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Detection of Carbapenemase Producers and Inhibitory Activity of *Pediococcus pentosaceus* YTPP 02 against Carbapenemase-Producing Bacteria

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Abstract

Carbapenem resistance caused by carbapenemase enzymes is of serious public health concerns globally, resulting in limited options for effective treatment. Rapid detection and exploring new agents are urgently required. This study aims to detect carbapenemase producers and to investigate the antibacterial potential of *Pediococcus pentosaceus* YTPP 02 against carbapenemase-producing *Acinetobacter baumannii* and *Klebsiella pneumoniae*. The carbapenemase producers were detected by a Nitro-beta test (NBT), a novel and rapid method, as well as by a combined disc with resazurin assay (CRA), and multiplex PCR. *P. pentosaceus* was isolated from pickled white radish on the MRS agar + 1 % $CaCO_3$. The 16s rDNA-based PCR and sequencing were used for species identification. The agar overlay assay, agar well technique, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were employed to determine antibacterial activity of *P. pentosaceus*. The NBT successfully detected carbapenemase in all 5 clinical isolates within 20 min, and they all were positive for metallo-β-lactamases in CRA techniques within 7 h. The multiplex PCR detected *VIM* in *A. baumannii* BUU 62715, co-presence of *NDM* and *OXA-48 like* genes in both *K. pneumoniae* strains and *NDM* in *A. baumannii* BUU 60056 and 30899. Both *A. baumannii* and *K. pneumoniae* isolates were resistant to carbapenems. The 16s rDNA sequencing indicated *P. pentosaceus*. This bacterium exhibited Gram-positive cocci in pairs and quadruplets. *P. pentosaceus* YTPP 02 showed antibacterial activity against *A. baumannii* BUU 62715 and *K. pneumoniae* BUU 10624, with an inhibition diameter of 19 mm. The cell-free supernatant demonstrated promising antibacterial activity against *A. baumannii* isolates, with inhibition zones ranging from 15.3 - 18.5 mm, and a MIC of 62.5 mg/mL. *P. pentosaceus* YTPP 02 showed promising antibacterial activity against carbapenemase-producing bacteria. This could be a good candidate for further study and development as a new agent for carbapenem-resistant bacteria.

Keywords: Novel detection, Nitro-beta test, Combined dice, Antibacterial activity, *Pediococcus pentosaceus*, Carbapenemase producer, *Acinetobacter baumannii*, *Klebsiella pneumoniae*

Introduction

An increase in the consumption of carbapenem antibiotics for the treatment of cephalosporins-resistant bacteria has consequently resulted in carbapenem-resistant bacteria, particularly in pathogenic gram-negative bacteria [1]. This issue is of great public health concerns worldwide, with high morbidity and mortality rates affecting both hospitalized patients and health professionals. Carbapenems are considered as the last resort antibiotics that are commonly reserved for the treatment of severe bacterial infections. Resistance to this class of antibiotics poses a difficulty in the selection of effective and administration of appropriate antibiotics, leading to higher risks for treatment failure and death [2]. Carbapenem-resistant Enterobacterales (CPE) and *Acinetobacter baumannii* are classified in the critical group of WHO Bacterial Priority Pathogens List (2024 update) of clinical importance that are required for research, development and strategies to prevent and control antimicrobial resistance (World Health Organization, 2024). The mechanisms of resistance to carbapenems are chiefly mediated by the production of carbapenemases, as well as the loss of specific porin channels, and theincreasing expression of efflux pumps [3]. Clinically important carbapenemases can be classified based on the Ambler classification system into class A, B and D [4]. Class A includes mostly *Klebsiella pneumoniae* carbapenemases (KPC) that can be inhibited by clavulanic acid (CA) and phenylboronic acid (PBA) [5]. Class B is the family of metallo- β-lactamases (MBL), including New Delhi MBL (NDM), imipenem-resistant phenotype (IMP) and Verona integrin-encoded MBLs (VIM). Due to this class of enzymes are zinc-dependent enzymes, they can be inactivated by Ethylenediaminetetraacetic acid (EDTA) [6]. Class D is a group of oxacillinase (OXA) with carbapenemase activity, including OXA-48 and OXA-48 like carbapenemases [7]. Rapid detection of carbapenem resistance in gram-negative bacteria could improve treatment efficiency and reduce treatment failure [8].

Lactic acid bacteria (LAB) have extensively been used in several daily industries and food preservatives to prevent food spoilage [9,10]. LAB are known to produce a variety of beneficial substances, including antimicrobial compounds (also known as bacteriocins) [11]. *Pediococcus pentosaceus* is classified as LAB belonging to the genus *Latilactobacillus*. *P. pentosaceus* is a gram-positive cocci, forming pairs or quadruplets, non-motile, catalase-negative and aerobic to microaerophilic bacterium [12]. This bacterium has attracted interest due to it possessing numerous biological functions including antioxidant, anti-inflammation, anti-tumor and cholesterol-lowering effects [13]. Similar to other LAB, *P. pentosaceus* can inhibit the pathogenic bacteria that colonize and cause infection in the human gut [14].

The available effective antibiotics for the treatment of carbapenem resistance are scarce, and there are no studies on the anti-bacterial potential of *P. pentosaceus* against carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumonia*. Therefore, research and development of novel rapid diagnostic techniques and antibacterial agents that are effective and safe for treating carbapenem-resistant pathogens are of research importance. The present study, therefore, develops and utilizes rapid and simple methods for detection of carbapenemase-producing bacteria, as well as investigated anti-bacterial activity of *P. pentosaceus* against carbapenem-resistant *A. baumannii* and *K. pneumonia*.

Materials and methods

Reagents and bacterial isolates

Ceftazidime (CAZ), cefotaxime (CTX), meropenem (MER), ertapenem (ERT), cloxacillin (CX), clavulanic acid (CA) and other chemical reagents were purchased from Sigma-Aldrich, Germany. Mueller-Hinton broth (MHB) and agar (MHA) and MRS medium (HiMedia, India) were prepared using distilled water. A total of 5 bacterial isolates used in this study were obtained from Chon Burin hospital, including *Acinetobacter baumannii* BUU 62715, 60056 and 30899, and *Klebsiella pneumonia* BUU 30761 and 10624. *Escherichia coli* ATCC 25922 was used as a reference strains. The organisms were transferred between institutions and handled according to standard biosafety guideline and regulations.

Detection of β-lactamase-producing bacteria by a Nitro-beta test

In this study, we developed a novel simple and rapid method called the Nitro-beta test to detect and

discriminate different types of β-lactamases, including extended-spectrum β-lactamases (ESBL), AmpC β-lactamases and carbapenemases within 20 min. The Nitro-beta test is the modified version of the Nitro-Carba test developed by Teethaisong *et al.* [15]. Briefly, 1 inoculation loop (10 µL in size) of an overnight-grown bacterial colony was dispensed in 500 µL of bacterial cell lysis buffer in a microcentrifuge tube for extraction of β-lactamases from bacterial cells. The samples were mixed vigorously for 2 min prior to adding 100 µL of extracted β-lactamases to wells containing 50 µL each of sterile water (control), 1,600 µg/mL CA, 800 µg/mL CX and 160 µg/mL ERT in a separate well. Following shaking gently and incubation for 5 min, 50 µL of nitrocefin at a concentration of 500 µg/mL was added to each well as aforementioned above. The colorimetric change from yellow to red was monitored within 20 min. The color change from yellow to red in the positive control denoted the presence of all types of β-lactamases, while no color change in the well containing CA (ESBL inhibitor) or CX (AmpC inhibitor) in comparison to control denoted positive for ESBL or AmpC, respectively. For carbapenemase positive, the color changed from yellow to red in the well containing ERT.

Detection of carbapenemase-producing bacteria by a combined disc with resazurin agar

Resazurin is a common dye to monitor the growth of viable cells, including bacteria, by changing the assay color from blue to pink (resorufin). The combined disc assay along with resazurin chromogenic agar plate was previously proven to have good diagnostic performance by showing high sensitivity and specificity in the differentiation of MBL and KPC carbapenemases produced by Enterobacteriaceae [16]. To execute this assay, briefly, 10 µg MER disc, 750 µg EDTA disc and 1 mg CA disc were prepared according to a previous study [16]. The antimicrobial disc susceptibility test was performed by spreading evenly a sterile swab soaked with 0.5 McFarland standard bacterial suspension over the surface of Mueller-Hinton agar containing resazurin. Four discs including MER, MER + EDTA, MER + CA and MER + CA + EDTA, were placed equidistantly on the agar surface. The inhibition zone diameter of each disc was measured after incubation for 7 h at 37 °C. The results were interpreted as follows; EDTA synergy (MER + EDTA − MER and MER + EDTA + CA – MER + CA, subtracted inhibition zone ≥ 5 mm) denoted a positive for MBL, while CA synergy (MER + CA – MER and MER + EDTA + CA – MER + EDTA, subtracted inhibition zone ≥ 5) denoted a positive for KPC.

Detection of carbapenemase genes by multiplex PCR

Genes encoding carbapenemases including *blaNDM*, *blaKPC*, *blaVIM*, *blaOXA-48* and *blaIMP* in the isolates of *A. baumannii* and *K. pneumoniae* were characterized by multiplex PCR technique according to previous reports [15,17,18]. The primer sets, concentration and amplicon size of each gene are depicted in **Table 1**.

To extract DNA and multiplex PCR analysis. An overnight bacterial culture was pelleted by centrifugation at 6,000 rpm, and the DNA was extracted using GF-1 Bacterial DNA Extraction Kit (Vivantis, Malaysia). The PCR reaction was carried out using GoTaq® Green Master Mix (Promega Cooperation, USA) in a total reaction of 25 µL comprising of green master mix, 100 ng DNA template, mixture of primers and nuclease-free water. The PCR thermocycle (Bio-Rad, T100 thermocycler) was set as follows; initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 1 min and final extension for 5 min. The PCR product was separated by 2 % agarose gel electrophoresis containing SYBR Safe DNA staining dye at 100 V for 45 min. The amplicon was visualized under ChemiDoc Touch image system (Bio-Rad, USA).

Table 1 Primer sequences for detection of carbapenemase family genes [17].

Target genes	Sequences (5′→3′)	Primer concentration (µM)	Product size
bla$_{\text{NDM-type}}$ (F)	CCTCAACTGGATCAAGCAGG	0.4	341
bla$_{\text{NDM-type}}$ (R)	CCTTGCTGTCCTTGATCAGG	0.4	
bla$_{\text{KPC-type}}$ (F)	CATTCAAGGGCTTTCTTGCTGC	0.4	538
bla$_{\text{KPC-type}}$ (R)	ACGACGGCATAGTCATTTGC	0.4	
bla$_{\text{VIM-type}}$ (F)	GATGGTGTTTGGTCGCATA	0.5	390
bla$_{\text{VIM-type}}$ (R)	CGAATGCGCAGCACCAG	0.5	
bla$_{\text{OXA-48-like}}$ (F)	GCTTGATCGCCCTCGATT	0.6	281
bla$_{\text{OXA-48-like}}$ (R)	GATTTGCTCCGTGGCCGAAA	0.6	
bla$_{\text{IMP-type}}$ (F)	TTGACACTCCATTTACDG	0.6	139
bla$_{\text{IMP-type}}$ (R)	GATYGAGAATTAAGCCACYCT	0.6	

Y = C or T; D = A, G or T

Isolation and characterization of *P. pentosaceus*

The pickled white radish was purchased locally from the market in Chon Buri Province, Thailand. The isolation procedures were carried out in accordance with previous studies [19,20]. The sample was mixed in 1 mL sterile normal saline, serially diluted and spread (100 µL) evenly on MRS agar supplemented with 1 % $CaCO_3$. The plates were cultured anaerobically at 37 °C for 48 h. The different bacterial colonies, such as color, morphology, margin and size, were selected for further identification by Gram staining and molecular identification by 16s rDNA-based polymerase chain reaction (PCR) using 27F and 1492R universal primers. The PCR product was purified for sequencing, and the nucleotide sequences were compared in the nucleotide database of National Center for Biotechnology Information (NCBI). The phylogenetic tree based on 16s rDNA sequence was constructed using MEGA 11 software by analyzing the neighbor-joining cladogram, CrustalW alignment and bootstrap with 1,000 replications

Screening of antibacterial activity of *P. pentosaceus* YTPP 02 by an agar overlay assay

A single colony on MRS agar was selected to grow anaerobically in MRS broth at 37 °C for 48 h. The suspension of *P. pentosaceus* was measured by spectrophotometry at an absorbance of 600 nm. The suspension was subsequently diluted to O.D. 0.1 prior to dropping 5 µL on MRS agar, then they were incubated at 37 °C for 24 h before adding 0.7 % soft-MHA containing carbapenemase-producing bacteria, with a final concentration of 0.5 standard McFarland equivalent (1.5×10^8 CFU/mL). The plate was additionally incubated for a further 24 h. The antibacterial activity of *P. pentosaceus* was determined by measuring the diameter of the inhibition zone. The experiments was performed in triplicate.

Screening of antibacterial activity of *P. pentosaceus* YTPP 02 by an agar well assay

A 48 hours-culture of *P. pentosaceus* was centrifuged at 6,000 rpm to collect the cell-free supernatant (CFS). The CFS was lyophilized to obtain a dry powder. The CSF powder was dissolved in sterile distilled water to a concentration of 500 mg/mL. To test its antibacterial activity against drug-resistant bacteria, an overnight culture of carbapenemase-producing isolates were adjusted to 0.5 standard McFarland prior to confluently inoculating with a sterile cotton swab. Agar plugs were created and removed to create wells to which was added 40 µL of CSF. The inhibition zone diameters were measured after the agar plate was incubated at 37 °C for 24 h. The experiment was carried in triplicate.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

MICs of CSF and antibiotics, including ceftazidime (CAZ), cefotaxime (CTX), ertapenem (ERT) and meropenem (MER) against carbapenemase producers was executed using a broth microdilution assay in accordance with Clinical and Laboratory Standards Institute (CLSI) for antimicrobial susceptibility testing. The lowest concentration showing no visible growth from a spectrophotometry measurement at 600 nm following incubation at 37 °C for 24 h was noted as the MIC.

MBC determination was performed by aliquoting 100 µL from the well showing no visible growth or the concentration above MIC to plate on MHA. The concentration showing no growing colony on the agar plate after incubation at 37 °C for 24 was denoted as the MBC.

Statistical analysis

The data from at least 3 independent experiments were expressed as mean ± S.D. The statistical analysis was performed by IBM SPSS Statistics 20 using the 1-way ANOVA with Tukey's HSD post hoc test. The $p < 0.05$ was considered as statistically significant difference.

Results and discussion

Detection of β -lactamase-producing bacteria by a Nitro-beta test

The Nitro-beta test (NBT) successfully detected the presence of carbapenemase in all 5 clinical isolates within 20 min, while the non- β-lactamase-producing *E. coli* ATCC 25922 showed no colorimetric change from yellow to red (**Figure 1**). This finding confirmed the absence of β-lactamase in the reference strain of *E. coli* ATCC 25922. Regarding to the Nitro-Carba test, a previous method developed by Teethaisong *et al.* [15], showed high sensitivity and specificity, particularly using ERT (100 % both) in the detection of carbapenemase-producing Enterobacteriaceae. The ERT was able to discriminate carbapenemases from other class of β-lactamases. The Nitro-beta test is the derivative of Nitro-Carba test in order to extend the exploitability to differentiate other types of β-lactamases including ESBL and AmpC by incorporating the specific inhibitors in the experiment. However, ESBL and AmpC producers are still required to validate the assay performance in detection and discrimination of distinct β-lactamases.

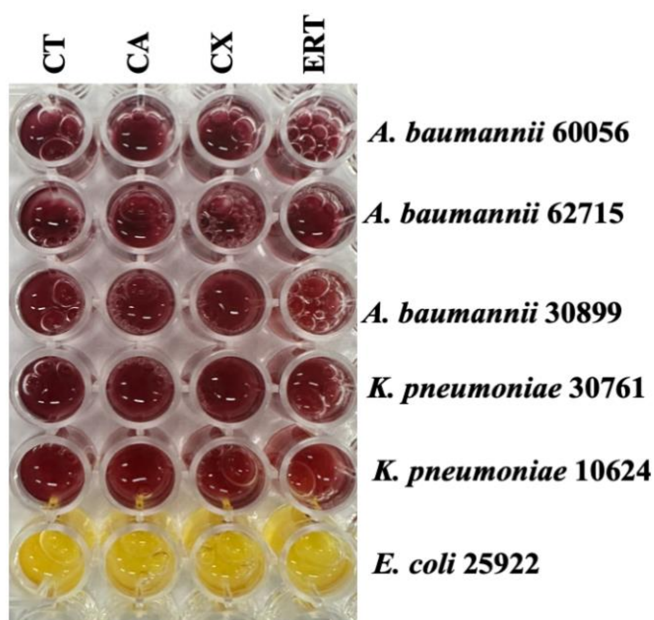

Figure 1 Detection and discrimination of β-lactamase producers using a Nitro-beta test. The results were monitored within 20 min. CT = control; CA= clavulanic acid; CX = cloxacillin; ERT = ertapenem. Yellow represents no hydrolysis of nitrocefin, and red indicates hydrolysis of nitrocefin.

Rapid or early detection of antibiotic-resistant bacteria, particularly carbapenem resistance in gram-negative bacteria plays a vital role in restricting the dissemination of recalcitrant bacteria to healthcare professionals and other patients. Further, the accurate and rapid diagnostic method could improve the rate of successful treatment and reduce treatment failure and death [21]. The conventional detection methods of carbapenem resistance are based on antimicrobial susceptivity profile either by disc diffusion or MIC. These assays usually have a turnaround time of approximately 18 - 24 h. Another drawback is that it cannot discriminate distinct resistance mechanisms [22]. The NBT assay developed in the present study is superior to those of antimicrobial susceptibility tests, especially in turnaround time and in detecting β-lactamase-mediated resistance in gram-negative bacteria. It could be a promising technique for rapid detection and discriminate distinct types of β-lactamases in clinical settings**.**

Detection of carbapenemase-producing bacteria by a combined disc with resazurin agar plate

The present study employed the phenotypic method using a combined disc with specific inhibitors, along with a resazurin chromogenic agar plate for early detection and differentiation of distinct types of carbapenemases including MBL and KPC within 7 h as illustrated in **Figure 2(A)**. Based on inhibition zone diameter and interpretation criteria, the result showed that all 5 clinical isolates were resistant to MER (inhibition zone diameter ≤ 14 mm), while the reference strain *E. coli* 25922 was susceptible to the MER disc (≥ 23 mm). The zone diameters of all 5 clinical isolates were resumed in MER supplemented with EDTA, which is the MBL inhibitor (**Figure 2(B)**). These findings indicated that all 5 clinical isolates produced MBL. A combined disc with a resazurin chromogenic agar plate showed high sensitivity and specificity in the detection of MBL and KPC [16]. By comparing to multiplex PCR used to detect the carbapenemase-encoding gene in 5 clinical isolates, the results corresponded to this experiment and all 5 clinical isolates used in this study harbored MBL-encoding gene (VIM and NDM)

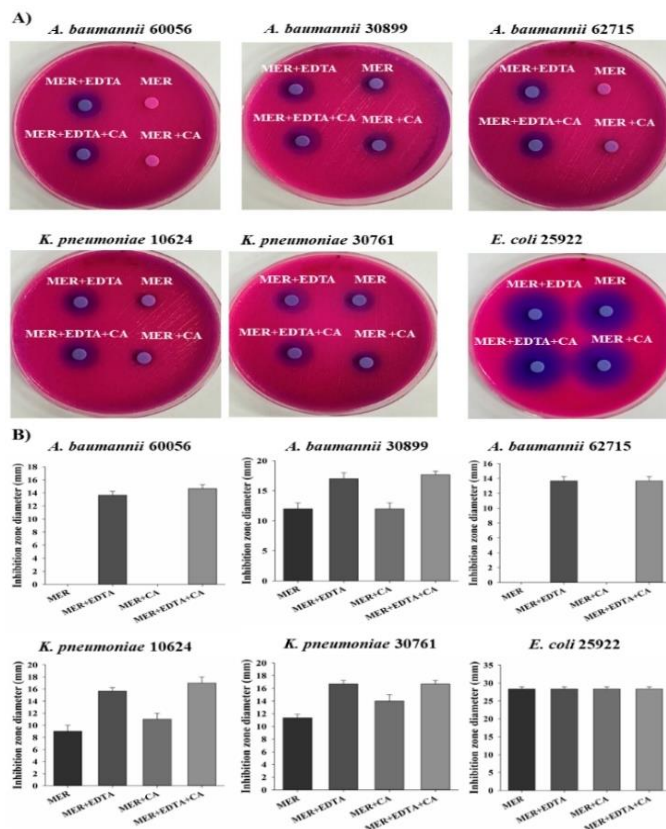

Figure 2 (A) a combined disc with resazurin chromogenic agar technique for rapid detection of carbapenemase-producing *A. baumannii* and *K. pneumoniae*. (B) the inhibition zone diameters of meropenem (MER, 10 µg/disc), meropenem (10 µg/disc) + clavulanic acid (CA, 1 mg/disc), meropenem (10 µg/disc) + EDTA (750 µg/disc) and meropenem (10 µg/disc) + EDTA (750 µg/disc) + clavulanic acid (1 mg/disc).

Multiplex PCR for detection of carbapenemase genes

The multiplex PCR specifically targeting carbepenemase-enconding genes was employed to simultaneously detect 5 distinct carbapenemase genes in drug-resistant bacteria. The results demonstrated that *A. baumannii* BUU 62715 carried VIM-type carbapenemase. Two isolates of *K. pneumoniae* exhibited co-presence of OXA-48 like and NDM carbapenemase. In *A. baumannii* BUU 30899 and 60056, NDM gene was detected (**Figure 3**).

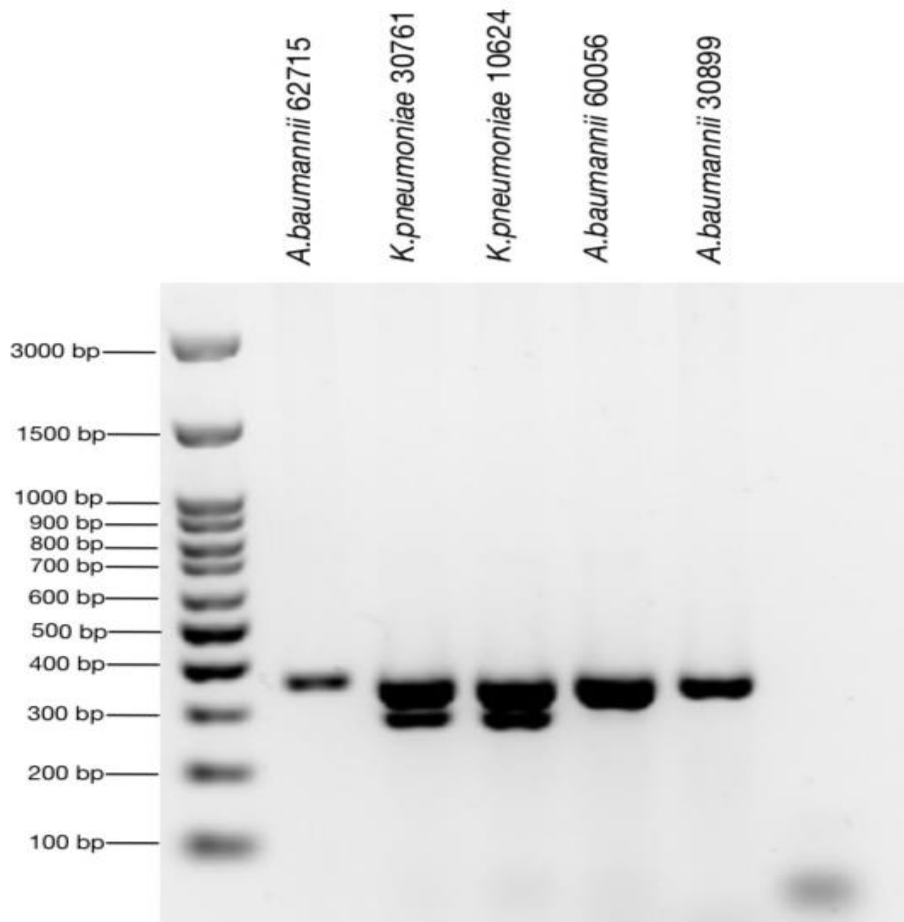

Figure 3 Agarose gel electrophoresis for analysis of multiplex PCR for detection of carbapenemase-encoding genes. The PCR amplicons were separated by 2 % agarose gel electrophoresis. BH 100 bp DNA Ladder H3 RTU (Bio-Helix, Germany) was used as a DNA marker.

Characterization and Morphology of *P. pentosaceus* YTPP 02

The result from PCR targeting 16s rDNA, sequencing and nucleotide BLAST analysis confirmed the *P. pentosaceus* (99.80 % identity to *P. pentosaceus* strain 4K116, GenBank accession no. OM942991.1). This bacterium was designated as *P. pentosaceus* YTPP 02. The result for gram staining showed gram-positive characteristics, cocci-shaped in pairs and tetrads (**Figure 4**). *P. pentosaceus* can be found in different sources, but fermented foods, such as pickled, dairy products, are the rich source of *P. pentosaceus*. *P. pentosaceus* is mostly considered and confirmed as a probiotic that provides several beneficial activities to human health. Food-derived *P. pentosaceus* is thought to be safer than other sources [23]. The Phylogenetic tree analysis indicated that *P. pentosaceus* YTPP 02 is closely related to the species of *P. pentosaceus* strain CK116, as well as strain CK116 (**Figure 5**).

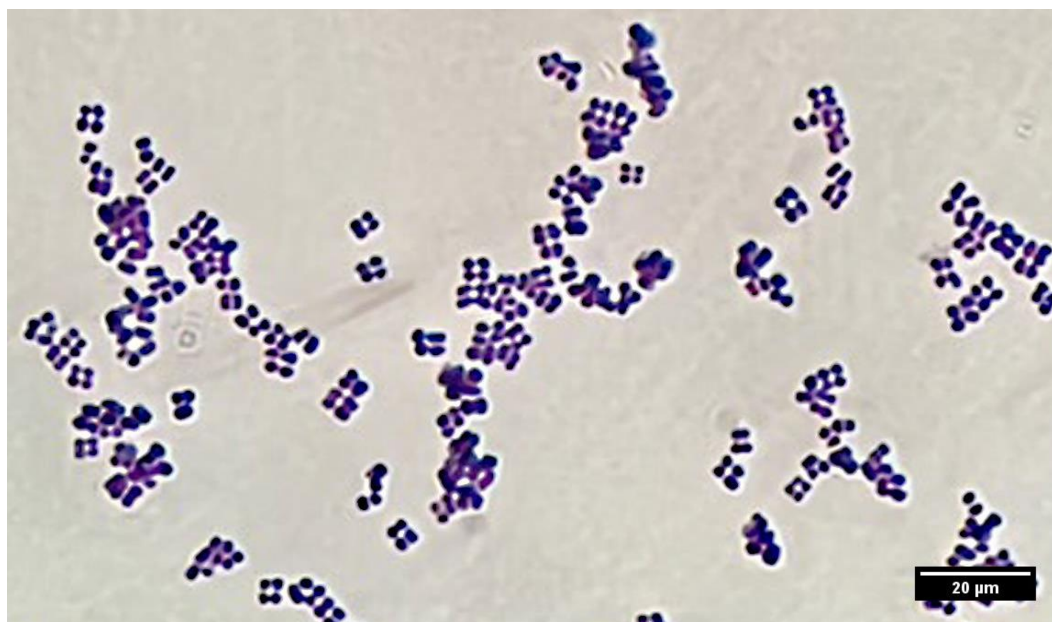

Figure 4 Gram-staining and morphology of *P. pentosaceus* YTPP 02. Scale bar = 20 and magnification = 1,000×.

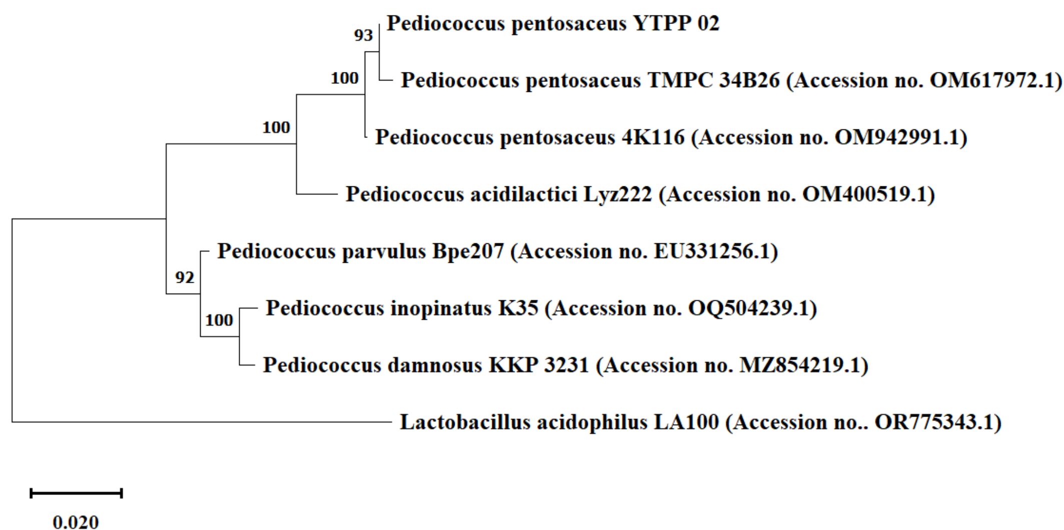

Figure 5 Phylogenetic tree of *P. pentosaceus* YTPP 02 based on 16s rDNA nucleotide sequence compared to distinct strains in the Genus *Pedicococus* and *Lactobacillus*. The scale bar denotes 0.02 % divergence.

Antibacterial activity of *P. pentosaceus* YTPP 02 by an agar overlay assay

The agar overlay assay showed that *P. pentosaceus* had high antibacterial potential in inhibiting antibiotic-resistant bacteria, including *A. baumannii* BUU 62715, 60056 and 30899, and *K. pneumoniae* BUU 30761 and 10624 (**Figure 6(A)**). The highest inhibition diameters were observed against *A. baumannii* BUU 62715 and *K. pneumoniae* BUU 10624, with inhibition zones of 19.67 ± 2.08 and 19.33 ± 2.31 mm, respectively, and they were not significantly different from the result of *A. baumannii* 60056 and *K. pneumoniae* 3076. These inhibitions were significantly higher than *A. baumannii* BUU 30899 ($p < 0.05$) (**Figure 6(B)**). *P. pentosaceus* YTPP 02 is effective against carbapenemase-producing bacteria.

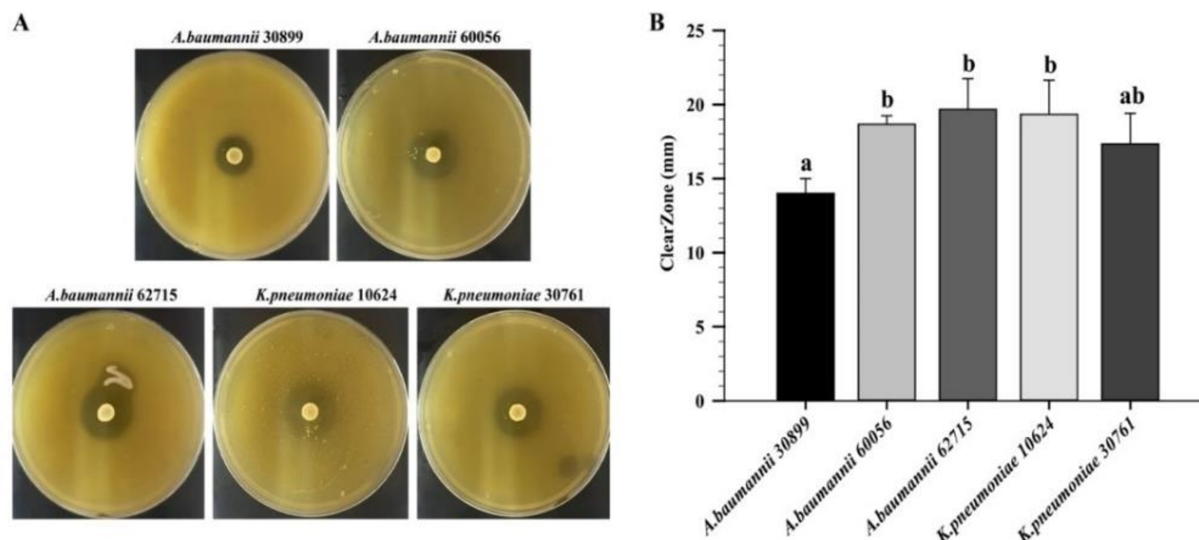

Figure 6 (A) Antibacterial activity of *P. pentosaceus* YTPP 02 by agar overlay assay against antibiotic-resistant bacteria, (B) and inhibition zone diameters against *A. baumannii* BUU 62715, 60056 and 30899, and *K. pneumoniae* BUU 30761 and 10624. The data from 3 independence experiments were expressed as mean ± S.D. and analyzed by 1-way ANOVA with Tukey's HSD. Different superscript letter represents statistical significant difference ($p < 0.05$).

Antibacterial activity of *P. pentosaceus* YTPP 02 by an agar well assay

The agar overlay assay showed that *P. pentosaceus* had high antibacterial potential in inhibiting antibiotic-resistant bacteria, including *A. baumannii* BUU 62715, 60056 and 30899, and *K. pneumoniae* BUU 30761 and 10624 (**Figure 6(A)**). The highest inhibition diameters were observed against *A. baumannii* BUU 62715 and *K. pneumoniae* BUU 10624, with inhibition zones of 19.67 ± 2.08 and 19.33 ± 2.31 mm, respectively, and they were not significantly different from the result of *A. baumannii* 60056 and *K. pneumoniae* 3076. These inhibitions were significantly higher than *A. baumannii* BUU 30899 ($p < 0.05$) (**Figure 6(B)**). *P. pentosaceus* YTPP 02 is effective against carbapenemase-producing bacteria.

To determine the antibacterial activity of the secreted substance by *P. pentosaceus* YTPP 02, the CFS was collected and lyophilized to evaluate by an agar well assay. The results showed that CFS showed antibacterial activity against *A. baumannii* BUU 62715, 60056 and 30899, and *K. pneumoniae* BUU 30761 and 10624 (**Figure 7(A)**). The inhibition zone diameters against *A. baumannii* BUU 62715, 60056 and 30899 were 15.3 ± 0.58, 17 ± 3.61 and 18.5 ± 1.5 mm. The antibacterial activity of CFS against *K. pneumoniae* isolates were found to be significantly lower than those of *A. baumannii* strains ($p < 0.05$) (**Figure 7(B)**). Regarding antibacterial activity of *P. pentosaceus*, it has been reported that this bacterium showed an antibacterial effect by producing bacteriocins or bacteriocin-like substances [24]. In our study, *P. pentosaceus* exhibited antibacterial activity against antibiotic-resistant bacteria. The antibacterial activity of CFS determined by the agar well diffusion suggested that *P. pentosaceus* could produce antimicrobial substances. Our findings are consistent with previous studies that *P. pentosaceus* possesses antibacterial activity and produces antimicrobial substances or bacteriocin-like substances [14,24].

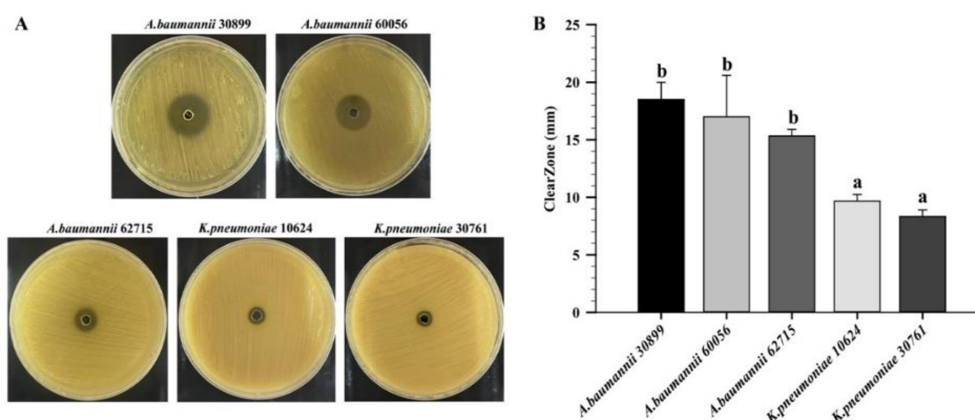

Figure 7 (A) Antibacterial activity of cell-free supernatant (CFS) of *P. pentosaceus* YTPP 02 by an agar well assay against antibiotic-resistant bacteria, and (B) inhibition zone diameters against *A. baumannii* BUU 62715, 60056 and 30899, and *K. pneumoniae* BUU 30761 and 10624. The data were expressed as mean ± S.D. and analyzed by 1-way ANOVA with Tukey's HSD. Different superscript letter represents statistically significant difference ($p < 0.05$).

MIC and MBC determination

The results of MIC determination showed that CSF of *P. pentosaceus* YTPP 02 inhibited the growth of *A. baumannii* isolates, with a MIC value of 62.5 mg/mL, while the MIC for *K. pneumoniae* was 125 mg/mL. The antibacterial susceptibility profile of these 5 strains were found to be resistant to CAZ, CTX, MER, ERT, with MIC values in the resistance range in accordance with CLSI guidelines. These findings indicated that all 5 bacterial isolates used in the present study were highly resistant to practically prescribed antibiotics, such as CAZ and CTX, for treatment of the infection caused by *A. baumannii* and *K. pneumoniae*. The MBC value in killing 3 strains of *A. baumannii* was 125 mg/mL, while the MBC for *K. pneumoniae* isolates was 125 mg/mL.

Table 2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of cell-free supernatant, cefotaxime, ceftazidime, meropenem and ertapenem against carbapenem-resistant clinical isolates.

Bacterial isolates	MIC					MBC
	CSF (mg/mL)	CAZ (µg/mL)	CTX (µg/mL)	MER (µg/mL)	ERT (µg/mL)	CSF (mg/mL)
A. baumannii BUU 62715	62.5	> 512^{R}	> 512^{R}	64^{R}	512 ^{R}	125
A. baumannii BUU 60056	62.5	256^{R}	128^{R}	128^{R}	512 ^{R}	125
A. baumannii BUU 30899	62.5	128^{R}	128^{R}	16^{R}	128 ^{R}	125
K. pneumoniae BUU 30761	125	256^{R}	> 512^{R}	16^{R}	256 ^{R}	250
K. pneumoniae BUU 10624	125	> 512^{R}	> 512^{R}	64^{R}	256 ^{R}	250
E. coli ATCC 25922	62.5	≤ 0.06^{S}	≤ 0.06^{S}	0.016^{S}	0.016 ^{RS}	125

CSF = cell-free supernatant of *P. pentosaceus* YTPP 02; CAZ = Ceftazidime; CTX = Ceftotaxime; R = resistant as interpreted in according to CLSI guideline.

Conclusions

The Nitro-beta test developed in this study is a simple and rapid technique for early detection and discrimination of β-lactamases, particularly carbapenemase producers within 20 min. The combined disc method with resazurin agar plate detected MBL in all clinical isolates within 7 h. Regarding the antibacterial potential of *P. pentosaceus* YTPP 02, it inhibited the growth of carbapenemase-producing *A. baumannii* and *K. pneumonia.* The present study

suggests *P. pentosaceus* YTPP 02 produces antimicrobial substances to inhibit the growth of resistant bacteria. This could be a good candidate for development as a new agent for antibiotic-resistant bacteria. However, the identification and purification of antimicrobial substances and the mechanism of action should be further investigated.

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