JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2018. Vol. **12**(3), p. 1473-1481

Isolation and Characterization of Antibiotic-producing Endophytic Bacteria from *Citrus aurantifolia* Swingle

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http://dx.doi.org/10.22207/JPAM.12.3.51

(Received: 15 May 2018; accepted: 20 July 2018)

Research on the isolation and characterization of antibiotics-producing endophytic bacteria from *Citrus aurantifolia* leaves has been done. The isolation process was carried out by spreading spread plate isolation technique, screening of antibiotic-producing bacteria was done by disc dilution method and characterization of antibiotic-producing bacteria was done by microscopic, macroscopic and 16S rRNA analysis methods. The result of this research is four endophytic bacteria isolates that potentially produces antibiotic compounds. First, isolates with the CA01 code of fermentation fluids have inhibitory activity against *Streptococcus mutans* bacteria. Second, isolate with the code CA 02 able to inhibit growth of *Vibrio chloreae* bacteria. Third, isolates with the CA 03 code were able to inhibit bacterial growth of *Salmonella thypii* and *E. faecalis*. Fourth, isolate with CA 04 code has inhibitory activity on *Salmonella thypii* and *Salmonella thyposa* bacteria. The microscopic, macroscopic and 16S rRNA characterization for antibiotic-producing endophytic bacteria results were *Bacillus cereus* RNS_01 (code CA01), *Pantoea agglomerans* ZFJ-15 (Code CA 02), *Bacillus subtilis* 55C1-1 (Code CA 03) and *Bacillus pumilus* SH-B11 (Code CA 04).

Keywords: Endophytic bacteria, *Citrus aurantifolia*, antibiotic, 16S rRNA.

Antibiotics had been used since 1928 for disease preventive and treatment of infectious diseases. Nowadays, the use of antibiotics is increasing along with the increase of new infectious disease cases and resistance increase of some bacteria that cause the infection against the antibiotics used. Most antibiotics that are commercially used are synthetic antibiotics that are prone to trigger resistance to pathogens, especially bacteria[1,2,3]. This situation led to the need for exploration to find a source of new and more potent natural antibiotics. One of the natural source antibiotics that widely developed recently by various research institutions is the endophytic bacteria that live mutually in the host plant[4,5].

Several types of endophytic bacteria that have been successfully isolated and are known to produces active compounds with antibiotic[6], antimalaria[7] and antifungal[8,9] properties. The ability of endophytic bacteria to produce these active compounds is a potential that can be developed to replace the process of discovery of active compounds by extracting plants, especially medicinal plants[10]. One disadvantage of obtaining an active compound from the plant is it requires longer time and more complex process with smaller yield compared to extracting active compound from bacteria after a fermentation process.

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One of the plants that has antibiotic potential is the *Citrus aurantofolia* plant. Natural content of *Citrus aurantifolia* plant that it has secondary metabolite in the form of essential oil. It was reported by many researchers that the essential oil of lime leaves has antibacterial and antifungal properties[11,12]. In society, lime leaves was used to treat skin diseases, sore throat and sprue. Some study also reported that lime leaves used as anti-inflammatory (anti-inflammatory) and mouthwash, because the leaf is known also contains essential oils[13,14].

The essential oils antibacterial activity is caused by active compounds contained in essential oils which can inhibit or kill bacterial or fungal growth[15,16]. The compounds produced by the essential oil of lime leaves include limonene, β pinen, sabinene, (E) -β-Ocimene, α-pinen, myrcene, linalool, geranial, neral, citronellol, geranilacetate, nerilacetate, geraniol, and anti fungal nerol[17]. Given the potential of active compounds contained in Citr*us aurantifolia* plants, it was suspected that endophytic bacteria living in citrus plants can also produce the same active compounds or almost the same as those produced by the original plant. There is not much research has been done related to endophytic bacterial isolation on *Citrus aurantifolia* plant. So this research is done to obtained the endophytic bacteria isolate from *Citrus aurantifolia* plant which able to produces antibiotic compound, followed by bacterial characterization and antibacterial activity test.

METHODS AND MATERIALS

Tools and Materials

Tools used in this research were petri dish, bunsen lights, mortar, stanfer, ose, beaker glass, erlenmeyer, test tube, vortex, autoclave, Laminar Air Flow cabinet, incubator cabinet. Material used were medium Nutrient Broth (NB), Nutrient Agar (NA), disc paper, NaCl 0.85% solution, alcohol, sodium hypochlorite, aquadest.

Sample Collection

Leaves of ***Citrus aurantifolia*** **Swingle** were taken from the fruiting plant, cut with sterile knife and then washed with sterile aquadest, then it was put in plastic bags and placed in refrigerator (cooled temperature ±10^{o}C)[18].

Sterilization of Surface from Plant Organs

Collected leaves was cut for approximately 1 cm^2. Leaves cut then desinfected with etanol 70% for 1 minute, natrium hipoklorit 2% for 6 minutes, etanol 70% for 30 seconds to remove the natrium hipoklorit and then washed using sterile aquadest[19].

Isolation, Purification and Gram Coloration of Endophytic Bacteria Isolates from *Citrus aurantifolia* Leaves

Sterile leaves was finely grounded using a sterile mortar and then inserted into a serial dilution of 0.85% NaCl solution and vortex. Each serial dilution was inoculated into TSA medium and benomil fungicide as many as 1 µL mL-1 was added with an antifungal spread plate method[18]. It was incubated at 27^{0}C for 1 to 3 x 24 hours. The colonies of grown bacteria was observed morphologically and purified. Pure bacteria isolates then pass through Gram staining[19].

Characterization of *Citrus aurantifolia* Leaf Endophytic Bacteria Isolates using 16S rRNA

Characterization of bacterial isolate by 16S rRNA method was done at the Laboratory of Industrial Microbiology of LIPI Biotechnology Research Center, Bogor. The base order was checked and edited using the Bioedit Sequence Alignment Editor (*http://www.mbio.ncsu.edu/BioEdit/bioedit.html*). Equality analysis was done by using the Basic Local Alignment Tool at the National Center For Biotechnology Information (*http://www.ncbi.nlm.nih.gov*). While the evolution analysis was done by using ClustalW2 Phylogenetic Tree (*www.ebi.ac.uk*).

Antibiotic Activity Test from Fermentation Fluid of Endophytic Bacteria Isolate *Citrus aurantifolia* Leaf

The test was done towards test bacteria obtained from UAAC Bacteria Culture Center Biotechnology Laboratory, UPT Sumber Daya Hayati, Andalas University, Padang. Test bacteria that were used in this research are *Streptococcus mutans* ATCC 25175, *Staphlococcus aureus* ATCC 25923, *Eschericia coli* ATCC 25922, *Enterobacter faecalis* ATCC 29212, *Micrococcus luteus* ATCC 10240, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Vibrio chloreae* INABA, *S.epidermidis* ATCC 12228, *Salmonella thypii, Salmonella thyposa* NCTC 786 *Salmonella thypimurium* ATCC 14028 and Methicillin-Resistant *Staphylococcus aureus* (MRSA).

Each endophytic bacteria isolate was grown into Nutrient Broth medium for 24 hours at 27^0C with agitation of 120 rpm. Subsequently, it was centrifuged at 10,000 rpm for 15 min and the supernatant formed was tested for its activity using a diffusion-agar disc paper. Each of the test bacteria was planted into Nutrient Agar medium by spread plate method and disc paper which immersed with bacterial supernatant was placed, incubated at 27^0C for 48 hours. The inhibition zone formed indicates the formation of antibiotic compounds produced by endophytic bacteria after the fermentation process[10].

RESULT AND DISCUSSION

After isolation and screening of endophytic bacteria from *Citrus aurantifolia* leaf, as much as seven bacterial colonies were detected to produce antibiotic compounds. The morphology of these seven bacteria turned out to be grouped into four colonies of different bacteria. Based on the results of characterization using Gram staining method and microscopic observation, it was shown that the four isolated bacteria were all in the form of bacillus, three bacteria from Gram positive group and one Gram negative (Table 1). The number of bacteria that grow in the medium is relatively small, this is due to environmental factors and the organ taken only leaves. It also known that the presence of endophytic bacteria in plants is influenced by environmental factors and plant organs[3,19].

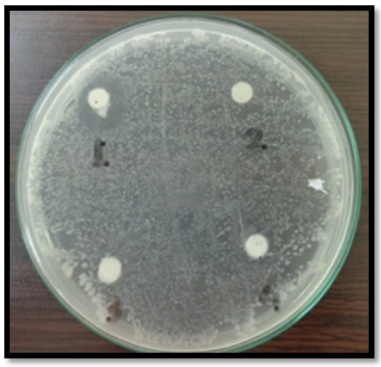

Fig. 1. Observation of endophytic bacterial fermentation fluid inhibitory zone towards Streptococcus *mutans* test bacteria

The data in Table 1 shows that the macroscopic and microscopic characteristics of antibiotic-producing endophytic bacteria isolates from *Citrus aurantifolia* leaves are very diverse. This is shows that the presence of bacteria contained in leaf tissue is also relatively large and diverse as well. In Table 1, it was also shown that all isolated antibiotic-producing bacteria colony were in circular form, three endophytic bacterial isolates were Gram-positive i.e CA 01, CA 03, CA 04 whereas CA 03 Gram was negative with all bacil cell foem. It is known that theoretically the colony's distinction from bacteria is a characteristic of a particular species[20,21].

Furthermore, Table 2 shows the results of antibiotic activity test from antibiotic-producing endophytic bacteria from *Citrus aurantifolia* leaf. It is demonstrated that endophytic bacterial isolate may inhibit the growth of some test bacteria. Inhibitory power of each isolate bacteria is different, as CA1 isolate bacteria positive to be able to inhibit *Streptococcus mutans* bacteria, CA 02 isolate bacteria can inhibit *Vibrio chloreae* bacteria, CA 03 can inhibit *E. faecalis* bacteria and CA 04 can inhibit *Salmonella thypii* and *Salmonella thyposa* bacteria. It is characterized by

Table 1. Macroscopic and microscopic results of antibiotic-producing endophytic bacteria isolate from *Citrus aurantifolia* leaves

No.	Isolate	Macroscopic observation of the colony				Microscopic observation of the colony	
	Code	Form	Color	Border	Elevasion	Gram	Cell form
1.	CA 01	Circular	White	Undulate	Convex	Positif	Bacil
2.	CA 02	Circular	White	Entire	Umbonate	Negative	Bacil
3.	CA 03	Circular	Cream	Entire	Convex	Positif	Bacil
4.	CA 04	Circular	Cream	Entire	Umbonate	Positif	Bacil

Table 2. Profile of antibiotic activity of the bacterial fermentation of endophytic bacterial isolates from *Citrus aurantifolia* leaves

No	Isolate Code	Antibiotic activity against bacaterial test														
		S.thypii	*S. thyposa* NCTC 786	*S. thypimurium* ATCC 14028	*E.coli* ATCC 25922	*E.faecalis* ATCC 29212	*S.mutan* ATCC 25175	*M.luteus* ATCC 10240	*V.chloreae* INABA	*B.subtilis* ATCC 6633	*MRSA*	*P. aeruginosa* ATCC 27853	*S. epidermidis* ATCC 12228	*S. aureus* ATCC 25923
1.	CA 01	-	-	-	-	-	+	-	-	-	-	-	-	-
2.	CA 02	-	-	-	-	-	-	-	+	-	-	-	-	-
3.	CA 03	+	-	-	-	+	-	-	-	-	-	-	-	+
4.	CA 04	+	+	-	-	-	-	-	-	-	-	-	-	-

the activity of each isolate that have the same ability with its host plant. This ability is antibacterial, because the host plant have secondary metabolite compound that is essential oil which have ability as antibacterial. It has been reported by previous researchers that *Citrus aurantifolia* plant in addition to benefits for health, it also containing essential oil with antibacterial and antifungal properties[12].

Figure 1 showed the ability of the inhibitory power of the endophytic bacteria isolate fermentation fluid to one of the test bacteria, *Streptococcus mutans*. The results of this experiment showed that each endophytic bacterial isolate produces antibiotic compounds that different capabilities towards the test bacteria used (Table 2). This allegedly caused by the differences in the active compound and molecular structure of the resulting antibiotic compounds. Concerning this condition, further research on the purification and determination of the molecular structure of each antibiotic compound produced is needed. In another study it was reported that the essential oils and antioxidant content of *Citrus aurantifolia* leaf with volatile oils concentrations of 20%, 10%, 5%, 1% was able to inhibited the growth of *Staphylococcus aures, Bacillus subtilis, Enterobacter faecalis, Salmonella paratypii, E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Serratia marcescens*[23].

In Figure 2 it can be seen that endophytic bacterial isolates that have a large inhibitory ability was the CA 01 bacterial isolate that able to inhibit the *Streptococcus mutans*. This condition is shown by the clear zone formed. The clear zone was showed that the activity of CA 01 bacterial isolate has the content of bioactive compounds which is similar to the leaves of *citrus aurantifolia*. Ardani (2010) also mentioned that the essential oil contained in *C. aurantifolia* can inhibit the bacterium *Streptococcus mutans* for 0.06%[22].

The evolutionary analysis was performed using Clustal W2 Phylogenetic Tree (*www.ebi.ac.uk*). In this analysis, the outgroup used was *Escherichia coli* K12. The results showed that *Bacillus subtilis* 55-C1-1 usually has very close genetic relationship with *Bacillus pumilus* SH-B11 (Figure 3). From the results of Pairwise Sequence Alignment analysis both bacteria have the similarity of 93.9%. In this study, most of the

Table 3. Result of endophytic bacteria 16S rRNA sequence analysis

No.	Isolate Code	16S rRNA Sequence NCBI Result	BLAST (%)	Homology
1	CA 01	AGAGCTTGCTCTTATGAAGTTAGCGGC GGACGGGTGAGTAACACGTGGGTAA CCTGCCCATAAGACTGGGATAACTCC GGGAAACCGGGGCTAATACCGGATAAC ATTTTGAACCGCATGGTTCGAAATTGAAA GGCGGCTTCGGCTGTCACTTATGGATGGA CCCGCGTCGCATTAGCTAGTTGGTGAGGT AACGGCTCACCAAGGCAACGATGCGTAG CCGACCTGAGAGGGTGATCGGCCACACT GGGACTGAGACACGGCCCAGACTCCTAC GGGAGGCAGCAGTAGGGAATCTTCCGCA ATGGACGAAAGTCTGACGGAGCAACGCCG CGTGAGTGATGAAGGCTTTCGGGTCGTAAA ACTCTGTTGTTAGGGAAGAACAAGTGCTAG TTGAATAAGCTGGCACCTTGACGGTACCTAA CCAGAAAGCCACGGCTAACTACGTGCCAGC AGCCGCGGTAATACGTAGGTGGCAAGCGTT ATCCGGAATTATTGGGCGTAAAGCGCGCGCA GGTGGTTTCTTAAGTCTGATGTGAAAGCCCA CGGCTCAACCGTGGAGGGTCATTGGAAACT GGGAGACTTGAGTGCAGAAGAGGAAAGTGGA ATTCCATGTGTAGCGGTGAAATGCGTAGAGA TATGGAGGAACACCAGGTGGCGAAGGCGA CTTTTCTGGTCTGTAACTGAACACTGAAGGC CGCGAAAGCGTGGGGAGCAAACAGGATTAG ATACCCTGGTAGTCCACGCCGTAAACGATG AGTGCTAAGGGTTAGAGGGGTTTCCGCCC CTTAGTGCTGAAGTTAACGCATTAAGCAC TCCCCCTGGGGAGTACGGCCCCAAGCC TGAAACCTCAAAGAAATTGACCGGGGCC CCCAACAAGCGGGTGGAGCATGTGGT TTAATCCGAAGCAACGCGGAAGAACCTT ACCCAGGTCTTGACATCCTCTGACAAACC CTAGAGGATAGGCCTCTCTCCTTGGGGA CCAGAGGGCCAGGTGGGCCATGGTG GCGGTCACTCGTGGTGGTGAGATGTTGG GTTAATTCCGGCACCGAGGCCAACCTTTG TCCTTAGTGCCCACCATTAAGTTGGCCAT CCTAAGGTGATGCCCGGGGCCAACCCGG AGGAAGGTGGGGAGGAGGTCAATTCTTC ATCCCCCTTATGCCTTGGCTTCCCCACGT CTTCCAATGGACGGTCCAAAGAGCTCCAGG ACCGGGAGGGGGAGTTATTTTCATAAACCCT TTTTCAGTTGGGATTTTGGGCTCCAATTGCCT TCCAGGAAGCGGGAATCCTTGGTAATCGGGG ATCACCAGGCCGCGTGGAATAGGTTCCCGG CCCTGTTCCCCCCCCCCGGCCCCACCCGGGG ATTTGGTACCACCGGAAGTGGGTGGGGTACC TTTTTGGAGCCAGCCGCCTAAGG	*Bacillus cereus* RNS_01 (KT380683.1)	94
2	CA 02	GCAGTCGAGCGGAGTTGACGGAAAGCTTGCT TTCCTGATACTTAGCGGCGGACGGGTGAGTAA CACGTAGGCAACCTGCCCTCAAGCTTGGGAC AACTACCGGAAACGGTAGCTAATACCGAATAC TTGTTTTCTTCGCCTGAAGGAAACTGGAAAG ACGGAGCAATCTGTCACTTGGGGATGGGCCT GCGGCGCATTAGCTAGTTGGTGAGGTAACGG CTCACCAAGGCGACGATGCGTAGCCGACCTGA	*Pantoea agglomerans* ZFJ-15 (EU931554.1)	99

		GAGGGTGATCGGCCACACTGGGACTGAGACA CGGCCCAGACTCCTACGGGAGGCAGCAGTAGG GAATCTTTCCGCAATGGGCGAAAGCCTGACGG AGCAATGCCGCGTGAGTGATGAAGGTTTTCGG ATCGTAAAGCTCTGTTGCCAGGGAAGAACGCT TGGGAGAGTAACTGCTCCCAAGGTGACGGTAC CTGAGAAGAAAGCCCCGGCTAACTACGTGCCA GCAGCCGCGGTAATACGTAGGGGGCAAGCGTTG TCCGGAATTATTGGGCGTAAAGCGCGCGCAGGC GGTCATGTAAGTCTGGTGTTTAATCCCGGGGCTC AACCCCGGATCGCACTGGAAACTGCGTGACTTG AGTGCAGAAGAGGAGAGTGGAATTCCACGTGTA GCGGTGAAATGCGTAGAGATGTGGAGGAACACCA GTGGCGAAGGCGACTCTCTGGGCTGTAACTGACG CTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATT AGATACCCTGGTAGTCCACGCCGTAAACGATGAAT GCTAGGTGTTAGGGGTTTCGATACCCTTGGTGCCG AAGTTAACACATTAAGCATTCCGCCTGGGGAGTAC GGTCGCAAGACTGAAACTCAAAGGAATTGACGGG GACCCGCACAAGCAGTGGAGTATGTGGTTTAATTC GAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT CCAACTAACGAGGCAGAGATGCGTTAGGTGCCCT TCGGGGAAAGTTGAAACAGGTGGTGCATGGTTGT CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC CGCAACGAGCGCAACCCTTATATTTAGTTGCCAGC ATTTCGGATGGGCACTCTAAATAGACTGCCGGTG ACAAACCGGAGGAAGGTGGGGATGACGTCAAAT CATCATGCCCCTTATGACCTGGGCTACACACGTAC TACAATGGCCGGTACAACGGGCAGTGAAGCCGC GAGGTGGAACCAATCCTAAAAAGCCGGTCTCAG TTCGGATTGCAGGCTGCAACTCGCCTGCATGAA GTCGGAATTGCTAGTAATCGCGGATCAGCATGCC GCGGTGAATACGTTCCCGGGTCTTGTACACACC GCCCGTCACACCACGAGAGTTTATAACACCCGA AGTCGGTGGGGTAACCGCAAGGAGCCAGCCGC CGAAGGTGGGATAGAT		
3	CA 03	GCAGTCGAGCGGACAGATGGGAGCTTGCTCCCT GATGTTAGCGGCGGACGGGTGAGTAACACGTG GGTAACCTGCCTGTAAGACTGGGATAACTCCGG GAAACCGGGGCTAATACCGGATGGTTGTTTGAA CCGCATGGTTCAAACATAAAAGGTGGCTTCGGC TACCACTTACAGATGGACCCGCGGCGCATTAGC TAGTTGGTGAGGTAACGGCTCACCAAGGCAACGA TGCGTAGCCGACCTGAGAGGGTGATCGGCCACAC TGGGACTGAGACACGGCCCAGACTCCTACGGGAG GCAGCAGTAGGGAATCTTCCGCAATGGACGAAAG TCTGACGGAGCAACGCCGCGTGAGTGATGAAGGT TTTCGGATCGTAAAAGCTCTGTTGTTTAGGGAAGA ACCAAGTACCGTTCGGAATAGGGGCGGTACCTTG ACCGGTACCTAACCCAGAAAGCCACGGGCTAACT ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA AGCGTTGTCCGGAAATTATTGGGCGTAAAGGGCT CGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCC CGGCTCAACCGGGGAGGGTCATTGGAAACTGGGG AACTTGAGTGCAGAAGAGGAGAGTGGAATTCCAC GTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAAC ACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTG ACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGG ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAG TGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTG CAGCTAACGCATTAAGCACTCCGCCTGGGGAGTAC GGTCGCAAGACTGAAACTCAAAGGAATTGACGGG	*Bacillus subtilis* 55C1-1 (JN366797.1)	99

		GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATT CGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC ATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTC GGGGGCAGAGTGACAGGTGGTGCATGGTTGTCG TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC GCAACGAGCGCAACCCTTGATCTTAGTTGCCAGC ATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGA CAAACCGGAGGAAGGTGGGGATGACGTCAAATC ATCATGCCCCTTATGACCTGGGCTACACACGTGCT ACAATGGACAGAACAAAGGGCAGCGAAACCGCGA GGTTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGA TCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAAT CGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATA CGTTCCCGGGCCTTGTACACACCGCCCGTCACACC ACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTA ACCTTTTAGGAGCCAGCCGCCGAAGGTGGGACA GATGAT		
4	CA 04	GCTTGCTCCCGGATGTTAGCGGCGGACGGGTGA GTAACACGTGGGTAACCTGCCTGTAAGACTGGG ATAACTCCGGGAAACCGGAGCTAATACCGGATA GTTCCTTGAACCGCATGGTTCAAGGATGAAAG ACGGTTTCGGCTGTCACTTACAGATGGACCCGC GGCGCATTAGCTAGTTGGTGAGGTAACGGCTCA CCAAGGCGACGATGCGTAGCCGACCTGAGAGG GTGATCGGCCACACTGGGACTGAGACACGGCC CAGACTCCTACGGGAGGCAGCAGTAGGGAATC TTCCGCAATGGACGAAAGTCTGACGGAGCAAC GCCGCGTGAGTGATGAAGGTTTTCGGATCGTA AAGCTCTGTTGTTAGGGAAGAACAAGTGCAAG AGTAACTGCTTGCACCTTGACGGTACCTAACC AGAAAGCCACGGCTAACTACGTGCCAGCAGC CGCGGTAATACGTAGGTGGCAAGCGTTGTCCG GAATTATTGGGCGTAAAGGGCTCGCAGGCGGT TTCTTAAGTCTGATGTGAAAGCCCCCGGCTC AACCGGGGAGGGTCATTGGAAACTGGGAA ACTTGAGTGCAGAAGAGGAGAGTGGAATT CCACGTGTAGCGGTGAAATGCGTAGAGATGT GGAGGAACACCAGTGGCGAAGGCGACTCTCT GGTCTGTAACTGACGCTGAGGAGCGAAAGCG TGGGGAGCGAACAGGATTAGATACCCTGGTAG TCCACGCCGTAAACGATGAGTGCTAAGTGTTA GGGGGTTTCCGCCCCTTAGTGCTGCAGCTAA CGCATTAAGCACTCCGCCTGGGGAGTACGG TCGCAAGACTGAAACTCAAAGGAATTGACG GGGGCCCGCACAAGCGGTGGAGCATGTGG TTTAATTCGAAGCAACGCGAAGAACCTTACC AGGTCTTGACATCCTCTGACAACCCTAGAGA TAGGGCTTTCCCTTCGGGGACAGAATGACAG GTGGTGCATGGTTGTCGTCAGCTCCTGTCCT GAGATGTTGGGTTAAGTCCCCCAACGAGCG CAACCCTTGATCTTAGTTGCCAGCATTCAGTTG GGCACTCTAAGGTGACTGCCGGTGACAAACC GGAGGAAGGTGGGGATGACGTCAAATCATCA TGCCCCTTATGACCTGGGCTACACACGTGCT ACAATGGACAGAACAAAGGGCTGCGAGACC GCAAGGTTTAGCCAATCCCACAAATCTGTTCT CAGTTCGGATCGCAGTCTGCAACTCGACTGC GTGAAGCTGGAATCGCTAGTAATCGCGGATC AGCATGCCGCGGTGAATACGTTCCCGGGCCT TGTACACACCGCCCGTCACACCACGAGAGTT TGCAACACCCGAAGTCGGTGAGGTAACCTTT ATGGAGCCAGCCGCCGAAG	*Bacillus pumilus* SH-B11 (CP010997.1)	99

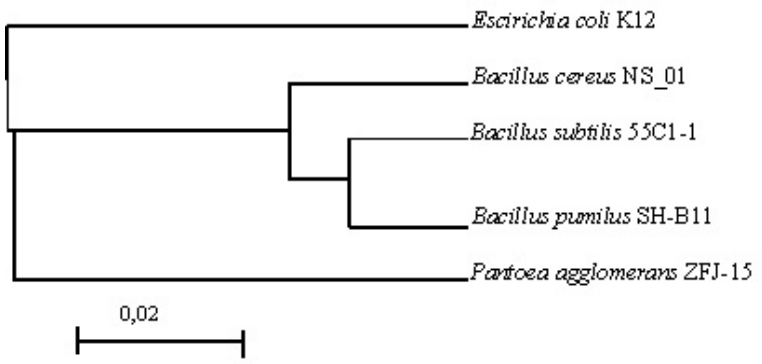

Fig. 2. Phylogenetic tree of 16S rRNA sequence of isolated antibiotic-producing endophytic bacterial in this research

bacteria obtained is from the *Bacillus* genera, this is because *Bacillus* existence is very abundant in the environment *Citrus aurantifolia* and *Bacillus* bacteria have the ability to penetrate into plant tissue[23,24].

CONCLUSIONS

As much as four endophytic bacterial isolates that could potentially produce antibiotic compounds was obtained. First, isolates with CA01 code has fermentation liquids with inhibitory activity against *Streptococcus mutans* bacteria. Second, isolate with code CA02 was able to inhibit the growth of *Vibrio chloreae* bacteria. Third, isolate with the CA03 code was able to inhibit bacterial growth, *Salmonella thypii* and *E. faecalis*. Fourth, isolate with CA04 code has inhibitory activity on *Salmonella thypii* and *Salmonella thyposa* bacteria. The results of microscopic, macroscopic and 16S rRNA characterization, the four endophytic bacteria that potentially producing the antibiotic were *Bacillus cereus* RNS_01 (code CA01), *Pantoea agglomerans* ZFJ-15 (Code CA 02), *Bacillus subtilis* 55C1-1 (Code CA 03) and *Bacillus pumilus* SH-B11 (Code CA 04).

ACKNOWLEDGEMENTS

The author would like to say special thanks to The Rector of Andalas University for his support this research under Professor Research Grant Project, Andalas University 2017, with Contract Number: 17/UN.16.17PP.HGB/LPPM/2017.

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