**BIODIVERSITAS**
Volume 24, Number 4, April 2023
Pages: 1971-1979

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240405

# Antimicrobial activities of endophytic bacteria isolated from *Ageratum conyzoides* Linn.

**NARUMON BOONMAN[1,♥], JARUWAN CHUTRTONG[1], CHANATE WANNA[1], SIRIPHAN BOONSILP[2], SUPATRA CHUNCHOB[3]**

[1]Department of Science, Faculty of Science and Technology, Suan Sunandha Rajabhat University. U-Thong nok Rd., Dusit, Bangkok 10300, Thailand. Tel./Fax.: +66-2160-1143, ♥email: narumon.bo@ssru.ac.th
[2]Department of Clinical Pathology, Faculty of Medicine, Vajira Hospital, Navamindradhiraj University. Kao Rd., Dusit, Bangkok 10300, Thailand
[3]Division of Conservation Biology, School of Interdisciplinary Studies, Mahidol University. Saiyok District, Kanchanaburi, 71150, Thailand

Manuscript received: 7 February 2023. Revision accepted: 3 April 2023.

**Abstract.** *Boonman N, Chutrtong J, Wanna C, Boonsilp S, Chunchob S. 2023. Antimicrobial activities of endophytic bacteria isolated from* Ageratum conyzoides *Linn. Biodiversitas 24: 1971-1979.* Endophytic bacteria were isolated from various parts of *Ageratum conyzoides* Linn. Total 35 isolates were obtained which were consisting of eight isolates from the roots (AconR1-AconR8), nine isolates from the stems (AconS1-AconS9), seven isolates from the leaves (AconL1-AconL7) and 11 isolates from the flowers (AconF1-AconF11). These endophytic bacteria were examined for their antimicrobial activities against human pathogenic bacteria and fungi. The AconR2 and AconR4 inhibited the growth of *Shigella flexneri* and *Salmonella enterica* ser. typhi, whereas *Escherichia coli* was only inhibited by AconR2. For the antifungal activities assay, all isolated endophytic bacteria revealed no effect on *Candida albicans*, while six endophytic isolates exhibited more than 80% mycelial growth inhibition against *Microsporum canis*. These highly effective isolates were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). The Bruker scores revealed that AconR2 and AconR4 were identified as *Pseudomonas aeruginosa*, AconS1 and AconS6 were classified as *Enterobacter asburiae*, Acon L5 and Acon F9 were identified as *Bacillus cereus*. The results indicated that these endophytic bacteria isolated from *A. conyzoides* effectively inhibited the growth of human pathogenic filamentous fungi and showed promise for further development as novel antifungal agents.

**Keywords:** Cross streak assay, dual culture technique, MALDI-TOF MS, *Microsporum canis*

## INTRODUCTION

During past few decades, research to find active compounds in natural products, broadly defined as chemicals produced by living organisms, has markedly increased (Sorokina and Steinbeck 2020). Numerous natural products have been reported from plants, animals, algae, planktons and microorganisms with widely divergent chemical structures and biological activities, including antimicrobial, anticancer, antioxidant, immunosuppressive, and anti-inflammatory properties (Carroll et al. 2022; Pham et al. 2019; Wanna 2019). Using naturally derived constituents as potential therapeutic applications for humans have clear advantages. Their chemical novelties can lead to drug candidates for complex targets, while their bi- and tri-dimensional complex structures that can be absorbed and metabolized in the human body (Strohl 2000). Moreover, the emerging health issues such as allergic reaction and hyperactivity have arisen from the use of synthetic compounds, therefore, consumers nowadays prefer non-toxic biological products produced from natural resources (Tang et al. 2020).

Using natural products to improve human health was documented in the sophisticated medicinal system of ancient Mesopotamia as early as 2900-2600 BC, with plants considered to be the most promising natural product sources (Siddiqui et al. 2014). Ethnobotanical information about medicinal plants from many countries reflects the use of traditional herbal plants as the primary therapeutic options of people around the world. Local wisdom is passed down from generation to generation for centuries (Pikulthong et al. 2022; Porras et al. 2021; Renna and Gonnella 2020; Van Wyk and Prinsloo 2018). Undoubtedly, early discoveries of potential natural products often focused on the extraction and examination of active compounds from the medicinal plants. Nowadays, it is established that living plant tissues harbor living microorganisms, collectively known as endophytes, which produce secondary metabolites that function as a biological defense for their plant host against various pathogenic microorganisms. Endophytes are, therefore, receiving significant interest from the scientific community due to their immense potential to contribute to the discovery of novel bioactive compounds (Alvin et al. 2014; Gautam 2014; Gouda et al. 2016).

*Ageratum conyzoides* Linn. is an annual erect herbaceous plant weed in the family Asteraceae, typically known as billy goat weed, goat weed or tropical whiteweed that grows to approximately 0.5-1 m in height. The roots are fibrous and shallow. The stems are green or red and covered with fine white hairs. The weak aromatic unpleasant smelling leaves are egg-shaped with a toothed margin and covered with fine hair. The flowers are purple, blue, pinkish or white with around 15 flower heads

arranged as a corymb in leaf axils or at the end of branches. The fruits are small brown one-seeded achenes with aristate pappi (Santos et al. 2016). This plant is normally classified as an invasive weed in agricultural lands and degraded areas, causing crop yield reductions and affecting biodiversity. However, it is widely utilized as a traditional medicine in various countries worldwide, for instance as wound and burn healing and as an anthelmintic, antimicrobial and anti-inflammatory agent as well as in the treatment of dysentery, diarrhea, pneumonia, rheumatism, malaria and skin diseases, etc., until it is commonly called "King of herbs" (Chahal et al. 2021). Later, several phytochemical studies have characterized alkaloids, flavonoids, tannins, coumarins, chromenes, sterols, triterpenes and saponosides from almost every part of this plant as phytoconstituents exhibiting diverse pharmacological properties (Yadav et al. 2019).

Fitriani et al. (2015) isolated endophytic bacteria, *Shewanella* and *Pseudomonas* from the roots of *A. conyzoides*. The crude extract of *Shewanella* exhibited the strongest antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* whereas the *Pseudomonas* extract showed the lower activity against only *E. coli* and *S. aureus*. GC-MS analysis of secondary metabolites revealed the existence of 2-amino-3-quinoline carbonitrile and boric acid in the crude extract of *Shewanella* while 9-octadecenoic acid and 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester were presented in the crude extract of *Pseudomonas*. Interestingly, endophytes from *A. conyzoides* are regarded as the treasure chest of natural products with medicinal importance waiting to be discovered. Therefore, this research aimed to isolate endophytic bacteria from various parts of *A. conyzoides* and to examine their growth inhibition activity against human pathogenic bacteria and fungi. This finding may demonstrate the potential to be a promising source of effective treatments for these infections.

## MATERIALS AND METHODS

### Plant collection and identification

The healthy and mature plants of *A. conyzoides*. were collected from an agricultural area in Tha Mai District, Chanthaburi, Thailand (12°37'28.088"N, 102°1'21.25.788"E) (Figure 1). Fifty fresh plants, consisting of roots to tips, were randomly collected from area of 4 acres and kept in sterile plastic bags with the roots immersed in sterile water. The plant samples were transferred to the laboratory within a few hours after collection.

The plant species was authenticated by a botanist at Bangkok herbarium, Plant Varieties Protection, Department of Agriculture, Bangkok, Thailand. A specimen of this plant was deposited at Bangkok herbarium with voucher number BK No. 084534.

### Surface sterilization and isolation of endophytic bacteria

Endophytic bacteria were isolated following the method described by Naoufal et al. (2018) and Sulistiyani et al. (2017) with some modifications. The whole plants were preliminarily cleaned with slow running tap water to remove adhering dirt and soil particles. Then, the plant parts were separated into 4 different parts, including roots, stems, leaves and flowers (Figure 2). Each plant organ was weighed to 10 g and washed twice with sterile distilled water. Surface sterilization was then carried out by immersion in 70% ethanol for 5 min followed by 2% sodium hypochlorite (NaOCl) for 5 min. Subsequently, the plant samples were rinsed with sterile distilled water five times, dried using sterile tissue paper and aseptically cut into small pieces.

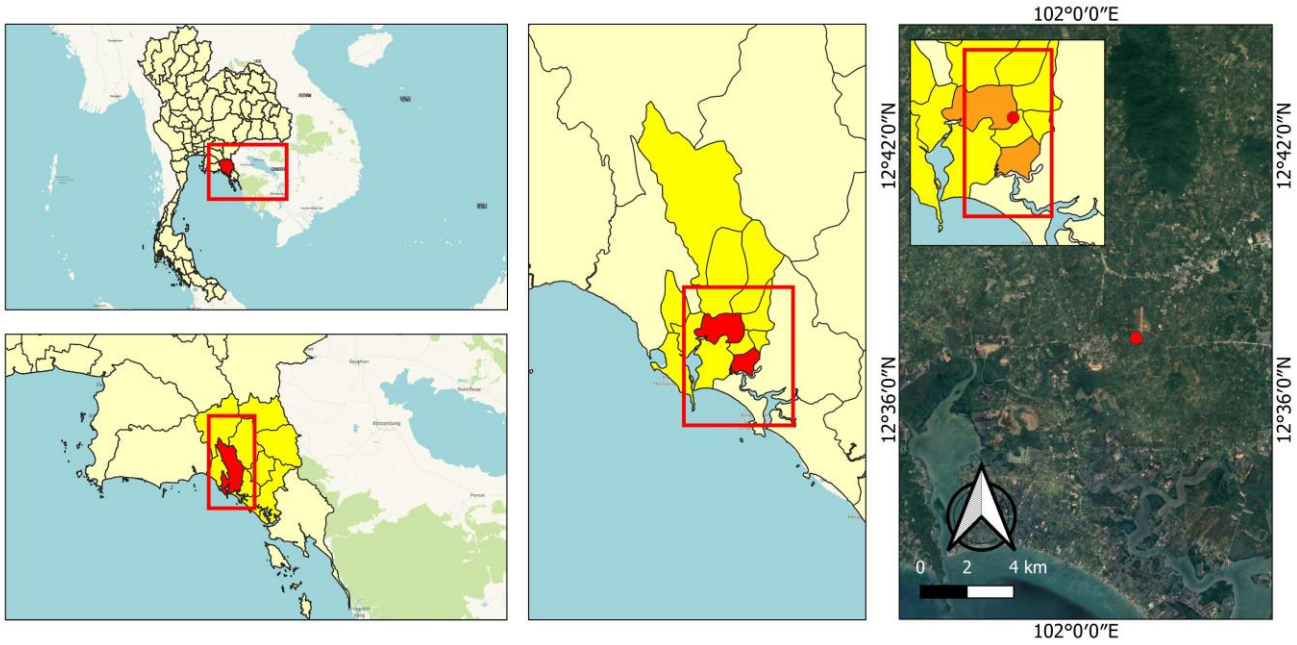



**Figure 1.** Plant specimen sampling site in Tha Mai District, Chanthaburi, Thailand


**Figure 2.** *Ageratum conyzoides* Linn. A. Whole plants; B. Roots; C. Stems; D. Leaves; E. Flowers

The sections were macerated with 10 ml of sterile 0.85% NaCl solution using a mortar and pestle and further homogenized by vortexing for 60 sec at high speed. Ten-folded serial dilutions were carried out for each sample separately using sterile nutrient broth (NB; Himedia, India) and 100 µl of each dilution were inoculated on nutrient agar (NA; Himedia, India) using the spread plate method. The plates were incubated at 28 ± 2°C in the dark and observed every day until there was colony growth. The surface sterilization procedure was validated by culturing aliquots of water from the last rinsing onto NA plates, incubating at the same temperature for endophytic isolation and checking for microbial growth. The isolated endophytic bacteria were preliminarily characterized according to their morphological characteristics. The distinct colony types were picked up and repeatedly streaked on fresh NA plates until pure cultures were obtained. All purified endophytes were subcultured in NA slants and stored in a refrigerator at 4°C for further studies.

**Preliminary characterization of endophytic bacteria**

All endophytic bacterial isolates were examined based on the macroscopic characters of their colonies characteristics growing on the NA plates (color, shape, margin, texture and elevation). Furthermore, the microscopic characterization was carried out by Gram's staining to determine the shape of cells and groups of bacteria (Bergey 1994).

**Pathogenic microorganisms**

Clinical isolates of Gram-negative bacteria including *Escherichia coli*, *Shigella flexneri* and *Salmonella enterica* ser. typhi, pathogenic yeast strain *Candida albicans* and pathogenic filamentous fungus *Microsporum canis* were used as test microorganisms in this study. All pathogenic strains were isolated from the patients at Vajira Hospital and then identified and maintained at the Microbiology Laboratory, Department of Clinical Pathology, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand. The bacterial strains were cultivated on NA medium while the fungal strains were grown on potato dextrose agar (PDA; Himedia, India).

**Evaluation of antibacterial activity**

*In vitro* antibacterial activity of the endophytic bacteria was determined by the cross streak assay as described by Balouiri et al. (2016) and Rajaram et al. (2020). Endophytic and pathogenic bacterial isolates from stock culture were reactivated on NA plates and incubated at 37°C for 24 h. One colony of the endophytic bacteria was picked up and seeded by a single streak in the center of a Mueller-Hinton agar (MHA; Himedia, India) plate. After incubation at 37°C for 72 h, the plate was seeded with the tested microorganisms by a single streak perpendicular to the central streak on the left and right sides, leaving a distance of 5 mm from the endophytic line on each side. After further incubation at 37°C for 24 h, the antibacterial interactions were analyzed by measuring the inhibition zone size using a Vernier caliper. The experiment was performed in triplicate.

**Evaluation of antifungal activity**

*C. albicans* from stock culture was subcultured on PDA plates and incubated at 37°C for 48 h and then cross streaked with each endophytic bacteria on a PDA plate using the same method as the antibacterial activity assay.

Antagonistic activity of the endophytic bacteria against the filamentous fungus was determined by the dual culture technique (Chen et al. 2012; Naoufal et al. 2018; Shirasangi and Hegde 2018). *M. canis* from stock culture was cultured on PDA plates at room temperature for 14 days. A mycelial block (5 x 5 mm) was cut off using a sterile scalpel and placed at a distance 2 cm away from the edge of each fresh PDA plate. Then, one colony of the actively growing endophytic bacteria was streaked in a

straight line on the PDA plate at the opposite side of the fungal block with a distance 2 cm away from the other edge. A PDA plate containing only a mycelial block without endophytic bacteria was used as the control. Three replications were maintained for each endophytic isolate. These plates were cultured at room temperature for 14 days and then the radial growth of *M. canis* in the control and treated plates was measured using a Vernier caliper. The percentage of mycelial growth inhibition was calculated using the following formula:

$$\% \text{ growth inhibition} = [(C - T)/C] \times 100$$

Where:

C is the area of the fungal colony on the control sample

T is the area of the fungal colony on the treatment sample with endophytic bacteria

**Identification of endophytic bacteria**

For identification of endophytic bacteria, the sample preparation procedure was performed according to Pomastowski et al. (2019) and Strejcek et al. (2018) with slight modifications. The endophytic bacteria were grown on NA plates overnight at 37°C. A single colony of bacteria was smeared as a thin film directly onto a MALDI target plate. The bacterial smear was overlaid with 1 µL 70% formic acid and allowed to dry completly. The bacterial extract on the MALDI target plate was then spotted with 1 µL of HCCA matrix solution (10 mg/ml α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) before drying in air at room temperature. The MALDI target plate with samples was analyzed using a microflex MALDI-TOF Mass Spectrometer (Bruker Daltonics, Germany). All mass spectra were measured automatically using Flex Control software according to the standard measurement method for microbial identification. MALDI BioTyper 3.1 software was used for bacterial classification according to the manufacturer's standard protocol (Bruker Daltonics, Germany). Bruker identification scores were derived using the standard Bruker algorithm. Bruker scores between 2.3 and 3.0 indicate very close relatedness, scores between 2.0 and 2.3 indicate close relatedness, and scores below 1.7 indicate low relatedness. Identification of each bacterial isolate was performed in triplicate.

**Statistical analysis**

Results of antibacterial and antifungal activities were expressed as mean ± standard deviation. Statistical analysis was determined by one-way ANOVA with Tukey's test to evaluated significant differences between groups using the IBM SPSS Statistics version 24. A statistically significant difference was determined as $P$-value < 0.05.

## RESULTS AND DISCUSSION

**Endophytic bacteria isolates**

Endophytic bacteria were isolated from various parts of *A. conyzoides*. A total of 35 endophytic bacteria were isolated and purified, divided into eight isolates (22.86%) from the roots (AconR1-AconR8), nine isolates (25.71%) from the stems (AconS1-AconS9), seven isolates (20.00%) from the leaves (AconL1-AconL7), and 11 isolates (31.43%) from the flowers (AconF1-AconF11). Each isolate of endophytic bacteria when cultured on a NA plate revealed a different colony morphology. Each endophytic bacteria isolate was examined by Gram's stain and observed under a microscope. The endophytes from roots included six Gram-negative bacilli, one Gram-negative coccus and one Gram-positive bacillus. All isolates from stems were Gram-negative with four cocci and five bacilli whereas all isolates from leaves were Gram-positive with one coccus and six bacilli. All endophytic bacteria isolated from flowers were bacilli with four Gram-negative and seven Gram-positive.

The results obtained indicated that endophytic bacteria thrived inside all parts of *A. conyzoides*. Endophytic bacteria benefited their host directly by improving plant nutrient uptake and by modulating growth and stress related phytohormones, and indirectly by targeting pests and pathogens with antibiotics, hydrolytic enzymes, or nutrient competition (Afzal et al. 2019). Moreover, the natural compounds produced by endophytic bacteria have several potential applications in pharmaceutical industry. Ecomycins, Pseudomycins, Munumbicins and Xiamycins are some examples of the novel antibiotics produced by the endophytic bacteria (Christina et al. 2013). Therefore, antimicrobial activity of the endophytic isolates obtained in this research against human pathogenic bacteria and fungi was examined to discover effective natural compounds against these infections.

**Antibacterial activity**

All 35 isolates of endophytic bacteria from *A. conyzoides* were screened for their antibacterial activity against three human pathogenic bacteria including *E. coli*, *S. flexneri* and *S. typhi* using the cross streak assay. The results showed that most endophytes were unable to inhibit the growth of human pathogenic bacteria. Only AconR2 was able to inhibit *E. coli* growth, with a mean inhibition zone of 3.92 ± 0.38 mm. For *S. flexneri*, only AconR2 and AconR4 exhibited the inhibition zones of 7.58 ± 1.26 mm and 6.75 ± 1.09 mm, respectively. In testing with *S. typhi*, the results were consistent with *E. coli* that only AconR2 and AconR4 had the inhibition zones of 7.25 ± 0.25 mm and 8.92 ± 2.67 mm, respectively (Table 1). Unfortunately, all endophytic bacteria isolated from the stems, leaves and flowers did not exhibit antagonistic effects against these pathogenic bacteria. This preliminary antibacterial screening found that the endophytic bacteria from *A. conyzoides* were not strongly effective against the human pathogenic bacteria. Therefore, MIC and MBC values were not further determined.

Endophytic bacteria in this study were less effective in inhibiting the growth of pathogenic bacteria. This may be because this research focused on testing against Gram-negative bacilli, thus their distinctive structure and the expression of antibiotic inactivating enzymes or non-

enzymatic pathways make them more resistant than Gram-positive bacteria (Breijyeh et al. 2020).

**Table 1.** Antibacterial and antifungal activities of the endophytic bacteria isolated from *Ageratum conyzoides*

| Isolate | Antibacterial activity | | | Antifungal activity | |
|---|---|---|---|---|---|
| | ***E. coli*** **inhibition zone (mm)** | ***S. flexneri*** **inhibition zone (mm)** | ***S. typhi*** **inhibition zone (mm)** | ***C. albicans*** **inhibition zone (mm)** | ***M. canis*** **growth inhibition (%)** |
| AconR2 | 3.92 ± 0.38 [b] | 7.58 ± 1.26 [b] | 7.25 ± 0.25 [b] | 0.00 ± 0.00 [a] | 87.00 ± 1.24 [ab] |
| AconR4 | 0.00 ± 0.00 [a] | 6.75 ± 1.09 [b] | 8.92 ± 2.67 [b] | 0.00 ± 0.00 [a] | 90.50 ± 1.20 [bc] |
| AconS1 | 0.00 ± 0.00 [a] | 0.00 ± 0.00 [a] | 0.00 ± 0.00 [a] | 0.00 ± 0.00 [a] | 81.38 ± 3.77 [a] |
| AconS6 | 0.00 ± 0.00 [a] | 0.00 ± 0.00 [a] | 0.00 ± 0.00 [a] | 0.00 ± 0.00 [a] | 84.16 ± 2.65 [a] |
| AconL5 | 0.00 ± 0.00 [a] | 0.00 ± 0.00 [a] | 0.00 ± 0.00 [a] | 0.00 ± 0.00 [a] | 94.70 ± 0.74 [c] |
| AconF9 | 0.00 ± 0.00 [a] | 0.00 ± 0.00 [a] | 0.00 ± 0.00 [a] | 0.00 ± 0.00 [a] | 92.92 ± 0.77 [c] |

Note: Data are means ± standard deviation of three replications; the same letter within the same column indicates no statistically significant difference

**Antifungal activity**

The antifungal activity of all 35 endophytic isolates was examined against *C. albicans* by cross streak assay and *M. canis* by the dual culture technique. Surprisingly, there was no endophytic bacteria showed an inhibition zone against *C. albicans*, possibly because *C. albicans* is a dimorphic fungus with complex growth processes. However, some bacteria have been reported to inhibit the growth of *C. albicans*, such as *P. aeruginosa* that produced phenazines and phospholipase C, which inhibited *C. albicans* only during the mycelial stage (Hogan and Kolter 2002) and *Lactobacillus rhamnosus* that reduced *C. albicans* hyphal differentiation and inhibited biofilm formation only in the early stages but had no significant effect on the mature biofilm (Matsubara et al. 2016).

On the other hand, six isolates of endophytic bacteria were highly effective in inhibiting the growth of *M. canis* with the percentage of mycelial growth inhibition greater than 80%. AconL5 and AconF9 were the most effective which inhibited the growth of *M. canis* at up to 94.70% and 92.92%, respectively followed by AconR4 and AconR2 that inhibited *M. canis* growth at 90.50% and 87.00%, respectively. AconS6 and AconS1 also inhibited *M. canis* growth at 84.16 % and 81.38%, respectively (Table 1, Figure 3 and Figure 4). These results concurred with previous studies which reported that endophytic bacteria in the *Bacillus* genus inhibited the growth of many filamentous fungi, such as *B. subtilis* ZZ120, isolated from the stems of *Prunus mume*, was active against many plant pathogenic fungi, including *Fusarium graminearum*, *Alternaria alternata*, *Rhizoctonia solani*, *Cryphonectria parasitica* and *Glomerella glycines*, by producing lipopeptides that altered fungal membrane permeability and lipid composition leading to the hyphal abnormality and chitinase enzymes that disrupted the fungal cell wall (Li et al. 2012). *B. mojavensis* SZMC 22228, isolated from mumijo, a traditional Mongolian medicine, inhibited the growth of dermatophytes, including *Microsporum canis*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Trichophyton tonsurans* by producing a chymotrypsin-like protease (Galgóczy et al. 2014).

**Identification of endophytic bacteria**

Six selected bacterial culture isolates were subjected to species identification by MALDI-TOF MS. Among these, two isolates (AconR2 and AconR4) were identified as *Pseudomonas aeruginosa*, two isolates (AconS1 and AconS6) were identified as *Enterobacter asburiae* and two isolates (AconL5 and AconF9) were identified as *Bacillus cereus* with the Bruker score values of >2.0 indicating close relatedness to the identified species. The obtained results were consistent with the colony and cellular morphologies of each isolate as shown in Table 2. MALDI-TOF MS of proteins in the 2000 to 20,000 m/z range is the clinical standard for microbial identification with the advantages of convenience, speed, cost-effectiveness and accuracy when compared with conventional biochemical methods (Tsuchida et al. 2020).

Considering the endophytic bacteria species, *B. cereus* had the most statistically significant inhibitory effect on *M. canis* followed by *P. aeruginosa* and *E. asburiae*, respectively (Figure 4). Interestingly, *Bacillus* spp. are the most widespread endophytic bacteria and dominant among the biocontrol agents due to their ability to form spores that allows them to grow and survive under various ecological stress conditions.

Species of the *Bacillus* genus are known to produce antifungal peptides with a wide range of activities are involved. Mechanism by which the peptides function is either to inhibit the fungal growth or to directly kill the pathogenic fungi by inhibiting the mycelial growth, affecting the spore germination, or causing the hyphae or spores to become swollen, deformed, twisted or broken (Li et al. 2021). Surfactin, iturin and fengycin are lipopeptide compounds derived from *Bacillus* spp.

The antifungal mechanism of surfactin is mainly to destroy the fungal lipid membrane and lyse the pathogenic fungi (Lima et al. 2018) and down-regulate the expression of several genes involved in ergosterol synthesis, morphogenesis or metabolism such as glycolysis, ethanol and fatty acid biosynthesis, thus affecting the physiological functions of the pathogenic fungi (Jakab et al. 2022) while the mechanism of iturin and fengycin is to change the surface tension of fungal cell membrane; this results in the leakage of $K^+$ and other important ions in the cell, eventually leading to fungal death (Banerjee et al. 2022;

Lei et al. 2019). Moreover, cell wall lyases including cellulase, glucanase, protease and chitinase produced by *Bacillus* spp. are particularly effective against the pathogenic fungi by causing damage to the fungal cell walls (Gomaa and El-Mahdy 2018). Another endophytic bacterium, *Pseudomonas* spp., has also been described in the synthesis of novel antifungal lipopeptides. Ecomycins derived from *P. viridiflava* are associated with some unusual amino acids such as homoserine and β-hydroxy aspartic acid in addition to common amino acids such as alanine, serine, threonine, and glycine which have significant bioactivities against a wide range of human fungal pathogens (Miller et al. 1998). Pseudomycins obtained from *P. syringae* contain non-traditional amino acids with strong activity against human and plant pathogenic fungi (Harrison et al. 1991). In addition, *Enterobacter* sp. also produced chitinase which can inhibit hyphal extension of various pathogenic filamentous fungi (Dahiya et al. 2005). The potentials of these plant-associated bacteria, therefore, make them highly attractive to extend the limits of antimicrobial activity in plants to become effective therapeutic agents of infectious diseases in humans.

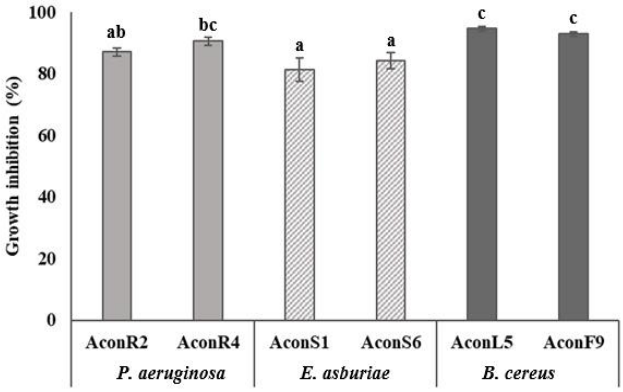



**Figure 4.** Percentage of mycelial growth inhibition of six effective endophytic isolates against *M. canis*

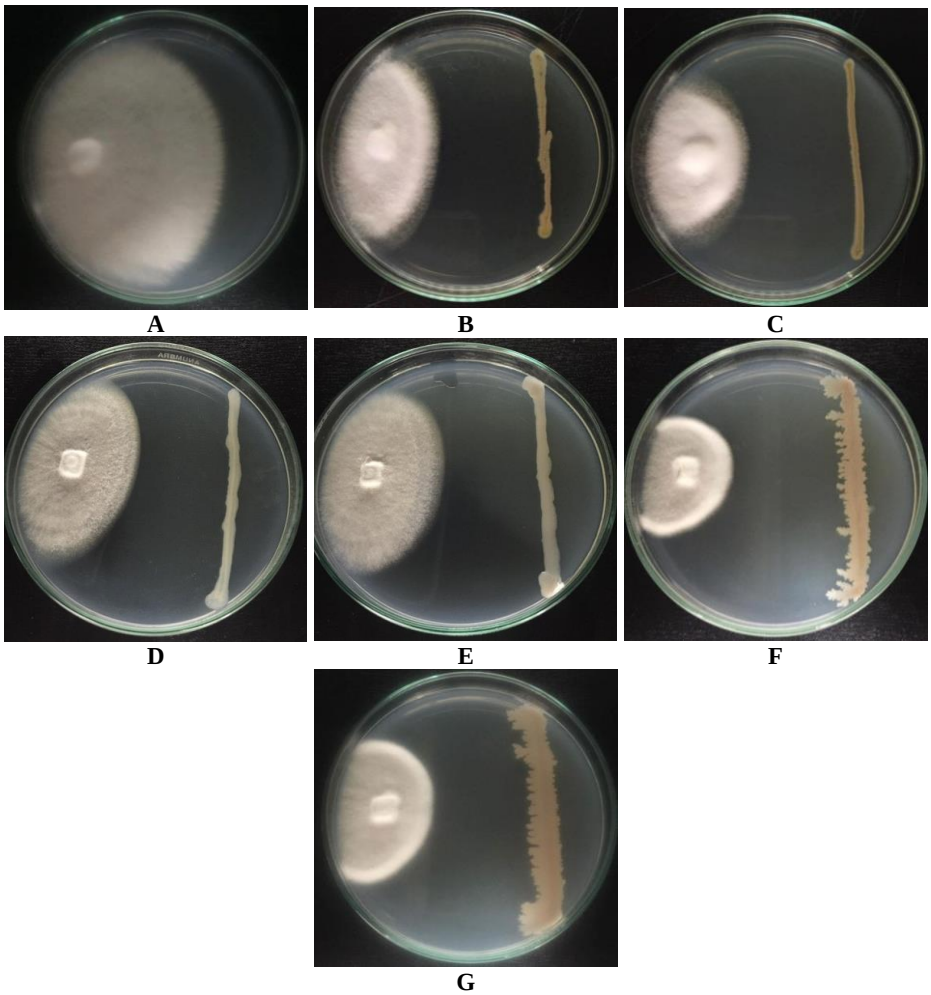
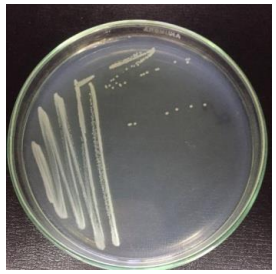
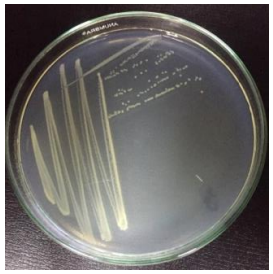
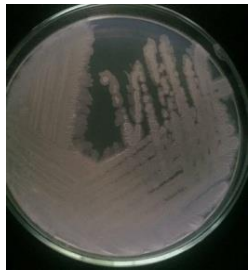
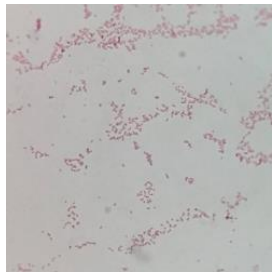
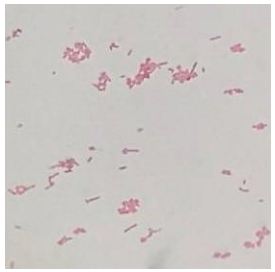
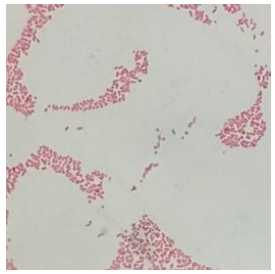
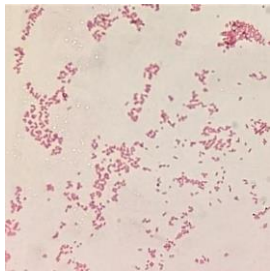
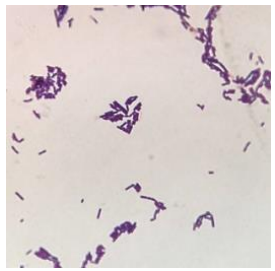
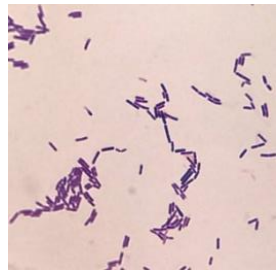



**Figure 3.** Antifungal activity against *M. canis* by dual culture technique. A. Control; B. AconR2; C. AconR4; D. AconS1; E. AconS6; F. AconL5; G. AconF9

**Table 2.** Colony and cellular morphology characteristics of six effective endophytic isolates

| Characteristics | Isolates | | | | | |
|---|---|---|---|---|---|---|
| | **AconR2** | **AconR4** | **AconS1** | **AconS6** | **AconL5** | **AconF9** |
| **Colony morphology** |  |  |  |  |  |  |
| Color | White | White | White | White | White | White |
| Shape | Circular | Circular | Circular | Circular | Irregular | Irregular |
| Margin | Entire | Entire | Entire | Entire | Erose | Erose |
| Texture | Smooth | Mucoid | Smooth | Smooth | Rough | Rough |
| Elevation | Raised | Raised | Raised | Flat | Flat | Flat |
| **Cellular morphology** |  |  |  |  |  |  |
| Gram's stain | Negative | Negative | Negative | Negative | Positive | Positive |
| Shape | Bacillus | Bacillus | Bacillus | Bacillus | Bacillus | Bacillus |
| **Identification** | *P. aeruginosa* | *P. aeruginosa* | *E. asburiae* | *E. asburiae* | *B. cereus* | *B. cereus* |

In conclusion, it can be considered that endophytic bacteria, including *P. aeruginosa*, *E. asburiae* and *B. cereus*, from *A. conyzoides* showed potentials as promising sources of natural products for filamentous fungal inhibition. However, their active compounds and mode of action require further studies. Moreover, other biological effects such as antioxidant, anticancer, anti-inflammatory or plant growth-promoting activities should also be further examined in order to be applied for a variety of benefits.

## ACKNOWLEDGEMENTS

This research was supported by a grant from Suan Sunandha Rajabhat University. We were grateful to Microbiology Laboratory Unit, Division of Central Laboratory and Blood Bank, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand for providing the MALDI-TOF Mass Spectrometer. We were also thankful Asst. Prof. Dr. Vachiraporn Pikulthong and Asst. Prof. Sirirat Phakpaknam in preparing a plant specimen for species authentication.

## REFERENCES

Afzal I, Shinwari ZK, Sikandar S, Shahzad S. 2019. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. Microbiol Res 221: 36-49. DOI: 10.1016/j.micres.2019.02.001.

Alvin A, Miller KI, Neilan BA. 2014. Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. Microbiol Res 169: 483-495. DOI: 10.1016/j.micres.2013.12.009.

Balouiri M, Sadiki M, Ibnsouda SK. 2016. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal 6 (2): 71-79. DOI: 10.1016/j.jpha.2015.11.005.

Banerjee S, Sen S, Bhakat A, Bhowmick A, Sarkar K. 2022. The lipopeptides fengycin and iturin are involved in the anticandidal activity of endophytic *Bacillus* sp. as determined by experimental and in silico analysis. Lett Appl Microbiol 75: 450-459. DOI: 10.1111/lam.13750.

Bergey DH. 1994. Bergey's manual of determinative bacteriology. Lippincott Williams & Wilkins, Baltimore.

Breijyeh Z, Jubeh B, Karaman R. 2020. Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules 25 (6): 1340. DOI: 10.3390/molecules25061340.

Carroll AR, Copp BR, Davis RA, Keyzers RA, Prinsep MR. 2022. Marine natural products. Nat Prod Rep 39: 1122-1171. DOI: 10.1039/D1NP00076D.

Chahal R, Nanda A, Akkol EK, Sobarzo-Sánchez E, Arya A, Kaushik D, Dutt R, Bhardwaj R, Rahman MH, Mittal V. 2021. *Ageratum conyzoides* L. and its secondary metabolites in the management of different fungal pathogens. Molecules 26: 2933. DOI: 10.3390/molecules26102933.

Chen N, Jin M, Qu HM, Chen ZQ, Chen ZL, Qiu ZG, Wang XW, Li JW. 2012. Isolation and characterization of *Bacillus* sp. producing broad-spectrum antibiotics against human and plant pathogenic fungi. J Microbiol Biotechnol 22 (2): 256-263. DOI: 10.4014/jmb.1107.07021.

Christina A, Christapher V, Bhore SJ. 2013. Endophytic bacteria as a source of novel antibiotics: an overview. Pharmacognosy Rev (13): 11-16. DOI: 10.4103/0973-7847.112833.

Dahiya N, Tewari R, Tiwari RP, Hoondal GS. 2005. Production of an antifungal chitinase from *Enterobacter* sp. NRG4 and its application in protoplast production. World J Microbiol Biotechnol 21: 1611-1616. DOI: 10.1007/s11274-005-8343-6.

Fitriani A, Ihsan F, Hamdiyati Y. 2015. Antibacterial activity of *Shewanella* and *Pseudomonas* as endophytic bacteria from the root of *Ageratum conyzoides* L. Asian J Appl Sci 3 (3): 415-420.

Galgóczy LN, Guba M, Sajben-Nagy EI, Vörös M, Takó M, Vágvölgyi C. 2014. Antidermatophytic effect of *Bacillus mojavensis* SZMc 22228 and its secreted chymotrypsin-like protease. Acta Biol Szeged 58 (2): 157-162.

Gautam AK. 2014. Diversity of fungal endophytes in some medicinal plants of Himachal Pradesh, India. Arch Phytopathol Plant Prot 47 (5): 537-544. DOI: 10.1080/03235408.2013.813678.

Gomaa EZ, El-Mahdy OM. 2018. Improvement of chitinase production by *Bacillus thuringiensis* NM101-19 for antifungal biocontrol through physical mutation. Microbiology 87: 472-485. DOI: 10.1134/S0026261718040094.

Gouda S, Das G, Sen SK, Shin HS, Patra JK. 2016. Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 7: 1538. DOI: 10.3389/fmicb.2016.01538.

Harrison L, Teplow DB, Rinaldi M, Strobel G. 1991. Pseudomycins, a family of novel peptides from *Pseudomonas syringae* possessing broad-spectrum antifungal activity. Microbiology 137 (12): 2857-2865. DOI: 10.1099/00221287-137-12-2857.

Hogan DA, Kolter R. 2002. *Pseudomonas-Candida* interactions: an ecological role for virulence factors. Science 296 (5576): 2229-2232. DOI: 10.1126/science.1070784.

Jakab A, Kovacs F, Balla N, Toth Z, Ragyak A, Sajtos Z, Csillag K, Nagy-Koteles C, Nemes D, Bacskay I, Pócsi I, Majoros L, Kovács AT, Renátó Kovács. 2022. Physiological and transcriptional profiling of surfactin exerted antifungal effect against *Candida albicans*. Biomed Pharmacother 152: 113220. DOI: 10.1016/j.biopha.2022.113220.

Lei S, Zhao H, Pang B, Qu R, Lian Z, Jiang C, Shao D, Huang Q, Jin M, Shi J. 2019. Capability of iturin from *Bacillus subtilis* to inhibit *Candida albicans* in vitro and in vivo. Appl Microbiol Biotechnol 103: 4377-4392. DOI: 10.1007/s00253-019-09805-z.

Li H, Wang X, Han M, Zhao Z, Wang M, Tang Q, Liu C, Kemp B, Gu Y, Shuang J, Xue Y. 2012. Endophytic *Bacillus subtilis* ZZ120 and its potential application in control of replant diseases. Afr J Biotechnol 11 (1): 231-242. DOI: 10.5897/AJB11.2839.

Li T, Li L, Du F, Sun L, Shi J, Long M, Chen Z. 2021. Activity and mechanism of action of antifungal peptides from microorganisms: a review. Molecules 26 (11): 3438. DOI: 10.3390/molecules26113438.

Lima WG, Parreira AG, Nascimento LA, Leonel CA, Andrade JT, Palumbo JM, Soares AC, Granjeiro PA, Ferreira JMS. 2018. Absence of antibacterial, anti-candida, and anti-dengue activities of a surfactin isolated from *Bacillus subtilis*. J Pharm Negative Results 9 (1): 27-32. DOI: 10.4103/jpnr.JPNR_11_17.

Matsubara VH, Wang Y, Bandara HMHN, Mayer MPA, Samaranayake LP. 2016. Probiotic lactobacilli inhibit early stages of *Candida albicans* biofilm development by reducing their growth, cell adhesion, and filamentation. Appl Microbiol Biotechnol 100 (14): 6415-6426. DOI: 10.1007/s00253-016-7527-3.

Miller M, Garton-Kenny GK, Redgrave R, Sears S, Condron C, Teplow T, Strobel S. 1998. Ecomycins, unique antimycotics from *Pseudomonas viridiflava*. J Appl Microbiol 84 (6): 937-944. DOI: 10.1046/j.1365-2672.1998.00415.x.

Naoufal D, Amine H, Ilham B, Khadija O. 2018. Isolation and biochemical characterisation of endophytic *Bacillus* spp. from Urtica dioica and study of their antagonistic effect against phytopathogens. Annu Res Rev Biol 28 (4): 1-7. DOI: 10.9734/ARRB/2018/43642.

Pham JV, Yilma MA, Feliz A, Majid MT, Maffetone N, Walker JR, Kim E, Cho HJ, Reynolds JM, Song MC, Park SR, Yoon YJ. 2019. A review of the microbial production of bioactive natural products and biologics. Front Microbiol 10: 1404. DOI: 10.3389/fmicb.2019.01404.

Pikulthong V, Bhirompan S, Dechkla M, Mokkapan K, Boonman N, Wanna C, Machana K, Phakpaknam S. 2022. Local herbs for pain relief in the area of Tumbon Khao Hin Son, Chachoengsao, Thailand. Biodiversitas 23 (10): 5012-5019. DOI: 10.13057/biodiv/d231007.

Pomastowski P, Złoch M, Rodzik A, Ligor M, Kostrzewa M, Buszewski B. 2019. Analysis of bacteria associated with honeys of different geographical and botanical origin using two different identification approaches: MALDI-TOF MS and 16S rDNA PCR technique. PLoS ONE 14 (5): e0217078. DOI: 10.1371/journal.pone.0217078.

Porras G, Chassagne F, Lyles JT, Marquez L, Dettweiler M, Salam AM, Samarakoon T, Shabih S, Farrokhi DR, Quave CL. 2021. Ethnobotany and the role of plant natural products in antibiotic drug

discovery. Chem Rev 121 (6): 3495-3560. DOI: 10.1021/acs.chemrev.0c00922.

Rajaram SK, Ahmad P, Keerthana SSS, Cressida PJ, Moorthy IG, Suresh RS. 2020. Extraction and purification of an antimicrobial bioactive element from lichen associated *Streptomyces olivaceus* LEP7 against wound inhabiting microbial pathogens. J King Saud Univ Sci 32 (3): 2009-2015. DOI: 10.1016/j.jksus.2020.01.039.

Renna M, Gonnella M. 2020. Ethnobotany, nutritional traits, and healthy properties of some halophytes used as greens in the Mediterranean basin. In: Grigore MN (eds.). Handbook of halophytes: From molecules to ecosystems towards biosaline agriculture. Springer, Cham. DOI: 10.1007/978-3-030-17854-3_100-1.

Santos RF, Nunes BM, Sá RD, Soares LA, Randau KP. 2016. Morpho-anatomical study of *Ageratum conyzoides*. Rev Bras Farmacognosia 26: 679-687. DOI: 10.1016/j.bjp.2016.07.002.

Shirasangi S, Hegde YR. 2018. Evaluation of bacterial endophytes against major fungal pathogens of groundnut under *in vitro* condition. J Pharmacogn Phytochem 7 (6): 1239-1243. DOI: 10.20546/ijcmas.2018.710.051.

Siddiqui AA, Iram F, Siddiqui S, Sahu K. 2014. Role of natural products in drug discovery process. Intl J Drug Dev Res 6 (2): 172-204.

Sorokina M, Steinbeck C. 2020. Review on natural products databases: where to find data in 2020. J Cheminform 12 (1): 1-51. DOI: 10.1186/s13321-020-00424-9.

Strejcek M, Smrhova T, Junkova P, Uhlik O. 2018. Whole-cell MALDI-TOF MS versus 16S rRNA gene analysis for identification and dereplication of recurrent bacterial isolates. Front Microbiol 9: 1294. DOI: 10.3389/fmicb.2018.01294.

Strohl WR. 2000. The role of natural products in a modern drug discovery program. Drug Discov 5 (2): 39-41. DOI: 10.1016/s1359-6446(99)01443-9.

Sulistiyani S, Ardyati T, Winarsih S. 2017. Antimicrobial and antioxidant activity of endophyte bacteria associated with *Curcuma longa* rhizome. J Exp Life Sci 6 (1): 45-51. DOI: 10.21776/ub.jels.2016.006.01.11.

Tang DYY, Khoo KS, Chew KW, Tao Y, Ho SH, Show PL. 2020. Potential utilization of bioproducts from microalgae for the quality enhancement of natural products. Bioresour Technol 304: 122997. DOI: 10.1016/j.biortech.2020.122997.

Tsuchida S, Umemura H, Nakayama T. 2020. Current status of matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) in clinical diagnostic microbiology. Molecules 25 (20): 4775. DOI: 10.3390/molecules25204775.

Van Wyk AS, Prinsloo G. 2018. Medicinal plant harvesting, sustainability and cultivation in South Africa. Biol Conserv 227: 335-342. DOI: 10.1016/j.biocon.2018.09.018.

Wanna C. 2019. Free radical scavenging capacity and total phenolic contents in peel and fleshy crude extracts of selected vegetables. Pharmacogn J 11 (6): 1351-1358. DOI: 10.5530/pj.2019.11.209.

Yadav N, Ganie SA, Singh B, Chhillar AK, Yadav SS. 2019. Phytochemical constituents and ethnopharmacological properties of *Ageratum conyzoides* L. Phytother Res 33 (9): 2163-2178. DOI: 10.1002/ptr.6405.