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Research Article

CHARACTERIZATION of ENDOPHYTIC *Bacillus toyonensis* BESP21 PRODUCING ANTIBIOTICS

Feskaharny Alamsjah, Anthoni Agustien *

Deparment of Biology, Andalas University, Kampus Limau Manis, Padang, Indonesia

*Corresponding Author Email: anthoniagustien@sci.unand.ac.id

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ABSTRACT

This study aims to determine the character of potential antibiotic-producing endophytic bacteria isolates and to determine the type of endophytic bacteria. Research carried out includes the characterization of endophytic bacteria and biomolecular identification of bacteria. The results showed that bacterial growth was up to 22 hours, which phase was stationary at 18 hours. The optimum condition of endophytic microbes showed that bacteria at a temperature of 33^0 C; pH 7.0; and salinity at NaCl 0.5%. Biomolecular identification using 16S rRNA method, based on phylogenetic and evolutionary distance, BESP21 isolates have a very close relationship with *Bacillus toyonensis* BSP21.

Keywords: characterization, *Bacillus toyonensis* BESP21, endophytic, antibiotics

INTRODUCTION

Endophytic bacteria are bacteria that live in in host plant tissue without causing it symptoms of the disease[1]. It is known that certain types of endophytic bacteria have the ability to produce secondary metabolites in the form of active compounds that are antibiotic[2]. The ability of endophytic microbes to produce secondary metabolites in accordance with their host plants is a very large and reliable opportunity to produce secondary metabolites from endophytic microbes isolated from the host plant[3]. The ability of endophytic bacteria to produce active compounds in the form of antimicrobials is a potential that can be developed considering that generally active compounds are obtained by extracting plants, especially plants drug. To obtain active compounds from plants more complicated time and process are needed than if extracting active compounds from bacteria[4]. Preliminary research found that BESP21 bacterial isolates isolated from *Piper sarmentosum* betel plants which live wildly in the forest of Biology education and research at Andalas University, Padang, Indonesia, have the ability to produce antibiotics that are broad-spectrum and potentially indicated, to know the character and type of bacteria from the BESP21 isolate. Biomolecular applications to detect and identify microorganisms with certain molecular markers such as 16S rRNA or its coding genes up to now are more often used to explore bacterial diversity and analyze bacterial community structures[5]. The identification of endophytic bacteria in medicinal plants was carried out using the 16S rRNA and universal primers methods used as DNA amplifiers[6].

MATERIALS AND METHOD

Profile of microbial growth and antibiotic activity

After bacterial rejuvenation in the NA medium, an inoculum was made by inoculating the culture into 50 ml of the production medium with the composition per 1 L medium containing 3% corn water immersion, 3% sucrose, 0.5% CaCO3, 0.2% MgSO4, FeSO4 0.1%, ZnSO4 0.01% at pH 7.0. The cultures were incubated at room temperature, agitation 150 rpm for 24 hours. Five ml of inoculum was inoculated on Erlenmeyer containing 95 ml of antibiotic production medium. Sampling as much as 1 ml of culture every two hours interval for 24 hours fermentation. Determined the number of bacteria by pour plate method and also tested the antibiotic inhibition of *E. coli* and *S. aureus*.

Bacteria endophytic abiotics conditions

The character of isolates against abiotic effects was carried out by bacterial isolates grown on a liquid medium with variations in growth factors such as temperature (30, 33, 35 and 37^0 C), pH of the medium (pH 4, 5, 6, 7 and 8) and salinity (NaCl 0 .1; 0.5; 1.0; 3.0 and 5.0 %). Then the bacterial population was determined by the spectrophotometric method at a wavelength of 600 nm. The data obtained illustrates the optimum conditions of temperature, pH and salinity for the growth of each bacterium.

DNA Isolation and Amplification of DNA of Endophytic Bacteria

Identification was carried out using molecular analysis based on 16S rDNA fragments in bacteria. Genomic DNA was isolated following the modified Pitcher *et al* (1989) method. As much as 1 ose of bacteria is inserted into a tube containing TE buffer, then the tube is flipped back until homogeneous and centrifuged for 5 minutes at a speed of 13000 rpm. Then added 50uL of lysozyme 50µg / ml and incubated at 37° C for 30 minutes. Add successively an extraction buffer of 300 ul (shake until homogeneous), 150ul sodium acetate (then let stand for 10 minutes), and 500 ul chloroform (then back and forth in a row). The tube is then centrifuged at 10,000 rpm for 10 minutes. The supernatant was transferred to a new 1.5 mL tube. Add isopropanol in a ratio of 1: 1 (supernatant: isopropanol). The tube was centrifuged again at 13,000 rpm for 10 minutes. Remove the supernatant. DNA pellets were washed with 70% ethanol, centrifuged at 13,000 rpm for 10 minutes. The tube containing the

DNA genome is dried and added 50 ul TE 1x. Amplification of 16S rDNA fragments was carried out using GoTaq (Promega) with 27F primers (5'-AGAGTTTGATCCTGGCTCAG-3 ') and 1492R (5'-GGTTACCTTGTTACGACTT-3')[7,8].

DNA base sequences

PCR products that have been purified using a DNA purification system kit and carried out squeezing at INACC, LIPI, Cibinong. DNA base sequences then analyzed for similarity were carried out using the Basic Local Alignment Tool at NCBI. Evolution analysis is carried out using the Clustal, Bio Edit and MEGA5 programs. The results obtained can show the type of betel endophytic bacteria.

RESULTS AND DISCUSSION

Growth of BESP21 endophytic bacteria isolates and antibiotic activity

The bacterial isolate BESP21 in its lifetime consists of several phases, in the first phase BSP21 isolates in the lag phase, which is for 2 hours (0-2 hours incubation), this means that the isolation period in the growth media for two hours. While the duration of the exponential phase in isolates of endophytic bacteria BESP21 for 16 hours (2-18 hours incubation). Isolate of BESP21 endophytic bacteria with a duration of 4 hours in the stationary phase (18-22 hours incubation) (Fig.1). In the lag phase, bacterial cells are in a state of adaptation, which in this phase is characterized by conditions for the absence of cell growth. In the exponential phase, cells are very actively dividing, so that the number of living cells is more than that of dead cells, in this phase the cell growth rate is very high. The difference in the exponential duration of each isolate is due to the factors that affect the growth of these bacteria in each type. These factors include pH, temperature, composition of growth media.

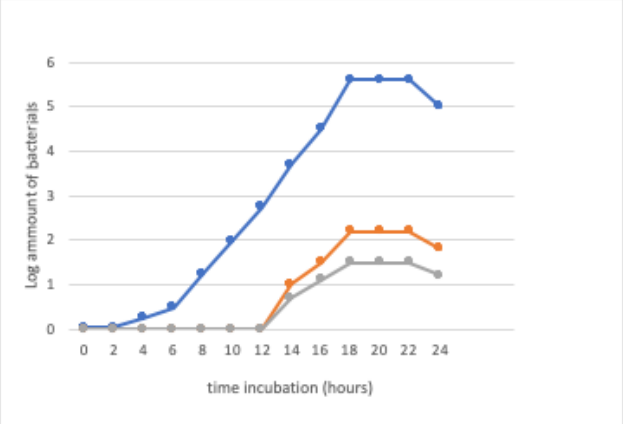

Fig.1: Growth curve of isolates of endophyte bacteria BESP21; bacteria, antibiotic activity against *E. coli*; antibiotic activity against *S. aureus*

Besides other factors that influence it are the generation time of each of these isolates can be different, depending on the genes responsible for cell division. In the stationary phase, it is characterized by the number of bacterial cells that live in balance with those of dead cells and the formation of secondary metabolites. The length of the stationary phase depends on the nutrients that are still present, the secondary metabolites formed which can affect the life of the bacteria. Based on the growth curve, it can be said that to isolate antibiotics from culture was carried out at 18 hours of incubation.

Effect of temperature on bacterial growth

Isolate of BESP21 endophytic bacteria can live in a temperature range in the range of 30 to 37^0 C, this shows that based on temperature, bacterial isolates include groups of mesophilic bacteria or bacteria that live at moderate temperatures. The optimum temperature condition of BESP21 isolate is at 33^0 C. The temperature factor is one of the important abiotic factors for bacterial life, this is because temperature can affect metabolism that occurs in bacterial cells, especially against the catalytic work of enzymes in anabolism and catabolism in cells. The optimum temperature of each bacterial isolate is the result of all the temperatures needed by cells in their life activities. *Bacillus toyonensis* isolated from tea plant rhizosphere soil of Nilgiri Hills, India have an optimum temperature of 37 C in producing IAA hormone[9].

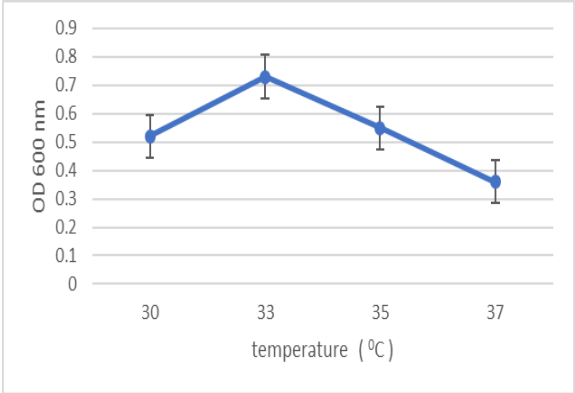

Fig.2: Effect of temperature on bacterial growth

Effect of pH on bacterial growth

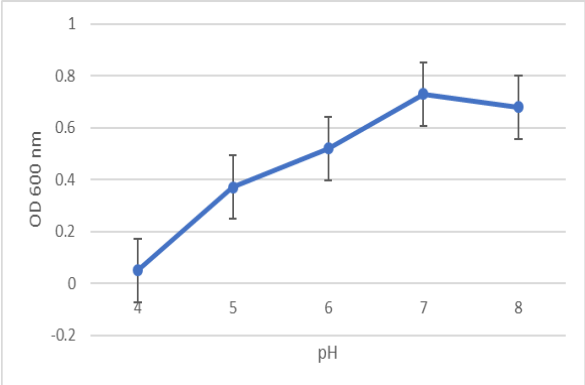

Fig.3: Effect of pH on bacterial growth

Effect of pH of the medium on the growth of endophytic bacterial isolates, where bacterial isolates can live from intervals of pH 4.0 to 8.0 (Fig. 3). This shows that bacteria can live in an acidic, neutral or alkaline condition. Isolate of BESP21 endophytic bacteria optimum pH conditions at pH 7.0. This means that enzymatic catalytic work in bacterial cells is best at pH 7.0 or working in a neutral pH condition. Although bacterial isolates can live at pH 4 or in an acid conditions, the number of cells is small and significant when compared to pH 7 or neutral environment. The pH factor is one of the main factors for bacterial life, because it involves enzymatic reactions that occur in the bacterial cell. Enzymes which are mostly proteins consisting of amino acids, the mechanism of catalytic action of the enzyme is strongly influenced by level of ion hydrogen. pH of microbial growth

media affects cells where redox reactions affect enzymatic reactions and assimilation of nutrients[10].

Effect of salinity on bacterial growth

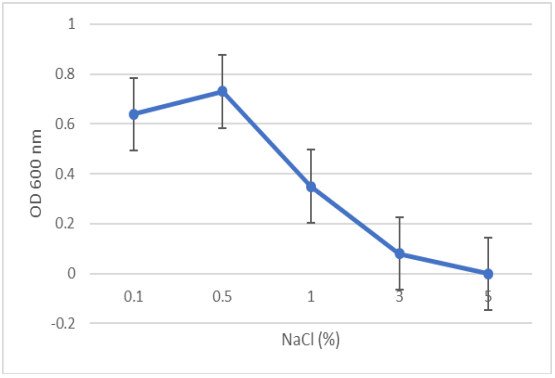

Fig. 4: Effect of salinity on bacterial growth

BESP21 endophytic bacteria isolates can live at 0.1 - 3% NaCl concentration, but cannot live at 5% NaCl. The survival of bacterial isolates against the salinity environment is very dependent on the mechanism of the cell membrane in maintaining the balance of NaCl concentration. A very high concentration of NaCl will cause the solution in the cell to exit the cell or plasmolysis, causing bacterial cells to die. 0.5% NaCl is the most optimal salinity level for isolates of BESP21 endophytic bacteria. Low concentration salinity can cause immediate dissolution of substances that cause loss of physical stress cells. Whereas media with high salinity concentration causes water flow followed by an increase in compatible solutes such as proline, glutamate, glycine betaine, ectoine and trehalose[11].

Identification of bacteria by 16S rRNA method

Figure 5 shows that BESP21 endophytic bacteria isolates had the closest kinship relationship with *Bacillus toyonensis* LAMA 1111, *Bacillus cereus* IAM 11 and *Bacillus toyonensis* K22.1 and *Bacillus weihenstephanensis* TF3 with 98% similarity values. After biochemical testing and alignment of various sequences, BESP21 isolates can be said to be closer to *Bacillus toyonensis* K22.1, so isolates of BESP21 endophytic bacteria are *Bacillus toyonensis* strain BESP21.

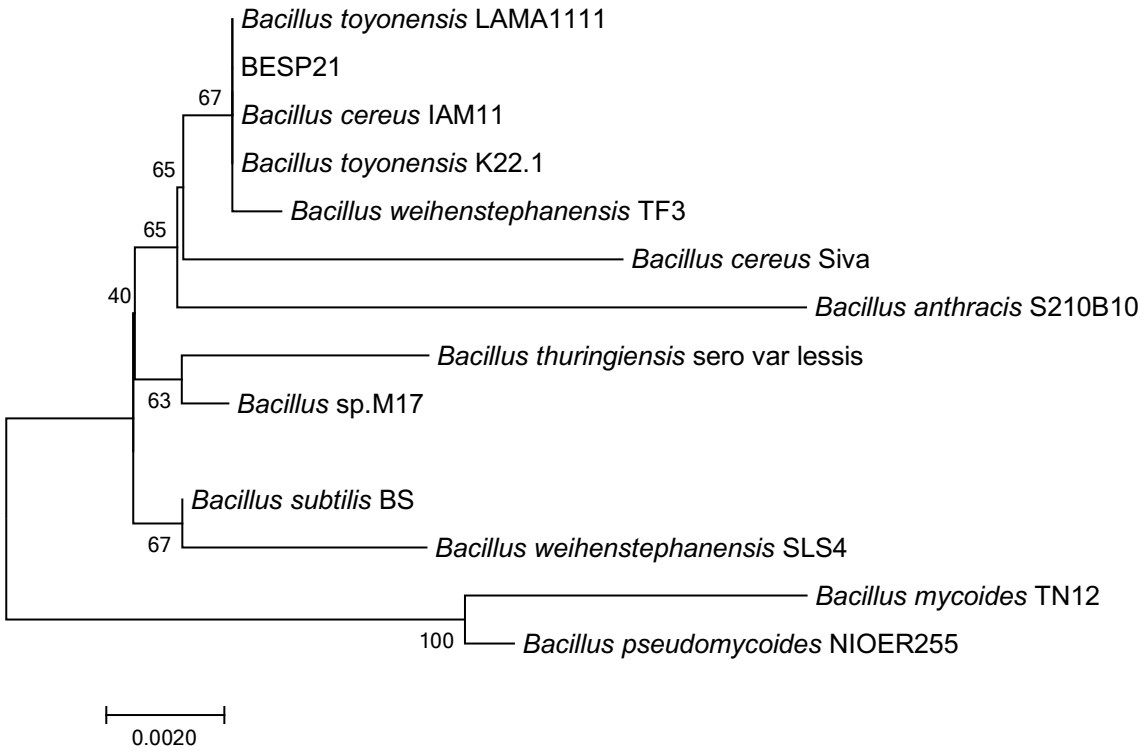

Fig. 5: Phylogenetic tree isolates BESP21 and other types of bacteria

Exploration of betel endophytic microbes in the North-East region of India, found *Muscodor albus* which is the endophytic microbes of *Piper nigrum* with have strong antibiotic activity[12]. Endophytic bacteria from betel *P. colubrinum* and *P. nigrum* also produce antibiotics[13]. Fourteen green betel or *P. betle* endophytic bacteria that live in the Bogor region with the highest number of isolates from leaf organs with one potential BS1 (*Pseudomonas* sp.) Isolate which can inhibit the growth of three pathogenic bacteria[14].

CONCLUSION

The results showed that bacterial growth was up to 22 hours, which phase was stationary at 18 hours. The optimum condition of endophytic microbes for temperature, pH and salinity showed that bacteria at a temperature of 33^0 C; pH 7.0; and salinity at NaCl 0.5%. Biomolecular identification using 16S rRNA method, based on phylogenetic and evolutionary distance, BESP21 isolates have a very close relationship with *Bacillus toyonensis* BSP21.

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