Isolation and molecular identification of Endophytic bacteria from Noni fruits (*Morinda citrifolia* l.) and their antibacterial activity

Sogandi i* and P Nilasari

1Department of Pharmacy, University of 17 Augustus 1945, Jakarta, 14356, Indonesia

*E-mail: sogandi@uta45jakarta.ac.id

Abstract. Endophytic bacteria are microorganisms that exist in the system of plant tissues such as fruits, leaves, twigs, roots and they can form colonies without causing damage to the plant. One potential medicinal plant has endophytic bacteria are Noni fruits (*Morinda citrifolia* l). This study aimed at isolating endophytic bacterial from Noni fruits (*Morinda citrifolia* l), screening endophytic bacteria, determining antibacterial activity of potential endophytic bacteria, identifying 16S rRNA of potential endophytic bacteria, and detection of an antibacterial compound using GCMS. Endophytic bacteria were successfully isolated from Noni fruits and disc-diffusion methods were used to screen for antibacterial activity against pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*, and *Streptococcus mutans*). The result of bacterial activity showed isolate ACP1, ACP2, ACP4, and ACP6 were produced antibacterial compounds. The activity shown with the formation of a clear zone and ACP6 isolate could inhibit four pathogenic bacteria with the highest clear zone. Molecular identification was investigated used PCR amplification 16S rRNA gene. The result showed that the potential isolate ACP6 (Acc. No. MH915674.1) belonging as Enterobacter cloacae with 99% sequence similarities and according to GCMS analysis, ethyl acetate fraction from secondary metabolite ACP6 contains Pyrazine as a bioactive compound. These findings suggest that the identified strains may contribute to the search for new sources of antibacterial substances.

1. Introduction

Increased cases of infectious diseases caused by pathogenic bacteria have an impact on the increasing incidence of pathogenic bacteria against commercial antibiotics that are currently widely used. Pathogenic bacteria that are resistant to antibiotics include *Staphylococcus aureus* and *Pseudomonas aeruginosa* [1, 2]. Indonesia is a country that has high biodiversity and vast tropical rainforest area which are potential to be sources of new antibiotics. One of the plant species that have been widely used as medicine is noni plants. Noni fruit (*Morinda citrifolia* L.) contains various active compounds as antibacterial agents such as L-asperuloside, alizarin, acubin, and anthraquinone [3, 4]. Based on previous research, noni fruit extract has the effectiveness to inhibit the growth of several bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella*, and *Shigella dysenteriae* [4, 5, 6]. However, the problem is the process of taking these bioactive compounds requires quite a lot of biomass. The latest alternative solution that can be done to overcome these problems is by utilizing endophytic bacteria in plant tissues. Endophytic bacteria are microbes that live in the internal tissues of living plants without causing real negative effects directly. The nature of endophytic bacteria that do not
have a negative impact on plant tissue indicates the possibility of a mutualism symbiotic relationship between endophytic bacteria and their hosts [7, 8]. High-level plants contain several endophytic bacteria which are able to produce biological compounds or secondary metabolites which are thought as a result of coevolution or genetic transfer (genetic recombination) from their host plants into endophytic bacteria [9]. The physiology of high-level plants originating from the same species will be different if grown in different environments, thus it can be said that the diversity of endophytic bacteria is very high. Based on these considerations, endophytic bacteria can be a source of various new secondary metabolites that have the potential to be developed in the medical, agricultural and industrial fields [10, 11].

Endophytic bacteria can produce rare and important bioactive compounds for their host plants so that the need to grow rare and long lifespan plants will be reduced, thus biodiversity in the world can be protected [11]. Bacteria that are used as a source of biological products will facilitate the process and reduce production costs so that ultimately produce products at lower prices. The ability of endophytic bacteria to produce secondary metabolites in accordance with their host plants is a very large and reliable opportunity to produce secondary metabolites [12]. Various studies regarding the potential of bioactive compounds contained in endophytic bacteria have been carried out. Some types of endophytic bacteria that can produce broad-spectrum antibiotics are endophytic bacteria isolated from *Grevillea pteridifolia* plant where these bacteria produce kakadumycin which is efficacious as an anti-malaria agent [13]. Endophytic bacterium *Pestalotiopsis microspora* isolated from Taxus plants can produce paclitaxel which is efficacious as an anticancer [14]. Furthermore, *Pseudomassaria* sp produces secondary metabolites that work like insulin and has been shown to reduce blood glucose in rats [15, 16].

In contrary, metabolites of noni fruits reported have bioactivity as antibacterial agents [17, 18]. Our study was aimed to isolate endophytic bacteria, identify endophytic bacteria from noni fruit (*Morinda citrifolia* L.), know the antibacterial activity, and analyze antibacterial compounds produced by potential endophytic bacterial isolates.

2. Materials

The tools and materials used in this study were glassware including test tubes, Erlenmeyer flasks, Petri dishes, measuring cups, drop pipettes, ovens, and micropipettes. It was also used analytical balance, UV-vis spectrophotometer, vortex, incubator, refrigerator, magnetic stirrer, water baths, aluminum foil, Bunsen, laminar air flow, digital cameras, Polymerase Chain Reaction (PCR) machines, separating funnels, and GC-MS (Gas Chromatography-Mass Spectrometer) machines, *Streptococcus mutans* ATCC 31987, *Staphylococcus aureus* ATCC 25323, *Escherichia coli* ATCC 25922, *Shigella dysenteriae* ATCC 13313, *Nutrient Agar*, *Nutrient Blood*, *Nutrient Broth* (HIMEDIA, India), sodium hypochlorite (NaOCl) 5.25%, nystatin, ethanol, chloroform, n-hexane, ethyl acetate, ampicillin, distilled water, NaCl (Merck, Germany), primer 16s rRNA (IDT, Singapore) 27F (5'-AGAGTTTGATCMTGGCTCAG-3'), 1492R (5'-TACGGYTACCTTGTTACGACT-3'), PCR Clean up and Gel extraction (Zymo Research).

3. Methods

3.1. Isolation of Endophytic bacteria

In this study, noni fruit was obtained from the farm of BALITRO, Bogor. The fruits were surface sterilized following the protocol by Suhando et al. [19] with few modifications. At first fresh fruit were washed with running tap water, then surface sterilized with 2% sodium hypochlorite for 5 min and washed with distilled water at least three times. Noni fruits sample finally washed with 70% ethanol and then evaporated. After evaporation of ethanol from Noni fruits surface, they were cut into small pieces (1 × 1 cm) and placed on the surface of nutrient agar plates supplemented with nystatin and incubated at 37 °C. The plates were checked each day after inoculation and after 3 days of incubation, the endophytic bacteria from fruit sample were taken, purified, numbered, transferred separately to nutrient agar slants and kept at 4 °C for further use.
3.2. Determine of potential isolate
For selecting potential endophytic, all the endophytic isolates were grown in 10 ml of nutrient broth medium for 48 h at 37°C with mild shaking (150 rpm). Cell-free supernatant (CFS) was prepared by centrifugation at 12000 g, 4°C for 5 min [20]. The antibacterial activity was determined against four pathogens bacteria; *Streptococcus mutans* ATCC 31987, *Staphylococcus aureus* ATCC 25323, *Escherichia coli* ATCC 25922 and *Shigella dysenteriae* ATCC 13313. The antibacterial testing was carried out by using disc diffusion method [21]. *Streptococcus mutans* was grown on Blood Agar (BA), while another bacterial were grown on Nutrient Agar (NA) medium. Negative control was nutrient broth sterile and ampicillin (10µg/disc) was used as the positive control. The bacterial test was mixed with sterile nutrient agar and transferred into Petri dishes to give a solid plate. The discs (diameter 6 mm) containing 30 microliter supernatant and loaded onto surface agar plates and then were incubated for 24 h at 37 °C. All sample and controls were performed in two replications. Diameters of clear zone inhibition were measured at the end of incubation. Endophytic bacteria which showed the highest clear zone against the bacterial test set as potential isolate.

3.3. Strain identification of Potential Isolate
Strains isolate potential were identified using 16S rRNA gene. Total genomic DNA was extracted using a DNA Extraction Kit (ZymoResearch) as described by the manufacturer. With genomic DNA as the template, a portion of the bacterial 16S rRNA gene (±1400bp) was amplified with universal primers (27F and 1492R) designed by Jiang *et al.* (2006) [22]. The 25µl of PCR mixture (Bioline) contained 12.5µl MyTaq Red Mix 2x, 1µl primer forward, 1µl primer reverse, and 1µl of genomic DNA with concentration 100 ng/µl as a template, and PCR grade water to make up the volume. A negative control (PCR mixture without DNA template) was included for each PCR reaction. Amplifications were carried out in an Agilent Sure cycle using the following conditions: 95 °C for 3 min, 35 denaturation cycles at 95 °C for 15 s, annealing temperatures 52 °C 30 s, primer extension at 72 °C for 45 s followed by a final extension at 72 °C for 3 min. In each case, the PCR product was run on 1% agarose gel containing floroafe, isolated and purified using Zymo Spin Column PCR purification kit (Zymo Research). Sequencing process was made at 1st Base Asia, Malaysia, furthermore Sequence of DNA was edited using Chromas Pro software and alignment sequence by DNA MAN software and aligned with the sequences in the GenBank by basic local alignment search tool (BLAST) program to reveal the sequence homology with the closely related organism. 16s RNA sequence of ACP6 was deposited in GenBank under accession number MH915674.1. Phylogenetic analysis using MEGA 7.0 software to determine the level of genetic relationship. This study used to neighbor-join for statistical method, a bootstrap method for a test of phylogeny with a number of bootstrap replications was 1000. The phylogenetic analysis was constructed using MEGA 7.0 software package [23].

3.4. Growth of Potential Isolate
Sub-cultured of potential isolate in the nutrient broth medium for 14h at 37 °C with shaking 150 rpm. A 500 µl aliquot of the potential isolate was cultured to a 500 ml nutrient broth medium in a 1L flask and incubated at 37 °C for 48h, optical density (600nm) were taken every 2 h.

3.5. Production of Crude Extract
Cell-free supernatants for the antibacterial assay was prepared by growing ACP6 isolate (1%, v/v) into 300 mL nutrient broth medium and incubated at 37 °C, shaker 150 rpm for 32 h (early stationer phase). The cells were harvested with centrifuged at 12000 g 10 min. Cell-free supernatant was extracted with ethanol, and then fractionation using ethyl acetate, Hexane, and chloroform. Furthermore, the extract was evaporated at 40 °C using the oven (Memmert).
3.6. Antibacterial Assay

The antibacterial activity of ethanol, ethyl acetate, hexane, and chloroform extracts of secondary metabolite potential isolate against *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, and *Shigella dysenteriae* was analyzed using disc diffusion Method [24]. In brief, the Nutrient Agar (Himedia, India) plates were prepared by pouring into sterile plates 20 ml of the molten media containing bacterial test. After 15 min, 30 µl supernatants were then spotted onto paper discs surface (diameter 6 mm; Macherey Nagel, Germany) and put on agar plates surface. After diffusion of the extracts into medium plates were incubated at 37 ºC. For negative control, the discs were loaded with dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA) alone whereas ampicillin (10 µg/disc; Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control. After incubation, the zone of inhibition around the disc was measured in millimeter using a transparent ruler. The measurements were performed in triplicates to determine the mean of the inhibition zone.

Minimum inhibitory concentration (MIC) test was carried out by microdilution technique against the bacterial test with various concentration of the extracts (3.12; 6.25; 12.50; 20; 25; and 30%). The extracts were dissolved in DMSO (dimethyl sulfoxide). Positive controls were prepared to contain only inoculum and sterile DMSO solutions without extracts for assuring no antibacterial effect. Negative controls were also prepared with extract solutions and sterile medium to ensure they were not contaminated. The MIC of a substance defined as the lowest concentration where bacterial growth was inhibited. These determinations were performed in triplicates.

3.7. GCMS Analysis of Bioactive Compound

Extract of secondary metabolite bacteria potential was isolated from ethyl acetate fraction for determination of bioactive compound as an antibacterial agent. Chemical analysis was conducted using gas chromatography coupled with mass spectrometry (GCMS Agilent Technologies 7890) equipped with an HP ultra 2 Capillary Column (30 m × 0.20 mm LD, 0.11 µm film thickness). The column temperature, 250°C, carrier gas helium linear gas velocity, 30 cm/sec; split ratio, 1/30, ion source temperature, 230°C and interface temperature, 280°C. The identification of chemicals was performed in comparison with database and confirmed using authentic standard samples.

4. Results and Discussion

4.1. Determination of Noni Fruit

Determination is done on the part of Noni fruit which in this study was used as a source to isolate endophytic bacteria. Determination aims to ensure that the plants used in the study are correct. Based on the results of the determination carried out, it is known that the noni fruit used in the study is *Morinda citrifolia* L.

4.2. Isolation of Endophytic Bacteria

Isolation of endophytic bacteria from Noni fruit samples begins with a method of surface sterilization [25]. Surface sterilization method is used to remove epiphytic microorganisms that are attached to plant samples [26]. Addition of nystatin to the isolation of NA medium functions as an antifungal agent [27]. Six isolated endophytic bacteria were selected based on morphological characteristics while namely ACP1, ACP2, ACP3, ACP4, ACP5, and ACP6. This result is consistent with Lodewyckx *et al*. [28] which argue that endophytic bacteria can be isolated from the roots, leaves, stems, flowers, fruits, and seeds. Endophytic bacteria are abundant in the roots and the number decreases in the stems and leaves [29]. The diversity of endophytic bacteria determined from various plant conditions as host plants for endophytic bacteria themselves [30]. This explains that physiological factors influence the growth of host plants also affect the diversity of endophytic bacteria. These physiological factors include soil structure, plant age, geographical distribution, time of sampling [31] and types of plant tissue [32].
4.3. **Determine of Potential Isolate**

All the pure isolates of endophytic bacteria were screened for their antibacterial activity by disc diffusion methods. Antibacterial activity assay revealed that crude extracts from six endophytic bacteria can inhibit the growth of both Gram-positive and negative bacterial test. In this study, *Streptococcus mutans* and *Staphylococcus aureus* as Gram-positive bacteria, while *Escherichia coli* and *Shigella dysenteriae* as Gram-negative bacteria. Strobel and Daisy (2003) were revealed that endophytes bacteria could be possibly synthesized pharmacologically active compounds as a result of their close endophytic host association and interactions [7]. In additional, endophytic bacteria *Pseudomonas* and *Bacillus* have also been reported to have antimicrobial activity like as on their host [33]. Therefore ACP6 isolate was inhibited all bacterial test, it is assumed that the dominancy an organism related to the production of secondary metabolites which are important as a chemical defense. Furthermore, ACP6 isolate was selected for further identification.

4.4. **Production of Secondary Metabolite**

Measurement of Cell density and antibacterial compound production are shown in Figure 1. Increased biomass of isolate ACP6 was shown with an OD 600 nm which increased from 0.1 at the measurement of the 2nd hour to reach a peak of 0.8 in the 32nd hour and after that, it gradually decreased until the OD value of 0.5 when incubation is continued for 48 hours.

![Figure 1. Growth of biomass ACP6 isolate.](image)

The growth of biomass ACP6 isolate showed that the 2nd hour is the beginning of the log phase. Meanwhile, the exponential phase occurs between the 8th to 28th-hour incubation. After the 28th hour, the isolate experienced a stationary phase until the 32nd-hour incubation. Base on this result, secondary metabolites isolate ACP6 was produced after incubation at least 28 h due to secondary metabolites are commonly produced in late growth phase [34], secondary metabolites are not essential for the growth of the culture but serve survival functions in nature [35]. This result also supported Simanjuntak et al. (2002) stated, if secondary metabolites from bacterial usually produced at the end exponential phase or at the beginning stationary phase [36]. The death phase of isolate ACP6 began to be seen after 40 hours where the OD<sub>600</sub> gradually decreased until 0.5 at 48th-hour incubation. Furthermore, secondary metabolite production from ACP6 isolate was harvested at the early exponential phase which after 32 h of incubation when cells biomass was at optimum OD (Fig 1).
4.5. Antibacterial Activity

Extraction of secondary metabolites potential isolate ACP6 was carried out using chloroform, n-hexane, ethyl acetate, and 70% ethanol. Furthermore, the extract was retested with pathogenic bacteria. Antagonistic activity test results of antibacterial compounds from various solvents showed that bacterial extract using ethyl acetate showed a broad spectrum of antibacterial activity, the extracts inhibited the growth of Gram-positive and Gram-negative bacteria with the best activity because it was able to inhibit all pathogenic bacteria test with the highest inhibitory zone (Fig.2). The ethyl acetate extracts showed antibacterial activity against *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli* and *Shigella dysenteriae* with clear zone diameter of 12, 13, 10 and 9 mm respectively. This antibacterial activity was lower than the positive control (ampicillin) with inhibition zone diameter 16 mm.

![Inhibitor Zone (mm)](image_url)

**Figure 2.** Antibacterial activity of extracts with various solvents against the bacterial test.

The formation of inhibitory zones around the disc indicates the inhibitory activity of extracts ACP6 isolates against pathogenic bacteria. This is in accordance with the statement of [37] which states that endophytic microbial isolates are said to have antimicrobial activity if a clear zone is formed around microbial isolates grown on media that have been inoculated by pathogenic bacteria. The difference in diameter of the inhibitory zone can be caused by the content of different antibacterial compounds [38, 39] and the cell wall diameter of pathogenic bacteria [40]. Some bacterial extract showed minimum antibacterial activity. These extracts are thought to actually contain active compounds yet the amount is very small or contains other active compounds [41] such as indole acetate acid (IAA) and enzymes [42, 43].

Minimum Inhibitory Concentration (MIC) ethyl acetate extract of secondary metabolite ACP6 isolate originating from noni fruit (*Morinda citrifolia* L.) against the growth of *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, and *Shigella dysenteriae* were ≤ 20%, ≤ 12.5%, ≤ 20%, and ≤ 6.25% respectively. According to Aligiannis *et al.* (2001) [44], the extracts can be classified based on MIC results in strong inhibitors: MIC up to 10%, moderate inhibitors, MIC between 10 and 30% or weak inhibitors, and MIC above 30%. In general, ethyl acetate extract from secondary metabolite ACP6 was the strong growth inhibitors for *Shigella dysenteriae* and moderate for *Streptococcus mutans*, *Staphylococcus aureus*, and *Escherichia coli*. Generally in this study revealed Gram-negative bacteria were more resistant to the extracts than Gram-positive bacteria. Although it is not possible to propose a general hit for the resistance, this higher resistance could be attributed to the outer membrane [45, 46].
4.6. Molecular identification of endophytic bacterial isolate ACP6

From totally were 6 isolates, ACP6 isolate was selected as potential isolates. Furthermore, isolation of the genome from this isolate was carried out. Genomic isolation was carried out according to the method of Zhu et al. (1993) as described in the research procedure [47]. The isolated genome was amplified using the 16S rRNA gene using a PCR machine. PCR results were then electrophoresed using 0.8% agarose gel at 100 volts for 30 minutes. Agarose gel was then visualized under UV light and DNA bands were obtained on the gel with a size of ± 1500 bp (Figure 3).

![Figure 3. Gel electrophoresis of PCR product 16s rRNA gene amplification.](image)

After purification, the 16S rRNA gene sequence was determined. The partial sequences of 16S rRNA obtained are aligned with the sequences of bases in the NCBI database. Sequencing of ACP6 isolate that has been aligned shows that the 16S rRNA gene in the isolate is ± 1370 bp (base pair) then 16s rRNA gene sequence of ACP6 isolate was registered to the Genbank NCBI database by Enterobacter sp obtaining accession number of MH915674.1. This supported by the reconstruction of phylogenetic trees to determine the relationship between species. Bacterial sequence alignment with several other bacterial sequences taken from the NCBI-GenBank database is done using crystal X2. Then the alignment results were entered into NJPlot to reconstruct phylogenetic trees. The results of phylogenetic tree reconstruction showed that isolate ACP6 had the closest relationship with Enterobacter cloacae strain CX-43 (accession number: MH368381.1), Enterobacter cloacae strain CX-42 (accession number: MH368380.1), Enterobacter hormaechei strain CW3 (accession number: KU867635.1), Enterobacter hormaechei strain S13 (accession number: CP031565.1) The 16S rRNA sequence-based phylogenetic tree analysis revealed that ACP6 belonged to the family Enterobacteriaceae and grouped with Enterobacter cloacae clade (Fig. 4).
Figure 4. A cladogram resulted from the neighbor-joining tree of ACP6 isolate.

*Enterobacter cloacae* is a Gram-negative bacterium, has anaerobic fluctuations, rod-shaped and motile. If *Enterobacter* sp is grown on artificial media, it can change glucose and eventually formed acid and gas. These bacteria reduce nitrate to nitrite. These bacteria can form capsules, citrate, and acetate which can be used as the only carbon source [48].

*Enterobacter* sp is also a bacterium producing protease, amylase, and cellulase enzymes. Protease producing bacteria can generally be used as probiotics that provide benefits to humans and animals because they can suppress the growth of pathogenic bacteria [49, 50]. Shankar *et al.* (2006) explained that *Enterobacter* sp has a habitat that is not known until now, yet *Enterobacter* sp is widespread in the environment, food, water, soil, and vegetables [51]. *Enterobacter* sp has been reported as an endophytic and associated fungi in plants [52, 53]. Khalifa *et al.* (2016) have been isolated new metabolite from endophytic *Enterobacter cloacae* strain MSR1 [54]. The results revealed that *Enterobacter cloacae* consumed certain carbohydrates such as glycerol, D-xylene, D-maltose, and esculin melibiose as a sole carbon source and certain amino acids such as arginine. In addition, Akinsanya *et al.* (2015) also reported that endophytic bacteria *Enterobacter* have been isolated from *Aloe vera*. Crude extract and ethyl acetate fractions of the metabolites have antimicrobial activities against pathogenic bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella Typhimurium*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Escherichia coli*, *Streptococcus pyogenes*, and *Candida albicans*, with inhibition zones ranging from 6.0 till 57 mm [55].

4.7. Identification compound produced by endophytic isolate

A total of 16 secondary metabolites were identified from isolates ACP6 using GCMS which can be classified into several groups. However, not all of these compounds have antibacterial activity. The compounds with the highest concentration were 2-Oxabicyclo [3.3.1] Non-6-en-3-one, 4-Hydroxyendo with a content of 26.15% at a retention time of 27.565. The dominant compounds detected in GCMS analysis were Pyrazine compounds and their derivatives with retention times of 28,475 (8.20%), 28,600 (5.11%), 28,641 (4.63%), 28,786 (8.68%), 31,034 (7.72%) and 31,171 (12.10%). In addition, there are also other class compounds namely Methyl mercapto propylamine, Sulfide, Pheniletanamine, and Cyclopentanone. Pyrazine is a compound with nitrogen-containing heterocyclic components and is the result of interactions between α-dicarbonyl while amines are interactions between α-amino groups of
amino acids. The role of amino acid chains in the formation of pyrazine is important, especially the part of the chain containing nitrogen atoms such as glutamine, asparagine, and lysine [56].

Pyrazine has been widely reported to have antibacterial activity, including the one proposed by Sikine at al. (2017) that the derivatives of Pyrazine, namely Pyrido [2,3-b] can inhibit the growth of Staphylococcus aureus (0.078 mg/ml), Bacillus cereus (0.078 mg/ml), Escherichia coli (0.625 mg/ml), and Salmonella typhi (1.25 mg/ml). In addition, the synthetic compound Pyrazine-2-Carbohydrazide has antibacterial activity against Gram-negative and Gram-positive bacteria such as Staphylococcus aureus, Bacillus subtilis, Salmonella typhi and Escherichia coli [57]. Pyrazine in the form of Hydrazinium salt is also reported to have stronger antibacterial activity compared to acidic compounds and antibiotics from the Cotrimoxazole group when tested on Escherichia coli, Salmonella typhi and Vibrio cholera [58].

5. Conclusion
This study revealed endophytic bacteria Enterobacter cloacae strain ACP6 (MH915674.1) was isolated from Noni fruits (Morinda citrifolia), ethyl acetate fractions of the metabolites showed high antibacterial activity against Streptococcus mutans, Staphylococcus aureus Escherichia coli, and Shigella dysenteriae. Furthermore, ACP6 isolate was produced pyrazine as bioactive compounds and for future exploration important to investigation and characterization of their bioactive compounds that may be of use in the medical and food industries.

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