is serious in solanaceous crops (such as chili, tomato, potato, eggplant, etc.) and crops in other families (such as ginger, cucumber, marigold, Siam tulip, etc.). Even though *R. solanacearum* has been classified as a single species, its extensive diversity revealed by phylogenetic analyses has been widely acknowledged. Due to the differences in numerous strains of *R. solanacearum* in host range, physiological properties, pathogenicity, and geographic distribution, *R. solanacearum* is recognized as a species complex (Hayward, 1991; Fegan and Prior, 2006). Since *R. solanacearum* was discovered over 100 years ago, various strategies have been developed for controlling bacterial wilt disease for decades, such as physical, chemical, biological, and cultural methods (Yuliar *et al*., 2015). However, having been hampered by the persistence of the pathogen to survive for years in wet soil, water ponds, or asymptomatic hosts, and by the broad diversity of the pathogen strains, the available disease managements remain limited in success (Wang *et al*., 2023). Even though using disease-resistant cultivars is considered an effective method of disease control, the available bacterial wilt-resistant cultivars have been reported their effectiveness in specific geographic and/or strains of *R. solanacearum* resulting in unstable resistance. Moreover, no chemical pesticides are known to provide worldwide effective control of this soil-borne pathogen (Hayward, 1991; Yuliar *et al*., 2015). The most commonly used chemical treatment was fumigation of contaminated soil with methyl bromide before this pesticide was recognized as an ozone-depleting chemical and was phased out by most countries in the early 2000s (Yuliar *et al*., 2015). In recent years, biological control has gained more attention as an alternative disease management strategy because of its environment-friendly viewpoint (Wang *et al*., 2023).

The most reported biological control agent (BCA) against bacterial wilt is antagonistic bacteria (Wang *et al*., 2023). The biocontrol mechanism employed by these bacteria is caused by various interactions, such as competition for nutrients and root colonization area, antibiosis, parasitism, and induced systematic resistance of plant hosts. Many BCAs have been reported to produce metabolites with antimicrobial activity, such as antibiotics, biosurfactants, cell wall-degrading enzymes, lipopeptides, or volatile organic compounds (Bonaterra

et al., 2022; Wang *et al*., 2023). A majority of bacteria having antagonistic activity against *R. solanacearum* belong to the genus *Bacillus* and *Pseudomonas*, which mostly focused on controlling the bacterial wilt of tomatoes (Wang *et al*., 2023). Dowarah *et al*. (2021) isolated endophytic bacteria from chili seed (cv. Firingi) in India and obtained 6 antagonistic bacteria against bacterial wilt, which were *B. subtilis* KJ-2, *B. velezensis* KJ-4, *B. amyloliquefaciens* WK-2, *B. subtilis* WK-3, *Leuconostoc mesenteroides* KP-1, and *Lactococcus lactis* LB-3. All six endophytic bacteria showed the ability to control tomato bacterial wilt at the seedling stage. Only two strains, KJ-2 and WK-2, exhibited growth-promoting of tomato plants. Kashyap *et al*. (2021) reported their best five rhizobacteria isolated from rhizosphere soil of chili plants in five climate zones of India. *Pseudomonas fluorescens* PDS1, *B. subtilis* BDS1, *B. cereus* UK4, *B. amyloliquefaciens* UK2, and *B. subtilis* KA9 demonstrated both biocontrol potential against chili bacterial wilt and growth-promoting of chili plants (cv. Pusa Jwala).

The prerequisite for success in controlling soil-borne pathogens by antagonistic bacteria is competitive colonization of host roots and survival in the rhizosphere (Dowarah *et al*., 2021). Furthermore, numerous factors may affect the efficacy of biocontrol, such as host cultivar type, host age, biotic and abiotic factors, chemical residues in soil, nutrient availability, climate, etc. Problems with inconsistent biocontrol efficacy in field performance and even lack of efficacy of BCAs have been frequently reported, including BCAs against bacterial wilt (Bonaterra *et al*., 2022; Wang *et al*., 2023). Therefore, efforts are still required to obtain novel BCAs to control bacterial wilt.

The objective of this study was to obtain highly potential BCAs for controlling bacterial wilt disease in chili. Thus, rhizobacteria from the rhizosphere soil of healthy chili were isolated and randomly selected to screen their antagonistic activity against *R. solanacearum.* The biological control efficacy was observed both *in vitro* and *in vivo*. In addition, the plant growth-promoting traits and antagonist-related lytic enzyme productions of the obtained antagonistic bacteria were preliminarily studied.

Materials and methods

Bacterial strains isolation from rhizosphere soil of chili plants

Chili rhizosphere soil samples were collected from healthy chili from various fields in Thailand. The samples were labelled and stored at 4 °C until used. About 10 grams of each soil sample was mixed with 90 ml of sterilized distilled water in a 250 ml Erlenmeyer flask and shaken at 150 rpm for 1 hour.

Afterward, serial dilution of the soil suspension was prepared at up to 10^{-6} dilution. The 100 µl of the diluted suspension from 10^{-4} to 10^{-6} were spread onto nutrient agar (NA, peptone 5 g, beef extract 3 g, and agar 15 g per l). After two days incubated at 28 °C, colonies with different colony morphology were selected and cross-streaked to a new NA plate. The isolated strains were cultivated at 28 °C in nutrient broth (NB) with shaking at 150 rpm for 24 hours. The strains were kept on NA plate and stored for further use at 4 °C. For long-term storage, the bacterial cultures were preserved at -80 °C in 20% (v/v) glycerol.

Pathogen and culture condition

Ralstonia solanacearum DOA-BC1954 was kindly provided by the Plant Protection Research and Development Office, Department of Agriculture (DOA), Thailand. *R. solanacearum* was cultivated at 28 °C, 150 rpm, in nutrient glucose broth (NGB, NB adding 10 g of glucose) for 24 hours. The pathogen was maintained on nutrient glucose agar (NGA) and stored at 4 °C for further use.

In vitro screening of antagonistic bacteria against R. solanacearum

Screening of the isolated bacteria for antagonistic activity against *R. solanacearum* under *in vitro* conditions was carried out by dual-culture assay. The cell suspension of *R. solanacearum* was adjusted to an optical density at 600 nm (OD_{600}) of 0.2 before swabbing throughout NA surface with a sterile cotton swab. After that, a sterile paper disc, 5 mm in diameter, was placed onto the NA surface and 10 µl of each isolated bacterial suspension adjusted to OD_{600} of 0.2 was dropped on the disc. The same amount of NB medium was used as a negative control. These plates were incubated at 28 °C for 24 hours before the diameter of *R. solanacearum* inhibition zone was measured (Thongwai and Kunopakarn, 2007). All tests were repeated at least three times with four replicates for each isolated strain.

Identification of the antagonistic bacteria

The isolated strains showing antagonistic activity against *R. solanacearum* were primarily identified by observing the morphology of their single colony and Gram staining. By molecular identification, genomic DNA of the antagonistic bacteria was extracted using the Presto™ Mini gDNA Bacteria Kit (Geneaid Biotech Ltd., Taiwan). The 16S rDNA gene was amplified using primer pair 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and U1492R (5'-

GGTTACCTTGTTACGACTT -3') (Tan *et al*., 2013). Amplified PCR products were purified and sequenced by Macrogen Inc. (South Korea). Obtained sequencing results were analyzed by comparing them with known sequences in the National Center for Biotechnology Information (NCBI) database using the nucleotide BLAST tool. Phylogenetic trees were constructed by the maximum likelihood method in MEGA 11.0 software.

Biocontrol efficacy assays in greenhouse

Chili seedlings (*Capsicum annuum* cv. Jinda) at the age of 30 days were used in biocontrol efficacy assays carried out in a greenhouse. All greenhouse experiments included at least 10 plants per treatment, and each experiment was repeated five times. Cell suspensions of the antagonistic strains and *R. solanacearum* were diluted with NB to OD_{600} of 0.2. The chili plant in a pot was treated with the pathogen and antagonistic strains by the soil-pouring method. 10 ml suspensions of the pathogen and each antagonistic strain (D402-5(3), M601-4, or KJB01) were poured at the same time into the chili pot. Plants treated with 20 ml of NB served as a blank control. Besides, plants inoculated with 10 ml of *R. solanacearum* suspension and 10 ml of NB served as RS control. After that, the plants were observed daily for 12 days after inoculation. The disease index was recorded based on a scale of 0 – 4 as described by McLaughlin and Sequeira (1988). Disease incidence and biocontrol efficiency were calculated according to Xue *et al*. (2009):

Disease incidence = [Σ (The number of diseased plants in this index × Disease index)/(Total number of plants investigated × The highest disease index)] × 100%.

Biocontrol efficacy = [(Disease incidence of control – Disease incidence of antagonist-treated group)/Disease incidence of control] × 100%.

Detection of lytic enzyme production and plant growth promotion (PGP) traits

The bacterial suspension was adjusted to OD_{600} of 0.2 before being tested. Production of two antagonist-related lytic enzymes, protease and cellulase, were tested. Skim milk agar medium (Masi *et al*., 2021) and carboxymethyl cellulose (CMC) medium (Hankin and Anagnostakis, 1977) were used for protease and cellulase activity detection, respectively. For PGP traits detection, phosphate solubilizing and nitrogen fixing abilities of antagonistic strains were tested. Pikovskaya's agar with added tricalcium phosphate (Patel *et al*., 2017) and Burk's N-free agar (Thamkhongdee *et al*., 2019) were used for phosphate solubilizing and nitrogen fixing tests, respectively. Briefly, a sterile disc was

placed on the surface of tested media before 10 µl of the cell suspension was dropped on the disc. After incubation of the agar plate at 28 °C for 2 to 7 days, the diameter of hydrolytic zone around the disc was measured. For cellulase detection, the hydrolytic zone was stained with Congo red before observation. In the case of nitrogen fixing test, the diameter of colony grown around the disc was measured.

Statistical analysis

Data were analyzed using SPSS software version 29 (SPSS, Chicago, IL, USA). Greenhouse experiments were conducted in a completely randomized design. Statistically significant differences between multiple groups were analyzed by using a one-way analysis of variance (ANOVA) and the least significant difference (LSD) test at $P < 0.05$.

Results

In vitro antagonistic activity of isolated bacteria against Ralstonia wilt

Rhizosphere soil of healthy chili plants was collected from various chili fields in central, eastern, and northeastern regions of Thailand. Rhizobacteria were isolated on NA medium. About 118 bacterial isolates showing different colony morphologies were selected for testing their antagonistic activity against *R. solanacearum* DOA-BC1954, the high virulence strain in chili. A dual-culture assay revealed the best three isolated strains that were able to inhibit the growth of the pathogen (Table 1 and Figure 1). The strongest antagonistic strain, D402-5(3), was isolated from Ratchaburi provinces, with an average inhibition zone diameter of 2.63 cm. Strains M601-4 and KJB01 exhibited inhibition zone diameters of 2.50 cm and 2.33 cm, respectively. All three strains are Gram-negative bacteria. Their colony morphology on NA medium is shown in Table 1.

Molecular-based identification of three antagonistic bacteria

To identify the genus and species of the three antagonistic strains, D402-5(3), M601-4, and KJB01, their 16S rDNA genes were amplified, sequenced, and analyzed by the nucleotide BLAST tool in NCBI. It was found that D402-5(3) and KJB01 were highly similar to *Pseudomonas sp.* CMR5 (accession no. FJ652623.1) with 97.82% identity and *Pseudomonas sessilinigenes* CMR12a (accession no. CP027706.1) with 96.98% identity, respectively. Strain M601-4 had the highest homology with *Acinetobacter baumannii* AYP-A2 (accession no.

CP024124.1) which was 97.59% identity. Phylogenetic trees based on 16S rDNA genes were constructed by MEGA 11.0 software. Figure 2A revealed that the 16S rDNA sequence of strain KJB01 was clustered into one branch with *Pseudomonas sessilinigenes*. The 16S rDNA sequence of strain M601-4 was clustered into one branch with *Acinetobacter baumannii* (Figure 2B).

Table 1. *In vitro* inhibition of growth of *Ralstonia solanacearum* by antagonistic isolated bacteria, their colony morphology, Gram staining result, and the soil sample collection place

Isolated strain	Gram staining	Colony morphology on NA medium				Inhibition zone (cm)[1/]	Rhizosphere soil source
		Colour	Form	Margin	Surface		
D402-5(3)	-	Milky white	Circular	Entire	Smooth glistening	2.63 ± 0.35	Chom Bueng District, Ratchaburi Province
M601-4	-	Milky white	Circular	Entire	Smooth	2.50 ± 0.24	Surin City, Surin Province
KJB01	-	Yellowish	Circular	Entire	Smooth glistening	2.33 ± 0.11	Na Yai Am District, Chanthaburi Province

[1/] Values given are the diameter of *R. solanacearum* inhibition zone deducted from the diameter of a disc (5 mm) which is displayed as the means ± SD of triplicate measurement.

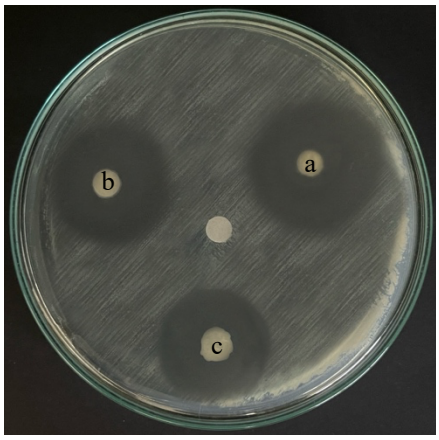

Figure 1. Antagonistic activity of the isolated strains obtained from dual-culture assay: The letters a, b, and c indicated the tested discs of D402-5(3), M601-4, and KJB01, respectively. The disc at central of plates served as negative control by adding NB medium instead of cell suspension.

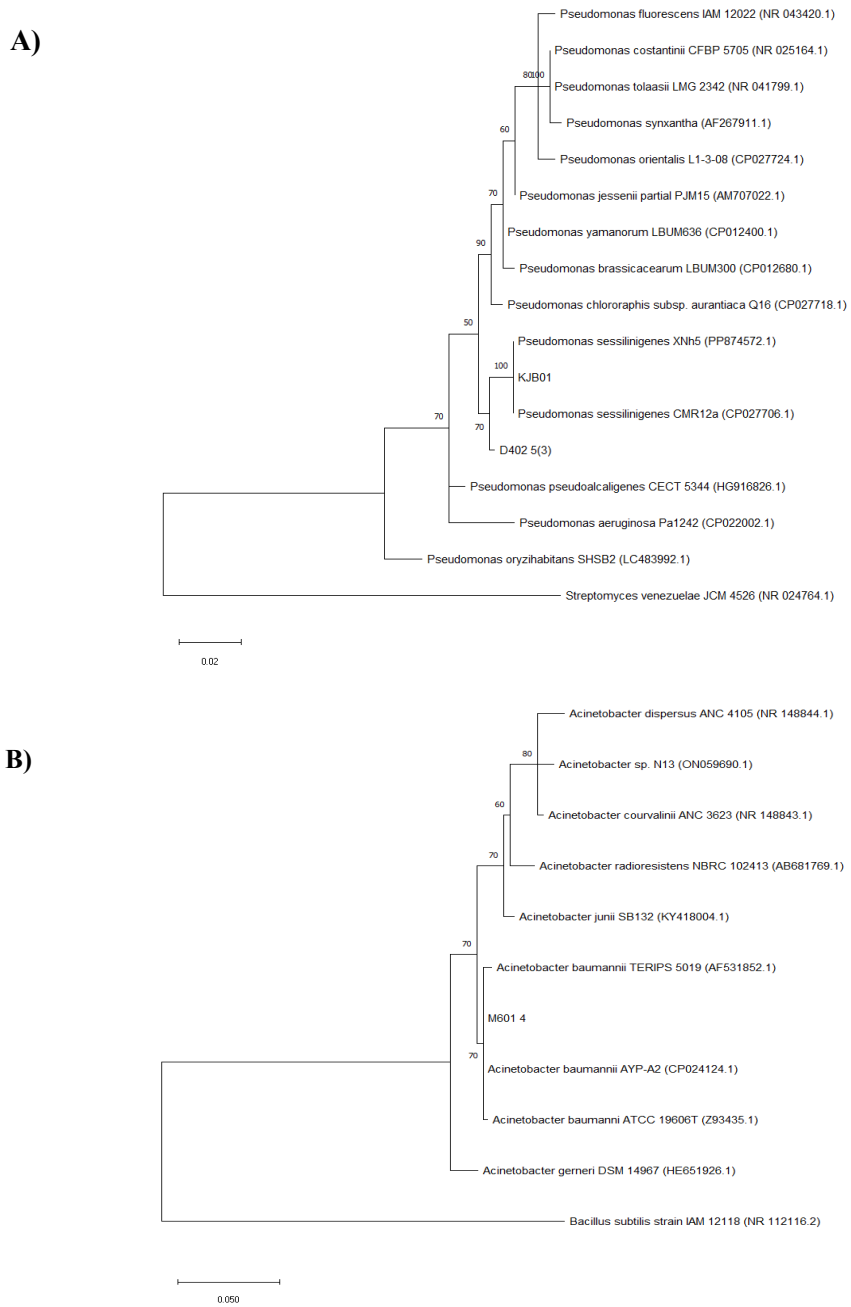

Figure 2. Phylogenetic tree established by the maximum likelihood method, based on 16S rDNA gene sequences of A) strains D402-5(3) and KJB01, B) strain M601-4, and related bacteria strains in the NCBI database. The numbers at branch are the confidence level generated by bootstrap analysis (1000). The scale bar indicates the evolutionary distance between strains.

Chili bacterial wilt biocontrol efficacy of three antagonists

In greenhouse experiments, the biological control activities of *Pseudomonas* sp. D402-5(3), *P. sessilinigenes* KJB01, and *A. baumannii* M601-4 on chili bacterial wilt were evaluated by the soil-pouring method. After 4 days of inoculation, no wilt symptoms were observed from all three antagonistic strain treatments compared with RS control (only *R. solanacearum* inoculation) showing wilt symptoms at disease incidence of 39.33% (Figure 3). All chili plants in blank control (pouring with NB) were healthy during the observation period. On 12th day post-inoculation, the chili plant treated with D402-5(3) exhibited the lowest disease incidence of 24.0%, followed by M601-4 and KJB01 (Figures 3 and 4). D402-5(3) showed the highest biocontrol efficacy of 73.12%, followed by M601-4 and KJB01 with biocontrol efficacies of 58.19% and 43.2%, respectively, on the 12th day post-inoculation (Table 2).

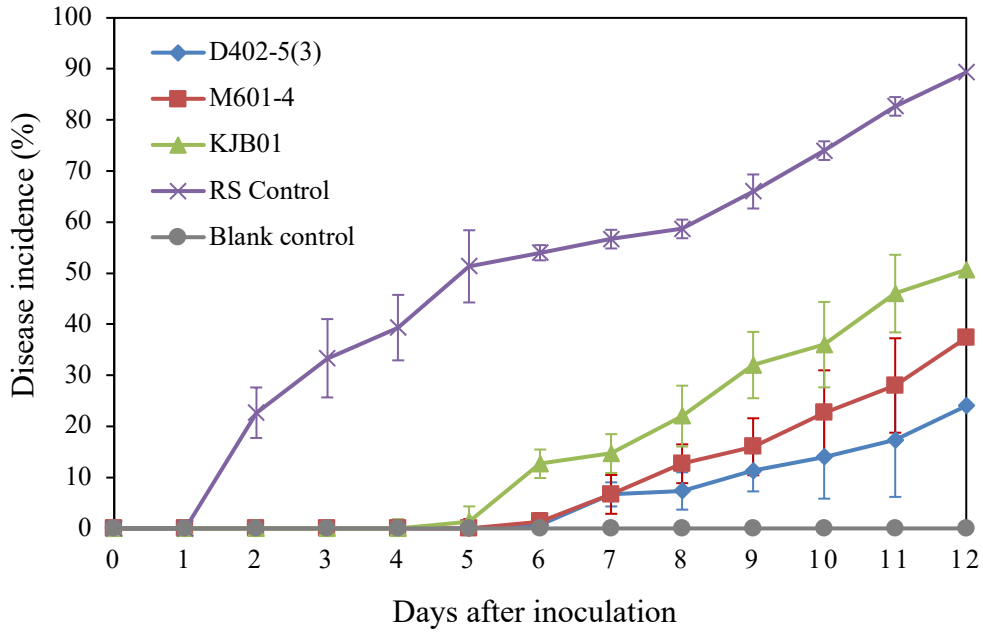

Figure 3. Day-wise progression of chili bacterial wilt disease incidence in different treatment groups. Blank control, NB treated control group; RS control, *Ralstonia solanacearum* (RS) treated without antagonist strain; D402-5(3), RS treated with strain D402-5(3); M601-4, RS treated with strain M601-4; KJB01, RS treated with strain KJB01. Error bars represent the SD values from the means of measurements conducted in at least three independent experiments.

Figure 4. Photographs of chili plants of each treatment after 12 days of inoculation: A) Blank control: NB treated control group; B) RS control: RS treated without antagonist strain; C) D402-5(3): RS treated with strain D402-5(3); D) M601-4: RS treated with strain M601-4; and E) KJB01: RS treated with strain KJB01.

Table 2. Efficacy of three antagonistic bacteria strains for biological control of *Ralstonia solanacearum* on chili after inoculation by the soil-pouring method on the 12th day post-inoculation

Treatment	Disease incidence (%)[1]	Biocontrol efficacy (%)[1]
RS control	89.3 ± 1.82[a]	0.00[a]
D402-5(3)	24.0 ± 11.15[b]	73.12 ± 12.1[b]
M601-4	37.3 ± 9.24[bc]	58.19 ± 10.89[bc]
KJB01	50.6 ± 7.6[c]	43.2 ± 8.20[c]
Blank control	0.00[d]	-

[1]/ Means ± SD within the same column followed by different letters indicate significant differences between treatments by LSD test at $P \leq 0.05$

Table 3. Production of antagonist-related lytic enzymes and plant growth promotion traits in three antagonistic bacterial strains

Strain	Genus and species	Cellulase[1]	Phosphate[2]	Nitrogen[3]	Protease[4]
D402-5(3)	*Pseudomonas* sp.	++			+++
M601-4	*Acinetobacter baumannii*		+++	++	
KJB01	*Pseudomonas sessilinigenes*				+++

[1]/Diameter of hydrolytic zone: + < 10 mm; ++ = 10 -13 mm; +++ > 13 mm.
[2]/Diameter of hydrolytic zone: + < 3 mm; ++ = 3 - 5 mm; +++ > 5 mm.
[3]/Diameter of colony: + < 3 mm; ++ = 3 - 5 mm; +++ > 5 mm.
[4]/Diameter of hydrolytic zone: + < 15 mm; ++ = 15 – 18 mm; +++ > 18 mm.

Detection of lytic enzyme productions and PGP traits

Two antagonist-related lytic enzyme productions and two PGP traits of the three antagonistic strains were detected to primarily study their antagonistic mechanism and their ability to promote plant growth. The results showed that

Pseudomonas sp. D402-5(3) was able to produce cellulase and protease, *P. sessilinigenes* KJB01 produced only protease, and *A. baumannii* M601-4 could solubilize phosphate and fix nitrogen (Figure 5 and Table 3).

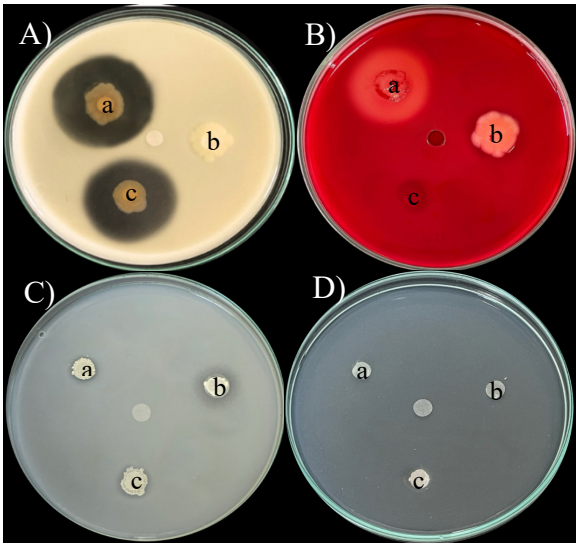

Figure 5. Detection of antagonist-related lytic enzyme productions and plant growth promotion traits in three antagonistic bacterial strains: A) protease detection; B) cellulase detection; C) phosphate solubilizing detection; and D) nitrogen fixing detection. The letters a, b, and c indicated the tested discs of D402-5(3), M601-4, and KJB01, respectively. The disc at central of the plates served as a negative control by adding NB medium instead of cell suspension.

Discussion

Biological control by using antagonistic bacteria is an efficient and environment-friendly approach that was regarded as a sustainable alternative method for plant disease management (Bonaterra *et al*., 2022). The key to success in the biological control of plant diseases is to obtain a strong biological control agent (BCA). In this study, we paid attention to bacterial wilt disease of chili caused by *R. solanacearum* because chili is Thailand's economically important crop which had been seriously damaged by this pathogen. The first step leading to successful biocontrol of plant disease is competitive colonization of host roots by antagonistic bacteria, especially for *R. solanacearum,* which invades the host plants through root wounds or opening gaps (Hayward, 1991; Yuliar *et al*., 2015; Dowarah *et al*., 2021). Therefore, we collected rhizosphere soil of healthy chili plants from various fields in central, eastern, and northeastern areas of Thailand as a source of chili rhizosphere bacteria. The isolated bacteria with different

colony morphology were selected for further *in vitro* screening on their antagonistic activity against *R. solanacearum* using a dual-culture assay. The results showed that the best obtained three antagonistic bacteria were strains D402-5(3), M601-4, and KJB01. Analysis of their 16S rDNA gene sequences by the nucleotide BLAST tool and phylogenetic tree construction showed that strains D402-5(3), M601-4, and KJB01 were highly similar to *Pseudomonas* sp., *Acinetobacter baumannii*, and *Pseudomonas sessilinigenes*, respectively. Genus *Bacillus* and *Pseudomonas* were frequently reported as antagonistic bacteria against *R. solanacearum*. Achari and Ramesh (2014) isolated bacteria from the xylem of healthy eggplant, chili, and *Solanum torvum* Sw. Among 55 identified isolates, the isolates with high biocontrol activity were identified as strains of *Bacillus* sp., *Streptomyces* sp., *Staphylococcus* sp., *Enterobacter* sp., and *Agrobacterium* sp. Mohammed *et al*. (2020) reported six species of *Pseudomonas*, isolated from the rhizosphere of tomato plants, having the ability to inhibit growth of *R. solanacearum*. On the contrary, only *Acinetobacter* sp. Xa6, isolated from the forest soil, has been reported so far as a bacterial wilt antagonist (Xue *et al*., 2009). *Acinetobacter* sp. Xa6 also exhibited colonization capacity on tomato roots.

From our greenhouse experiments, all three strains were able to suppress wilt disease and prolong the appearance of the symptom in chili plants (cv. Jinda). Chili plants treated solely with *R. solanacearum* (RS control) began wilting on the 4th day after inoculation. The highest biocontrol efficacy against chili bacterial wilt of 73.12% was obtained from *Pseudomonas* sp. D402-5(3), followed by *A. baumannii* M601-4 and *P. sessilinigenes* KJB01 with a biocontrol efficacy of 58.19% and 43.2%, respectively. Hu *et al*. (2010) reported endophytic bacterium *B. amyloliquefaciens* Bg-C31 isolated from mangrove showing bacterial wilt biocontrol efficacy about 60–80% in chili pots (cv. Zhongjiao No. 11) conducted in Guangdong province, China. Arwiyanto *et al*. (2010) described *Pseudomonas putida* Pf-20 isolated from the rhizosphere of *Mimosa invisa* in Indonesia. Strain Pf-20 inhibited the growth of *R. solanacearum* with an inhibition zone diameter of 1.20 cm and was able to control bacterial wilt in chili (cv. Lokal) with biocontrol efficacy of about 42 – 60%. Two rhizobacteria, *P. fluorescens* PDS1 and *B. subtilis* KA9 isolated from chili rhizosphere in India, provided *R. solanacearum* inhibition zone diameters of 2.24 and 2.01 cm, respectively (Kashyap *et al*., 2021). Biocontrol efficacy of 71.11 and 65.97% was obtained from strains PDS1 and KA9 carried out under glasshouse conditions using chili cv. Pusa Jwala. Even though it is difficult to compare the biocontrol efficacies between BCAs that were experimentally conducted in different conditions (e.g., the virulence of *R. solanacearum*, a cultivar of chili, climate, etc.), we may still evaluate *Pseudomonas* sp. D402-5(3) as a strong potential BCA against chili bacterial wilt.

By *in vitro* detection of lytic enzyme productions and plant growth-promoting (PGP) traits, the results showed that *Pseudomonas* sp. D402-5(3) was able to produce cellulase and protease, while *P. sessilinigenes* KJB01 produced only protease. In addition, *A. baumannii* M601-4 could solubilize phosphate and fix nitrogen, which showed potential to promote host plant growth. Many reports revealed that cellulase and protease degrade the cell walls of many phytopathogenic fungi. Besides, bacterial protease is known to enhance plants' immune system, which allows the plants to withstand stresses (Dowarah *et al*., 2021; Bonaterra *et al*., 2022). Due to its high production of cellulase and protease, strain D402-5(3) will be further studied for antagonistic activity against chili pathogenic fungi such as *Colletotrichum* sp., *Fusarium oxysporum*, and *Choanephora* sp.

In conclusion, the present study showed that three Gram-negative bacteria isolated from the rhizosphere soil of chili plants from different areas in Thailand had strong antagonistic activity against *R. solanacearum*. The results from the *in vitro* antagonistic assay and *in vivo* biocontrol efficacy assay suggested that *Pseudomonas* sp. D402-5(3), *P. sessilinigenes* KJB01, and *A. baumannii* M601-4 have the potential as biological control agents against chili bacterial wilt. All three strains, especially D402-5(3), should be further explored for antagonistic activity against other phytopathogens in the future. Moreover, it is interesting to study biocontrol efficacy from a combination of these strains.

Acknowledgements

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