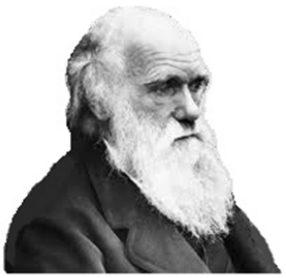
Available online at www.worldnewsnaturalsciences.com



World News of Natural Sciences

An International Scientific Journal

WNOFNS 31 (2020) 1-8 EISSN 2543-5426

Antimicrobial Potency from Endophytic Bacteria of Bignay Plant (*Antidesma bunius* (L.) Spreng.) Against Pathogenic Bacteria

Ida Indrawati*, Nia Rosiana, Andita Fitri Mutiara Rizki, Nabila Nada

Biology Department, Faculty of Mathematics and Life Sciences, Universitas Padjajaran, Jatinangor,Sumedang, Jawa Barat 45363, Indonesia

*E-mail address: ida.indrawati@unpad.ac.id

ABSTRACT

The need for new drugs is increasing in the antibiotic field. However, synthetic antibiotics often have a bad impact on body health, hence, in this paper, endophytic bacteria is investigated as an antibiotic source. The purpose of this research is to gain an understanding of the antibacterial potency of the endophytic bacteria associated with the Bignay plant (*Antidesma bunius*) against pathogenic bacteria that is Gram-negative i.e *Salmonella thypimurium* ATCC 14028, *Escherichia coli* ATCC 35218 and Gram-positive i.e *Staphyllococcus aureus* ATCC 6538. This research used the descriptive method and consists of endophytic bacterial isolation, identification, and antimicrobial testing. The endophytic bacterial isolation from fruit and stem of Bignay plant was performed by using the pour plate method, while the bacteria was identified by applying a biochemical test method using Vitek 2.0. The antimicrobial test was performed by way of paper disc diffusion. The resulting endophytic bacteria isolated from the fruit and stem of the Bignay plant are *Bacillus pumilus, Bacillus amyloliquefaciens, Bacillus cereus, Bacillus* sp.1, and *Bacillus* sp.2. The most potential endophytic bacteria as antimicrobial against pathogenic bacteria were found to be *Bacillus pumilus, Bacillus* sp.1, and *Bacillus* sp.2.

***Keywords*:** Endophytic, Antibacteria, *Antidesma bunius,* Gram stain

1. INTRODUCTION

Recently, pathogenic bacteria causing human infection has become a global health problem, and this is one factor in the high rates of death throughout the entire world. In addition,

(Received 28 March 2020; Accepted 20 April 2020; Date of Publication 22 April 2020)

the level of virulence of pathogenic microorganisms are also increasing. Moreover, the need to use antibiotic substances at greater that prescribed dose is also one of the problems of bacterial resistance against commonly used antibiotics.

Therefore, a new type of antibiotic derived from natural sources is needed. One of these is the utilization of endophytic bacteria. Endophytic bacteria are organisms that live in the tissue and do not cause disease in its host plants (Anjum and Chandra, 2015). Bacterial endophyte has the potential to be developed as a source of new antibiotics (Purwanto, 2014).

Utilization of endophyte antibacterials could come from plants that are located in Indonesia. The existing biodiversity in Indonesia is a natural resource that is very valuable, particularly for the benefit of Indonesian society, however, the richness and biodiversity has not been fully explored, so that the potential of natural biological resources in Indonesia is still not yet exploited optimally.

2. EXPERIMENTAL / RESULT (MATERIALS AND METHODS)

The experiment was conducted using a descriptive method that consists of 3 stages: 1) endophytic bacterial isolation, 2) identification and 3) antimicrobial test. Material used in this research is the bacteria found on Bignay (*Antidesma bunius*) stems and fruit, the bacteria test culture *Staphyllococcus aureus* ATCC 6538, *Salmonella thypimurium* ATCC 35218, *Escherichia coli* ATCC 35218, and *Bacillus cereus*. Other materials used in this research include water pepton, water fuschin, alcohol 70 and 96%, akuades sterile antibiotic amoxicillin, Buffer Pepton Water, gentian violet, lugol, immersion oil, physiological NaCl 0.85%, 0.5% NaOCl,), paper disk, filter paper, Nutrient Agar (NA), Nutrient Broth (NB), Blood Agar, Salmonella-Shigella Agar (SS Agar), Triptyc Soy Agar (TSA), and Vitek Card.

2. 1. Endophytic Bacterial Isolation from Bignay Stems and Fruit

Isolation of Endophytic Bacteria from Bignay (*Antidesma bunius*) stem and fruit samples was performed by first washing these in flowing water to eliminate macroscopic impurities. The samples were subsequently soaked in alcohol 96% solution for 2 minutes, then in a solution of NaOCl 0.5% for 5 minutes to remove superficial microbial epiphyte and environmental contaminants. Bacterial isolation was performed using the Pour Plate method, taking the 3 last dilutions for further processing. As a result, we were able to obtain 10 isolates of bacteria (5 from the fruit and 5 isolates from the stem).

Table 1. Macroscopic Characteristics of Endophytic Bacteria Isolates

Part of Plant	Isolate	Colony Form	Color	Surface	Edge	Elevation
Fruit	6B-A	Irregular	White	Smooth	Wavy	Convex
Fruit	6B-B	Round	White	Rough	Scalloped	Convex
Fruit	6B-D	Round	White	Rough	Serrated	Convex

Fruit	6B-E	Round	White	Rough	Serrated	Convex
Fruit	7B-H	Round	White	Smooth	Scalloped	Flat
Stem	5T-A	Round	White-Yellowish	Smooth	Flat	Convex
Stem	5T-B	Round	White	Smooth	Flat	Convex
Stem	5T-C	Round	White	Smooth	Wavy	Convex
Stem	7T-D	Round	White-Yellowish	Smooth	Flat	Convex
Stem	6T-F	Round	White-Yellowish	Smooth	Flat	Convex

2. 2. Identification of Endophytic Bacteria

The bacterial endophyte was identified by means of macroscopic morphology, (elevation, colony form, edges and surfaces), using Gram stain to determine Gram group of bacteria and biochemical identification by means of applying Vitek 2.0. From 10 bacterial endophyte isolates, we obtained 5 different species i.e., *Bacillus pumilus*, *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus* sp.1 and *Bacillus* sp.2.

Table 2. Results of Endophytic Bacteria Identification of Bignay Fruit and Stem samples

Part of Plant	**Isolate**	**Colony Form**
Fruit	6B-A	*Bacillus pumilus*
Fruit	6B-B	*Bacillus amyloliquefaciens*
Fruit	6B-D	*Bacillus* sp.1
Fruit	6B-E	*Bacillus* sp.2
Fruit	7B-H	*Bacillus amyloliquefaciens*
Stem	5T-A	*Bacillus cereus*
Stem	5T-B	*Bacillus cereus*
Stem	5T-C	*Bacillus cereus*
Stem	7T-D	*Bacillus cereus*
Stem	6T-F	*Bacillus cereus*

2. 3. Antimicrobial Activity Test

The antibacterial activity test was performed by using 5 different bacterial endophytes species from 10 species of bacteria found on the Bignay stems and fruit. The endophytic bacteria was tested for antibacterial activity by following the Kirby-Bauer method. Herein, paper discs were inserted into sterile vial bottles and endophytic bacterial suspension of each isolated species at 50 µl and equal to 0.5 McFarland ($1{,}5\times10^8$ CFU/ml) were added individually one endophyte to each paper disc. The culture was then incubated for 24 hours at a temperature 37 ºC. For examination purposes, 15 ml TSA media was added to a sterile petri dish and the agar was doped by placing 0.1 ml of pathogenic bacteria *S. aureus* ATCC 6538, *S. thypimurium* ATCC 14028, *E. coli* ATCC 35218, and *B. cereus* on its surface. The paper discs containing the incubated endophytic bacteria at 100% concentration were then placed on the surface of the agar medium, and the plate incubated for 24 hours at 37 ºC. The diameters of the zones of inhibition produced by bacterial endophyte were compared with the control of antibiotic amoxicillin 25 µ g.

Table 3. Inhibition Zone of Antibacterial Activity Test of Endophytic Bacteria from Bignay Plant (*Antidesma bunius* L.) (mm)

No.	Isolate	*S. thypimurium*	*S. aureus*	*E. coli*	*B. cereus*
1.	6B-A (*B.pumilus*)	14	24	16	22
2.	6B-B (*B.amyloliquefaciens*)	14	16	14	12
3.	6B-D (*Bacillus* sp.1)	11	13	10	13
4.	6B-E (*Bacillus* sp.2)	15	18	15	20
5.	5T-A (*B.cereus*)	11	13	12	10
6.	Control	34	48	12	24

B. pumilus (isolate 6B-A) can be categorized as having very strong sensitivity (a diameter of inhibition zone of more than 20 mm (Pan, et al, 2009) and a percentage of inhibited bacterial growth of 50%) against *S. aureus* ATCC 6538 (24 mm) and *B. cereus* (22 mm). Moreover, it had a strong sensitivity (a diameter of inhibition of 10-20 mm) against *E. coli* ATCC 35218 (16 mm) and *S. typhimurium* ATCC 14028 (14 mm). This was in contrast to the research conducted by Kuta, et al., (2009) wherein a species of *B.pumilus* produced inhibition zone diameter of 5 mm against *S. aureus*.

The *bacterial endophytes* also produced inhibition zones against *S. thypimurium* ATCC 14028, the largest inhibition zone diameter being that produced by the suspension of *Bacillus* sp. 2 (isolates 6B–E) (15 mm and a percentage of inhibited bacterial growth of 55.8%). Furthermore, *B. pumilus* (isolates 6B-A) and *B.amyloliquefaciens* (isolates 6B-B) produced equal inhibition zone diameters of 14 mm and had percentages of inhibited bacterial growth of

58.8% against *S. thypimurium* ATCC 14028, while the species *Bacillus* sp.1 and *B. cereus* produced inhibition zone diameters of 11 mm and percentages of inhibited bacterial growth of 67.6%.

In our study, the species *B. amyloliquefaciens* (isolates 6B-B) produced an inhibition zone diameter of 16 mm with percentage of inhibited bacterial growth of 66.7%. Again, this was in contrast to a study by Hui Sun, et al 2013 wherein *B. amyloliquefaciens* did not generate an inhibition zone against bacteria *S. aureus*. We also saw that *Bacillus* sp.2 produced an inhibition zone diameter of 18 mm, and a percentage of inhibited bacterial growth of 62.5%. In addition, *Bacillus* sp.1 and *B. cereus* produced inhibition zone diameters of 13 mm, with percentage of inhibited bacterial growth of 72.9%.

B. pumilus bacteria produce the secondary metabolite compound pumilacidin which is derived from lipopeptida. The compound pumilacidin is included in the antimicrobial peptides (AMPs) that include basitrasin, and which are a class of non-ribosomal peptide synthesisizers known to be effective in killing *Micrococcus luteus* and *S. aureus* (Chandra, et al, 2014). Furthermore, according to Bottone and Peluso (2003), *B. pumilus* bacteria are effective against fungi *Aspergillus* spp. and *Mucor* spp.

B. amyloliquefaciens are a Gram-positive bacterium group that produce potent antibacterial secondary metabolite amylociclycin compounds. The amylociclycin compounds are composed of iturin and fengisin (Scholz, et al, 2014). The compound iturin and fengisin belong to same class of non-ribosomal peptide synthesis as basitrasin. The antibacterial iturin and fengisin are antagonistic to pathogenic bacteria and to phytopathogenic fungi like *Aspergillus* spp. (Chandra, et al, 2014).

Bacillus sp. 1 has a strong sensitivity towards the four test bacteria. In our study, the diameter of inhibition zone was 13 mm against *S. aureus* ATCC 6538 and *B. cereus*, 11 mm against *S. typhimurium* ATCC 14028, and 10 mm against *E. coli* ATCC 35218. The bacterium *B. cereus* with yellow pigments also has strong sensitivity against the four pathogenic bacteria, including an inhibition zone of 13 mm against *S. aureus* ATCC 6538, 12 mm against *E. coli* ATCC 35218, 11 mm against *S. typhimurium* ATCC 14028, and 10 mm against *B. cereus*. We found that the diameter of inhibition zone which is produced by endophytic bacteria is inversely proportional to the percentage of bacterial growth, i.e., the larger the diameter of zones of inhibition generated, the smaller the percentage growth of bacteria.

The greatest diameter of inhibition zone was induced by a suspension of *B. pumilus* isolates (6B-A) against test bacteria *S. aureus* ATCC 6538 (24 mm), then against *B. cereus* (22 mm). *Bacillus* sp. 2 (isolates the E-6B) against *B. cereus* i.e. produced an inhibition zone of 20 mm, while the suspension of *B.amyloliquefaciens* against bacteria test *S. aureus* ATCC 6538 produced a diameter of inhibition zone of 16 mm.

This five species of endophytic bacteria has inhibition zones larger through suspension as compared to deposition and supernatant. This shows that endophytic bacteria suspensions produce more effective antibacterials as compared to deposition and supernatant production. However, a deposition of endophytic bacteria was found producing antibacterials against bacteria *S. aureus* ATCC 6538. What is more, Khandelwal and Khurana (2016) state that the concentration of proteins as antibacterial compounds in sediment is higher than the concentration of protein in the supernatant bacteria. In our study, the sediment extracts of the leaf produced inhibition zones, while the inhibition zone of the supernatant did not generate inhibition zones against *S. aureus*. Therefore, deposition produced inhibition zones against *S. aureus* ATCC 6538, which means that it is more effective as an antibiological. It should also

be noted that the difference in the rate of growth of the bacteria is influenced by secondary metabolite growth and that each species of bacteria has a different rate of growth.

3. CONCLUSIONS

Based on the research that has been done, it can be concluded that there are 10 bacterial endophytes that can be isolated from the stem and fruit of Bignay. In our work, we extracted 5 species, namely *Bacillus pumilus*, *Bacillus amyloliquefaciens*, *Bacillus* sp.1, *Bacillus* sp.2 and *Bacillus cereus*. Of those listed, bacterial endophyte *Bacillus pumilus* has the strongest antibacterial activity against pathogenic bacteria *S. aureus* ATCC 6538.

Acknowledgement

The authors would like to acknowledgement to Ministry of Research, Technology and Higher Education for providing Penelitian Dasar Unggulan Perguruan Tinggi who was supporting this work.

References

[1] Anjum Nawed and Ramesh Chandra. Endophytic Bacteria: Optimizaton of Isolation Procedure From Various Medicinal Plants and Their Preliminary Characterization. *Asian Journal of Pharmaceutical and Clinical Research* 8 (4) (2015) 233-238

[2] Baruzzi F., L. Quintieri, M. Morea, and L. Caputo. Antimicrobial Compounds Produced by Bacillus spp. and Applications in Food. *FORMATEX* (2011) 1102-1111

[3] Belmi, R. M., Joe, G., dan Myra, L. Tansengco. *Antidesma bunius* (Bignay) Fruit Exctract as an Organic Pesticide Against Epilachna spp. *Journal of Asian Scientific Research* 4 (7) (2014) 320-327

[4] Butkhup, L., and Samappito S. Changes In Physico-Chemical Properties, Polyphenol Compounds and Antiradical Activity During Development and Ripening of Maoluang (*Antidesma bunius* L. Spreng) Fruits. *Journal of Fruits and Ornamental Plant Research* 19 (1) (2011) 85-99

[5] Bottone, Edward, J and Richard, W.P. Production by Bacillus pumilus (MSH) of An Antifungal Compound That Is Active Against Mucoraceae and Aspergillus Species: Preliminary Report. *Journal of Medical Microbiology* 52 (2003) 69-74

[6] Chandra, Datta, S., Byung, W.Y., In-Cheol, Y., Young, T. H. Antimicrobial Peptides of the Genus Bacillus: A New Era For Antibiotics. *Canadian Journal of Microbiology* 61(2) (2014) 93-103

[7] Habib, R., Mominur, R., Kaiser, H., Obayed, R., Mohammed, A. S. Phytochemical Screening, Cytotoxicity, Antioxidant Capacity and Antibacterial Potentiality of Methanol Extract of Antidesma ghaesembilla Gaertn. *Advances in Natural and Applied Sciences* 5 (2) (2011) 69-74

[8] Hila, Naxhije, Ariola, D., Klementina, P., Sotir, M., Zheni, B., Erleta, P., and Aida, D. The Dynamics of Antimicrobial Resistance of Salmonella thypimurium Isolates. *Journal of IMAB* 17 (1) (2011)

[9] Hui, S., Yan, H., Qing, X., Renyuan, Y and Yongqiang, T. Isolation Characterization, and Antimicrobial Activity of Endophytic Bacteria from Polygonum cuspidatum. *African Journal of Micribiology Research* 7(16) (2013) 1496-1504

[10] Konrad, P., Adriana, E., Rosat and Grzegor, W. Staphylococcus aureus as an Infectious Agent: Overview of Biochemistry and Molecular Genetics of its Pathogenicity. *Journal Acta Biochimica Polonica* 56 (4) (2009) 597-612

[11] Kuta, Faruk A., Lohya, N and Priscilla, Y. O. Screening of *Bacillus* Species with Potentials of Antibiotics Production. *Applied Medical Informatic* 24 (1-2) (2009) 42-46

[12] Lizardo, R. C. M., Mabesa, L. B., Dizon, E. I. and Aquino, N. A Functional and Antimicrobial Properties of Iignay (*Antidesma bunius* L.Spreng.) Extract and its Potential as Natural Preservative in a Baked Product. *International Food Research Journal* 22(1) (2015) 88-95

[13] Orwa, C., Mutua, A.. Kindt, R., Jamnadass, R and Simans, A (2015). Agroforestry Database: A Tree Reference and Selection Guide Version 4.0 Http://www.worldagroforestry.org/af/treedb/

[14] Pan, X., Chen F., Wu, T., Tang, T,H., and Zhao, Z. The Acid, Bile Tolerance and Antimicrobial Property of Lactobacillus acidophilus NIT. *Journal Food Control* 20 (2009) 598-602

[15] Prihandani Suryatmiati, S., Poeloengan, M., Noor, S. M and Andriani. Antibacterial Test of Garlic (Allium sativum L.) Against Staphylococcus aureus, Escherichia coli, Salmonella typhimurium and Pseudomonas aeruginosa in Improving Food Safety. *Agricultural Informatics* 24 (1)(2015) 53-58

[16] Purwanto, Safra, U.M., Pasaribu, F.H., Bintang, M. Isolation of Endophytic Bacteria from Green Betel Plants (Piper betle L.) and its Potential as Producer of Antibacterial Compounds. *Curr. Biochem* 1 (1) (2014) 51-57

[17] Puspitasari, E. and Ulfa, E. U. Cytotoxicity Test of Buni Fruit (*Antidesma bunius* L. Spreng) Methanol Extract on Hela Cells. *Journal of Basic Sciences* 10 (2) (2009) 181-185

[18] Scholz, R., Joachim, V., Anto, B., Zhiyuan, W., Yueqiu, H., Kristin, T., Torsten, S., Stefanie, H., Peter, L., Rainer. B. Amylocyclicin, a Novel Circular Bacteriocin Produced by Bacillus amyloliquefaciens FZB42. *Journal of Bacteriology* 196 (10) (2014) 1842-1852

[19] Shaikh, Sibhghatulla, Jamale Fatima, Shazi Shakil, Syed Mohd. Danish Rizvi, and Mohammad Amjad Kamal. Antibiotic Resistance and Extended Spectrum Beta-lactamases: Types, Epidemiology and Treatment. *Saudi Journal Biol Sci*. 22(1) (2015) 90-101

[20] Tallent, Sandra M., Kristin M. Kotewicz, Errol A. Strain and Reginald W. Bennett. Efficient Isolation and Identification of Bacillus cereus Group. *Journal of Aoac International* 95 (2) (2012) 446-451