International Journal of Agricultural Technology 2023 Vol. 19(2):505-516
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Antibiotic biosynthesis gene of *Bacillus velezensis* as a bioagent for controlling blackleg and soft rot of potato

Kumvinit, A.[1,2] and Akarapisan, A.[1,2*]

[1]Division of Plant Pathology, Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand, 50200; [2] Center of Excellence on Agricultural Biotechnology: (AG-BIO/MHESI), Bangkok, Thailand, 10900.

Kumvinit, A. and Akarapisan, A. (2023). Antibiotic biosynthesis gene of *Bacillus velezensis* as a bioagent for controlling blackleg and soft rot on potato. International Journal of Agricultural Technology 19(2):505-516.

Abstract A total of 80 bacteria were isolated from the rhizospheres of healthy and asymptomatic potato plants. The bacterial isolates were screened by the co−inoculation method and it was found that strain CR74 had high antagonistic activities against bacterial blackleg and soft rot of potato caused by *P. carotovorum* subsp. *brasiliense*, *D. dadantii*, *P. putida* and *B. altitudinis*. Strain CR74 was identified as *Bacillus velezensis* through its 16S *rRNA* and *gyrA* genes. Strain CR74 inhibited synthesis of violacein and degraded the natural violacein of *Chromobacterium violaceum* ATCC 12472^T. The antibiotic biosynthesis genes of strain CR74 were detected by sequence analysis. The results of PCR detection and sequencing showed the potential antibiotic genes of bacillomycin B, fengycin D, iturin A and surfactin. Therefore, *B. velezensis* strain CR74 is a potential biocontrol agents for the control of bacterial blackleg and soft rot on potato.

Keywords: *Bacillus velezensis*, Bacillomycin B, Fengycin D, Iturin A, Surfactin

Introduction

The *Bacillus subtilis* species complex, including *B. subtilis*, *B. velezensis*, *B. amyloliquefaciens*, *B. mojavensis*, *B. atrophaeus*, *B. sonorensis*, *B. licheniformis* and *B. vallismortis* produces endospores which result in a long shelf life of more than 2 years. Thus, the species have a desirable characteristic for being biocontrol agents (Borshchevskaya *et al*., 2013). In addition, *B. velezensis* is a close relative of *B. amyloliquefaciens* and *B. subtilis*. Many strains of *Bacillus* species have been presented to suppress the growth of fungal and bacterial pathogens in plants. Some of these bacteria which enhance plant growth are related as plant growth-promoting rhizobacteria (PGPR) (Stein, 2005). PGPR can increase plant growth by providing plant growth hormones and nutrients, and by reducing the effects of plant pathogens. The antagonistic

* **Corresponding Author:** Akarapisan, A.; **Email:** angsana.aka@gmail.com

bacteria *Bacillus* spp. Which can suppress plant pathogens are often applied as biocontrol agents to control plant diseases. They also have the ability of soil to suppress the activity of soil-borne pathogens by different mechanisms. The PGPR mechanisms concerned in this process can include production of phytohormones (auxin, gibberellins and cytokinin), nitrogen fixation, mineral and phosphate solubilization, and degrading enzymes production (proteases, amylases and hemicelluloses) (Myo *et al*., 2019; Rabbee *et al*., 2019; Li *et al*., 2020). *Bacillus* spp. produces lipopeptides and showed the highest antagonistic activity against plants pathogens (Aleti *et al*., 2016). *Bacillus* spp. produced lipopeptides which are highly active surface molecules and include surfactin, iturin and fengycin (Kearns *et al*., 2004). In addition, the production of antibiotic compounds contributes to the suppression of plant pathogens. For example, *B. amyloliquefaciens* inhibited pathogen sporulation and growth, and secreted secondary suppressive metabolites including iturin A, surfactin and fengycin (Lin *et al*., 2018). Based on genomic analyses of *B. velezensis* strain FZB42 (formerly *B. amyloliquefaciens* strain FZB42), it can secrete many secondary lipopeptide metabolites such as iturin, surfactin and fengycin which is a desirable characteristic for a biocontrol agent (Chowdhury *et al*., 2015). *Bacillus velezensis* could be used as potential biocontrol agent and plant growth promoter; thus promote economic and agricultural development.

The rhizosphere of plants contains, along with AHL (*N*-Acyl homoserine lactonase) producing pathogens, AHL degrading bacteria, which are termed as quorum quenching (QQ). Numerous *Bacillus* spp. were found to produce AHL-lactonase enzymes which shutting down the expression of the virulence in bacterial pathogens (Raafat *et al*., 2019). QQ has been suggested as a disease control strategy and is classified according to the mechanism of the enzymatic reaction. QQ enzymes of *Bacillus* spp. were first identified from attenuated virulence in *Erwinia carotovora* as quorum quenchers through the *aiiA* gene (Rasmussen and Givskov, 2006; Li *et al*., 2008). The *aiiA* genes that encode AHL-lactonase enzyme hydrolyzes the homoserine lactone ring and inactivates the quorum sensing (QS) system by AHL signal degradation from *Bacillus* sp. (Kalia, 2013). In addition, QS-systems are the primary quorum sensing signals, which moderate virulence factors production in many pathogenic microorganisms (Torres *et al*., 2013). Furthermore, *Bacillus* spp. have been developed as biological control agents against bacterial plant diseases in agriculture. In this study, we isolated a biocontrol agent and showed its inhibitory effects on the bacterial diseases of blackleg and soft rot of potato. The study aimed to determine whether the potential inhibitory mechanism of antagonistic bacteria against bacterial pathogens occurs through the detection of

lipopeptides such as baciliomycin, fengycin, iturin and surfactin gene, and to assess their control effect by AHL signal degradation.

Materials and methods

Screening and co-inoculation experiment for antagonistic activity

Bacteria were isolated from the rhizosphere of healthy and asymptomatic potato plants using the serial dilution method. One g of potato root was suspended in 99 ml of sterile distilled water in 250 ml flasks and was shaken at 180 rpm for 1 hour. The solution was diluted four times. A 200 µl volume from dilutions 2, 3 and 4 were spread on the nutrient agar (NA) and incubated at 28 ℃ for 2 days. Then, single bacterial colonies were transferred to new plates.

Bacterial pathogens on potato included *Pectobacterium carotovorum* subsp. *brasiliense* strain PY1 and *Dickeya dadantii* strain CK211 (blackleg and soft rot disease), *Pseudomonas putida* strain CK223 and *Bacillus altitudinis* strain CK507 (soft rot disease) were reported by Kumvinit and Akarapisan (2019). One ml of bacterial pathogens cell concentrations was calculated (OD_{600} = 10^8 cfu/ml) and was transferred to 99 ml of NA, and then was poured into petri-dishes. Then, 20 µl of antagonistic bacteria (10^8 cfu/ml) were evaluated using paper disc method. Paper discs (6 mm diameter) containing the antagonistic bacterial suspensions were plated on the agar. The plates were incubated at 28 ℃ for 48 hours and the inhibition zone was measured and selected.

For co-inoculation experiments, as described by Esmaeil *et al*. (2011). The bacteria were prepared in sterile 0.85% NaCl to 10^8 cfu/ml. The bacterial pathogen suspension (10 µl) was mixed with the antagonistic bacteria suspension (10 µl) for 2 hours. After that, the tubers were injected with mixed suspension. The potato tubers were incubated at room temperature for 4 days. The experimental design was a Complete Randomized Design (CRD) with 4 replications. The tubers were cut longitudinally after the incubation period.

Phylogenic analysis

For DNA extraction, bacteria were cultivated in Luria Bertani broth at 28 ℃ overnight and then extracted using a modification of the protocols of Cheng and Jiang (2006). The universal primer of the 16S *rRNA* gene (16S ribosomal RNA) was amplified using the primer 16SF and 16SR for amplification of a 1,500 bp region. Approximately 50 ng DNA template was added to 25 µl Quick Taq HS DyeMix (TOYOBO CO., LTD.) as well as 20

μM of each primer and added with distilled water to create a final volume of 50 μl. The thermal cycling conditions were carried out with an initial denaturation at 94 ℃ for 5 min, followed by 35 cycles consisting of 30 s at 94 ℃, 64 ℃ for 30 s, 72 ℃ for 2 min and followed by a final extension at 72 ℃ for 10 min in a PCR machine.

The partial *gyrA* gene (subunit A of gyrase DNA) was amplified using the primer set: forward primer gyrAF and reverse primer gyrAR for the amplification of a 970 bp region (Chun and Bae 2000). PCR program included an initial denaturation at 94 ℃ for 5 min, followed by 35 cycles consisting at 94 ℃ for 30 s, 60 ℃ for 30 s, 72 ℃ for 1 min and final extension at 72 ℃ for 10 min. The PCR products were purified and sequenced using an ABI PrismTM 3730xl DNA sequencer by fluorescent dye-termi-nator sequencing (Bio Basic Inc). Sequence alignment and analysis of genes were performed using the Clustal W program in the MEGA10 program and then compared with available sequences using BLASTn in GenBank. Phylogenetic trees were constructed of a neighbor-joining method.

AHLs degradation assay

The analysis for degradation was conducted using the LB medium at pH 6.5. The N-hexanoyl homoserine lactone (C6-HSL) was applied as the primary target particle of the degradation analysis. C6-HSL, Natural NAHL, produced by *Chromobacterium violaceum* strain ATCC 12472 was used (Yates *et al*., 2002). Screening of bacterial isolates for N-Acyl homoserine lactonase (AHLs) degradation activity used *C. violaceum* strain ATCC® 12472™ as the biosensor for AHL detection and synthesis of violacein.

The strains of antagonistic bacteria were streaked as lines on LB solid medium and incubated at 25 ℃ for 48 hrs. The biosensor *C. violaceum* strain ATCC 12472™ was calculated by measuring OD600 = 0.1 (10^6 cfu/ml) and was spotted (10 μl) at a distance of 0.5 cm from the antagonistic bacterial line and incubated at 25 ℃ for 24 hrs. For testing on LB liquid medium, 20 μl of antagonistic bacterial suspension of concentration 10^8 cfu/ml and 20 μl of *C. violaceum* ATCC 12472^T were dropped into 1 ml of LB. The tubes were incubated at 25 ℃ for 48 hrs. The experiment was performed with 4 replications.

Detection of lipopeptide antibiotic genes by specific primer

The antibiotic genes were amplified in a PCR using the primers listed in Table 1. The target antibiotic genes included Bacillomycin B (*bamB*), Fengycin

D (*fenD*), Iturin A (*ituA*) and Surfactin (*SrfAA*). The PCR amplification consisted of initial denaturation at 95 ℃ for 5 min, followed by 35 cycles of denaturation 45 s at 95 ℃, annealing temperature as described in Table 1 for 45 s, extension 1 min at 72 ℃, and final extension 72 ℃ for 10 min (Athukorala *et al*., 2009; Joshi and McSpadden-Gardener, 2006; Rahman, 2012). The sequences were used for alignment and deposited in the GenBank database.

Table 1. PCR primers and annealing temperatures for the detection experiment

Primer	Sequence (5'-3')	Size(bp)	Antibiotic (Target gene)	Annealing (℃)
BACA-F BACA-R	TGAAACAAAGGCATATGCTC AAAAATGCATCTGCCGTTCC	482	Bacillomycin B (*bamB*)	58
FEND-F FEND-R	CCTGCAGAAGGAGGAGAAGTGAAG TGCTCATCGTCTTCCGTTTC	293	Fengycin D (*fenD*)	58
ITUD1F ITUD1R	GATGCGATCTCCTTGGATGT ATCGTCATGTGCTGCTTGAG	647	Iturin A (*ituA*)	58
SRF-F SRF-R	ATGAAGATTTACGGAATTTA CCACTCAAACGGATAATCCTGA	201	Surfactin (*SrfAA*)	52

Results

Screening and co-inoculation experiment for antagonistic activity

The rhizosphere bacteria from healthy and asymptomatic potato plants were collected from potato fields in Chai Prakan and Fang Districts, Chiang Mai Province, Thailand in 2019 to 2020. A total of 80 bacteria were isolated and then pre-screened with *P. carotovorum* subsp. *brasiliense* strain PY1 using paper disc method. Isolates FP27, CF58, CR74 and CR75 effectively inhibited the growth of bacterial pathogen strain PY1. Thereafter, the four antagonistic isolates were screened by the co–inoculation method on potato tubers. Strain CR74 had high antagonistic activities against bacterial blackleg and soft rot of potato strain PY1, CK211, CK223 and CK507 (Figure 1).

Treatment with the antagonistic strain resulted in wound diameters of 0.310, 0.101, 0.086 and 0.104, respectively. While, inoculation with only bacterial pathogens produced wound diameter of 2.693, 2.398, 1.172 and 1.042, respectively. The CR74 strain was most potent in reducing tissue maceration and inhibiting invasion of wounds by the bacterial pathogens.

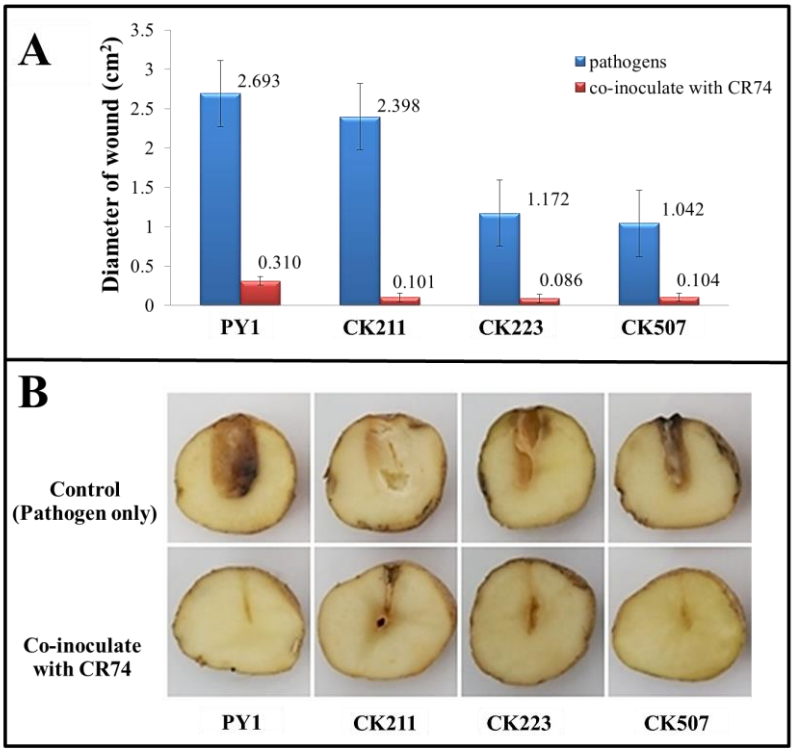

Figure 1. Co-inoculation experiment with the bacterial soft rot and blackleg of potato (strain PY1 = *P. carotovorum* subsp. *brasiliense*, strain CK211 = *D. dadantii*, strain CK223 = *P. putida* and strain CK507= *B. altitudinis*) for 4 days after incubation. A) Data were expressed as the average of four replicates with the bar indicating the standard error of the mean. P values were calculated at $P < 0.05$ as compared to the treatment. B) Attenuation of the maceration capacity of bacterial pathogens by CR74

Phylogenetic analysis

Strain CR74 was identified as *Bacillus velezensis* by using 16S *rRNA* and the *gyrA* genes analyzed to determine phylogenetic assignments with accession numbers MN818626 and MN850875, respectively. The results shown in Figure 2, of a BLAST search determined that the 16S *rRNA* gene sequences in GenBank database were over 99% similar to *B. velezensis* strain CBMB205 (NR116240.1). While, the *gyrA* gene sequence grouped closely with *B. velezensis* strain NRRL BD-545 (EU138622.1) (Figure 3).

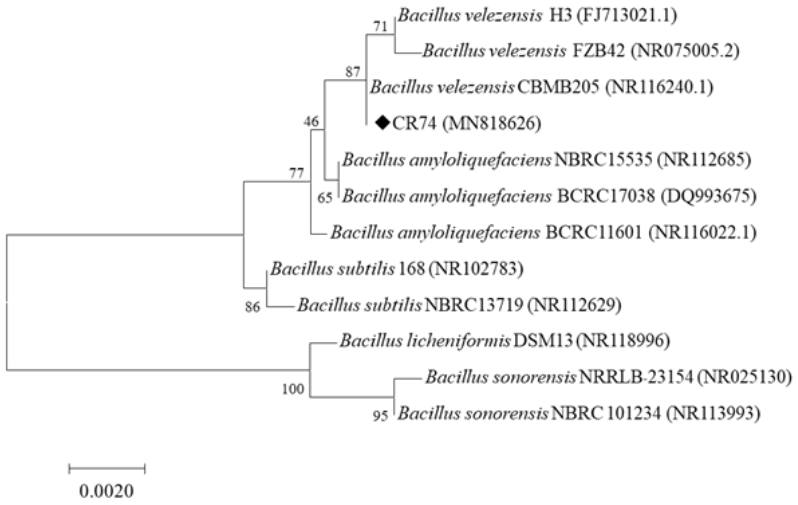

Figure 2. Phylogenetic tree depand on neighbor-joining tree method of the 16S ribosomal RNA (16S *rRNA*) gene of strains CR74. The percentage of replicate trees indicated in the bootstrap test (1000 replicates)

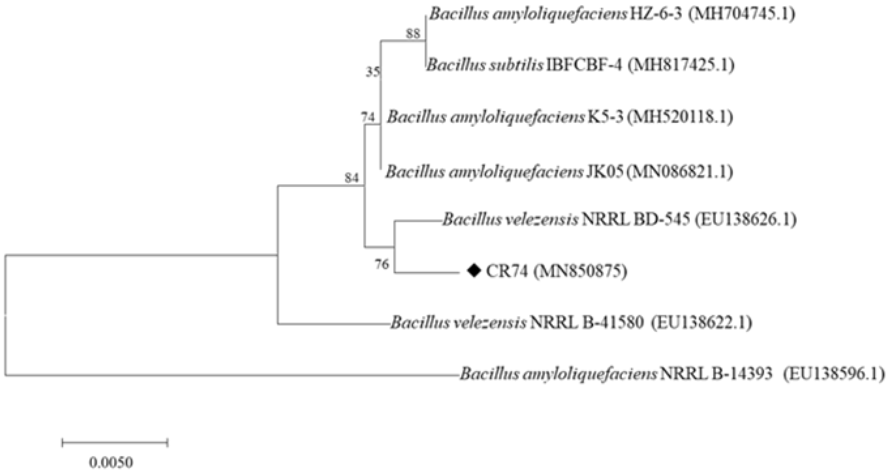

Figure 3. Phylogenetic tree depand on neighbor-joining tree method of subunit A of DNA gyrase (*gyrA*) gene sequences of strains CR74. The percentage of replicate trees indicated in the bootstrap test (1000 replicates)

AHLs degradation assay

Chromobacterium violaceum ATCC 12472^{TM} was used as the biosensor strain for the detection of degraded natural AHL (C6-HSL) and production of

the violacein pigment. Antagonistic bacteria of *B. velezensis* strain CR74 were tested with *C. violaceum* ATCC 12472^{TM} on LB medium. Strain CR74 was capable of inhibiting violacein synthesis and degraded natural NAHL (C6-HSL) after incubation at 25°C for 48 hrs on LB solid medium and LB liquid medium (Figure 4). Inhibition of violacein was due to AHL-lactonase production. Moreover, AHL-lactonase hydrolyzed AHL secreted by Gram-negative bacteria and thereby attenuated the symptoms caused by the plant pathogens tested.

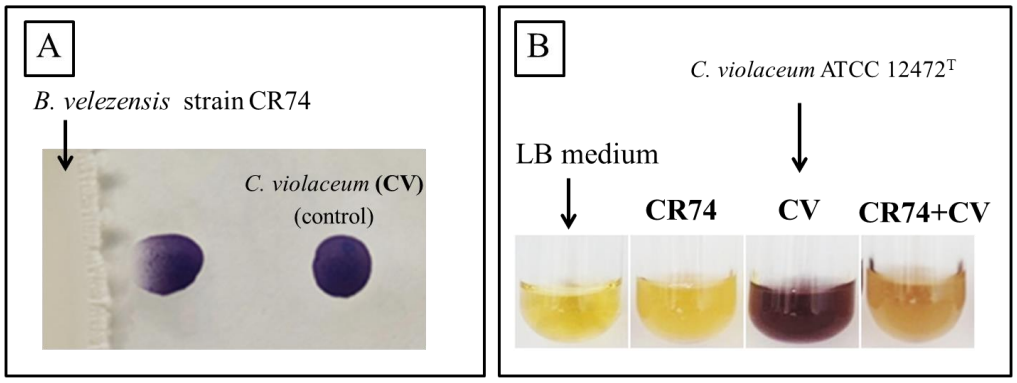

Figure 4. Testing of the antagonistic bacteria's AHL degradation activity using the biosensor *Chromobacterium violaceum* ATCC 12472^{TM} which produces the violacein pigment

Detection of lipopeptide antibiotic genes by specific primers

PCR amplification using the primers of baciliomycin B (*BymB* gene), fengycin D (*FenD* gene), iturin A (*ituA* gene) and surfactin (*srfAA* gene) indicated that *B. velezensis* strain CR74 amplified 482, 293, 647 and 201 bp, respectively. When the sequences were analyzed using a BLASTx algorithm-based program and aligned in the GenBank database, the proteins clustered at 99-100% with the *Bacillus subtilis* group including *B. subtilis*, *B. amyloliquefaciens* and *B. velezensis*. The protein analysis of sequence data from the existing BLAST database showed that the *bamB*, *FenD*, *ituA* and *srfAA* genes were distributed among *B. velezensis* (WP172614726, WP003153889 and WP163068998), *B. amyloliquefaciens* (ALF16457, ALF62663, ALF622667 and WP165881847) and *B. subtilis* (AAY34397, AAY34407, AVN84851 and QDP14442) (Figure 5).

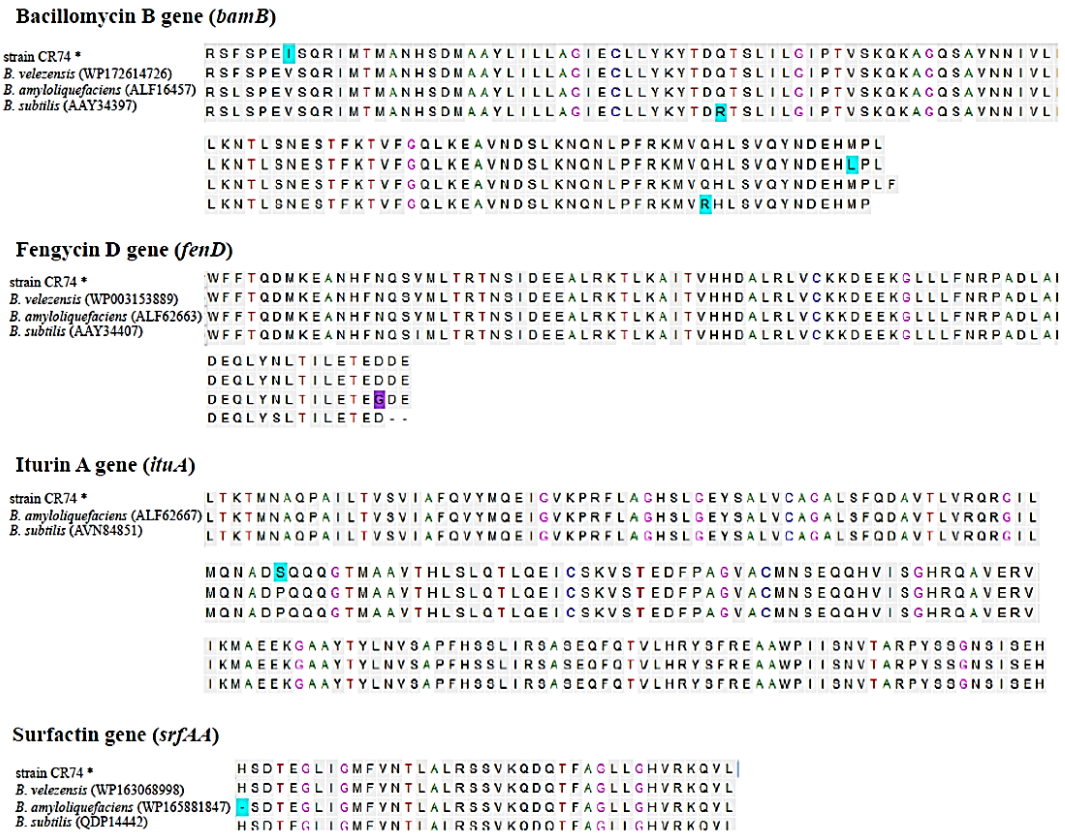

Figure 5. Antibiotic genes of *B. velezensis* strain CR74 compared with *B. velezensis, B. amyloliquefaciens* and *B. subtilis* on GenBank

Discussion

The 80 bacterial isolates that were examined for their antagonistic activity against the bacterial pathogens of blackleg and soft rot of potato showed varying degrees of antagonism *in vitro*. In this study *B. velezensis* strain CR74 showed the highest degree of inhibition using paper disc assay and the co-inoculation method. Strain CR74 reduced tissue maceration area when compared to the bacterial blackleg and soft rot of potato. Esmaeil *et al*. (2011) found that *Bacillus* strain DMA133 reduced maceration of potato tuber tissues by *P. carotovorum* subsp. *carotovorum* strain EMPCC. Hoda and Kamal (2016) reported that *P. atrosepticum* caused potato maceration and was inhibited by *B. subtilis* strain Bs3. Furthermore, biocontrol activities directed at the bacterial soft rot disease of potato have been reported in several bacterial competitors from the *Bacillus* genus (Czajkowski *et al*., 2012; Rahman *et al*., 2012).

Antibiotics are produced by the Gram-positive endospore-forming bacteria. Peypoux *et al*. (1999) reported that surfactin is a grateful surfactant frequently utilized as an antibiotic. Lin *et al*. (2018) investigated whether biological control can serve as an alternative approach, and found that *B. amyloliquefaciens* strain Ba01 inhibited the potato common scab pathogen and secreted secondary metabolites such as surfactin, fegycin and iturinA. The current study of strain CR74 detected antibiotic genes by sequence analysis using the GenBank database including the bacillomycin B, fengycin D, iturin A and surfactin genes. Also, strain CR74 the degraded the violacein pigment of *C. violaceum* ATCC 12472^{TM} on LB medium. AHL-lactonase plays a role in quorum sensing systems; AHL signals particles to support the manufacture of virulence metabolites in several microorganisms and has a role in the establishment of biofilms by Gram-negative bacteria (Torres *et al*., 2013; Lade *et al*., 2014). Three targets could intercept AHL-based quorum sensing including alteration of AHLs molecules or enzymatic degradation, interference with signal receptors and inhibition of AHL synthesis by blocking synthase proteins (Koch *et al.*, 2005; Geske *et al*., 2008). However, biocontrol agents have also been observed to control various plant diseases attributed to bacteria. Bacillus species have been recorded to manufacture AHL lactonase (Lade *et al*., 2014). Garge and Nerurkar (2016) also reported that 97 isolates of bacteria were screened for AHL degradation using the biosensor *C. violaceum* strain CV026. The results found that 20 isolates could reduce violacein production. The *Bacillus* sp. strains As30, Gs42 and Gs52 were found to potentially reduce the *Pectobacterium* soft rot disease.

Moreover, the antagonistic bacteria can produce secondary metabolites active against pathogens including hydrolytic enzymes, plant growth promoters and also antibiotic lipopeptides. Strain CR74 was reported by Kumvinit and Akarapisan (2020) to produce hydrolytic enzymes (protease, amylase, cellulase and lipase), plant growth promotion (IAA (total-auxin), siderophores, phosphate solubilization), surfactin antibiotic detected by LCMS-QTOP, and biofilm formation to support the biocontrol properties.

In Conclusion, *B. velezensis* strain CR74 suppressed bacterial blackleg and soft rot of potato. This bacterium was capable of degrading the violacein pigment of *C. violaceum* ATCC 12472^{TM} and had AHL-degradation. Furthermore, Sequence analysis detected the presence of antibiotic genes coding for bacillomycin B, fengycin D, iturin A and surfactin which indicated a potential for biocontrol.

Acknowledgements

This research was partially supported by the Innovation Agriculture Research Center, Faculty of Agriculture, Chiang Mai University and the Center of Excellence on Agricultural Biotechnology: (AG-BIO/MHESI), Bangkok, Thailand.

References

Aleti, G., Lehner, S., Bacher, M., Compant, S., Nikolic, B., Plesko, M., Schuhmacher, R., Sessitsch, A. and Brader, G. (2016). Surfactin variants mediate species-specific biofilm formation and root colonization in *Bacillus*. Environmental Microbiology, 18:2634-2645.

Athukorala, Y., Ahn, G. N., Jee, Y. H., Kim, G. Y., Kim, S. H. and Ha, J. H. (2009). Antiproliferative activity of sulfated polysaccharides isolated from an enzymatic digest of *Ecklonia cava* on the U-937 cell line. Journal of Applied Psychology, 21:307-314.

Borshchevskaya, N. L., Kalinina, N. A. and Sineokii, S. P. (2013). Design of a PCR test based on the *gyrA* gene sequence for the identification of closely related species of the *Bacillus subtilis* group. Applied Biochemistry and Microbiology, 47:646-655.

Cheng, H. R. and Jiang, N. (2006). Extremely rapid extraction of DNA from bacteria and yeasts. Biotechnology Letters, 28:55-59.

Chowdhury, P. S., Hartmann, A., Gao, X. and Borriss, R. (2015). Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42 – a review. Frontiers in Microbiology, 780:1-11.

Chun, J. and Bae, K. S. (2000). Phylogenetic analysis of *Bacillus subtilis* and related taxa based on partial *gyrA* gene sequences. Antonie Van Leeuwenhoek, 78:123-127.

Czajkowski, R., de Boer, W. J., van Veen, J. A. and van der Wolf, J. M. (2012). Characterization of bacterial isolates from rotting potato tuber tissue showing antagonism to *Dickeya* sp. biovar 3 *in vitro* and *in planta*. Plant Pathology, 61:169-182.

Esmaeil, M., Badraldin, E. S. T. and Vittorio, V. (2011). Virulence attenuation of *Pectobacterium carotovorum* using N-Acyl-homoserine Lactone degrading bacteria isolated from potato rhizosphere. The Plant Pathology Journal, 27:242-248.

Garge, S. S. and Nerurkar, S. A. (2016). Evaluation of quorum quenching *Bacillus* spp. for their biocontrol traits against *Pectobacterium carotovorum* subsp. *carotovorum* causing soft rot. Biocatalysis and Agricultural Biotechnology, 9:48-57.

Geske, D. G., O'Neill, C. J. and Blackwell, E. H. (2008). Expanding dialogues: from natural autoinducers to non-natural analogues that modulate quorum sensing in Gram-negative bacteria. Chemical Society Reviews, 37:1432-1447.

Hoda, H. E. and Kamal, A. M. A. (2016). Combination of different antagonistic bacteria to control of potato blackleg disease caused by *Pectobacterium atrosepticum* under greenhouse and field condition. International Journal of Phytopathology, 5:53-43.

Joshi, R. and McSpadden-Gardener, B. B. (2006). Identification and characterization of novel genetic markers associated with biological control activity in *Bacillus subtilis*. Phytopathology, 96:145-154.

Kalia, V. C. (2013). Quorum sensing inhibitiors: an overview. Biotechnology Advances 31, 224-245.

Kearns, D. B., Chu, F., Rudner, R. and Losick, R. (2004). Genes governing swarmimg in *Bacillus subtilis* and evidence for phase variation mechanism controlling surface motility. Molecular Microbiology, 52:357-369.

Koch, B., Liljefors, T., Persson, T., Nielsen, J., Kjelleberg, S. and Givskov, M. (2005) The LuxR receptor: the sites of interaction with quorum-sensing signals and inhibitors. Microbiology, 151:3589-3602.

Kumvinit, A. and Akarapisan, A. (2019). Characterization of blackleg and soft rot from potato in northern Thailand. Journal of Phytopathology, 167:655-666.

Kumvinit, A. and Akarapisan, A. (2020). Surfactin production, biofilm formation and antagonistic activity against phytopathogens by *Bacillus velezensis*. Asian Journal of Plant Pathology, 14:27-35.

Lade, H., Paul, D. and Kweon, H. J. (2014). N-Acyl homoserine lactone-Mediated quorum sensing with special reference to use of quorum quenching bacteria in membrane biofouling control. BioMed Research International, 162584:1-25.

Li, X., Du, G. and Chen, J. (2008). Use of enzymatic biodegradation for protection of plant against microbial disease. Biotechnology, 4:1-12.

Li, Y., Qin, L., Roberts, P. D., Hu, X., Xie, L., Gu, C., Liao, X., Han, P. and Liao, X. (2020). Biological fertilizer containing *Bacillus subtilis* BY-2 for control of *Sclerotinia sclerotiorum* on oilseed rape. Crop Protection, 138:105340.

Lin, C., Tsai, C-H., Chen, P-Y., Wu, C-Y., Chang, Y-L., Yang, Y-L. and Chen, Y.L. (2018). Biological control of potato common scab by *Bacillus amyloliquefaciens* Ba01. PLoS ONE, 13:1-17.

Myo, M. E., Liu, B., Ma, J., Shi, L., Jiang, M., Zhang, K. and Ge, B. (2019). Evaluation of *Bacillus velezensis* NKG-2 for bio-control activities against fungal diseases and potential plant growth promotion. Biological Control, 134:23-31.

Peypoux, F., Bonmatin, J. M., Wallach, J. (1999). Recent trends in the biochemistry of surfactin. Applied Microbiology and Biotechnology, 51:553-563.

Raafat, M. M., Ali-Tammam, M. and Ali, E. A. (2019). Quorum quenching activity of *Bacillus cereus* isolate 30b confers antipathogenic effects in *Pseudomonas aeruginosa*. Infection and Drug Resistance, 12:1583-1596.

Rabbee, F. M., Ali, S. M., Choi, J., Hwang, S. B., Jeong, C. S. and Baek, K-H. (2019). *Bacillus velezensis*: A valuable member of bioactive molecules within plant microbiomes. Molecules 24:1-13.

Rahman, M. M., Ali, M. E., Khan, A. A., Akanda, A. M., Uddin, M. K., Hashim, U. and Hamid, S. B. (2012). Isolation, characterization and identification of biological control agent for potato soft rot in Bangladesh. The Scientific World Journal. pp:1-6.

Rasmussen, T. B. and Givskov, M. (2006). Quorum-sensing inhibitors as anti-pathogenic drugs. International Journal of Medical Microbiology Supplements, 296:149-161.

Stein, T. (2005). *Bacillus subtilis* antibiotic: structures, syntheses and specific function. Molecular Microbiology, 56:845-857.

Torres, M., Romero, M., Prado, S., Dubert, J., Tahrioui, A., Otero, A. and Llamas, I. (2013). *N*-acylhomoserine lactone-degrading bacteria isolated from hatchery bivalve larval cultures. Microbiological Research, 168:547-554.

Yates, E. A., Philipp, B., Buckley, C., Atkinson, S., Chhabra, S. R., Sockett, R. E., Goldner, M., Dessaux, Y., Camara, M., Smith, H. and Williams, P. (2002). N-Acyl homoserine lactones undergo lactonolysis in a pH, temperature and acyl chain length-dependent manner during growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. Infection and Immunity, 70:5635-5646.

(Received: 17 August 2022, accepted: 30 December 2022)