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Microbial Community Structure and Endophytic microbes of *Sorghum bicolor* in gradient nutrient-N application

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Abstract

Soil microbial community structure and endophytic bacteria in plant play key role on plant growth promotion. Growth of *Sorghum bicolor* is also influenced by soil and endophytic microbes. Applying either compost or fertilizers may affect community member of soil microorganism, but after now there is few information dealt with this issue. Methods for monitoring soil microorganism and plant are mandatory to study the effect of soil augmentation on plant growth. So that we can know whether or not microorganism are affected by the nutrients, or the soils microorganisms are not adversely affected by the fertilizer application. In this study, we are introducing the methods of soil bacterial community structure analysis as the way to monitor soil microorganism status, and as a method to study interlink between fertilizer application, soil microbial community structure and growth of *Sorghum bicolor*.

Since microorganisms not only found in soil but also in plant as endophytic or saprophytic microorganism microorganism, and they may responds quickly to environmental changes, and play role on plant growth, therefore we also isolate them to study their possible role on plant growth promotions and increase plant growth under water shortage conditions. We will use the information for optimal fertilizer applications. It is well known that soil bacteria and endophytic associated microorganism contribute to plant survival under critical condition therefore those microbes are tested for their role as biocontrol agent of *Sorghum bicolor*.

To study the effect of fertilizer application, 3 cycles of *Sorghum bicolor* plantation have been carried out in Cibinong, and Katingan test plot, and conduct metabarcoding analyses of 16S rDNA of bulk soil during flowering stage. The culture dependent through isolation of soil microorganism and endophytic microbes in plant are also performed. Minor community member of soil bacteria was fluctuative but major microbial groups was stable after 2 cycles of *Sorghum bicolor* plantation. Fertilizer application not adversely affect major component of soil microbial community structure, which might suggest appropriate application of fertilizer not adversely affect microbial community



structure. We have selected 78 endophytic bacteria from stem and root of sweet sorghum plants. All the 78 isolates were tested for antagonist activity by direct confrontation method. The mechanisms of fungal inhibition of selected isolates were investigated for antifungal compounds production and lytic enzymes activity. Antifungal compounds production was checked by detecting the presence of NRPS and PKS gene. Lytic enzymes activity of the bacteria was evaluated by their ability to produce cellulase, chitinase, and protease. Selected bacteria were identified using molecular analysis based on 16S rRNA gene. 14 out of 78 tested isolates showed antagonistic activity. Two isolates designated as ACNM5 and ACNM6 were able to inhibit all four fungal strains. Only one bacterial extract, designated as ACNM4 showed fungal inhibition and 4 bacteria designated as ACIL1, ACNM4, ACNM6 and ATNM4 produced antifungal compounds via NRPS pathway. Ten isolates were able to produce hydrolytic enzymes. Two isolates, designated as BTIL3 and BTIL6 showed cellulose, chitinase, and protease activity. Nine of 14 isolates were able to produce protease. Therefore, we assumed that the mechanism to inhibit fungal growth was mostly done by their proteolytic ability. Based on 16S rRNA gene analysis, showed that the genus Burkholderia was potential as biocontrol agent.

Keywords: endophytic microbes, fertilizer application, 16S RNA metabarcoding analysis, soil bacterial community structure, Sorghum bicolor



Revegetation of Degraded Grassland with Sorghum Plants Using Anorganic and Organic Fertilizer Application in Cibinong and Katingan of Indonesia

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Abstract

Revegetation implies planting trees, shrubs and other plants in disturbed/degraded areas. Sorghum plant could grow at many condition of lands, and has many uses such as for human food source (seeds), animal feed (leaves), source of bioethanol, sugar and important material (stem). In combination with many species of intercropping plants, sorghum plants were cultivated 3 cycles and 2 cycles in Imperata cylindrica grassland of Cibinong (West Java) and Katingan (Central Kalimantan), respectively in 2016 until 2018. The objective of these revegetations was to improve the productivity of degraded grassland through planting sorghum by applying anorganic and organic fertilizer. The methods of the revegetation were (1) conducted soil chemical analysis, (2) designed the site plots according to the research treatments, i.e. completely randomized design, (3) cultivated and evaluated the sorghum plants. Treatments of fertilizer included the uses of compost, inoculated microbes and dosage of N fertilizer, i.e. 0%, 25%, 50%, 75% and 100%. The results of soil chemical analysis before and after sorghum cultivation, plant growth, total biomass, leaf chlorophyl content, sugar content of stem juice and production of sorghum plants as well as N content of the sorghum plants will be presented and discussed in detail furthermore.

Keywords: Revegetation, Degraded land, Sorghum, N Fertilizer, Organic Fertilizer



Development of Novel Molecular Techniques for the Evaluation of Soil and Plant Status

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Abstract

Applying appropriate amount of fertilizers is critical to support vigorous growth of crop plants. Methods for monitoring soil and plant status are mandatory, so that we can know whether or not the plants are satisfied with the nutrients, or the soils are not adversely affected by the fertilizer application. In this study, we are trying to establish the methods of soil bacterial community structure analysis as the way to monitor soil status, and the methods of biomarker-based diagnosis of nutritional stress of sorghum plants.

Since soil microorganisms respond quickly to environmental changes, their community structures are used to assess the soil condition. However, to date only limited information is available about the effects of agricultural practices on soil bacterial community structure. We thus cultivated sorghum on the plots that had not been used before, then performed 16S RNA metabarcoding analysis. The results from two seasons suggested that the sorghum cultivation indeed affected the bacterial community structure, but the change was smaller than the seasonal changes. Biodiversity of soil bacteria was not significantly affected by the cultivation. We also found that automated ribosomal intergenic spacer analysis (ARISA) can be used to analyze the bacterial community structures.

As the index of nutritional sufficiency of plants, we looked for the parameters reflecting internal nitrogen status of hydroponically-cultured sorghum seedlings, then identified the expression of certain genes and the leaf SPAD values to respond early to nitrogen limitation. We are now examining if these biomarker candidates are applicable to the plants grown outside on soil as the practical samples.

Keywords: automated ribosomal intergenic spacer analysis (ARISA), 16S RNA metabarcoding analysis, nutrient stress, soil bacterial community structure, *Sorghum bicolor*

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Studies and Development of Indonesian Rice and Sorghum Cultivars with High Lignin Content

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Abstract

Lignin has potentials to be utilized as raw materials for energy, chemical and medical industries. As the major sources of natural and renewable lignin are plants, breeding strategies to produce biomass plants with high lignin content are desirable. Meanwhile, the vast area of marginal under-cultivated land in Indonesia can be utilized sustainably for the biomass production for that purpose. In this project we are focusing on two approaches for lignin production, the first is the screening and breeding of drought tolerant, high biomass, and high lignin content sorghum (Sorghum bicolor) and the second is the studies of the function of transcription factors (TF's) predicted to play roles in lignin biosynthesis by genetic engineering using rice as a model system. Sorghum has the potential to be cultivated for different purposes such as food, feed or energy sources. The ability of sorghum to grow in marginal areas prone to abiotic stresses, such as drought, better than other crops, makes it an ideal crop to be cultivated in those mainly alang-alang (Imperata cylindrica) dominated marginal non-productive areas, which can be found all over Indonesia. For that purpose, we screened 30 Indonesian sorghum genotypes under field and glass house conditions to assess their productivities, biomass and total lignin productions. The drought tolerant status of the sorghum genotypes were estimated by bioassay under PEG6000 in a growth chamber and their physiological responses under drought were observed by measuring their water use efficiency, leaf water potential and photosynthetic rates. We are now attempting to breed high biomass and high lignin content sorghum lines. In addition, we are also studying the roles of 3 TF's from rice (Oryza sativa) and Arabidopsis thaliana, indicated to have roles in cell wall depositions for their functions in lignin biosynthesis in rice in collaboration with Professor Toshiaki Umezawa and his team at Kyoto University. Transformations of 2 TF's have been performed to japonica rice cv Nipponbare and javanica rice cv Rojolele. Putative transgenic rice lines overexpressing the TF's have been obtained and molecular characterizations are being carried out. The lignin contents of the overexpressing rice lines will be determined.

Keywords: marginal land, lignin, drought, rice, sorghum, transcription factors



Selection and Breeding of Grass Plants with High Carolific Biomass

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Abstract

Sustainable use of deteriorated grasslands left after the tropical deforestation has been of increasing concern in many Southeast Asian countries including Indonesia. The reclamation of the grasslands through converting them to biomass crop fields is one of the promising solutions to this problem, which is in line with the direction of Sustainable Development Goals (SDGs). Grass biomass crops, such as Sorghum, generally show much higher biomass productivity than trees, and hence are potent lignocellulosic biomass feedstocks. To promote their applications to bio-based materials and chemicals, utilization characteristics of their lignocelluloses is needed to be improved via breeding approaches. In the present study, with bioengineering techniques such as CRISPR/Cas9 genome-editing to manipulate expressions of enzymes and transcription factors affecting the content and/or structure of lignin, a major component of lignocellulosic biomass, we strive to generate transgenic rice (Oryza sativa) plants with enhanced biomass heating values, which will provide beneficial information for breeding grass biomass plants. In parallel, using near-infrared (NIR) spectroscopy, we work for development of a screening system for selecting Sorghum crop varieties, aiming at rapid identification of genetic resources that show superior lignin characteristics.

Keywords: CRISPR/Cas9, grass biomass plants, heating value, lignin, rice, Sorghum



Enhancement of Grass Plants Particleboards Properties Glued with Natural Adhesives

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Abstract

Grass plants such as alang-alang (Imperata cylindrica) and sorghum (Sorghum bicolor) have been tried as alternative materials for particleboard due to the decreasing of wood from natural forest as the main raw material of the particleboard industry. However some of the grass plants particleboard properties are needed to be improved. Therefore experiments to enhance the board properties were done. Among them are addition of sucrose to citric acid as adhesive of alang-alang particleboard, addition of catalyst to citric acid as adhesive of sorghum bagasse particleboard, and combination of sengon wood and sorghum bagasse particles as raw material for particleboard. Effects of citric acid and sucrose ratio of 25: 75, 20:80, 15:85, 10:90, 5:95 were tried with adhesive content of 20% and pressing condition of 200°C for 10 mins for alang-alang particleboard. Results showed that addition of sucrose enhanced the particleboard properties. One of the disadvantages of using citric acid and sucrose is that high temperature (200°C) should be applied. In order to decrease the pressing temperature, 1-3% of ZnCl₂ catalyst was added to accelerate the curing reaction of citric acid-sucrose adhesive of sorghum bagasse particleboard. Thermal analysis DSC and TGA, and FT-IR and insoluble matter analysis were used to investigate the effect of catalyst to the curing behavior of citric acid-sucrose adhesive. Results of DSC and TGA analysis showed that ZnCl₂ decreased the curing temperature, enthalpy reaction and activation energy. The particleboard properties that prepared using the pressing temperature of 180°C and 1% ZnCl₂ catalyst are met the type 8 board of the JIS standard. Ratios of sengon wood : sorghum bagasse particles of 100:0, 75:25, 50:50, 25:75, 0:100 were tried to prepare particleboard using citric acid concentration of 15% and 20%. The properties were tested in accordance to JIS A 5908. The results showed that higher ratio of sengon wood improved the internal bond strength, while the higher ratio of sorghum bagasse improved other properties such as thickness swelling, and bending strength. All board properties were met the JIS standard.

Keywords: citric acid, alang-alang leaves, sorghum bagasse, particleboard, properties enhancement



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Patent Mapping : Study Case of Sorghum Biomass Particle **Board Product**

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Abstract

Initiating development from natural resources transform into primarily product, preserve many opportunity in utilized potential useful in diversification model of product especially wood. Development of environmentally friendly particleboard made from sweet sorghum bagasse has recently attracted attention. On the other hand, patent information has been used mainly for patent document searches and patent clearance searches, including prior art searches and infringement searches. Although a patent document naturally includes a lot of information, by using multiple patent documents at the same time, it is possible to take new approaches which could reveal new information that would otherwise not be available. Patent Map defined as "Patent information collected for a specific purpose of use, and assembled, analyzed and depicted in a visual form of presentation. This information contains selected themes for research and development, pick out new ideas, and gain an understanding of competitors' technology development, or technology foresight. In this study, patent mapping is used for analyzing sorghum particle-board based.

Keywords : Patent Mapping, Sorghum Biomass Particle Board, Analyzing



Potential of Wood-Based Materials using Sorghum Bagasse and Citric Acid

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Abstract

The aim of this study is to clarify the effective manufacture conditions of woodbased materials composed of grass plants and bio-based adhesives. We have been investigated relationship between the manufacture conditions and the material properties as to particleboard and molding using sorghum bagasse and citric acid. According to our research results, the optimum manufacture conditions of the particleboard were citric acid content of 20wt%, pressing temperature of 200°C and pressing time of 10min. In addition, sucrose addition as an adhesive component was found to be effective to improve the mechanical properties. The physical and mechanical properties of the particleboard bonded with citric acid and sucrose satisfied the requirement of 18type, JIS A 5908. The biological durability was similar to that of particleboards bonded with synthetic resin adhesives. In the molding, effects of adding method and content of citric acid on the physical and mechanical properties were investigated. As the adding method, powder and solution additions were attempted. It was clarified that the optimum citric acid content was different by the adding method.

Keywords: sorghum bagasse, particleboard, molding, citric acid, manufacture condition





Poster Presentation