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In vitro antibacterial activity of panduratin A and its derivatives against vancomycin-resistant *Entercoccus faecium* clinical isolates

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ABSTRACT: Discovery and development of antibiotics against vancomycin-resistant *E. faecium* (VRE*fm*) is of high priority. The aim of this study was to investigate the therapeutic potential of panduratin A, a bioactive compound isolated from *Boesenbergia rotunda*, due to its antibacterial activity against Thai clinical isolates of VRE*fm*. Susceptibility testing of panduratin A using a microbroth dilution method revealed a minimum inhibitory concentration (MIC) of 2 µg/ml against all 39 VRE*fm* isolates tested. The antibacterial effect of panduratin A was bacteriostatic in most isolates. The time-kill data revealed that panduratin A at 1 × MIC inhibited bacterial growth for at least 24 h. The inhibitory effect was more pronounced compared with 1 × MIC linezolid, a standard antibiotic for VRE*fm* treatment. All VRE*fm* isolates from this study exhibited vanA-type resistance supporting a crucial role of this type of vancomycin resistance in Thailand. Additionally, the antibacterial activity of the modified chemical structure of panduratin A reveals that a modification of the hydroxyl group(s) of panduratin A structure by either mono- or bis-alkylation abolished the antibacterial activity against VRE*fm*. The present study reveals a potential use for panduratin A in the treatment of, at the least, vanA positive VRE*fm* infection.

KEYWORDS: multidrug-resistant bacteria, flavonoids, vancomycin, linezolid, enterococci

INTRODUCTION

Infections caused by certain multidrug-resistant bacteria have become a global public health threat [1]. One of these bacteria is in the genus, *Enterococcus*, which are Gram-positive, round-shaped bacteria that can be found in the digestive tract of almost all land animals [2]. The most common species that cause human infections such as urinary tract infections, bacteremia, intra-abdominal infections, and endocarditis are *Enterococcus faecium* and *Enterococcus faecalis* [3]. Enterococcal infections can be difficult to treat because of the propensity of the bacteria to develop resistance to many antibacterial agents [4]. Originally, the standard treatment for these infections commonly used a combination of penicillin and aminoglycosides [5]. However, resistance to aminoglycosides quickly developed [5]. Subsequently, the glycopeptide, vancomycin, which was effective against aminoglycoside-resistant enterococci, became a standard treatment [6]. The target of vancomycin is the D-alanyl-D-alanine (D-Ala-D-Ala) terminus of the growing lipid-PP-disaccharide-pentapeptide that is involved in cell wall synthesis [7]. The binding of vancomycin to its target results in inhibition of transpeptidases, thus preventing the elongation and cross-linking of the peptidoglycan matrix [8]. The overuse of vancomycin has led to the emergence of vancomycin-resistant enterococci (VRE) since 1980s. The resistance occurred due to the modification of its target, the D-Ala-D-Ala terminus of peptidoglycan [2]. Currently, VRE infections are categorized as a serious threat by the United States Centers for Disease Control [9]. VRE was reported to be the cause of approximately 5–50% of healthcare-associated infections in the United States and European Union/European Economic Area countries [10]. The rising incidence of VRE infections is a challenge in healthcare settings, thus, infection control measures have been recommended by the Healthcare Infection Control Practices Advisory Committee (HICPAC) [11].

Panduratin A, a chalcone compound, is extracted from the rhizomes of *Boesenbergia rotunda*. Many studies have shown that the compound has several pharmacological activities such as anti-proliferative and anti-inflammatory properties [12–14], a nephroprotective effect against drug-induced kidney injury [15] and inhibition of renal cyst enlargement in polycystic kidney disease [16]. In addition, antibacterial activity of panduratin A has been demonstrated against *Prevotella intermedia*, *Prevotella loescheii*, *Pro-*

phyromonas gingivalis, *Propionibacterium acnes*, *Streptococcus mutans*, and clinal strains of *Staphylococcus* including methicillin-resistant *Staphylococcus aureus* (MRSA) [17, 18]. Previous studies also showed that panduratin A has *in vitro* antibacterial activity against vancomycin intermediate/resistant *E. faecalis* and *E. faecium* [18]. Although antibiotics such as linezolid, tigecycline, and daptomycin can be used for the treatment of vancomycin resistant enterococcal infections, the overall prevalence of multidrug-resistant enterococci has been reported [19]. It is of interest whether panduratin A has an antibacterial activity against *E. faecium* isolates that are highly resistant to vancomycin. Although the antibacterial activity of panduratin A against VRE isolated from patients in Korea in year 2010 was reported [18], it is uncertain whether the resistance characteristics of VRE isolates may have changed from the past and/or differences in genetic properties between the Korean and Thai isolates. It is imperative to test the antibacterial activity of panduratin A in VRE isolated from Thai patients. Due to the urgent need to develop antibiotics against vancomycin-resistant *E. faecium* (VRE*fm*) [17], the present study determined the antibacterial activity of panduratin A and its derivatives against VRE*fm* isolated from patients admitted to a Thai tertiary hospital. In addition, the contribution of the vanA- and vanB-type resistance to the antibacterial activity of panduratin A was investigated.

MATERIALS AND METHODS

Microorganisms

Forty-four enterococcal clinical isolates obtained from the Department of Clinical Pathology, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand, including: 39 VRE*fm*, 4 vancomycin-susceptible *E. faecium*, and 1 vancomycin-susceptible *E. faecalis* were used in the study. *E. faecalis* ATCC 29212 was used as a reference control strain. These isolates were kept in 1 ml skim milk in 10% glycerol at −20 °C until use. The research protocol received approval from The Institutional Board of the Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand (COE: 024/2023 X). The research follows the international guidelines for human research protection as stated in the Declaration of Helsinki, The Belmont Report, CIOMS Guideline, International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

Chemicals

Vancomycin and linezolid were purchased from Sigma-Aldrich (USA). Difco™ Mueller Hinton Broth (MHB) and Brain Heart Infusion broth (BHI) were obtained from Thermo Fisher Scientific (USA). Panduratin A was isolated from the extract of *Boesenbergia rotunda*. Panduratin A with the purity of 98% (HPLC) was used in this study. All other chemicals were analytical grade and purchased from commercial sources.

Antibacterial susceptibility test

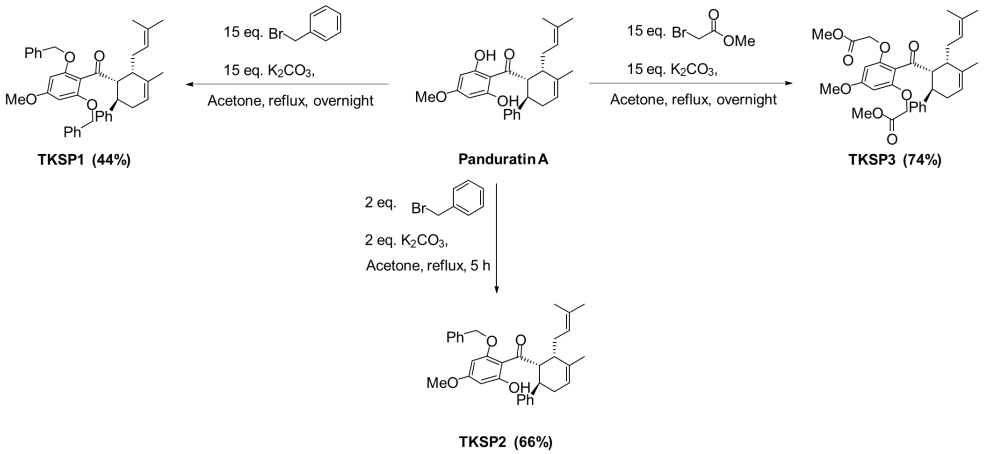
Broth microdilution assays were performed to determine the MIC of vancomycin, linezolid, and panduratin A against enterococci according to a protocol described by The Clinical and Laboratory Standards Institute (CLSI), 2022 [20, 21]. To prepare the bacterial inoculum, isolated bacterial colonies obtained after overnight incubation at 37 °C were resuspended in 3 ml MHB. The bacterial suspension was adjusted to achieve OD_{600} at approximately 0.08–0.1. A 1:100 dilution of the adjusted bacterial suspension was added into the wells containing various concentrations of antibacterial agents/compounds. The final concentrations of vancomycin and panduratin A ranged from 0.5–128 and 0.5–16 µg/ml, respectively. The microtiter plates were incubated at 37 °C for 24 h. The MIC was determined based on the lowest concentration of antibacterial agents/compounds which showed no visible growth of the bacteria. To determine the minimum bactericidal concentration (MBC), 1 µl from each well with no visible growth was streaked on blood agar plates. The plates were incubated at 37 °C for overnight. The MBC was determined as the lowest concentration of antibacterial agents/compounds with no colony growth.

Time-kill assay

A colony isolated from an overnight culture of VRE*fm* isolate no. G315/4 was resuspended in 5 ml BHI broth and incubated at 37 °C overnight. The overnight culture (250 µl) was inoculated into 250 ml of fresh BHI broth and incubated at 37 °C for 5–6 h until an OD_{600} of approximately 0.12–0.13 was achieved. The bacterial cells were treated with either 1 × MIC of linezolid, 1 × MIC of panduratin A, or 2 × MIC of panduratin A. Samples from each flask were collected to perform colony counting every 15 min for the 1st h, at 2 h, at 2-h intervals between the 2nd h and the 8th h, at 4-h intervals between the 8th h and the 16th h, and at the 24 h.

DNA extraction and polymerase chain reaction (PCR) amplification

The DNA extraction was performed as described in Depardieu et al [22]. The cell pellet was obtained from 3 ml of the overnight culture by centrifugation at 15 000×*g* for 5 min, resuspended in 250 µl solution containing 50 mM Tris-hydrochloride pH 8, 10 mM EDTA, and 7% sucrose with lysozyme, and incubated at 37 °C for 20 min. After the incubation, the suspension was lysed with 1.25% sodium dodecyl sulfate (SDS) for 10 min on ice. RNase (20 µg/ml, USB, USA) was then added and incubated at 37 °C for another 30 min. Phenol-chloroform extraction was performed



Scheme 1: Synthesis of TKSP1, TKSP2, and TKSP3.

3 times, followed by 2 chloroform extractions. The DNA pellet was obtained by ethanol precipitation and washed with 70% ethanol. The air-dried DNA pellet was resuspended in 10 mM Tris (pH 8). PCR amplification of the *E. faecalis* and *E. faecium ddl* genes encoding D alanine:D-alanine ligase was performed to verify the species of enterococci [23]. For *E. faecium*, PCR amplification of the *vanA* and *vanB* genes was performed to identify the vancomycin-resistance gene types [22]. The amplification reactions contained 1x PCR buffer, 1.5 mM $MgCl_2$, 40 pmol of forward and reverse primers (Table S1), 50 µM dNTPs, 2U of *Taq* DNA polymerase (Invitrogen, USA), and 200 ng of enterococci genomic DNA as a template. Amplification was performed as follows: 3 min at 94 °C and 30 cycles of amplification consisting of 30 s at 94 °C, 30 s at 54 °C, and 1 min at 72 °C, with a final extension for 7 min at 72 °C.

Isolation of panduratin A and semi-synthesis of its derivatives

Panduratin A was isolated from dried rhizomes of *Boesenbergia rotunda* as previously described [24]. Panduratin A with a purity of 98% as determined by HPLC was used in this study. Three derivatives (TKSP1, TKSP2, and TKSP3) were synthesized as summarized in Scheme 1.

Data analysis

Data was expressed as mean ± standard deviation (SD). Statistical analyses were performed using one-way ANOVA followed by the Tukey test for multi-group comparisons. Statistically significant differences were considered at less than 0.05 ($^*p < 0.05$).

RESULTS

Verification of *Enterococci*

The species of *Enterococcus* was verified by PCR amplification using primers specific to the *ddl* genes of *E. faecium* and *E. faecalis*. The expected sizes of the *ddl* amplified products were 1,091-bps for *E. faecium* and 495-bps for *E. faecalis* [22]. Genomic DNA of vancomycin susceptible *E. faecalis* isolates, ATCC 29212 and S155, was used as a control in the amplification experiments. The results using the *E. faecium ddl* gene specific primers confirmed that 43 isolates are *E. faecium*, whereas no amplified products were obtained when genomic DNA of *E. faecalis* was used as a template (Fig. 1, lanes 39 and 42). On the other hand, an approximately 500-bps amplified product was observed in PCR reactions using the genomic DNA of *E. faecalis* and *E. faecalis ddl* gene specific primers (data not shown). In addition, the vancomycin susceptibility of the isolates was tested. The vancomycin MICs for 39 isolates of *E. faecium* were all higher than 128 µg/ml, whereas 4 isolates were confirmed as vancomycin-susceptible *E. faecium* (Table 1).

Antibacterial activity of panduratin A against vancomycin-resistant *E. faecium*

To evaluate the antimicrobial activity of our panduratin A against the clinical isolates of *E. faecium* obtained from the hospital, the MICs for panduratin A were determined using microbroth dilution assays. The concentrations of panduratin A used in this study ranged from 0.5–16 µg/ml. The results showed that of 44 isolates tested, i.e. 39 isolates of VRE*fm*, 4 isolates of vancomycin susceptible *E. faecium* (Table 2), and 1 isolate of vancomycin susceptible *E. faecalis* S155, (data not shown), all exhibited a MIC of 2 µg/ml. These results suggested that panduratin A has antibacterial activity against VRE, especially *E. faecium*. The MBCs for panduratin A against the isolates ranged from 4 µg/ml (6 isolates) to >16 µg/ml (33 isolates). These results indicate that panduratin A exhibits both bacteriostatic and bactericidal activities against the VRE*fm* isolates.

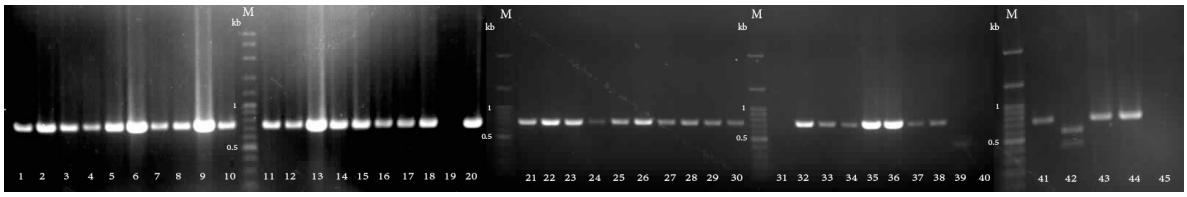


Fig. 1 Amplified products of genomic DNA of *E. faecium* isolates. Agarose gel electrophoresis (1.5%) of *E. faecium ddl* amplified products. Lanes 1–38, 40–41, and 43–45 contain amplified products using genomic DNA templates of *E. faecium* isolates G1821, G1836, G1092, G1277, G1312, G1349, G1616, G1453, G1093, G601, G833, G354, G382, G369, G598, G346, G338, G381, G1659, G1635, G1260, G1136, G1112, G1242, G1981, G1498, G1288, G1359, G949, G1692, G1366, G1771, G41, G315/4, G545, G351/6, G248/6, G350/6, G1111, G551/5, S47/5, 193/3, and G291, respectively. Lanes 39 and 42 contain no amplified products, using genomic DNA templates from *E. faecalis* isolates ATCC 29212 and S155, respectively. M indicates a 100-bp marker (Biotechrabbit, Germany).

Table 1 MIC for vancomycin against each clinical isolates of *E. faecium*.

	MIC > 128 μg/ml	MIC ⩽0.5 μg/ml
E. faecium	G1821, G1836, G1092, G1277, G1312, G1349, G1616, G1453, G1093, G601, G833, G354, G382, G369, G598, G346, G338, G381, G1635, G1260, G1136, G1112, G1242, G1981, G1498, G1288, G1359, G949, G1692, G1771, G41, G315/4, G545, G351/6, G248/6, G350/6, G551/5, S47/5, G193/3	G1659, G1366, G1111, G291

The time-kill curves for panduratin A

The killing effect of panduratin A was determined and compared with that of linezolid, an antibiotic drug generally used to treat vancomycin-resistant enterococci and staphylococci [25]. Time-kill assays were performed at 1 × MIC and 2 × MIC of panduratin A (MIC=2 μg/ml) and linezolid (MIC=2 μg/ml) against VRE*fm* clinical isolate no. G315/4. The results showed that the reduction in CFU/ml of VRE*fm* isolate no. G315/4 was approximately one log unit after 12-h exposure to panduratin A. Linezolid and panduratin A only inhibited growth of the bacteria; however, panduratin A seemed to be slightly more effective than linezolid especially at 2- and 12-h after treatment (Fig. 2, pink line with #).

Identification of vancomycin-resistance gene types of the VRE*fm* clinical isolates

The vancomycin resistance of VRE is associated with resistance genes such as *vanA*, *vanB*, *vanD*, *vanE*, and *vanG* genes [22]. We tested whether our panduratin A susceptible VRE*fm* isolates contained the 2 common vancomycin resistance types, vanA and vanB. Genomic DNA from each of the *E. faecium* isolates were used as templates for PCR amplification using *vanA* and *vanB* specific primer pairs. The *vanA* amplified products were detected in all VRE*fm*, whereas the amplified products were not detected when genomic DNAs of vancomycin susceptible *E. faecium* isolates no. G1659, G1366, G1111, and G291 were used as a template (Fig. 3, lanes 19, 31, 40, and 45, respectively). The amplified products of one of the vanA-type VRE*fm*, isolate no. G315/4, was purified and sent for DNA sequencing by U2Bio, Thailand. The obtained nucleotide sequence was aligned to the *vanA* gene of plasmid pSJ245, belonging to *E. faecium* strain SJ248, using the BLAST program (National Center for Biotechnology Information, NCBI, USA). The result revealed 96% similarity with 2% gaps (Fig. 4). These results suggested that all vancomycin resistant isolates obtained from the hospital are vanA-type resistant and are susceptible to panduratin A treatment. It should be noted that no amplified products were detected using the *vanB* specific primers (data not shown).

Antimicrobial effect of derivative of panduratin A against VRE*fm*

We, next, explored the antimicrobial activity of the panduratin A derivatives, TKSP1–3, on VRE*fm*. As shown in Scheme 1, both hydroxyl groups on the phenyl ring were alkylated either by a less polar benzyl

Table 2 MIC and MBC for panduratin A against VRE*fm*.

VRE*fm* isolate tag no.	MIC/MBC (μg/ml)	Antibacterial type
G1821, G1836, G1092, G1277, G1312, G1349, G1616, G1453, G1093, G601, G833, G354, G382, G598, G346, G338, G381, G1260, G1136, G1112, G1242, G1498, G1288, G1359, G949, G1692, G1771, G41, G315/4, G545, G351/6, G350/6, G551/5	2/>16	Bacteriostatic
G369, G1635, G1981, G248/6, S47/5, G193/3	2/4	Bactericidal

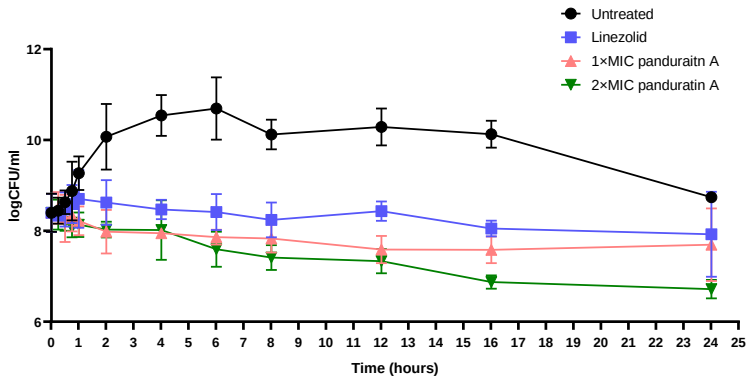


Fig. 2 Time-killing effect of panduratin A and linezolid against vancomycin-resistant *E. faecium* isolate no. G315/4. The MIC for panduatin A and linezolid was 2 µg/ml. Data are shown as the mean ± SD from three-independent experiments. $^{*}p < 0.05$ compared with treated bacteria whereas $^{\#}p < 0.05$ compared with linezolid-treated bacteria.

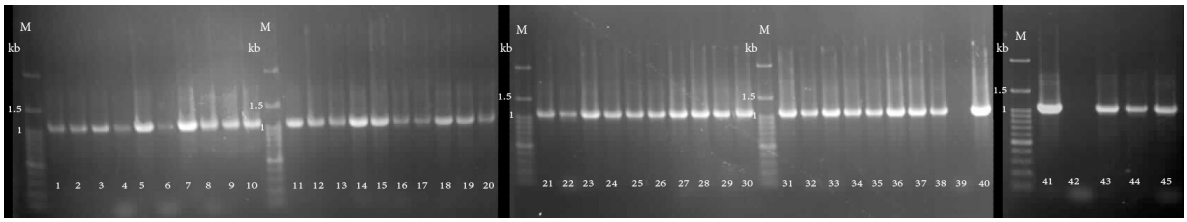


Fig. 3 Expression of *vanA* in clinical isolates of Enterococci. Agarose gel electrophoresis of *vanA* amplified products. Lanes 1–18, 20–30, 32–38, 41, and 43–44 contain the amplified products using genomic DNA of *E. faecium* isolates no. G1821, G1836, G1092, G1277, G1312, G1349, G1616, G1453, G1093, G601, G833, G354, G382, G369, G598, G346, G338, G381, G1635, G1260, G1136, G1112, G1242, G1981, G1498, G1288, G1359, G949, G1692, G1771, G41, G315/4, G545, G351/6, G248/6, G350/6, G551/5, S47/5, and 193/3, respectively, as a template. No amplified products are detected when genomic DNA of vancomycin susceptible *E. faecium* isolate no. G1659, G1366, G1111, and G291 were used as a template (lanes 19, 31, 40, and 45, respectively). Lanes 39 and 42 contain no amplified products of approximately 700 bp when genomic DNA from *E. faecalis* ATCC 29212 and vancomycin susceptible *E. faecalis* isolate no. S155 were used as a template. M indicates a 100-bp marker.

bromide (TKSP1) or a more polar methyl bromoacetate (TKSP3) group. Moreover, a mono-benzylated analogue, TKSP2, was prepared in order to affirm the significance of the hydroxyl group on the aromatic ring. As shown in Table 3, the antimicrobial activity of all alkylated derivatives vanished. These results support the notion that the hydroxyl group on the phenyl ring is required for the antimicrobial activity of panduratin A against VRE*fm*.

DISCUSSION

Many studies have proposed the use of panduratin A as an antibacterial therapy [12]. Although the antibacterial activity of panduratin A against VRE has been reported [18], the study was conducted more than ten years ago. The resistance characteristics of VRE isolates could be changed. Since WHO has announced that the development of new antibiotics for the treatment of VRE*fm* infection is a high priority [26], the present study reveals the therapeutic potential of panduratin A for the treatment of patients with VRE*fm* infections.

This study demonstrates the susceptibility of 43 Thai VRE*fm* isolates to panduratin A with MICs of 2 µg/ml irrespective of their susceptibility to vancomycin. These MIC values were somewhat compa-

Table 3 MIC and MBC for panduratin A and its derivatives against each clinical isolates of *E. faecium*.

VRE*fm* isolate tag no.	MIC/MBC (µg/ml)		
	TKSP1	TKSP2	TKSP3
G1821, G1836, G1092, G1277, G1312, G1349, G1616, G1453, G1093, G601, G833, G354, G382, G369, G598, G346, G338, G381, G1635, G1260, G1136, G1112, G1242, G1981, G1498, G1288, G1359, G949, G1692, G1771, G41, G315/4, G545, G351/6, G248/6, G350/6, G551/5, S47/5, G193/3, G1659, G1366, G1111, G291	>16/>16	>16/>16	>16/>16

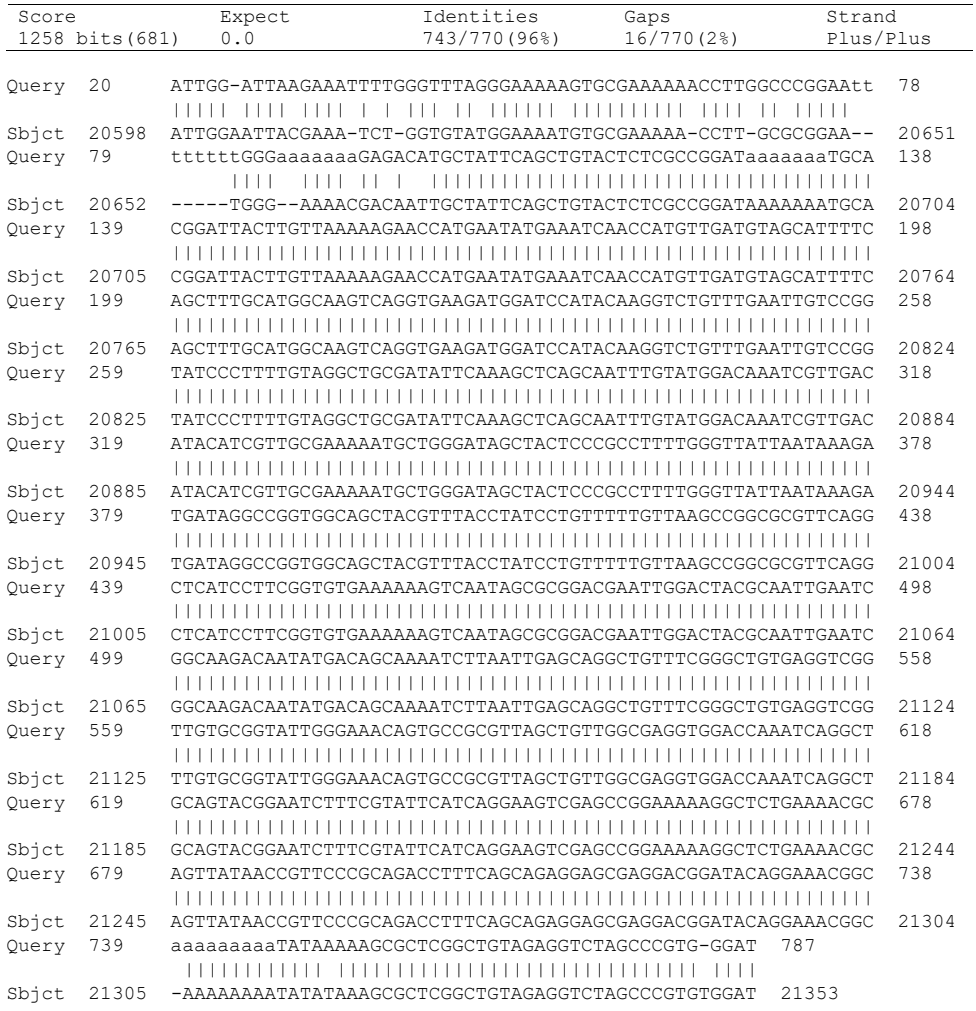


Fig. 4 The nucleotide sequence alignments of the *E. faecium* G315/4 *vanA* amplified products and the *vanA* gene of the reference plasmid, pSJ245 of *E. faecium* strain SJ248. The amplified product is used as the Query, and the plasmid is used as the Subject.

rable with the previous report [18]. The previous study showed that panduratin A had a bactericidal effect against clinical isolates of VRE*fm* with an MBC of 8 µg/ml [18]. Interestingly, our results showed that panduratin A had a bacteriostatic effect on most VRE*fm* isolates (33 out of 39) with MBCs ⩾16 µg/ml, whereas it showed only a bacteriocidal effect against the remaining 6 VRE*fm* isolates. The bacteriostatic effect of panduratin A has been supported by the time-kill data for the representative isolate, G315/4. The different results of our study compared to the previous study [18] might result from either different characteristics for the Korean versus the Thai VRE*fm* isolates, or the characteristics of VRE*fm* isolates having changed from year 2010 to 2023. These results imply that the different sources of the VRE*fm* isolates, and the passage of time might affect the panduratin A susceptibility of VRE. The bacteriostatic activity of panduratin A against VRE*fm* is similar to that of linezolid, a prototypic antibiotic drug for VRE that suppresses protein synthesis by preventing the formation of the 70S initiation complex [27]. As for the mechanism of action of panduratin A, a study in *Porphyromonas gingivalis*, a Gram-negative anaerobic bacterium, used transmission electron microscopy to investigate cell morphology after treatment with 4 µg/ml of panduratin A (1 × MIC) compared to untreated cells [28]. The results showed that cells treated with panduratin A exhibited disintegration of the cell surface and the separation of the cytoplasmic membrane from the cell wall. This led to speculation that panduratin A might cause leakage of intracellular constituents, especially the bioactive enzymes that are essential for propagation, leading to cell death. Therefore, we propose

that the mechanism of action of panduratin A against enterococci could be similar to that in *P. gingivalis*. However, we do not know why panduratin A exhibited bacteriostatic activity against most of our VRE*fm* isolates. Further studies are needed to explain the bacteriostatic effect of panduratin A observed in these isolates of VRE*fm*.

Panduratin A itself holds promise as an antibacterial against VRE*fm*. Interestingly, modification of panduratin A structure by either mono- or bis-alkylation of its hydroxyl group(s) abolished its antibacterial activity against VRE*fm*. These data indicate that the hydroxyl groups on the phenyl ring are crucial for the antibacterial effect and, therefore, any structural modification to improve the antibacterial efficacy of panduratin A should be done at other pharmacophores.

VRE types are classified according to differences in the genes encoding ligase and are denoted as: vanA, vanB, vanC, vanD, vanE, vanF, vanG, vanL, vanM, and vanN cluster types [29]. Among these resistance types, vanA and vanB are the most common cluster types among clinical isolates of VRE from humans [30]. Our data showed that panduratin A has antibacterial activity against all *vanA*-positive VRE*fm* isolates (39 clinical isolates). These results indicate that vanA-type resistance is predominant in our tested VRE*fm* isolates and panduratin A could be a useful antibacterial agent against VRE*fm* infection. Other studies from Northeastern [31] and Southern [32] parts of Thailand also found only *vanA*-positive VRE*fm* clinical isolates. It is not clear why this and the other studies could not detect the expression of *vanB* in any of the clinical isolates tested. It could be that *vanA*-positive VRE*fm* are more adapted to these hospital niches. However, there were many reports from Europe of a shift in outbreaks caused by *vanA*-positive VRE*fm* to *vanB*-positive VRE*fm* over time [33]. A switch from vanA predominance to vanB predominance has also been observed in Rajavithi hospital since 2005 [34]. So far, the shift from vanA to vanB positive isolates cannot be explained. It could be that vanB might have been transferred to a plasmid that is more efficient in terms of horizontal transfer of the vanB cluster type compared to the vanA cluster type.

In conclusion, panduratin A has antibacterial activity against VRE*fm* isolated from hospitalized Thai patients. Panduratin A could inhibit the growth of both *vanA*-positive and *vanA*-negative VRE*fm*. The hydroxyl groups are required for its antibacterial activity. Although the present study does reveal the therapeutic potential of panduratin A for the treatment of VRE*fm* infected patients, an investigation probing the efficacy of panduratin A in an *in vivo* model is required. In addition, the antibacterial activity of panduratin A against VRE*fm* containing other vancomycin resistance genes encoding ligase and vancomycin-resistant *E. faecalis* (VRE*fl*) should be investigated.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.020.

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Appendix A. Supplementary data

Table S1 DNA primers used in this study.

Primer	Sequence	Position	Size (bp)
ddl E. faecium F	GAGTAAATCACTGAACGA	1–18	1,091
ddl E. faecium R	CGCTGATGGTATCGATTCAT	1,091–1,072	
ddl E. faecalis F	CACCTGAAGAAACAGGC	206–222	475
ddl E. faecalis R	ATGGCTACTTCAATTTCACG	680–661	
vanA F	GGGAAAACGACAATTGC	176–192	732
vanA R	GTACAATGCGGCCGTTA	907–891	
vanB F	ACGGAATGGGAAGCCGA	169–185	647
vanB R	TGCACCCGATTTCGTTC	815–799	