

Research Article

ISOLATION OF THE ENDOPHYTIC BACTERIA IN *Andrographis paniculata* Nees GROWING WILD IN HAU GIANG PROVINCE

^{1,*} Huynh Van Truong, ¹Hoang Minh Tu, ²Huynh Gia Bao, ³Tran Doan Hau, ³Lam Quang Nhut, ³Doan Van Dien

¹Can Tho University of Medicine and Pharmacy, Vietnam.

²Medical student of Can Tho University of Medicine and Pharmacy, Vietnam.

³Master student of Can Tho University of Medicine and Pharmacy, Vietnam.

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ABSTRACT

Background: According to studies the endophytic bacteria, *Andrographis paniculata* Nees has antibacterial properties. Therefore, the isolation of the endophytic bacteria strains in *Andrographis paniculata* Nees was carried out in order to find some antibacterial strains that contribute to replacing synthetic antibiotics. **Research subjects and methods:** Eighteen of the endophytic bacteria strains were isolated from the roots, stems and leaves of *Andrographis paniculata*. **Results:** The results of investigation of antibacterial ability on 3 strains of *Escherichia coli*, *Aeromonas hydrophila* và *Staphylococcus aureus* showed that 6 strains were resistant to *Escherichia coli*, 4 strains were resistant to *Aeromonas hydrophila* and 3 strains were resistant to *Staphylococcus aureus* bacteria. Of which, 2 strains were resistant to both *Escherichia coli* and *Aeromonas hydrophila* strains, The strain was resistant to both *Escherichia coli* and *Staphylococcus aureus* strains. **Conclusion:** Two strains of bacteria TR2 and TL3 were identified as *Enterobacter ludwigii* CSR19B with 96% similarity and *Kosakonia radicincitans* DSM 16656 with 95% similarity.

Keywords: *Enterobacter ludwigii*, antibacterial, *Kosakonia radicincitans*, the endophytic bacteria, *Andrographis paniculata* Nees.

INTRODUCTION

Scientifically known as *Andrographis paniculata* Nees, belongs to the Acanthaceae family, and used to be familiar to every family medicine cabinet, clinic and even some hospitals in our country. It is used as an alternative to antibiotics for many diseases associated with fever caused by both bacteria and viruses. In international medical journals, a number of clinical studies have been published on the treatment effects of *Andrographis paniculata* Nees such as influenza, a control experiment of Burgos *et al.*, (1999). *Andrographis paniculata* Nees is a medicine for cold and flu that works better than known treatments and has no harmful side effects for patients. Besides, there are studies on medicinal plants with antibacterial properties such as: *Weedelia chinensis*, *Houttuynia cordata*, *Phyllanthus urinaria*, etc. These have been studied to show that they have antibacterial activity because they contain essential oils that are aldehydes and other ceton derivatives such as methyl n-nonyl ceton, L-decanal, L-dodecanal. The terpenes group includes the substances α -pinene, camphen ... with the effect of killing *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Shigella*, *Salmonella*, *E.coli* (Do Tat Loi, 2006; Shu-Chen *et al.*, 2008).

Microorganisms living endogenously in plants or in the root zone and including in medicinal plants help stimulate plant growth because they have the ability to fix nitrogen, decompose phosphorus, synthesize growth hormones and other compounds with the ability to directly inhibit a number of plant diseases or to stimulate plants to produce secondary metabolic compounds that help plants resist plant pathogens. In particular, some strains of microorganisms endogenous to medicinal plants can produce antibacterial compounds when they live inside medicinal plants. The groups of microorganisms capable of this include species of the general *Azospirillum*, *Herbapirillum*, *Gluconacetobacter*, *Klebsiella*...

Then, we conducted the study to isolate bacterial strains that live endogenously in *Andrographis paniculata* Nees growing wild in Hau Giang province.

RESEARCH SUBJECTS AND METHODS

Research subjects

Isolation of the endophytic bacteria strains from *Andrographis paniculata* Nees in Hau Giang with good properties such as the antibacterial ability.

Research means

Time - Place of implementation

Time: from December 2022 - April 2023

Place: Phat Truong Medical Laboratory, Laboratory of Can Tho University of Medicine and Pharmacy

Tools and equipment

Lame, Lammelle, Vortex machine, Microbiology grow cabinet, Microbiological incubator, Sample shaker, PCR machine.

Material

The whole roots, stems and leaves of *Andrographis paniculata* Nees in Hau Giang Province.

The *E.coli* strain was provided from the Institute of Biotechnology Research & Development, Can Tho University.

Aeromonas hydrophila strain was provided from College of Aquaculture and Fisheries, Can Tho University.

Staphylococcus aureus strain was provided from the Institute of Biotechnology Research & Development, Can Tho University.

*Corresponding Author: Huynh Van Truong,
Can Tho University of Medicine and Pharmacy, Vietnam.

Chemicals

Chemicals used to process samples: sterile distilled water, 70% alcohol, 3% H₂O₂. Chemicals used to stain Gram bacteria: Iodine, Fushin, Crystal violet, 70% alcohol, sterile distilled water.

- The medium for isolating endophytic bacteria and investigating the antibacterial ability of the isolated bacteria with PDA medium formula.
- Surveying medium for phosphate solubilization with NBRIP medium (Nautiyal, 1999)

16S rDNA sequencing

Sequencing uses an automated sequencer. The 16S rDNA sequences of the bacterial strains were compared with the 16S sequences on the gene bank using the BLAST

Statistical processing

The data in the experiments were processed by Microsoft Office Excel 2010 and Statgraphics 16.2 software for statistical analysis.

RESEARCH RESULTS AND DISCUSSION

Isolation results and colony characteristics of bacterial strains

Bacterial isolation results

From the roots, stems and leaves of the wild-growing *Andrographis paniculata* Nees in Hau Giang province, 18 endophytic bacteria strains were isolated on PDA medium. Most of these bacterial strains were distributed in all 3 parts of *Andrographis paniculata* Nees. Isolated bacteria were most concentrated in the roots. There are 9 strains. The endophytic bacteria were isolated from leaves with 6 strains and from leaves with at least 3 strains. These endophytic bacteria share a common characteristic of growth and development under microaerobic conditions. When inoculated on semi-solid NFb medium, the bacteria grew into a pellicle ring (white light ring on the medium), 2-4 mm from the surface of the medium. Some strains changed the color of the original NFb medium (Figure 1).

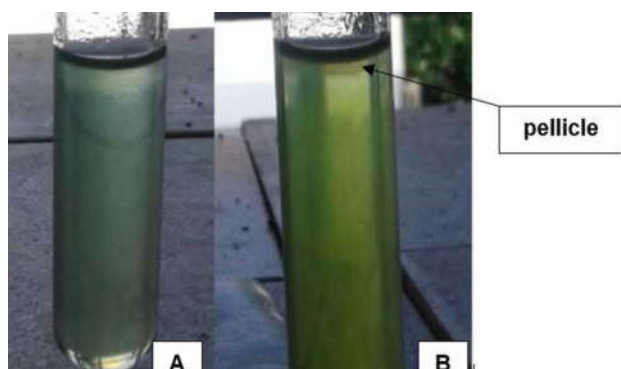


Figure 1. Bacterial growth and the forming of a pellicle in NFb medium

- *Note: A. Bacteria do not change the color of the medium
B. Bacteria make the medium turn green

This result is also consistent with the reports of authors such as Nguyen Huu Hiep *et al.*, (2013) on endophytic bacteria in banana, Cao Ngoc Diep and Phan Thi Nha (2011) on endophytic bacteria in pineapple, Dao Thanh Hoang (2014) on endophytic bacteria in high yielding rice.

Bacterial strains isolated on PDA medium from roots, stems, and leaves of the wild-growing *Andrographis paniculata* Nees in Hau Giang.

Colony characteristics

After culturing on PDA medium and isolating pure strains, it was found that most of the bacterial strains grow very quickly, the time for the strains to develop into colonies is 12 hours, and the latest one is 24 hours. Regarding the morphological characteristics of the colonies

Colony color: Most colonies are opaque white (10/18), ivory white (4/18), transparent white (2/18) and yellow (2/18).

Colony shape: Most of the colonies are round (15/18), the rest are irregular (3/18).

Colony cover form: Most of them are full cover (16/18), the rest are lobed (2/18)

Colony buoyancy: most of the colonies had tissue buoyancy (14/18), the rest were colonies with a slight sloped buoyancy (4/18).

Colony size: Colony diameters of isolated strains ranged from 1 to 8mm after inoculation on solid PDA medium, incubated at 30°C for 24 hours. The characteristics of color, shape and size of colonies are shown in the figure 2

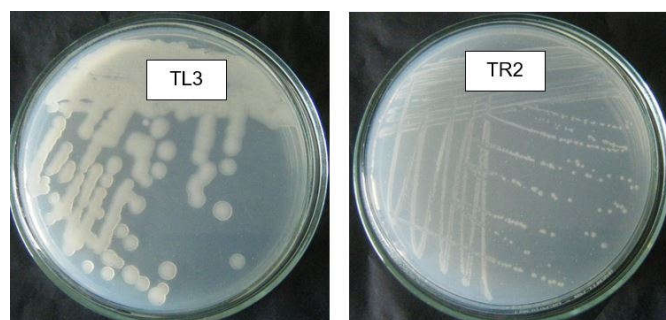


Figure 2. Bacterial colonies isolated on PDA medium

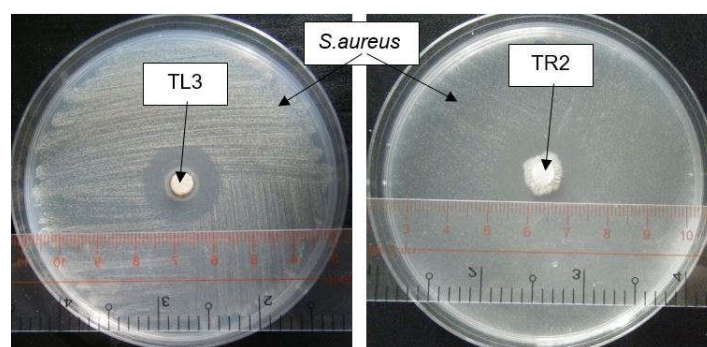


Figure 3. Diameter of the sterile ring of strains TL3 and TR2 on *Staphylococcus aureus*

*Note:

Strain TR2: colonies are opaque white, round, intact cover, tissue buoyancy, dry

Strain TL3: colonies are yellow, round, intact cover, tissue buoyancy, mucilaginous.

Antibacterial ability of isolated bacterial strains

All bacterial strains were tested for their antibacterial ability with 3 types of pathogenic bacteria: *E. coli*, *Aeromonas hydrophila* and *Staphylococcus aureus*. Resistance to *E. coli*, *A. hydrophila* and *Staphylococcus aureus* of isolated bacterial strains was demonstrated through the formation of a light ring around a filter paper impregnated

with bacterial solution on PDA medium that was spread with pathogenic bacteria. Then, it was the observation of the formation of light rings for 3 consecutive days.

Resistance to *Escherichia coli*

On the 3rd day, the size of the sterile ring of all strains decreased, but the TR2 strain increased slightly from 10 mm to 11 mm (equal to the size of the sterile ring on the first day). The strain with the best antibacterial performance was TL3 with a sterile ring diameter of 14 mm.

Resistance to *Aeromonas hydrophila* bacteria

When investigating the antibacterial ability of endophytic bacteria strains isolated with *Aeromonas hydrophila* bacteria causing disease in fish, the results showed that there were 2 bacterial strains (TR2 and TL3) that were able to create light rings around the colonies only 3 day after incubation, indicating that these 2 strains were all resistant to *Aeromonas hydrophila*.

Resistance to *Staphylococcus aureus* bacteria

On the 3rd day, the TR2 strain remained the same at 11 mm and TL3 strain is still the highest performing strain with 14 mm.

DISCUSSION

In general, when investigating the antibacterial ability of 18 bacterial strains isolated from the roots, stems and leaves of *Andrographis paniculata* Nees, 6 strains were resistant to *E. coli*, 4 were resistant to *A. hydrophila* and 3 strains are resistant to *S. aureus*. In particular, there are 2 strains (TR2 and TL3) with antibacterial ability against both pathogenic bacteria *E. coli* and *A. hydrophila*, and *S. aureus*.

Results of identification of some bacterial strains by PCR

After amplifying the DNA of the bacterial strains, these two strains were sent for sequencing and identification. Using NCBI BLAST N tool to compare homologous sequences with those on the gene bank (Table 1)

Table 1. Results of identification of promising bacterial strains isolated from *Andrographis paniculata* Nees

No.	Bacterial strain	Identification result	Similarity (%)
1	TR2	<i>Enterobacter ludwigii</i> strain CSR19B	96
2	TL3	<i>Kosakonia radicincitans</i> strain DSM 16656	95

Sequence of 16S-rRNA encoding gene of TR2 strain

DNA fragment of 1260 bp TR2 strain has a 96% homomorphous ratio with the DNA sequence of *Enterobacter ludwigii* strain CSR19B.

Comparing research results on some characteristics of *Enterobacter ludwigii* strain, it was found that *Enterobacter ludwigii* strain isolated from the root nodule of legume *Lupinus hirsutus* L. (also known as Lupines bean, widely grown in South America) has the ability to fix nitrogen, synthesize IAA in the medium with or without the addition of L-tryptophan and the ability to dissolve insoluble phosphorus, help to promote growth of shoot length and dry weight for rice plants when tested (Arab *et al.*, 2013). On the other hand, the *Enterobacter ludwigii* strain isolated from the Sago palm tree was resistant to *E.*

coli (K.L. Labrador *et al.*, 2014). *Enterobacter ludwigii* bacteria have both the ability to fix nitrogen, synthesize IAA, solubilize insoluble phosphate and resist to some pathogens. The TR2 strain endogenous in *Andrographis paniculata* Nees roots also has a homomorphism rate of 96% with the DNA sequence of *Enterobacter ludwigii*. In particular, the TR2 strain was found to be resistant to *E. coli* and *A. hydrophila*, which proves that the TR2 strain is a promising bacterial strain that can be used in cultivation, medicine and fishery can become a potential microbial antibiotic, which can replace some synthetic antibiotics.

16S-rRNA encoding gene sequence of TL3 strain

DNA fragment of 1331 bp TL3 has a 95% homomorphous ratio with the DNA sequence of *Kosakonia radicincitans* strain DSM 16656 (also known as *Enterobacter radicincitans* strain DSM 16656).

According to A. K. Brock *et al.*, (2012), *Kosakonia radicincitans* bacteria help improve the growth rate of plants and the yield of crops in agriculture, have the ability to synthesize IAA, fix nitrogen and dissolve insoluble Phosphate, and helps plants fight some other potential pathogens. The survey showed that the endogenous TL3 strain in *Andrographis paniculata* Nees also has the characteristics of nitrogen fixation, IAA synthesis, and high solubility of insoluble phosphorus, which helps plants grow and develop better. Especially, this strain is resistant to *A. hydrophila* fish disease bacteria with sterile ring reaching 14 mm on the 3rd day after inoculation.

CONCLUSIONS

Eighteen bacterial strains were isolated from the roots, stems and leaves of *Andrographis paniculata* Nees. There are six strains of bacteria that are resistant to enteric pathogens *E. coli*, four strains resistant to fish disease bacteria *A. hydrophila*, 3 strains of bacteria resistant to human pathogenic bacteria *S. aureus*. Two promising bacterial strains with good antibacterial ability, TR2 and TL3, were identified, respectively, as *Enterobacter ludwigii*, CSR19B strain and *Kosakonia radicincitans* strain DSM 16656, with homomorphous rates of 96% and 95%.

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