Consortium solid formula of bacillus spp. to control bacterial wilt on chili plants

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Abstract. Due to its high economic worth and ability to be consumed in both fresh and processed forms, chili (Capsicum annum L.) is a fruit that is frequently used as a vegetable. Pests and plant diseases, such as bacterial wilt caused by Ralstonia syzygii subsp. Indonesiensis, are the causes of reduced chili productivity. Plants will eventually perish as a result of symptoms like withering of the leaf tips that eventually spread to the undersides of the leaves. The lower vascular tissue and roots of the affected chili plants appear brown. Bacterial wilt is an important disease of chili caused by *Ralstonia syzigii* subsp. *indonesiensis* and can reduce yield by up to 90%. The research aimed to obtain the best solid formula consortium of *Bacillus* spp. for controlling *R. syzygii* subsp. *indonesiensis* on chili peppers. The study consists of 2 stages: 1). Production of a consortium combination of *Bacillus* spp. in solid waste 2). Test the ability of the consortium of *Bacillus* spp. in solid waste to control bacterial wilt in chili plants. The study used a completely randomized design that consisted of 19 treatments and four replications. Each treatment was introduced on chili seeds and seedlings, while the inoculation of *R. syzygii* subsp. *indonesiensis* was conducted on chili aged 35 days after planting. The results showed that the best solid formula consortium of the *Bacillus* spp. for controlling bacterial wilt (*R. syzigii* subsp. *indonesiensis*) on chili plants is the Bran + Bagasse formula for four weeks of storage.

1 Introduction

Chili (*Capsicum annum* L.) is a fruit often used as a vegetable because it has high economic value and is consumed in fresh or processed form [1]. Factors causing low chili productivity are pests and plant diseases, including bacterial wilt by *Ralstonia syzygii* subsp. *indonesiensis* [2]. Symptoms such as wilting of the tops of the leaves then spread to the underside of the leaves, and the plants become wilted; eventually, the plants will die. The attacked chili plants show that the lower vascular tissue and roots look brown [3].

Bacterial wilt control has been carried out through cultural measures, resistant varieties, sanitation, and crop rotation [4], and chemical disease control using bactericidal [5]. However, the use of bactericidal is not only economically ineffective. However, it can

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potentially cause adverse environmental impacts [6], so alternative control is needed by utilizing microorganisms as biological control agents [7].

One currently developed biological agent for controlling plant pathogens is from the Plant Growth Promoting Rhizobacteria (PGPR) group [8]. One of the biological agents classified as PGPR is *Bacillus* spp. [9]. The *Bacillus* spp group. which have been extensively studied as biological agents, namely *B*. *thuringiensis*, *B. cereus*, and *B. toyonensis* [10]. The use of *Bacillus* sp. singly causes *Bacillus* sp. cannot to last long and is not optimal either as a biocontrol agent or as a bioactivator [11]. Bacillus spp. can inhibit the growth of the fungus Fusarium oxysporum. Furthermore, B. toyonensis strain AGBE1.2.TL has the potential as a growth promoter for chilli plants and can control anthracnose disease [12].

The study aimed to obtain a solid formula for the consortium of *Bacillus* spp. for controlling bacterial wilt (*Ralstonia syzygii* subsp. *indonesiensis*) on chili plants.

2 Materials and methods

2.1 Time and place

The research was carried out from January to April 2023. This research was conducted at the Microbiology Laboratory and the Experimental Garden of the Faculty of Agriculture, Universitas Andalas

2.2 Implementation

2.2.1 Rejuvenation of B. cereus strain SLBE3.1AP and B. toyonensis strain AGBE 1.2.TL

Bacteria is a collection of Dr. Yulmira Yanti, S.Si., MP., namely *B. cereus* strain SLBE3.1AP and *B. toyonensis* strain AGBE 1.2.TL. Microtube isolates were rejuvenated using the scratch method on TSA medium, then incubated for 2x24 hours.

2.2.2 Confirmation of B. cereus strain SLBE3.1AP and B. toyonensis strain AGBE 1.2.TL

Confirmation of *B. cereus* strain SLBE3.1AP and *B. toyonensis* AGBE 1.2.TL strain was carried out by Gram test to determine whether the bacteria were gram-negative or positive. 3% KOH solution was dripped on a glass slide, then one single colony of pure cultures of *B. cereus* strain SLBE3.1AP and *B. toyonensis* strain AGBE 1.2.TL was taken with an ose needle and mixed [13]. Then a hypersensitivity reaction (HR) test was carried out using a suspension of *B. cereus* strain SLBE3.1AP and *B. toyonensis* strain AGBE 1.2.TL with a density of 108 CFU/ml infiltrated into the undersurface tissue of *Mirabilis jalappa* leaves and incubated for 2x24 hours [14].

2.2.3 Propagation of the Bacillus spp. (B. cereus strain SLBE3.1AP and B. toyonensis strain AGBE 1.2.TL)
Consortium

Bacillus spp. multiplied in liquid culture aged 2 x 24 hours was taken, put into 25 ml NB medium in culture bottles (volume 50 ml), and incubated on a rotary shaker for 24 hours. Furthermore, 1 ml of preculture results were transferred into 49 ml sterile coconut water in a

culture bottle (100 ml volume). The second stage of the main culture, the *Bacillus* spp consortium, was created by combining two compatible *Bacillus* spp. Furthermore, 1 ml of liquid culture of each species of *Bacillus* spp. (preculture results) was transferred into 23 ml of sterile coconut water in a culture bottle (50 ml) and incubated for 2x24 hours on a rotary shaker with a speed of 150 rpm at room temperature [12]. The population density was determined by comparing the turbidity of the bacterial suspension with that of McFarland's scale 8 (estimated population density of 10^8 cells ml-1) [14].

2.2.4 Multiplication of solid formula carrier materials for Bacillus spp (B. cereus strain SLBE3.1AP and B. toyonensis strain AGBE 1.2.TL)

Solid waste carrier materials are bagasse, rice bran, and rice straw, mashed using a blender. Each carrier material was put into a Schott bottle with a volume of 100 ml totaling 9.5 g. Then 0.5 grams of sucrose (5% of the total media weight) was added to each formula and sterilized by autoclaving at 121°C with 1 atm pressure for 15 minutes. After that, the formula ingredients were cooled, and 5 ml of main culture suspension 10^8 cells/ml was added. *Bacillus* spp.

2.2.5 Inoculum preparation of R. syzygii subsp. indonesiensis

The source of the inoculum was obtained from chili plants with bacterial wilt symptoms. Isolation of *R. syzygii* subsp. *indonesiensis* by removing the bacterial mass from the plant's vascular tissue by cutting the base of the plant stem and putting it in sterile distilled water for 10 minutes. The bacterial mass was cultured purely by streaking on a TZC medium to determine the virulent colonies of *R. syzygii* subsp. *indonesiensis*, then incubated for 2x24 hours.

2.2.6 Pathogenicity Test

Pathogenicity test of *R. syzygii* subsp. *Indonesiensis*, namely wounding, and watering of of *R. syzygii* subsp. *Indonesiensis* suspension on chili plant roots aged 35 DAP. Loosening the soil first, the roots are cut on both sides of the plant using scissors. Furthermore, 20 ml of of *R. syzygii* subsp. *Indonesiensis* suspension with a density of 107 cells/ml was sprinkled on the roots of the chili plants that had been injured [10].

2.2.7 Propagation of R. syzygii subsp. isolates indonesiensis

Bacteria were propagated using the streaking technique on TZC media, then incubated for 2x24 hours. Isolate *R. syzygii* subsp. *indonesiensis* was suspended using sterile distilled water with a density of 10^7 cells/ml then McFarland's solution scale seven was used as a comparison.

2.2.8 Preparation of planting media

The planting medium used was a mixture of soil and manure (2:1 v/v). The planting medium is put in a plastic bag and then sterilized in a boiler for 1 hour at 100^0C. The planting medium was put 5 g into a pottray for seeding and 10 kg into a polybag for planting [10].

2.2.9 Introduction of formulation for Bacillus spp. Consortium

Formula introduction of *Bacillus* spp. was carried out two times. Introductions were made to chili seeds and seedlings.

2.2.10 Inoculation of R. syzygii subsp. isolates Indonesia

The bacteria *R. syzygii* subsp. *indonesiensis* was inoculated on chili plants aged 35 DAP by cutting the roots on both sides of the plant at a distance of 5 cm from the stem. After that, 30 mL of the bacterial suspension of *R. syzygii* subsp. *indonesiensis*, with a population density of 10^7 cells/ml, was sprinkled on the plants [15].

2.2.11 Observation

Observations were made on the viability test of the *Bacillus* spp consortium solid waste formula, disease development, chili seedling growth, and plant growth. Observation of disease development includes the incubation period, incidence, and severity. Observation of seedling growth included field emergence, seedling height (cm), number of seedling leaves, wet seedling weight, and dry seedling weight. Observations on the growth of chili plants included plant height (cm), number of leaves, and first flower appearance. Data were analyzed using analysis of variance, if significantly different, then continued with Duncan's New Multiple Range Test (DNMRT) at a 5% level.

3 Results and discussion

All consortia of *Bacillus* spp. in different solid waste storage extended the incubation period compared to the negative control (Table 1)

Table 1. The incubation period of bacterial wilt on chili peppers introduced by the consortium of *Bacillus* spp. in solid waste with different storage times.

Treatment		Emerging Incubation (days after incubation)
Formula Carrier	**Storage (Week)**	
Bagasse + Rice Straw	6	-
Bran + Rice Straw	6	-
Bran + Bagasse + Rice Straw	4	-
Bagasse + Rice Straw	4	-
Bran + Rice Straw	4	-
Bran + Bagasse	4	-
Bran + Bagasse	6	40,25
Bran + Bagasse + Rice Straw	0	40,25
Septromisin	-	38,50
Bran + Bagasse + Rice Straw	8	38,50
Bran + Rice Straw	8	38,50
Bagasse + Rice Straw	0	38,50
Bran + Rice Straw	0	38,50
Bagasse + Rice Straw	8	36,75

Bran + Bagasse	8	36,75
Bran + Bagasse + Rice Straw	6	36,75
Bran + Bagasse	0	36,75
Negative Control	-	12,25

*(-) Indicates the plant does not cause symptoms until the last day of observation (42 dai)

The *Bacillus* spp. consortium in different storage solid wastes suppressed the incidence of bacterial wilt disease and significantly differed from the negative control (Table 2). Treatment of Bran + Rice Straw 4 and 6 weeks storage, Bran + Bagasse + Rice Straw 4 weeks storage, and Bran

+ Bagasse 4 weeks storage are formulas that have the potential to reduce the incidence of bacterial wilt disease and show significantly different effects compared to negative control and streptomycin with a disease incidence rate of 0.00%. The highest incidence of bacterial wilt was found in the negative control and streptomycin, namely 100% (Table 2).

Table 2. The incidence of bacterial wilt in chili plants introduced by the *Bacillus* spp. consortium. in solid waste with different storage times.

Treatment		Disease Incidence (%)
Formula Carrier	Storage (Week)	
Negative Control	-	100,00 a
Septromisin	-	100,00 a
Bran + Bagasse + Rice Straw	8	75,00 ab
Bran + Rice Straw	0	50,00 ab
Bran + Bagasse	0	50,00 ab
Bagasse + Rice Straw	8	50,00 ab
Bran + Bagasse	8	50,00 ab
Bran + Bagasse + Rice Straw	6	50,00 ab
Bran + Bagasse	6	50,00 ab
Bagasse + Rice Straw	0	50,00 ab
Bagasse + Rice Straw	6	25,00 ab
Bran + Rice Straw	8	25,00 ab
Bagasse + Rice Straw	4	25,00 ab
Bran + Bagasse + Rice Straw	0	25,00 ab
Bran + Rice Straw	6	0,00 b
Bran + Bagasse + Rice Straw	4	0,00 b
Bran + Rice Straw	4	0,00 b
Bran + Bagasse	4	0,00 b
CV=16.12		

* Numbers followed by the same lowercase letter in the same column are not significantly different according to DNMRT at the 5% level.

The *Bacillus* spp. consortium in different storage solid wastes suppressed the severity of bacterial wilt disease and significantly differed from negative controls. Treatment Rice Bran + Rice Straw without storage, 4 weeks, 6 weeks and 8 weeks, Bran + Bagasse without storage, 4 weeks, 6 35 weeks and 8 weeks, Bagasse + Rice Straw without storage, 4 weeks, 6 weeks

and 8 weeks, Bran + Bagasse + Rice Straw without storage, 4 weeks, 6 weeks and 8 weeks are treatments that have the potential to reduce the severity of bacterial wilt and show significantly different effects compared to negative controls with a disease severity value of 0.00-19.14%. Negative control was the treatment with the highest bacterial wilt disease severity, with a disease severity value of 100% (Table 3).

Table 3. The severity of bacterial wilt in chili plants introduced by the Bacillus spp. consortium on solid waste with different storage times (42 dai)

Treatment		**Disease Severity (%)**	**Criteria**
Formula Carrier	**Storage (Week)**		
Negative Control	-	100,00 a	dead
Septromisin	-	19,5 b	light
Bran + Rice Straw	0	19,14 b	light
Bran + Bagasse	0	18,55 b	light
Bran + Bagasse	8	13,16 b	light
Bagasse + Rice Straw	0	11,92 b	light
Bran + Bagasse + Rice Straw	6	9,98 b	light
Bran + Bagasse + Rice Straw	8	9,58 b	light
Bagasse + Rice Straw	8	6,56 b	light
Bran + Bagasse + Rice Straw	0	5,5 b	light
Bran + Bagasse	6	5,43 b	light
Bran + Rice Straw	8	5,07 b	light
Bagasse + Rice Straw	6	1,09 b	light
Bagasse + Rice Straw	4	0,98 b	light
Bran + Rice Straw	6	0,00 b	health
Bran + Bagasse + Rice Straw	4	0,00 b	health
Bran + Rice Straw	4	0,00 b	health
Bran + Bagasse	4	0,00 b	Health
CV= 13.55			

* Numbers followed by the same lowercase letter in the same column are not significantly different according to DNMRT at the 5% level.

The *Bacillus* spp consortium in different storage solid wastes increased the height of chili plants and significantly differed from the positive control (Table 4). treatment Bran + Bagasse +Rice Straw 4 weeks storage, Bran + Rice Straw 4 weeks storage, Bran + Bagasse +Rice Straw 0 weeks storage, Sugarcane Bagasse + Rice Straw 4 weeks storage, Bran + Rice Straw 6 weeks storage, Bran + Rice Straw Sugarcane dregs stored for 4 weeks, Bagasse + Rice Straw stored for 8 weeks, and Bran + Rice Straw stored for 8 weeks are treatments that have the potential to increase plant height with seedling heights ranging from 11.20-11.55 cm. The lowest chili plant seed height was found in the positive control, which was 9.33 cm (Table 4).

Table 4. Height of chili seedlings introduced to the *Bacillus* spp. consortium on solid waste with different storage times (21 das)

Treatment		Seedling Height (cm)
Formula Carrier	**Storage (Week)**	
Bran + Bagasse +Rice Straw	4	11,55 a
Bran + Rice Straw	4	11,55 a
Bran + Bagasse +Rice Straw	0	11,50 a
Bagasse + Rice Straw	4	11,48 a
Bran + Rice Straw	6	11,45 a
Bran + Bagasse	4	11,28 a
Bagasse + Rice Straw	8	11,23 ab
Bran + Rice Straw	8	11,20 ab
Bran + Bagasse +Rice Straw	8	10,88 abc
Bran + Bagasse	6	10,88 abc
Bagasse + Rice Straw	0	10,88 abc
Bran + Rice Straw	0	10,88 abc
Bagasse + Rice Straw	6	10,70 abc
Bran + Bagasse	0	10,63 abc
Bran + Bagasse	8	10,33 bc
Bran + Bagasse +Rice Straw	6	10,33 bc
Septromisin	-	10,05 cd
Positive Control	-	9,33 d
CV =5.09		

* Numbers followed by the same lowercase letter in the same column are not significantly different according to DNMRT at the 5% level.

The *Bacillus* spp. consortium in different storage solid wastes increased the number of chili seedling leaves and significantly differed from the positive control (Table 5). Treatment of Bran + Rice Straw storage for 4 weeks, Bran + Bagasse + Rice Straw without storage, 4 weeks, and 8 weeks had the potential to increase the number of leaf seedlings. It significantly differed from the positive control and 39 streptomycin with a leaf number range of 6,25-7, 00 strands. The lowest number of chili plant seed leaves was found in the positive control, namely 5.25 leaves (Table 5)

Table 5. The number of chili seedlings introduced to the *Bacillus* spp consortium on solid waste with different storage times (21 das).

Treatment		Number of Leaves (strands)
Formula Carrier	**Storage (Week)**	
Bran + Rice Straw	4	7,00 a
Bran + Bagasse +Rice Straw	8	6,25 ab
Bran + Bagasse +Rice Straw	4	6,25 ab
Bran + Bagasse +Rice Straw	0	6,25 ab
Bran + Bagasse	8	6,00 bc

Bran + Bagasse	6	6,00 bc
Bagasse + Rice Straw	4	6,00 bc
Bran + Bagasse	4	6,00 bc
Bagasse + Rice Straw	8	5,75 bc
Bagasse + Rice Straw	6	5,75 bc
Bran + Bagasse	0	5,75 bc
Bagasse + Rice Straw	0	5,50 bc
Bran + Rice Straw	8	5,50 bc
Bran + Bagasse +Rice Straw	6	5,50 bc
Bran + Rice Straw	6	5,50 bc
Bran + Rice Straw	0	5,50 bc
Streptomycin	-	5,50 bc
Positive Control	-	5,25 c
CV=8.47		

* Numbers followed by the same lowercase letter in the same column are not significantly different according to DNMRT at the 5% level.

The *Bacillus* spp. consortium in different solid waste storage on chili plants was able to extend the incubation period and reduce the incidence and severity of bacterial wilt disease compared to the negative control (inoculated with *Ralstonia syzigii* subsp. *indonesiensis*) and Streptomycin. Four treatments that have the potential to control *Ralstonia syzigii* subsp *indonesiensis* until the end of the observation (42 dai), namely Formula Bran + Bagasse stored for 4 weeks, Bran + Rice Straw stored for 4 weeks and 6 weeks, and Bran + Bagasse + Rice Straw stored for 4 weeks. It was allegedly due to the nutrient content of the carrier material supports the bacteria in maintaining its population during storage, which completeness of nutrition is the key to the success of each treatment in controlling plant pathogens and *Bacillus* spp. also produces secondary metabolites, such as antibiotics, siderophores, bacteriocins, and extracellular enzymes so that they can inhibit the growth of *Ralstonia syzigii* subsp. *indonesiensis*. *Bacillus* spp. can also induce plant resistance compounds and act as a Plant Growth Promoting Rhizobacteria (PGPR) [16]. Four formulations of *Bacillus* spp. capable of inhibiting the growth of the pathogen *Xanthomonas oryzae* pv. *oryzae,* which shows the occurrence of an antibiosis mechanism in which *Bacillus* spp., in the process of metabolism, produces antibiotic compounds that are secreted when bacteria form a stationary phase and produce enzymes such as chitinase enzymes, mycobacillin, bacitracin and others [17]. Furthermore, tofu dregs and tofu dregs + corn cobs are the best formulae for reducing root rot disease (*Sclerotium rolfsii*) in tomato plants [18].

Introduction to the *Bacillus* spp. consortium in different storage solid waste on chili seeds can increase the seedlings' height and the number of leaves of chili seedlings compared to the positive control. The best treatment to increase the growth of chili seedlings was the Bran + bagasse treatment which was stored for 4 weeks. It was thought to be due to the content of the carrier material, which provided nutrients for *Bacillus* spp. in interactions with plants. It is in accordance with the statement regarding solid waste containing organic carbon (carbohydrates) and organic nitrogen (proteins and amino acids), which are both used as a source of energy and for the growth of *Bacillus* spp. further supported by the statement of Asmin and Karimuna [19], Rice straw is an organic material that can bind Fe^{2+} ion solution, so K^+ has a great opportunity to be absorbed by plant roots and become a source of plant nutrients. Utilizing bagasse as organic material has the potential to become compost which can replace inorganic fertilizers and is beneficial for plant growth [20]. Bran provides

nutrients that are very important for plant growth [21]. *Bacillus* spp. reported that as a bacteria inducing plant resistance and as a Plant Growth Promoting Rhizobacteria (PGPR), which can increase plant growth [22].

4 Conclusion

Bacillus spp. .consortium formula. in solid waste, the best for controlling bacterial wilt by *Ralstonia syzigii* subsp. *indonesiensis* and increasing the growth and yield of chili plants, namely the Bran + Bagasse formula for 4 weeks storage.

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