Exploration of endophytic bacteria for inducing plant growth and diseases resistance to vascular streak dieback (*Ceratobasidium theobromae*) on cacao

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Abstract. Indonesia is the third largest cocoa production in the world after Ghana and Ivory Coast. The yield loss of cacao is drastically limited by the cacao diseases. Endophytic bacteria has been reported plays important role in inducing plant growth and diseases resistance. The aim of this research is to explore the endophytic bacteria capable to induce plant growth and diseases resistance to vascular streak dieback on cacao. The research step included: (1) isolation of endophytic bacteria from cacao tissues; (2) selection of endophytic bacteria as inducer of plant growth; (3) selection of endophytic bacteria as inducer of cacao plant resistance to *Ceratobasidium theobromae*; and (4) identification of potential isolates. Isolation of endophytic bacteria from Southeast Sulawesi, West Sumatera, and West Java resulted 288 bacterial isolates, and 40 of them induced strongly cacao seed germination. Further characterization on inducing plant growth characters and production of metabolite compound resulted 15 bacterial isolates for further analysis. Analysis phytoalexin compound produced by cacao seedling treated with endophytic bacteria revealed 10 isolates increases the relative ratio of Cyclohexene, 2-Methoxy-guaiacol, and 2,3- Dihydrobenzofuran, indicated inducing plant resistance to *C. theobromae*. Molecular identification showed highly similarity to *Bacillus cereus*, *Pseudomonas geniculata*, *Stenotrophomonas rhizophila*, and *S. maltophilia*, respectively.

Keywords: biological control, induced systemic resistance, defense respond

1. Introduction
Cocoa is the third largest internationally traded commodity after sugar and coffee [1]. Indonesia is ranked as the 3rd largest cocoa producing country in the world [2]. Vascular Streak Dieback (VSD) in coca plants caused by *Ceratobasidium theobromae* have been reported causing 3-60% yield loss. The spores (basidiospores) of this fungus is spread by the wind and initially infects very young leaves at the flushing stage. After penetration through leaves, subsequent developments lead to petioles and ultimately to the branches of plants and colonizing xylem tissue causes browning discoloration [3]. *C. theobromae* is known to be obligate (cannot be cultured on artificial media). Therefore, detailed research on this fungus is also still very limited. Molecular analysis is the main tool in the study of taxonomy and epidemiology. However, interesting things were reported by Samuel et al. 2012 [4] that based on ITS sequence analysis (Internal Transcribed Spacer) and phylogenetic studies of this fungus...
have a very high closeness to Ceratobasidium so that the pathogen causing VSD which was previously referred to as Oncobasidium theobromae was proposed as C. theobromae species.

This disease is difficult to control with conventional techniques because it infects the xylem tissue of protected plants. The use of fungicides can cause negative impacts (environmental pollution, emergence of pathogenic resistance, and killing of non-target organisms). Meanwhile the use of resistant varieties and technical culture was allegedly not able to control VSD in the field.

In order to support the reduction of the use of pesticides and environmentally friendly agricultural practices, pest and disease control has been developed with biological control based on the use of natural enemies or biological agents. Biological control of plant pathogens is based on the mechanism of antibiosis, competition, hyperparasitism and induction of resistance in host plants. Endophytic bacteria, one of the beneficial biological agents that inhabit plant tissues where C. theobromae colonize and cause disease in plants, can be developed as an environmentally friendly control technique. Konate et al. 2015 [5] reported that many endophytic bacteria were successfully isolated from the roots and stems of cocoa seedlings with an abundance of about 4.8 x 10^3 g^-1 plant tissue. The composition of endophytic bacterial species includes the genus Bacillus (67%), Clostridium (15%), Actinomycetes (11%) and Pseudomonas (7%). Based on these results, this study aims to obtain endophytic bacteria that can trigger plant growth and induce plant resistance to C. theobromae attacks.

2. Materials and methods

2.1. Sampling and isolation of endophytic bacteria

Sampling was carried out on healthy cacao plants among the expanse of cacao plants that were attacked by VSD disease. Plant parts taken include the shoots, leaves, and branches of cocoa in healthy plants that are between the cocoa plants that show symptoms of Vascular Streak Dieback. Sampling locations were carried out in three locations each on the islands of Sumatra, Sulawesi and Java. Samples that have been taken are placed in a cooler to avoid damaging plant tissue during the trip.

Isolation of endophytic bacteria from the shoots, leaves and branches of the cocoa plant was carried out based on the method of Hallmann et al. 1997 [6] and Munif et al. 2012 [7]. Parts of the plant that had been surface sterilized were macerated and added with sterile water at a ratio of 1:10, then the mixture and plant tissue were shaken on a shaker with a speed of 150 rpm for 60 minutes. The liquid is taken as much as 1 ml, carried out serial dilution and spread on the appropriate culture media. Three culture media used were Nutrient Agar, Triptic Soy and Water-Yeast extract-Agar [8].

2.2. Screening of endophytic bacteria as antagonist agent candidate

2.2.1. Cocoa seed germination test. Application of endophytic bacteria in cocoa seeds was carried out by immersion method. Each of endophytic bacterial colony was grown in a suitable liquid medium for 12 hours on a 100 rpm incubator. Cocoa seed was immersed in endophytic bacterial suspension (10^8 - 10^9 cfu mL^-1) for 2 hours. As many as 50 cacao seeds that have been treated are grown on sterile filter paper that has been moistened with sterile aquades in a petri dish. Observations were made on the germination of cocoa seeds 7 days after treatment.

2.2.2. Hypersensitivity reaction test. Hypersensitivity reaction tests were carried out on the leaves of tobacco plants in the White Burley variety. Each single bacterial colony was cultured on 3 ml of NB media for 24 hours in a 100 rpm incubator. The bacterial suspension is infiltrated in tobacco leaves. Hypersensitivity reactions are observed 24-48 hours after inoculation [8].

2.2.3. Hemolysis test on blood agar. A pure culture oose from each 24-hour-old endophytic bacterial isolate was scratched on blood agar media. The endophytic bacterial isolate was incubated at 37 ºC for 48 hours. Observations were made on hemolysis activity which was marked by the formation of clear
zones around the isolates. Isolates with negative hemolysis activity were used for further treatment [9] [10].

2.2.4. **Chitinolytic activity test.** Chitinolytic activity test was carried out on the media of colloidal chitin 0.2% (K$_2$HPO$_4$ 4 g L$^{-1}$, KH$_2$PO$_4$ 0.7 g L$^{-1}$, NaCl 0.3 g L$^{-1}$, FeSO$_4$·7H$_2$O 0.01 g L$^{-1}$, MnCl$_2$ 0.001 g L$^{-1}$, yeast extract 3 g L$^{-1}$, and agar 20 g L$^{-1}$) [11]. A total of 5 µL of endophytic bacterial suspension was dripped on a 0.6 mm diameter sterile filter paper placed on the surface of colloidal chitin media. Incubation is carried out for 4-7 days at room temperature. Chitinolytic activity is indicated by the formation of clear zones around endophytic bacteria colony.

2.2.5. **Phosphatase production test.** The test was carried out to determine the ability of endophytic bacteria to produce phosphatase enzymes. Tests were carried out using Pikovskaya agar (glucose 5 g L$^{-1}$, Ca (PO$_4$)$_2$ 2.5 g L$^{-1}$, (NH$_4$)$_2$SO$_4$ 0.5 g L$^{-1}$, KCl 0.2 g L$^{-1}$, MgSO$_4$·7H$_2$O 0.1 g L$^{-1}$, MnSO$_4$ 0.001 g L$^{-1}$, FeSO$_4$ 0.001 g L$^{-1}$, yeast extract 0.5 g L$^{-1}$, and 20 g L$^{-1}$ agar) [12].

2.2.6. **Siderofor production test.** Detection of siderophore production by endophytic bacteria was carried out by growing each endophytic bacterial isolate on agar medium Chroma Azurol Sulfonate (CAS agar) and analysed [13].

2.2.7. **IAA production test.** This test was conducted to see the ability of endophytic bacterial isolates to produce the hormone indole acetic acid (IAA) in vitro according to the method developed by Reetha et al. 2014 [14]. Analysis of IAA hormone levels was carried out using the colorimetric method. Supernatant is taken as much as 2 mL, plus Salkowsky reagent as much as 1 mL or in a ratio of 2:1. Incubation was carried out for 60 minutes, absorbance measurements were carried out using a spectrophotometer at a wavelength of 530 nm.

2.3. **Endophytic bacteria as a trigger growth of cocoa seeds**
Application of endophytic bacteria in cocoa seedlings was carried out by soaking the cocoa seeds for 2 hours before planting and spraying suspension of endophytic bacteria (10$^8$-10$^9$ cfu mL$^{-1}$) when the seedlings were 6 weeks after planting. Cocoa seeds that have been treated with endophytic bacteria were planted in a mixture of soil media, sand, and manure with a ratio of 2:1:1. Observations were made on the parameters of growth of cocoa seedlings (plant height, number of leaves and stem diameter at 10 weeks after treatment.

2.4. **Analysis of phytoalexin on cacao seedling**
The phytoalexin compound was identified using Pyrolysis-GC/MS-Shimadzu GCMS QP2010 (Shimadzu, Japan) at Integrated and Proximate Chemistry Laboratory, Forest Research and Development Center, Bogor with sample preparation referring to the Chaves and Gianfagna 2007 [15]. The result of GCMS-pyrolysis analysis is the area of chromatogram and the relative concentration of the compound.

2.5. **Identification of potential endophytic bacteria**
Molecular Identification of endophytic bacterial was started with the isolation of bacterial DNA based on the method of Sambrook and Russel 2001 [16]. Amplification of 16S rRNA gene using GenAmp PCR machine system 9700 by using a pair of 16S rRNA universal primers (forward primer 27F: 5'-AGA GTT TGA TCM TGG CTC AG-3' and reverse primer 1429R: 5'-CGG TTA CCT TGT TAC GAC TT TT -3') with 1500 base pairs (bp) PCR products [17]. DNA fragments were sent to the First Base Laboratory, Malaysia for sequencing. The nucleotides sequence of 16S rRNA genes were processed using the BioEdit application version 7.2.6.1. Analysis of the level of homology or DNA similarity compared to other nucleotides in the GenBank Database was processed using the program Basic Local Alignment Search Tools (BLAST) [18].
3. Results

3.1. Endophytic bacteria isolates from cacao

Sampling of cacao tissue was taken from three provinces: Southeast Sulawesi (Labuea Village, North Moramo District (Latitude 04°06'0170" and Longitude: 122°33'4033") and Lebo Jaya Village, Konda District (Latitude: 04°05'6888" and Longitude: 122°28'1506"), Konawe Selatan Regency), West Sumatera (Taratak Village, Lubuk Sikarah District (South Latitude: 00°47'48,480" and Longitude 100°37'52,390") and Transad Village, Tanjung Harapan District (Latitude 00°45'24,720" and Longitude 100°38'20,647"), Solok Regency), and West Java (Ciadam Village, Mande District (Latitude: 6°45'10.2" and Longitude: 107°11’09.4"), Cianjur Regency). Isolation resulted 288 endophytic bacterial isolates for further characterization.

3.2. Selection of endophytic isolates as plant growth and diseases resistance inducer on cacao seedling

3.2.1. Effect of endophytic bacteria on cocoa seed germination. The selection of endophytic bacteria as a trigger for growth in cocoa seedlings was conducted by observing the effect of soaking cocoa seeds with isolates of endophytic bacteria on the germination of cocoa seeds. The treatment of 288 endophytic bacterial isolates on cacao seed showed variety respond i.e. inhibited, no effects, or increased cacao seed germination compared to controls (without the treatment of endophytic bacteria). Our results showed the average germination of cocoa seeds in the control was 80%). One example of germination of cocoa seeds treated with endophytic bacteria are shown in Figure 1.

Based on quantitative (percentage of cocoa seed germination) and qualitative (simultaneous germination) observations, as well as pathogenicity tests, 40 endophytic bacterial isolates were selected for further characterization. The treatment of selected endophytic bacteria showed 100% cocoa seed germination rates, and not pathogenic to plants and mammals (negative on HR and hemolysis test). Further characterization of 40 bacterial isolates as growth triggers (plant height, number of leaves, and stem diameter) and producing metabolites that are thought to be able to suppress C. theobromae (chitinase) and induce plant growth (phosphatase, siderophore, and indole acetic acid) are presented in Table 1.

The characterization of 40 isolates exhibited chitinolytic activity showed different plant growth effects. Further data revealed the 40 bacterial isolates which produced phosphatase, siderophore, and IAA as many as 13, 10, and 19 isolates, respectively.

The effect of the application of endophytic bacteria on the growth of cocoa seedlings was observed at 10 weeks after application. The test results showed that the endophytic bacteria application to cacao plant seedlings gave mixed responses to the growth parameters of cacao seedlings. The most prominent variable from the application of endophytic was plant height. PRBN13 isolates (isolates from Padang which were isolated from the twigs and bacteria were isolated using Nutrient Agar media) gave the highest addition of plant height (9.4 cm) compared to other isolates. However, some endophytic bacterial.
isolates provide inhibition of plant growth compared to controls such as isolate PRBN 23, PRBK 22 (Table 1).

| No | Isolates of Endophytic Bacteria | Diameter of Chitinolytic zone (cm) | Plant Height (cm) | Leaf Number | Diameter of Bassal Stem (cm) | Diameter of Phosphatase Zone (mm) | Diameter of Siderophore Zone (cm) | Indole Acetic Acid (µg/ml) |
|----|---------------------------------|----------------------------------|------------------|-------------|-----------------------------|-----------------------------------|---------------------------------|--------------------------|
| 1  | CPBN19                          | 1.20                             | 5.13             | 3.75        | 1.18                        | 0.00                              | 0.00                            | 7.00                     |
| 2  | CPBN22                          | 0.67                             | 0.58             | 5.25        | 0.78                        | 2.50                              | 0.00                            | 0.00                     |
| 3  | CPBN23                          | 0.83                             | 6.93             | 5.00        | 1.50                        | 0.00                              | 0.25                            | 28.00                    |
| 4  | CDBK8                           | 0.50                             | 5.13             | 3.75        | 1.08                        | 0.00                              | 0.00                            | 39.00                    |
| 5  | CRBT1                           | 0.73                             | 6.35             | 3.00        | 1.05                        | 6.98                              | 0.00                            | 12.00                    |
| 6  | CRBT2                           | 0.50                             | 8.13             | 5.00        | 0.43                        | 0.00                              | 0.00                            | 65.00                    |
| 7  | CRBT3                           | 0.73                             | 6.43             | 3.25        | 1.33                        | 2.10                              | 0.00                            | 46.00                    |
| 8  | CRBT4                           | 1.40                             | 3.53             | 2.75        | 0.83                        | 2.73                              | 0.00                            | 2.00                     |
| 9  | CRBT5                           | 0.93                             | 2.75             | 3.00        | 1.20                        | 3.23                              | 0.00                            | 0.00                     |
| 10 | CRBT6                           | 1.03                             | 5.20             | 3.75        | 0.88                        | 0.00                              | 3.40                            | 0.00                     |
| 11 | CRBT8                           | 0.77                             | 5.85             | 4.00        | 0.45                        | 0.00                              | 0.00                            | 22.00                    |
| 12 | CRBT9                           | 0.97                             | 3.28             | 3.75        | 1.30                        | 0.00                              | 0.00                            | 0.00                     |
| 13 | CRBT10                          | 0.73                             | 4.85             | 3.25        | 1.30                        | 0.00                              | 1.60                            | 0.00                     |
| 14 | PPBN4                           | 0.23                             | 3.25             | 0.75        | 1.05                        | 3.18                              | 0.00                            | 0.00                     |
| 15 | PPBT9                           | 0.83                             | 7.65             | 4.25        | 0.80                        | 7.18                              | 0.00                            | 27.00                    |
| 16 | PPBK15                          | 1.10                             | 2.6              | 3.50        | 1.85                        | 0.00                              | 2.00                            | 0.00                     |
| 17 | PPBK17                          | 1.03                             | 4.90             | 4.25        | 1.00                        | 0.00                              | 0.00                            | 0.00                     |
| 18 | PDBN6                           | 0.97                             | 6.73             | 4.76        | 1.03                        | 0.00                              | 4.10                            | 16.00                    |
| 19 | PDBT10                          | 0.73                             | 5.40             | 2.75        | 1.75                        | 1.95                              | 0.00                            | 12.00                    |
| 20 | PDBT14                          | 1.47                             | 5.70             | 2.50        | 0.45                        | 0.00                              | 0.00                            | 0.00                     |
| 21 | PDBT24                          | 0.87                             | 6.38             | 3.25        | 0.50                        | 0.00                              | 0.00                            | 0.00                     |
| 22 | PDBT29                          | 1.57                             | 6.35             | 4.75        | 1.03                        | 0.00                              | 0.00                            | 76.00                    |
| 23 | PRBN5                           | 0.33                             | 3.70             | 2.25        | 1.38                        | 0.00                              | 1.60                            | 0.00                     |
| 24 | PRBN13                          | 1.54                             | 9.40             | 5.50        | 0.78                        | 0.00                              | 0.00                            | 0.00                     |
| 25 | PRBN17                          | 1.33                             | 4.05             | 4.50        | 1.25                        | 0.00                              | 0.00                            | 0.00                     |
| 26 | PRBN19                          | 1.17                             | 4.10             | 4.00        | 1.18                        | 0.00                              | 0.00                            | 0.00                     |
| 27 | PRBN23                          | 1.40                             | 1.56             | 4.50        | 1.28                        | 0.00                              | 0.00                            | 83.00                    |
| 28 | PRBN25                          | 1.37                             | 3.55             | 1.50        | 0.73                        | 0.00                              | 0.00                            | 91.00                    |
| 29 | PRBK22                          | 0.43                             | 2.13             | 2.75        | 1.25                        | 0.00                              | 0.00                            | 39.00                    |
| 30 | SPBN3                           | 0.20                             | 7.10             | 3.25        | 0.93                        | 9.78                              | 1.30                            | 27.00                    |
| 31 | SPBN8                           | 0.63                             | 4.99             | 3.75        | 1.38                        | 7.45                              | 0.00                            | 0.00                     |
| 32 | SPBN14                          | 0.73                             | 3.48             | 4.50        | 1.38                        | 0.00                              | 3.20                            | 0.00                     |
| 33 | SPBT5                           | 1.52                             | 4.68             | 1.00        | 1.08                        | 0.00                              | 0.00                            | 11.00                    |
| 34 | SPBT13                          | 0.87                             | 3.78             | 3.00        | 0.35                        | 0.00                              | 0.00                            | 0.00                     |
| 35 | SPBK1                           | 0.42                             | 5.33             | 1.75        | 0.60                        | 9.60                              | 2.80                            | 0.00                     |
| 36 | SPBW3                           | 1.27                             | 7.55             | 4.00        | 0.73                        | 0.00                              | 0.00                            | 18.00                    |
| 37 | SPBW4                           | 1.17                             | 4.45             | 3.00        | 0.75                        | 5.20                              | 0.00                            | 0.00                     |
| 38 | SPBW5                           | 1.33                             | 3.30             | 1.75        | 0.95                        | 2.30                              | 3.20                            | 0.00                     |
| 39 | SPBW7                           | 1.33                             | 3.55             | 2.25        | 1.05                        | 9.28                              | 0.00                            | 65.00                    |
| 40 | SDBN6                           | 0.72                             | 6.78             | 4.75        | 0.88                        | 0.00                              | 0.00                            | 15.00                    |
| 41 | Control                         | -                                | 4.10             | 2.25        | 0.73                        | -                                 | -                               | -                        |
3.3. The endophytic bacteria as inducer of plant resistance

Effect of endophytic bacteria on plant resistance was done by measuring the percentage of relative concentrations of phytoalexin compounds which are included in the group of phenolic compounds namely Cyclohexene, 2-Methoxy-guaiacol, and 2,3-Dihydrobenzofuran. Phytoalexin compound content analysis was performed on 15 selected endophytic bacterial isolates based on their effect on the growth of cocoa seedlings, supporting characters (phosphatase production, siderophore, IAA etc.), origin of the location, and the type of culture media used when isolating with the assumption that there would be more diverse candidate bacteria as potential biological agents for further development. The fifteen isolates of endophytic bacteria that were analyzed for their effect on the production of phytoalexin compounds in cocoa seedlings were CPBN 19, CPBN 23, CRBT2, CRBT4, CRBT8, PPBT9, PDBN6, PDBT10, PDBT29, PRBN13, SPBN3, SPBT5, SPBK1, SPBTW3, CRBT4, CRBT8, PPBT9, PDBN6, PDBT10, PDBT29, PRBN13, SPBN3, SPBT5, SPBK1, SPBTW3 and SDBN6. The results of the analysis of the phytoalexin content of cocoa seedlings treated with 15 endophytic bacteria are presented in Table 2.

Table 2. Relative concentration of phytoalexin compound in cacao seedling treated with endophytic bacteria

| No. | Kode Isolat | Cyclohexene | 2-methoxy-guaiacol | 2,3-Dihydrobenzofuran |
|-----|-------------|-------------|-------------------|----------------------|
| 1   | CPBN19      | 0.00        | 2.43              | 4.67                 |
| 2   | CPBN23      | 0.00        | 1.79              | 5.22                 |
| 3   | CRBT2       | 0.93        | 1.45              | 0.00                 |
| 4   | CRBT4       | 0.37        | 1.46              | 6.63                 |
| 5   | CRBT8       | 0.00        | 1.69              | 3.13                 |
| 6   | PPBT9       | 0.00        | 1.49              | 0.00                 |
| 7   | PDBN6       | 1.08        | 0.00              | 0.00                 |
| 8   | PDBT10      | 0.53        | 0.00              | 0.62                 |
| 9   | PDBT29      | 0.00        | 1.79              | 5.32                 |
| 10  | PRBN13      | 0.00        | 0.83              | 4.96                 |
| 11  | SPBN3       | 2.34        | 1.46              | 4.68                 |
| 12  | SPBT5       | 0.00        | 0.00              | 3.11                 |
| 13  | SPBK1       | 1.71        | 2.49              | 4.68                 |
| 14  | SPBW3       | 1.08        | 0.00              | 5.01                 |
| 15  | SDBN6       | 0.50        | 0.00              | 4.62                 |
| 16  | K (Media)   | 0.30        | 0.00              | 2.32                 |

* Relative concentration in 29 secondary metabolites compound analysed by Pyrolysis- GC/MS.

Table 2 shows the difference response of cacao seedlings to the treatment of endophytic bacteria in producing phytoalexin. Some bacterial isolates were able to induce the production of three types of phytoalexin compounds (Cyclohexene, 2-Methoxy-guaiacol, and 2,3-Dihydrobenzofuran) such as CRBT4 and SPBK1 isolates. Meanwhile most of the endophytic bacterial isolates induced the production of 2 kinds of phytoalexin such as CPBN19, CPBN23, PDBT10 and others. A small portion of endophytic bacterial isolates could only induce one type of phytoalexin compound as shown by PPBT9, PDBN6, and SPBT5 isolates.

Based on the ability to induce phytoalexin compounds, 10 isolates were selected for further identification. Selected isolates were CPBN 19, CPBN 23, CRBT4, CRBT8, PDBT29, PRBN13, SPBN3, SPBK1, SPBW3 and SDBN6. The identification process is carried out molecularly by sequencing the nucleotide gene encoding 16S rRNA and then aligning with its equivalent in the database at GenBank.
3.4. *The endophytic bacteria as inducer of plant resistance*  
Molecular identification of all endophytic bacterial isolates were based on 16S rRNA gene sequences. The 16S rRNA gene amplification resulted at a size of ± 1500 base pairs (Figure 2). The size of this fragment is in accordance with the Fitriani report 2016, which performs amplification with the same primer [19].

![Figure 2: Visualization of 16S rRNA gene fragments resulting from DNA amplification of endophytic bacterial isolates from cocoa plants, 1 kb (M) marker.](image)

The analysis results of endophytic bacterial DNA nucleotides were then compared with other DNA nucleotide sequences database in GeneBank. DNA sequencing revealed that nucleotide sequence of 16S rRNA genes ranges from 1400 base pairs. Alignment of 16S rRNA gene nucleotides from the ten isolates of endophytic bacteria showed five isolates of endophytic bacteria, namely SPBN3, SPBW3, SDBN6, PRBN13, CRBT4, have a similarity level of 90 - 99% with *Stenotrophomonas rhizophila*. Isolates PDBT29, CPBN19, CRBT8 have a 99% similarity level with *Pseudomonas geniculata*. On the other hand SPBK1 isolates similar to *Bacillus cereus* with a 99% similarity level and CPBN23 had a 99% similarity level with *Stenotrophomonas maltophilia*, respectively (Table 3).

4. Discussion  
Isolation or exploration of biological agents is an important step in the study of biological control of plant diseases. Biological agents are isolated in areas that show many symptoms of disease, but among them there are healthy plants or the disease could not develop even though the plants are vulnerable [20]. In this area, the failure of pathogens to develop and cause disease is thought to be the only one due to the role of beneficial microbes associated with plants. One of these microbial groups is endophytic bacteria, a group of bacteria that has been widely studied and reported to have great potential in triggering plant growth and biological control by various mechanisms [21] [5].

In this study, 288 endophytic bacteria have been isolated from the shoots, leaves and thrunk of cocoa from Southeast Sulawesi, West Sumatra and West Java. The selection of plant parts for isolation of endophytic bacteria was adjusted with the aim of controlling Vascular Streak Dieback disease which attacks the cacao thrunk which begins with infection of shoots appear (flushing), continues to develop in leaf tissue and infected the xylem through the petiol. Bacterial isolates that were successfully isolated were expected to have the same niche as the *C. theobromae* pathogen so they could effectively control VSD.
### Table 3. Alignment analysis of nucleotide 16S rRNA genes to the nucleotide sequence database at GenBank.

| Isolates Code | Reference Species                      | Query Cover | Identity % | Accession Number |
|---------------|----------------------------------------|-------------|------------|------------------|
| SPBK1         | Bacillus cereus strain FPA3            | 100         | 99%        | JQ3085501        |
| CPBN19        | Pseudomonas geniculata strain NBG2     | 99%         | 99%        | HQ256559.1       |
| SPBN 3        | Stenotrophomonas rhizophila strain L20 | 100%        | 99%        | JN700136.1       |
| SPBW 3        | Stenotrophomonas rhizophila strain R5-338 | 99%      | 99%        | JQ659736.1       |
| CRBT 4        | Stenotrophomonas rhizophila Sal 40     | 100%        | 90%        | LC389484.1       |
| CRBT 8        | Pseudomonas geniculata strain DD166    | 99%         | 99%        | KT216620.1       |
| SDBN 6        | Stenotrophomonas rhizophila strain KUD | 100%        | 99%        | KC355333.1       |
| PDBT 29       | Pseudomonas geniculata strain R6-798 16S | 99%   | 99%        | JQ659864.1       |
| PRBN 13       | Stenotrophomonas rhizophila strain L18 | 99%         | 99%        | JN700131.1       |
| CPBN 23       | Stenotrophomonas maltophilia strain AA1 | 100%     | 99%        | CP018756.1       |

Screening of endophytic bacteria was started by looking at the effect on germination of cocoa seeds. The method of direct selection to plants and continued characterization in the laboratory is expected to get endophytic bacterial candidates that can really be applied to crops without causing negative impacts on cocoa plants. Many steps of screening included characterization and selection on the growth of cocoa seedlings and the production of phytoalexin compounds as an indication of the induction of plant resistance to *C. theobromae* obtained 10 endophytic bacterial isolates and the identification results showed four main species namely *B. cereus*, *S. rhizophila*, *S. maltophilia*, and *P. geniculata*, respectively. The four species of bacteria are commonly used in biological control of plant pathogens, growth promoters, and bioremediation. According to Omer 2016 [22], *P. geniculata* could stimulate the growth of corn plants because it produced the hormones IAA, siderophore, phosphatase, and cyanide acid (HCN). *S. maltophilia* have been reported suppressed brown rot in potatoes caused by *Ralstonia solanacearum* [23]. *S. rhizophila* also could stimulate tomato plant growth and suppress pathogenic fungi in the rhizosphere [24]. Wang et al. 2012 [25] reported that *B. cereus* inhibited the growth of *Aspergillus flavus* and can produced chitinase. Furthermore, the results of this study are expected to further development of biological agents for controlling *C. theobromae*, the causal agent of Vascular Streak Dieback Diseases on cacao.

### 5. Conclusion

Isolation and screening of endophytic bacteria from shoots, leafs, and thrunk of cacao from Southeast Sulawesi, West Sumatera, and West Java resulted 10 bacterial isolates that induced cacao seed germination and were not-pathogenic to plant and mammals. All of those isolates produced chitinase, phosphatase, siderophore, and indole acetic acid. Application of the bacteria induced the growth of cacao seedling and increased the relative ratio of phytoalexin compound (Cyclohexene, 2-Methoxy-guaiacol, dan 2,3-Dihydrobenzofuran) indicating cacao resistance to *C. theobromae*. Molecular identification based on nucleotide sequence of 16S rRNA genes revealed the endophytic bacteria are...
Bacillus cereus (1 isolate), Pseudomonas geniculata (3 isolates), Stenotrophomonas rhizophila (5 isolates), and Stenotrophomonas maltophilia (1 isolate), respectively.

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