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Technical Note

Isolation, diversity and antimicrobial activity of endophytic bacteria from *Piper retrofractum* Vahl

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Abstract: Bacterial endophytes associated with a Thai medicinal plant, *Piper retrofractum* Vahl, were investigated. Twenty-one isolates of endophytic bacteria were obtained from three different parts (root, leaf and stem) of *P. retrofractum*. According to amplified ribosomal DNA restriction analysis patterns and 16S rRNA gene sequence, the endophytic bacteria isolated in this study belong to 11 families, 14 genera and more than 18 species, with one uncultured isolate. Several of the bacteria are in the genera *Pseudomonas* and *Bacillus*. Two isolates, S-PR6Y and S-PR1Y, show antagonistic properties against methicillin-resistant *Staphylococcus aureus* (MRSA). A partially purified antibacterial agent from *Lysobacter* sp. (isolate S-PR6Y) shows remarkable stability to heat and pH. It has the potential for use as an alternative antibacterial agent for the treatment of infection with MRSA.

Keywords: endophytic bacteria, *Lysobacter* sp., *Piper retrofractum* Vahl, antibacterial activity, methicillin-resistant *Staphylococcus aureus*

INTRODUCTION

The frequency of the emerging of drug-resistant pathogenic bacteria, especially methicillin-resistant *Staphylococcus aureus* (MRSA), continues to increase, which has become a serious problem in public health globally. Since the first appearance of MRSA in 1960 [1], it has become widespread both in hospitals and intensive care units [2]. MRSA represents now more than 60% of *S. aureus* isolates in US-hospital intensive care units [3]. Novel antibacterial agents are urgently needed to combat this problem.

Endophytic bacteria are those that reside asymptomatically in plant tissues, mainly in intercellular and vascular tissues [4]. They have been isolated from a range of plant types which are

mainly crop plants such as rice [5], potato [6], carrot [7], tomato [8] and citrus [9], and largely studied for their plant-promoting activity. There have been only few reports on the antimicrobial activity of endophytic bacteria and they showed promising activity [9-11]. Moreover, studies on bacterial endophytes of medicinal plants are scarce [9, 12]. These endophytes might have potential use as an alternative antibacterial agent against MRSA in the future.

Piper retrofractum Vahl (syn. *Piper chaba* Hunter) or Dee Plee in Thai, belonging to the *Piperaceae* family, is widely distributed in tropical and subtropical regions of the world [13]. Its different parts have been used in traditional medicine as a stimulant, carminative, tonic, antifungal (stem), antihypertensive, muscle relaxant (whole plant), and colic (root) and for post-natal women (fruit) [14]. To the best of our knowledge, there have been no reports on endophytic bacteria from *P. retrofractum* Vahl.

In the present study the diversity of bacterial endophytes associated with this plant, *P. retrofractum*, is investigated and a selected isolate with promising anti-MRSA activity is identified and examined.

MATERIALS AND METHODS

Isolation of Endophytic Bacteria

Three different parts (root, stem and leaf) of fresh and healthy *Piper retrofractum* Vahl from the herb garden, Thammasat University, were used for bacterial isolation. The samples were surface sterilised to avoid contamination, and the endophytic bacteria were isolated only from inner plant tissues according to a method previously described with some modifications [5]. Briefly, the samples were washed with running tap water for 5 min., followed by immersion in 70% ethanol for 3 min. They were then washed with 2.5% sodium hypochlorite solution for 5 min., rinsed with 70% ethanol for 30 sec., and finally washed with sterile distilled water five times. To confirm that the disinfection process was successful, a 100-μL aliquot of the second water rinse was spread on a tryptic soy agar (TSA) + yeast extract/glucose (YEG) (0.2% glucose, 0.2% yeast extract, 1.5% bacto agar) plate and examined for bacterial growth after incubation at 28^{o}C for 72 hr. One gram of the surface-sterilised sample was homogenised in a mortar containing 5 mL of normal saline and subsequently incubated at 28^{o}C for 1 hr, followed by centrifugation at 2,500 rpm for 5 min. Then 100 μL of the supernatant were spread on the TSA + YEG medium and incubated at 28^{o}C for 3 days.

Screening of Isolated Bacteria for Antibacterial Activity

Single colonies representative of each colony type were screened for their antibacterial activity against an indicator strain MRSA, DMST 5199, by co-culture method [15]. Briefly, the plate containing a single colony was overlaid with 5 mL of soft agar (1.2% agar) inoculated with the MRSA cell suspension at a final concentration of ca. 10^5 CFU/mL. The plate was then incubated at 37^{o}C for 18 hr and the appearance of a clear zone showing the antagonistic activity was observed. MRSA was obtained from Department of Medical Sciences, Ministry of Public Health, Thailand.

Amplified Ribosomal DNA Restriction Analysis (ARDRA)

The total genomic DNA of isolated bacteria was extracted with MasterPure Gram Positive DNA Purification kit (Epicenter, USA). The partial bacterial 16S rRNA gene with a size of approximately 735 bp was amplified using a pair of primers 799f (position 781 through 799

according to *E. coli* number) and 1492r (position 1492 through 1510 according to *E. coli* number) [5]. The polymerase chain reaction (PCR) mixture (50 μL) was composed of 1x PCR Master mix (Promega, USA), 1.5 μL of each primer (10 pmol/μL), 5 μL of dNTP (2 mM), 4 μL of $MgCl_2$ (25 mM) and 3 μL of template DNA (100 ng/ μL). The PCR reaction conditions were: 94°C for 5 min., followed by 30 cycles of denaturation at 94°C for 1 min., annealing at 52°C for 45 sec., and elongation at 72°C for 1 min. before a final extension at 72°C for 8 min. PCR products were purified by E.Z.N.A. gel extraction kit (Omega Biotek, USA). ARDRA [5, 16] was used to analyse the diversity of isolated endophytic bacteria. Digestion of the PCR products was carried out at 37°C for 10 min. using one *Hae*III or *Rsa*I [5] and two restriction endonucleases (*Hha*I and *Hae*III) [16]. The restriction fragments were separated on a 2.5% agarose gel running in 1x Tris-Borate-EDTA buffer at 100 V. Representative isolates of the different ARDRA patterns were selected for full-length 16S rRNA gene sequencing (First Base Laboratories, Malaysia). The nucleotide sequence was compared with GenBank nucleotide database using Blastn search [17].

Preparation of Partially Purified Antibacterial Agent (PPA)

A 200-mL YEG broth was inoculated with 1% (10^6 CFU/mL) of an overnight culture of isolate S-PR6Y, which had been screened to have strong antibacterial activity. The culture was incubated at 25°C for 48 hr with continuous shaking at 200 rpm. Following cultivation, the supernatant of cell-free culture was obtained through centrifugation (Sorvall Biofuge, Mandel Scientific, Canada) at 6,000xg for 20 min. Ammonium sulphate (103.2 g) was added to the supernatant (200 mL) with stirring and the mixture was left overnight at 4°C, then centrifuged at 8,000xg for 40 min. The supernatant was discarded and the precipitate was dissolved in 10 mL of sterile distilled water and the solution was dialysed against 1.5 L of sterile distilled water for 24 hr. The active supernatant was subjected to sterile filtration and designated as PPA.

Determination of Antibacterial Activity and Spectrum of Inhibitory Activity

The agar-well diffusion method [18] was used to detect the antibacterial activity of PPA against MRSA and bacteriocin activity (AU/mL) was determined by serial dilution method [19]. The assay for each sample was done in triplicate. The PPA was used to assess the antibacterial activity spectrum against a total of 12 selected Gram-positive and Gram-negative test bacteria (Table 2) by using the agar-well diffusion method. Equal volume of sterile distilled water was used as control solution. The appearance of the inhibition zone was determined after 18 hr of incubation.

Enzyme Sensitivity and Stability

The PPA was treated with the following enzymes: trypsin, α-chymotrypsin and proteinase K (Sigma-Aldrich, USA) at 37°C for 1 hr with a final concentration of 1, 5 and 10 mg/mL respectively. After incubation, the reaction mixtures were boiled for 10 min. to inactivate the enzymes and the residual antibacterial activity against MRSA was measured by agar-well diffusion. The heat stability of the PPA was investigated by determining the residual antibacterial activity against MRSA after incubation at different temperatures ranging between 40-100°C for 30 and 60 min., and at 121°C for 15 min. To investigate the effect of pH, the residual antibacterial activity against MRSA was measured following pH adjustment of the PPA with 0.1 N NaOH or 0.1 N HCl and 1-hr incubation at 4°C.

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RESULTS

Isolation and Screening of Antagonistic Endophytic Bacteria

Twenty one endophytic bacteria with different colony morphology were successfully isolated from the root, stem and leaf of *Piper retrofractum* Vahl. No bacterial colony was observed on the TSA or YEG plate after the second water rinse. This indicated that the surface disinfection was successful. The highest number and diversity of endophytic bacteria were found mostly in the stem. All the endophytic bacteria were screened for antibacterial activity against MRSA by the co-culture method and two out of 21 bacterial isolates exhibited the activity. They were isolated from stem and designated as isolates S-PR6Y and S-PR1Y.

Diversity of *P. retrofractum* Endophytic Bacterial Isolates

The diversity of endophytic bacteria was assessed in samples from root, stem and leaf of *P. retrofrctum*. A collection of 21 endophytic bacterial isolates was first investigated using the ARDRA method. Although the use of one or two restriction enzymes does not fully determine the diversity, it is a preliminary screening which can give a fairly accurate estimation. The ARDRA patterns obtained with *Hae*III or *Hha*I and *Hae*III digestion have more bands than that with *Rsa*I digestion (Figures 1-3). According to ARDRA patterns, the 21 isolates can be grouped into 19 distinct representatives that were sampled for 16S rRNA gene sequence analysis. As shown in Table 1, most isolates belong to Proteobacteria (42.1%), followed by Firmicutes (26.3%), Actinobacteria (21%), Bacteroidetes (5.3%) and an uncultured bacterium (5.3%). The majority of endophytic bacteria isolated belong to the Gammaproteobacteria class (36.8%) and consist of bacteria in the families Enterobacteriaceae (10.5%), Pseudomonadaceae (10.5%), Moraxellaceae (5.3%) and Xanthomonadaceae (10.5%). The second most prevalent phylum is Firmicutes (26.3%), which is the only Bacilli class from the families Bacillaceae (21%) and Staphylococcaceae (5.3%). There is one major class among the isolates, which is identified as Actinobacteria, viz. Micrococcaceae (5.3%), Microbacteriaceae (10.5%) and Streptomycetaceae (5.3%). There is only one isolate each (5.3%) from the Flavobacteria class and Alphaprotobacteria class, which are from the families Flavobacteriaceae and Sphingomonadaceae respectively.

Determination of Inhibitory Spectrum

One gram-negative isolate S-PR6Y, with high anti-MRSA activity, was selected for further study. This strain was identified as *Lysobacter* sp. with 99% identity according to its partial 16S rRNA gene sequence. The PPA obtained from *Lysobacter* sp. isolate S-PR6Y showed antagonistic activity against gram-positive test bacteria including MRSA and vancomycin-resistant *Eneterococcus faecalis* (VRE) (Table 2). Based on the size of the inhibition halo, the most sensitive strains were *B. cereus*, *B. subtilis*, *Enterococcus* sp., *Staphylococcus aureus* and MRSA.

Enzyme Sensitivity and Stability

The PPA prepared from S-PR6Y was tested for its sensitivity to different proteolytic enzymes (trypsin, α-chymotrypsin and proteinase K). Partial inactivation was observed after treatment at a high concentration (10 mg/mL) of the proteolytic enzymes (Table 3). Temperature stability experiments revealed that the PPA was stable at high temperatures up to 121^{o}C for 15 min. (Table 3). As for pH sensitivity, the antibacterial activity of PPA was maintained at a high level within the pH range of 3.0-9.0 (Table 3).

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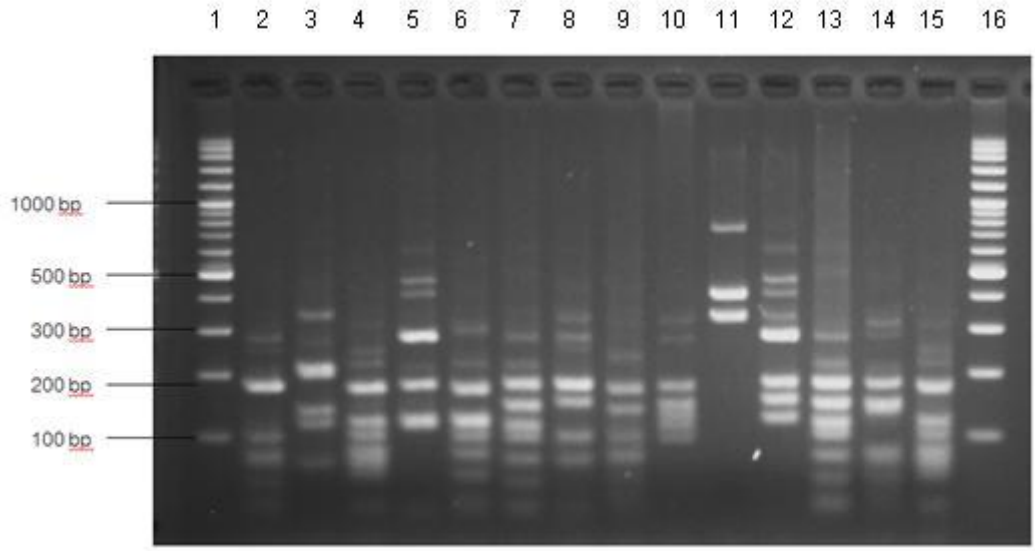

Figure 1. ARDRA patterns of 16S rRNA gene from representative isolates digested with *Hae*III and *Hha*I. Lanes 1 and 16: marker 1 kb; lanes 2-15: representatives of each group, i.e. isolates L-PR4T, L-PR3T, S-PR6Y, L-PR2T, S-PR2T, S-PR4T, S-PR4T, S-R8T, S-R10Y, S-R9T, S-R2Y, R-PR2T, S-PR1T, L-PR5T and S-PR6T respectively

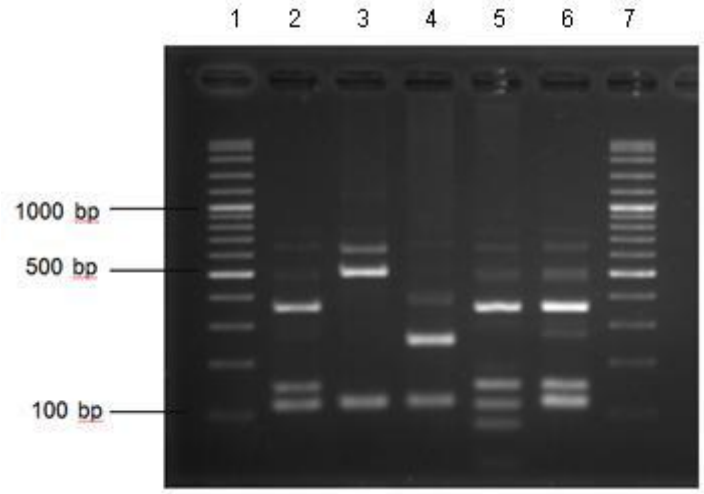

Figure 2. ARDRA patterns of 16S rRNA gene from some representative isolates digested with *Rsa*I. Lanes 1 and 7: marker 1 kb; lanes 2-6: representatives of each group, i.e. isolates L-PR4T, S-PR4T, S-PR10Y, S-PR9T and R-PR1Y respectively

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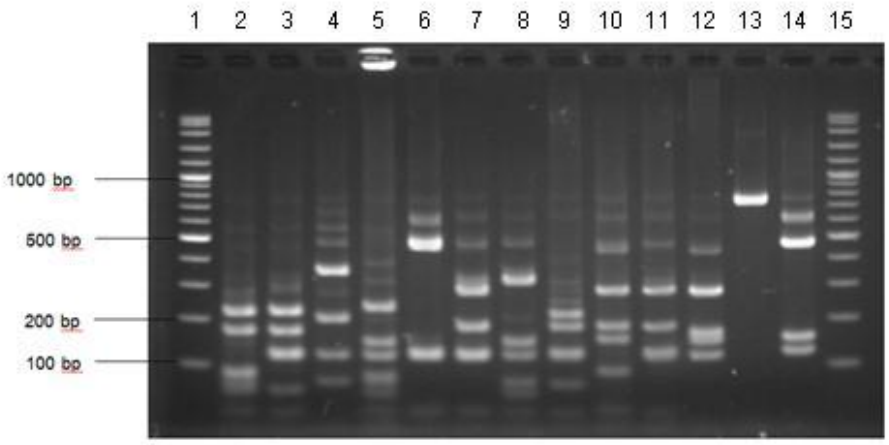

Figure 3. ARDRA patterns of 16S rRNA gene from representative isolates digested with *Hae*III. Lanes 1 and 15: marker 1 kb; lane 2-14: representative of each group, i.e. isolates S-PR6T, S-PR2T, L-PR3T, S-PR12Y, L-PR2T, L-PR4T, L-PR5T, S-PR5T, S-PR8T, S-PR10Y, S-PR9T, R-PR2Y and R-PR2T respectively

Table 1. Endophytic bacterial isolates obtained from *Piper retrofractum* Vahl

Identified taxum	Isolate	Accession no. of closest hit	Identity (%)
Actinobacteria			
Actinobacteria (class)			
Herbiconiux sp.	S-PR12Y	JQ723726.1	99%
Microbacterium sp.	L-PR2Y	KC853186.1	99%
Micrococcus lutues	L-PR5T	KC470045.1	100%
Streptomyces griseus	S-PR12T	GU569952.1	100%
Bacteroidetes			
Flavobacteria			
Chryseobacterium sp.	S-PR9T	JQ723708.1	99%
Firmicutes			
Bacilli			
Bacillus licheniformis	R-PR1T	KF148636.1	100%
Bacillus cereus	R-PR1Y	KF612021.1	100%
Bacillus flexus	R-PR2T	KC713922.1	100%
Bacillus gibsonii	L-PR2T	KC494308.1	100%
Staphylococcus hominis	R-PR2Y	KF436569.1	100%
Proteobacteria			
Alphaproteobacteria			
Sphingomonas sp.	S-PR10Y	JQ765413.1	99%
Gammaproteobacteria			
Enterobacter cloacae	S-PR5Y	KF478236.1	100%
Pantoea stewartii	S-PR4T	JN835507.1	100%
Lysobacter sp.	S-PR6Y	AB560626.1	99%
Xanthomonas sp.	S-PR8Y	KF358290.1	99%
Moraxella osloensis	L-PR4T	KC866294.1	100%
Pseudomonas fluorescens	S-PR2T	KF574011.1	100%
Pseudomonas sp.	S-PR1Y	KF358309.1	99%
Pseudomonas sp.	S-PR4Y	KC466153.1	99%
Pseudomonas sp.	S-PR2T	AB628275.1	99%
Uncultured bacterium	S-PR2Y	HM558243.1	100%

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Table 2. Inhibitory spectrum of PPA from *Lysobacter* sp. isolate S-PR6Y

Test bacteria	Source*	Growth medium	Inhibitiory activity†
Gram-positive			
Bacillus cereus	MT	TSA	++
Bacillus subtilis	ATCC6633	TSA	++
Enterococcus sp.	MT	TSAYE**	++
Vancomycin resistant *Enterococcus* (VRE)	DMST4737	TSAYE**	+
Staphylococcus aureus	MT	TSA	++
Methicillin resistant *S. aureus* (MRSA)	ATCC43300	TSA	++
Methicillin resistant *S. aureus* (MRSA)	DMST5199	TSA	++
Listeria monocytogenes	MT	TSAYE**	+
Gram-negative			
Ampicillin resistant *E.coli*	DMST19374	TSA	-
Salmonella typhimurium	MT	TSA	-
Shigella dysenteriae	MT	TSA	-
Shigella sonnei	MT	TSA	-

*ATCC = American Type Culture Collection; DMST = Department of Medical Sciences, Ministry of Public Health Thailand; MT = Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University

† (-) = no inhibition, (+) = mild inhibition (1-5 mm of inhibition zone), (++) = strong inhibition (more than 5 mm of inhibition zone)

**TSAYE = tryptic soy agar supplemented with 0.6% yeast extract

Table 3. Effects of enzymes, temperature and pH on PPA

Treatment and condition	**Residual activity (%)**
None (control)	100
Enzyme treatment	
Trypsin 1 mg/mL, 5 mg/mL	100
Trypsin 10 mg/mL	94.7
α–chymotrypsin 1 mg/mL, 5 mg/mL	100
α–chymotrypsin 10 mg/mL	90.6
Proteinase K 1 mg/mL, 5 mg/mL	100
Proteinase K 10 mg/mL	90.6
Temperature	
40-100^{o} C, 30-60 min.	100
121^{o} C, 15 min.	82
pH	
3.0 – 8.0	100
9.0	94

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DISCUSSION

Recently, endophytic bacteria have been isolated from a range of plant types including medicinal plants. They have attracted attention due to their potential use as plant growth promoters and anti-phytopathogenic agents while less research has been conducted on their therapeutic application as antimicrobial agents. In this study endophytic bacteria with antagonistic activity against MRSA have been isolated from a Thai medicinal plant, *P. retrofractum* Vahl, and some of its properties have been characterised. To our knowledge, this is the first report of the endophytic bacteria with anti-MRSA activity from *P. retrofractum*.

Twenty-one endophytic bacteria were successfully isolated from the root, stem and leaf of *P. retrofractum*. The stem gives the highest number and diversity of endophytic bacteria, which are most commonly found in roots as nodule-associated bacteria. According to ARDRA patterns and 16S rRNA gene sequence, the endophytic bacteria isolated in this study display considerable diversity. They are distributed among 11 families, 14 genera and more than 18 species with one uncultured isolate. Ten out of 14 genera, namely *Microccus*, *Microbacterium*, *Streptomyces*, *Bacillus*, *Staphylococcus*, *Sphingomonas*, *Enterobacter*, *Pantoea*, *Pseudomonas* and *Xanthomonas*, are bacteria commonly isolated from rhizospheric soil [5, 20-24]. Notably, some uncommon genera such as *Herbiconiux*, *Chryseobacterium*, *Moraxella* and *Lysobacter* are found in this study. Several endophytic bacteria isolated from *P. retrofractum* belong to the genus *Bacillus*, namely *B. licheniformis*, *B. flexus*, *B. thuringiensis* and *B. gibsonii*. *Bacillus* is found dominantly both inside and outside the rhizosphere of various plant species [11, 21, 25].

Among 21 isolates, S-PR6Y and S-PR1Y show antibacterial activity against MRSA, the gram-negative S-PR6Y possessing remarkable anti-MRSA activity. In a similar study by Ding et al [26], three novel indolosesquiterpenes isolated from *Streptomyces* sp. HKI0595, a bacterial endophyte of *Kandelia candel*, showed moderate to strong antimicrobial activities against MRSA and VRE by agar diffusion method. Munumbicins, antimicrobial compounds obtained from an endophytic bacterium, *Streptomyces* NRRL 30562 found in *Kennedia nigriscans*, also showed the anti-MRSA activity with an MIC of 2.5 μg/mL [27]. The isolate S-PR6Y was identified as a *Lysobacter* sp. with 99% identity according to its 16S rRNA gene sequence. It has been described as a ubiquitous inhabitant of soil and water and has gained interest as a rich source of extracellular enzymes and novel antibiotics [28, 29]. To our knowledge, this is the first report on the isolation of endophytic *Lysobacter* sp.

The PPA obtained from isolate S-PR6Y shows antagonistic activity against gram-positive test bacteria including MRSA and VRE. It is sensitive to all proteases at a high concentration, suggesting the proteinaceous nature or the presence of a peptide moiety in this antimicrobial substance. A high concentration of proteases used to inactivate the antimicrobial activity indicates that the antimicrobial agent from S-PR6Y might contain unusual amino acids. For example, cyclic antimicrobial peptides produced by *Bacillus* sp. containing unusual amino acids have been shown to be more resistant to proteases [30].

The antimicrobial agents from S-PR6Y are heat stable and active over a wide range of pH (3-9). The heat- and pH-stable properties have also been observed in other antibacterial peptides isolated from bacteria. Paracin 1.7 from *Lactobacillus paracasei* HD1-7 [31] and Bac-GM100 from *Brevibacillus brevis* GM100 [32] are extremely heat-stable and retain more than 80% of their original activity after 20 min. at 121°C and are active within a pH range of 3-10. The heat-stable property of low-molecular-weight-membrane active peptides is a characteristic of class II

bacteriocins, the ribosomally synthesised antimicrobial peptides produced by bacteria [33]. The stability over a wide range of pH and after heat exposure indicates that they can preserve their action at extreme conditions, a property which might have a potential application in agro-industries.

CONCLUSIONS

Twenty-one isolates of endophytic bacteria have been isolated from three different parts (root, leaf and stem) of a Thai medicinal plant, *Piper retrofractum* Vahl. They belong to 11 families, 14 genera and more than 18 species, with one uncultured isolate. *Lysobacter* sp. S-PR6Y from the stem produces an antibacterial fraction that is heat and pH stable. It shows promising activity against drug-resistant pathogenic bacteria, notably MRSA and VRE, which might be exploited as an alternative antibacterial agent for controlling MRSA in the future.

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REFERENCES

1. M. P. Jevons, A. W. Coe and M. T. Parker, "Methicillin resistance in staphylococci", *Lancet*, **1963**, *1*, 904-907.
2. D. J. Diekema, B. J. BootsMiller, T. E. Vaughn, R. F. Woolson, J. W. Yankey, E. J. Ernst, S. D. Flach, M. M. Ward, C. L. Franciscus, M. A. Pfaller and B. N. Doebbeling, "Antimicrobial resistance trends and outbreak frequency in United States hospitals", *Clin. Infect. Dis*., **2004**, *38*, 78-85.
3. H. W. Boucher and G. R. Corey, "Epidemiology of methicillin-resistant *Staphylococcus aureus*", *Clin. Infect. Dis*., **2008**, *46*, 344-349.
4. J. Hallmann, A. Quadt-Hallmann, R. Rodriguez-Kabana and J. W. Kloepper, "Interactions between *Meloidogyne incognita* and endophytic bacteria in cotton and cucumber", *Soil Biol. Biochem.*, **1998**, *30*, 925-937.
5. L. Sun, F. Qiu, X. Zhang, X. Dai, X. Dong and W. Song, "Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis", *Microb. Ecol.*, **2008**, *55*, 415-424.
6. B. B. Pageni, N. Z. Lupwayi, F. J. Larney, L. M. Kawchuk and Y. Gan, "Populations, diversity and identities of bacterial endophytes in potato (*Solanum tuberosum* L.) cropping systems", *Can. J. Plant Sci.*, **2013**, *93*, 1125-1142.
7. M. A. Surette, A. V. Sturz, R. R. Lada and J. Nowak, "Bacterial endophytes in processing carrots (*Daucus carota* L. var. *sativus*): Their localization, population density, biodiversity and their effects on plant growth", *Plant Soil*, **2003**, *253*, 381-390.
8. R. Upreti and P. Thomas, "Root-associated bacterial endophytes from *Ralstonia solanacearum* resistant and susceptible tomato cultivars and their pathogen antagonistic effects", *Front. Microbiol.*, **2015**, *6*, doi: 103389/fmicb.2015.00255.
9. S. Suhandono, M. K. Kusumawardhani and P. Aditiawati, "Isolation and molecular identification of endophytic bacteria from rambutan fruits (*Nephelium lappaceum* L.) cultivar Binjai", *HAYATI. J. Biosci.*, **2016**, *23*, 39-44.

10. S. Roy and D. Banerjee, "Isolation of antimicrobial compound by endophytic bacteria from *Vinca rosea*", *Int. J. Curr. Res.*, **2010**, *5*, 47-51.
11. C. Vagvolgyi, E. Sajben-Nagy, B. Boka, M. Voros, A. Berki, A. Palagyi, J. Krisch, B. Skrbic, N. Durisic-Mladenovic and L. Manczinger, "Isolation and characterization of antagonistic *Bacillus* strains capable to degrade ethylenethiourea", *Curr. Microbiol.*, **2013**, *66*, 243-250.
12. N. M. Zin, N. I. M. Sarmin, N. Ghadin, D. F. Basri, N. M. Sidik, W. M. Hess and G. A. Strobel, "Bioactive endophytic streptomycetes from the Malay Peninsula", *FEM. Microbiol. Lett*., **2007**, *274*, 83-88.
13. H. S. Lee, "Pesticidal constituents derived from Piperaceae fruits", *Agric. Chem. Biotechnol*., **2005**, *48*, 65-74.
14. S. Vinay, K. Renuka, V. Palak, C. R. Harisha and P. K. Prajapati, "Pharmacognostical and Phytochemical study of *Piper longum* L. and *Piper retrofractum* Vahl", *J. Pharm. Sci. Innov*., **2012**, *1*, 62-66.
15. J. S. Lee, M. J. Chung and J. G. Seo, "In vitro evaluation of antimicrobial activity of lactic acid bacteria against *Clostridium difficile*", *Toxicol. Res*., **2013**, *29*, 99-106.
16. M. K. Chelius and E. W. Triplett, "The diversity of Archaea and bacteria in association with the roots of *Zea mays* L.", *Microb. Ecol*., **2001**, *41*, 252-263.
17. NCBI, "Basic Local Alignment Search Tool (BLAST)", https://blast.ncbi.nlm.nih.gov/Blast.cgi (Accessed: August 2016).
18. J. R. Tagg and A. R. Mac-Given, "Assay system for bacteriocins", *Appl. Microbiol*., **1971**, *21*, 943.
19. S. P. Banerjee, K. C. Dora and S. Chowdhury, "Detection, partial purification and characterization of bacteriocin produced by *Lactobacillus brevis* FPTLB3 isolated from freshwater fish", *J. Food. Sci. Technol.*, **2013**, *50*, 17-25.
20. L. E. O. Costa, M. V. de Queiroz, A. C. Borges, C. A. de Moraes and E. F. Araújo, "Isolation and characterization of endophytic bacteria isolated from the leaves of the common bean (*Phaseolus vulgaris*)", *Braz. J. Microbiol*., **2012**, *43*, 1562-1575.
21. K. Wang, P. S. Yan, Q. L. Ding, Q. X. Wu, Z. B. Wang and J. Peng, "Diversity of culturable root-associated/endophytic bacteria and their chitinolytic and aflatoxin inhibition activity of peanut plant in China", *World J. Microbiol. Biotechnol.*, **2013**, *29*, 1-10.
22. S. Saidi, S. Chebil, M. Gtari and R. Mhamdi, "Characterization of root-nodule bacteria isolated from *Vicia faba* and selection of plant growth promoting isolates", *World J. Microbiol. Biotechnol.*, **2013**, *29*, 1099-1106.
23. R. Barzanti, F. Ozino, M. Bazzicalupo, R. Gabbrielli, F. Galardi, C. Gonnelli and A. Mengoni, "Isolation and characterization of endophytic bacteria from the nickel hyperaccumulator plant *Alyssum bertolonii*", *Microb. Ecol.*, **2007**, *53*, 306-316.
24. J. Han, D. Xia, L. Li, L. Sun, K. Yang and L. Zhang, "Diversity of culturable bacteria isolated from root domains of Moso Bamboo (*Phyllostachys edulis*)", *Microb. Ecol.*, **2009**, *58*, 363-373.
25. R. L. Melnick, C. Suárez, B. A. Bailey and P. A. Backman, "Isolation of endophytic endospore-forming bacteria from *Theobroma cacao* as potential biological control agents of cacao diseases", *Biol. Control*, **2011**, *57*, 236-245.
26. L. Ding, A. Maier, H. H. Fiebig, W. H. Lin and C. Hertweck, "A family of multi-cyclic indolosesquiterpenes from a bacterial endophyte", *Org. Biomol. Chem*., **2011**, *9*, 4029-4031.
27. A. Christina, V. Christapher and S. J. Bhore, "Endophytic bacteria as a source of novel

antibiotics: An overview", *Pharmacog. Rev.*, **2013**, *7*, 11-16.
28. A. Gökçen, A. Vilcinskas and J. Wiesner, "Biofilm-degrading enzymes from *Lysobacter gummosus*", *Virulence*, **2014**, *5*, 378-387.
29. H. Hamamoto, M. Urai, K. Ishii, J. Yasukawa, A. Paudel, M. Murai, T. Kaji, T. Kuranaga, K. Hamase, T. Katsu, J. Su, T. Adachi, R. Uchida, H. Tomoda, M. Yamada, M. Souma, H. Kurihara, M. Inoue and K. Sekimizu, "Lysocin E is a new antibiotic that targets menaquinone in the bacterial membrane", *Nat. Chem. Biol.*, **2015**, *11*, 127-133.
30. H. von Dohren, "Peptides", *Biotechnol.*, **1995**, *28*, 129-171.
31. J. Ge, Y. Sun, X. Xin, Y. Wang and W. Ping, "Purification and partial characterization of a novel bacteriocin synthesized by *Lactobacillus paracasei* HD1-7 isolated from Chinese sauerkraut juice", *Sci. Rep.*, **2016**, *6*, doi: 10.1038/srep19366.
32. M. Ghadbane, D. Harzallah, A. I. Laribi, B. Jaouadi and H. Belhadj, "Purification and biochemical characterization of a highly thermostable bacteriocin isolated from *Brevibacillus brevis* strain GM100", *Biosci. Biotechnol. Biochem.*, **2013**, *77*, 151-160.
33. B. Joseph, B. Dhas, V. Hena and J. Raj, "Bacteriocin from *Bacillus subtilis* as a novel drug against diabetic foot ulcer bacterial pathogens", *Asian. Pac. J. Trop. Biomed.*, **2013**, *3*, 942-946.