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"The Project for Producing Biomass Energy and Material through Revegetation of Alang-alang (*Imperata cylindrica*) Fields"

Effect of fertilization application on population and diversity of actinomycetes from rhizosphere soils of *Sorghum bicolor*

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Abstract

The aim of the study was to observe the effect of gradient-N fertilization, compost, microbial inoculant and its combination on population, diversity of actinomycetes, and their antibacterial and enzymatic activities from rhisosphere soils of *Sorghum bicolor*. Sorghum were cultivated in different fertilizers treatments. Fertilizer treatments including gradient-N chemical fertilizer alone (0%, 50% or 100% NPK) (TN0, TN50, TN100), gradient-N chemical fertilizer + microbial inoculant (TI0, TI50, TI100), gradient-N chemical fertilizer + compost (CN0, CN50, CN100), gradient-N chemical fertilizer + compost + microbial inoculant (CI0, CI50, CI100). Soil samples were collected from each rhizospheres of sorghum that has been treating with fertilization application for 100 days. The samples were air dried at room temperature for 7 days. One gram of each air-dried soil sample was pretreated with 0.05% sodium dodecyl sulfate (SDS). Humic acid vitamin agar (HVA) was used as selective media for isolation of actinomycetes. The application of different fertilizer treatments significant effected of population actinomycetes. The highest population of actinomycetes was isolated from rhizosphere soil with the application of CI50. The lowest population of actinomycetes was isolated from rhizosphere soil with the application of CN100 and CN50. A total 126 isolates of actinomycetes were obtained from 12 samples of rhizosphere soil from various treatment based on morphological characteristics. Among the 126 isolates, 95 isolates had aerial mycelium, and only 24 isolates produced soluble pigment into medium. The diversity of actinomycetes in all fertilizer treatments with addition of 50% NPK (except CI50) was higher than that in the other fertilizer treatments. Among the 126 isolates, 45 isolates showed antibacterial activity against *B. subtilis*. 54 isolates showed activity against *E. coli*, 101 isolates showed to produce cellulase, and 44 isolates showed produce phosphatase.

Keywords: Actinomycetes, *Sorghum bicolor*, fertilizer, antibacterial, enzyme activity

1. Introduction

Sorghum bicolor is multifunctional crop, not only for human food but also feed for animal, energy, and building material [1]. Sorghum more adaptable to drought stresses, low nutrient, soil pH, and can adaptation to marginal land [2]. Under the same stressed environment the adaptation and yield stability of sorghum is more enhanced than that of maize [2]. Sorghum can be an alternative crop that could be grown in non-productive land such as *Imperata* grassland [3]. Conversion of *Imperata* grassland to conservative agriculture land is considered one way to improve soil ecosystem [3]. In this research, we try converting *Imperata* grassland into agriculture field by planting the *Sorghum bicolor*. Those sorghum cultivated at Cibinong Science Center. Sorghum require mineral as the nutrient source of their growth and productivity. However, nutrient levels in soil of *Imperata* grassland are low in general [4]. It will be essential input of fertilizers to supplement plant mineral. There is possibility to use organic, nonorganic fertilizer, or combination both of them as an alternative nutrient source. The amount of

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fertilizer input would be determined as that balance for crop requirement. Applications fertilizers not only have effect directly enhance crop yield, but also have effect on the agriculture system such as degradation of soil and effect to soil microbial diversity.

The soil microorganism plays important roles in nutrient cycling process, decomposition and mobilization of organic that affects plant growth [5]. The soil microorganisms also produce antimicrobial agents and enzymes to invade soil borne pathogens [5]. Changes in microbial activity and composition can influence plant growth. Actinomycetes are aerobic and gram positive bacteria have genomes with high GC-content which widely distributed in soil [6]. Actinomycetes occur in plant rhizosphere soil and produce active compounds. They produce many secondary metabolites including plant growth hormone such as indole-3-acetic acid, hydrocyanic acid and siderophore [7]. Actinomycetes can protect roots by inhibition the development of soil borne pathogens by producing enzymes or producing antimicrobial compounds [8]. Many researchers have studied biological control of plant pathogens using *Streptomyces* spp. [9,10]. Therefore, the stability of the actinomycetes community is one important thing for the plant growth. So, the experiments were carried out to study the effect of gradient-N chemical fertilization, compost, microbial inoculation, and interaction of those factors on actinomycetes population, diversity, and their antibacterial and enzymes activities from rhizosphere soil of *Sorghum bicolor*

2. Methods

2.1. Experimental design and sample collection

Twelve treatments using different fertilizer applications were designed. Fertilizer treatments including gradient N-chemical fertilizer alone (0%, 50% or 100% NPK) (TN0, TN50, TN100), chemical fertilizer + microbial inoculant (TI0, TI50, TI100), chemical fertilizer + compost (CN0, CN50, CN100), chemical fertilizer + compost + microbial inoculant (CI0, CI50, CI100). All the fertilizers were added as basal fertilization before planting sorghum. Sorghum were cultivated at Cibinong Science Center, Cibinong, West Java Indonesia. Soil samples were collected from 12 plants sorghum 100 days old in each experimental plot. Later, the soil samples were air dried at room temperature for 3 days. The soil samples dried heat treatment at 50^{o}C for 15 minutes to depress the number of other bacteria [11]. The samples were ground with mortar and sieved, then 1 gr each samples used for isolation of actinomycetes.

2.2. Isolation of actinomycetes

Actinomycetes were isolated by SDS-Yeast Extract (SY) isolation method [12]. One gram of each samples were treated with 0.05% sodium dodecyl sulfate (SDS) at 40^{o}C for 20 minutes. Soil suspension was then diluted with sterile water (1:1000). Different aqueous dilutions (10^{-1} to 10^{-5}) of samples were spread onto the plates of Humic Acid Viatamin (HV) agar medium supplemented with cyclohexamide (50 mg/l) and nalidixic acid (20 mg/l) [13]. Then, the plates were incubated at 30^{o}C for 14 days. Colony of actinomycetes appeared was counted as colony forming units (CFUs). Typical colonies of actinomycetes were selected on morphological basis and purified onto yeast extract-starch agar (YSA) by restreaking. These YSA plates were then incubated at 30^{o}C for 14 days.

2.3. Morphological characterization

Morphology was observed after actinomycetes were cultured on YSA for 7 days. Actinomycetes were characterized by morphological criteria using eye and using light microscope. Aerial mycelium and production of soluble pigment were observed.

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2.4. Morphological characterization

Antibacterial screening: All the isolates actinomycetes were grown on YSA plates and incubated at 30°C for 7 days. Agar discs of well grown cultured of actinomycetes (6 mm diameter) were placed on plates seeded with each the test bacteria. The plates were then incubated at 37°C for 24 hours. Inhibition zones around the actinomycetes that indication of the antibacterial activity. Antibiotics screening was performed against *Bacillus subtilis* (InaCCB1) and *Eschericia coli* (InaCCB5). The cultures were obtained from Indonesian culture collection, Indonesian Institute of science.

Enzymatic screening: Actinomycetes that were grown on YSA plates for 7 days at 30°C were transferred to CMC and vikoskaya medium [14] and were incubated at 30°C for 7 days. The formations of clear zone around the actinomycetes that indication of enzyme activities (cellulase or phosphatase). For detection of cellulose degradation, CMC plates were flooded with 1% congo red solution. 1 M NaCl was then added until color disappeared.

2.5. Statistical analysis

Data were assessed by Duncan's multiple range tests, with probability, P=0.05. One-way ANOVA was used for analysis of significant difference among fertilizer treatments.

3. Result and Discussion

Isolates actinomycetes were successfully isolated from rhizosphere soils of sorghum by using SDS method isolation. The results showed that the application of different fertilizer treatments effected of population actinomycetes. The population of rhizosphere soil actinomycetes was significantly different among the fertilizer treatments. The colony-forming unit (CFUs) of actinomycetes in the CI 50 treatment was higher than that in the other fertilizer treatments and control. While, the lower actinomycetes population were observed in the CN100 and CN50 treatments. This clearly that compost addition with microbial inoculant and 50% NPK increased the actinomycetes population, while the application of the others fertilizer treatments without microbial inoculant decreased the actinomycetes population during the sorghum growth (Table 1). 50% NPK was optimal chemical fertilizer dose increased actinomycetes population under *Sorghum bicolor* growth. This contrast with the finding of Meena *et al* [15] and Zak *et al* [16] who reported that actinomycetes population was greater in soil after application of 100% NPK + 200 kg wellgrow grain ha^{-1}. Although many study reported that soil actinomycetes population increased in the application of manure compost [17] or manure compost with addition chemical fertilizer [18], but in this result showed that application of food resources (chemical fertilizers and compost) without addition microbial inoculant decreased actinomycetes population.

Different soil microorganisms had been extensively used as inoculants, including *Rhizobia*, *Azospirillum*, Mycorrhizal fungi, and biocontrol agents [19]. On-field inoculation *Phaseolus vulgaris* with two local rhizobial strains increased γ-proteobacteria, *α*-proteobacteria, *Firmicutes* and Actinobacteria [19]. Inoculation of *Sinorhizobium meliloti* strain L33 affected bacterial diversity in the rhizosphere of alfalfa by reducing the number of γ-proteobacteria and increasing the number of *α*-proteobacteria [19]. Another study reported population of actinomycetes was decreased during the entire maize growth period this might be caused the competition of carbon and nitrogen source between soil microorganisms and crops [20,21]. On the other hand, population of actinomycetes in the soil depends on group of actinomycetes. *Streptomycetes* group are faster growth actinomycetes than rare actinomycetes [22].

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Tabel 1. Actinomycetes population (cfu/g of soil) in the rizhosphere soil under *Sorghum bicolor* cultivation as affected by different fertilizer treatments.

Treatments	Root length (cm)
TI100	10 [f]
TI50	17 [d]
TI	8 [g]
TN100	18 [cd]
TN50	12 [e]
TN (control)	23 [b]
CI100	12 [e]
CI50	**28 [a]**
CI	19 [c]
CN100	5 [h]
CN50	5 [h]
CN	8 [g]

Note: TI: microbial inoculants; TN: chemical fertilizer (NPK); CI: compost + microbial inoculants; CN: compost; 100, 50, or 0 : NPK gradient 100 %, 50%, 0%.

A total 126 isolates of actinomycetes were obtained from 12 samples of rhizosphere soils from various treatment based on morphological characteristics. Among 126 isolates, 95 isolates have aerial mycelium, and only 24 isolates produce soluble pigment into medium. Application of fertilizers affected on microbial diversity. The result showed that gradient chemical fertilizer (NPK) influenced the diversity of actinomycetes. The diversity of actinomycetes in all fertilizer treatments with addition of 50% NPK (except CI 50) were higher than that in the other fertilizer treatments. The higher diversity of actinomycetes was in the TI50 followed in the CN50, and in the TN50. While, the lower diversity of actinomycetes was in the CN100 and CI100 followed in the control. Indicating that application compost addition with microbial inoculant and 50% NPK was optimal fertilizer increased actinomycetes diversity. Jangid et al. [23] repoted that bacterial diversity was higher in the soils amended with poultry litter than that treated with inorganic fertilizer. Animal compost increased bacteria diversity by increasing the carbon sources in the soil, thus improving the living condition for indigenous microbial population [24]. *Streptomyces* group was a group of actinomycetes were most commonly founded in soil.

Tabel 2. The diversity of rizhosphere soil actinomycetes in *Sorghum bicolor* cultivation

Treatment	Diversity of actinomycetes
TI100	9
TI50	18
TI (control)	7
TN100	8
TN50	13
TN	12
CI100	6
CI50	10
CI	12
CN100	6
CN50	15
CN	10
Total	126

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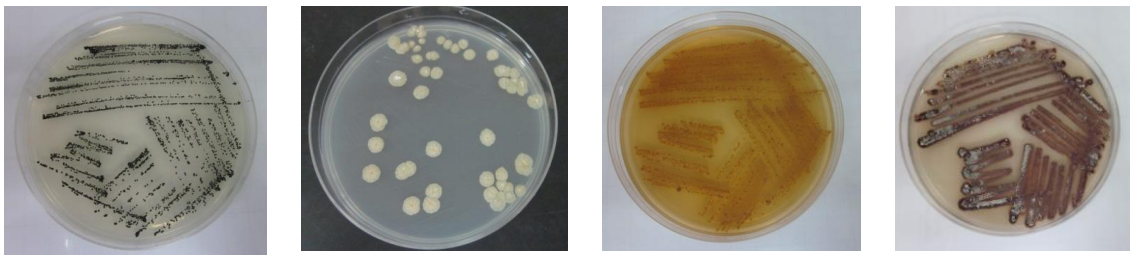

TN100(3) CI100(2) CN0(2) CI50(1)

Figure 1. Isolates actinomycetes isolated from rhizosphere soils of *Sorghum bicolor*

Rhizosphere soil Actinomycetes had activity against pathogenic bacteria and enzyme activities (capability to produce cellulase and phosphatase). Among the 126 isolates, 45 isolates showed antibacterial activity against *B. subtilis.* 54 isolates showed activity against *E. coli*, 101 isolates showed to produce cellulase, and 44 isolates showed produce phosphatase enzymes. Antibacterial and cellulase enzyme that were produced by actinomycetes have played important role to controlling soil-borne plant pathogens [25]. Antimicrobial activity as well as enzyme production capability the genus *Streptomyces* was dominant [26]. Actinomycetes have the capability to produce phosphatase. This enzyme is able to mineralize organic phosphates into inorganic phosphates that provides high P for plant [27].

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