Study of Plant Growth Promoting Bacteria from Coconut Coir Dust

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Abstract. Coconut coir dust or commercially known as cocopeat was a waste product of the coconut industry and was used as growing medium. Coconut coir dust as an environmentally sound peat substitute in soilless growing medium for crop production due to suitable physical and chemical properties. Plant growth-promoting bacteria (PGPB) are bacteria that can enhance plant growth and protect plants from disease and abiotic stresses through a wide variety of mechanisms. The objective of this study are to isolate and characterize PGPB from coconut coir dust including phosphate solubilization, indole acetic acid (IAA) production, protease enzyme and siderophore degradation activity. Total 40 isolates PGPB bacterial isolates were isolated and screened for their various activities and the result showed that 16 isolates have highest activity of PGPB. Four isolates in case of IAA production and CP.2AT isolate can produce IAA 28 ppm by High Performance Liquid Chromatography analysis, 3 isolates shown protease enzyme production (halo zone 1.6 – 2.2 cm), 5 isolates were confirmed have phosphate-solubilizing activity, 2 isolates have shown lignin degradation activity, 2 isolates were confirmed of cellulose degradation activity and 8 isolates have ability to production of siderophore. In addition composted coconut coir dust contain much more total of PGPB and lower C/N ratio than fresh coconut coir dust. Composting of coconut coir dust increased number of lignin and cellulose degradation bacteria. This results of this study indicated that coconut coir dust potentially for growing medium with high activity of plant growth promoting bacteria.

1. Introduction

Coconut coir dust or commercially known as cocopeat was a waste product of the coconut industry and was used as growing medium. Coconut coir dust as an environmentally sound peat substitute in soilless growing medium for crop production due to suitable physical and chemical properties [1]. Coconut coir dust is free from phytopathogens, easy to handle and transport, approximately 25% air-filled porosity (AFP) and 34% readily available water (RAW) with adequate water buffering capacity (WBC) [2]. Coconut coir dust is described as that brown, spongy particle of low weight which falls out when the fiber is shredded from the husk. The coir dust is about 70% of the weight of the coconut husk [3]. Coir dust is rich in lignin and tannins. Coconut coir dust was a local remaining material from manufacturing processes, popularly used as a growing medium for many ornamental plant production [4] because it had large water holding capacity, high contents of some nutrients (K, Na and Mg) [5].

Plant growth-promoting bacteria (PGPB) are bacteria that can enhance plant growth and protect plants from disease and abiotic stresses through a wide variety of mechanisms. The ability of diverse bacterial to promote plant growth occurs as a consequence of either direct or indirect mechanisms. Direct promotion of plant growth occurs when a bacterium either facilitates the acquisition of essential nutrients or modulates of level of hormones within a plant. Nutrient acquisition facilitated by PGPB typically includes nitrogen, phosphorus and iron. Modulation of hormone levels may entail PGPB (Plant Growth- Promoting Bacteria) synthesizing one or more of the phytohormones auxin, cytokinin and gibberellin. PGPB may promote plant growth directly usually by either facilitating resource acquisition
or modulating plant hormone levels. Indirect promotion of plant growth occurs when a PGPB decreases the damage to plants following infection with a phytopathogen including some soil fungi and bacteria. This usually occurs by the inhibition of the pathogen by the PGPB, decreasing the inhibitory effects of various pathogenic agents on plant growth and development, that is, by acting as biocontrol bacteria [6,7]. The aim of this research are to explore and study the potential of plant growth promoting bacteria activities from coconut coir dust.

2. Materials and methods

2.1 Qualitative analysis of plant growth promoting bacteria

Coconut coir dust (cocopeat) were collected from Riau, Indonesia. Cocopeat were added in sterile distilled water in ratio 1:10 (10 gram of cocopeat in 100 ml of distilled water) in the Erlenmeyer and homogenized by shaker for half an hour and decimally diluted in distilled water. Method of dilution was used spread plate technique. The last dilutions (1 x 10^{-4} dilution for phosphate solubilizing bacteria, siderophore and 1 x 10^{-5} dilution for enzyme protease activity and IAA producing bacteria) were used to spread on different specific cultural medium, namely skim milk agar medium for enzyme protease activity, Tryptic soya broth (TSB) and Pikovskaya agar medium [8] were used for isolation of IAA and phosphate solubilizing bacteria respectively. Blue agar with Chromo Azurol S (CAS) medium for siderophore activity. Lignin and cellulase activity were observed qualitatively on specific medium to know their degradation activity. The isolates were sub cultured on their specific medium for purification.

2.2 Phosphate Solubilizing and protease enzyme ability of bacterial isolates

Bacterial isolates were isolated for their phosphate solubilizing ability on pikovskaya medium. These medium were supplemented with insoluble phosphate Ca_{3}(PO_{4})_{2}. The solubilization zone were observed after 120 h of incubation at 30 °C. Skim milk agar medium was used for determination protease enzyme ability of isolates. The solubilization zone were measured after 72 h after incubation.

2.3 Siderophore production

Bacterial isolates were isolated for siderophore production on Chrome Azurol S (CAS) plates by spot inoculated and incubated at 37 °C for 24-48 hours. Halo zone were observed [9].

2.4 Analysis of Indole-3-acetic acid concentration

Four bacterial isolates (CP1C, CP2A, CP2C,CP2D) were grown on an Erlenmeyer containing liquid medium of TSB + 100 ppm Tryptophan. The bacterial culture was incubated for 72 hours at room temperature. The pH of the supernatant was adjusted to 2.8 with hydrochloric acid then the supernatant was extracted three times with equal volumes of ethyl acetate. The extract was evaporated to dryness and resuspended in 1 mL of ethanol. The samples were analyzed by high-performance liquid chromatography (HPLC) diode-array UV detection using a Symmetry C18 column at 280 nm. The mobile phase consisted of methanol/acetic acid/water (30:1.70, v/v/v) and the flow rate was 1.2 mL/min Standard solutions were prepared with pure indole-3-acetic acid [10].

3. Results and discussions

3.1 Population of plant growth promoting bacteria in the coconut coir dust

Total population of bacteria were observed in six medium. The result showed that coconut coir dust have bacteria with potential activities as plant growth promoting. This is indicated by the activity of bacterial on the specific medium. All bacterial isolates grew well on the specific medium. Forty isolates were obtained in various medium.
Table 1. Total population of plant growth promoting bacteria of coconut coir dust in various medium

| Sample          | Population ( CFU mL$^{-1}$) |
|-----------------|----------------------------|
|                 | NA   | PRO  | TSB  | PK   | CMC  | Poly R  |
| Cocopeat I (CP1) | 3.75x10$^7$ | 4.00x10$^7$ | 7.85x10$^7$ | 4.88x10$^7$ | 1.10x10$^8$ | 7.5x10$^6$ |
| Cocopeat II (CP2) | 9.59x10$^6$ | 1.50x10$^6$ | 8.25x10$^7$ | 4.88x10$^7$ | 4.68x10$^7$ | 3.33x10$^6$ |

3.2 Phosphate Solubilizing bacteria and Protease enzyme producing bacteria

Total 40 isolates PGPB bacterial isolates were isolated and screened for their various activities and the result showed that 16 isolates have highest activity of PGPB.

Table 2. Calcium phosphate solubilization by bacterial isolates in agar medium

| Bacterial isolate | Halo zone (cm) |
|-------------------|---------------|
| CP.1BPK           | 0.85          |
| CP.2APK           | 0.75          |
| CP.2EPK           | 0.72          |
| CP.1EPK           | 0.7           |
| CP.1APK           | 0.7           |

Five isolates were confirmed have phosphate-solubilizing activity. The ability to solubilize phosphate was observed by the formation of a halo zone around the colony after 72 h incubation. A halo zone will be formed when the bacterial isolates produce organic acid which will solubilize tri-calcium phosphate in the medium. Phosphate solubilizing bacteria render more phosphates into the soluble form that required for their growth and metabolism by secreting organic acids and/or enzymes (e. g. phosphatases), the surplus get the plants [11].

Figure 1. Phosphate solubilizing bacteria

The ability to produce protease enzyme was observed by the formation of a halo zone around the colony after 72 h incubation. Three isolates shown protease enzyme production are CP.1CPR (1.7 cm), CP.2APR (2.2 cm) and CP.2EPR (1.6 cm). Isolates which has protease enzyme as candidate for biocontrol agent. Proteolytic enzyme production was detected as formation of clear zone around the bacteria. Qualitative analysis of bacterial protease activity was carried out by growing bacterial isolates on the skim milk agar medium and the resulting clear zones were calculated.
The medium contains protein in the form of casein, which is milk protein that is not permeable to microorganism cell transport systems. Therefore, microorganisms produce extracellular proteases to hydrolyze casein to amino acids, transport it into cells, and use it in cells [12]. The clear zone formed in the medium indicates the casein hydrolytic reaction by extracellular proteases. Conversely, the absence of a clear zone in the medium indicates no casein hydrolytic reaction by extracellular proteases. Through the activity of these enzymes, plant growth promoting bacteria play a very significant role in plant growth promotion particularly to protect them from biotic and abiotic stresses [13,14].

3.3 Indole-3-acetic acid (IAA) producing bacteria
Four isolates in case of IAA production and CP.2AT Isolate can produce the highest IAA after 72 incubation by High Performance Liquid Chromatography analysis. The results originated from both qualitative and quantitative assays of IAA reflected the ability of four tested microorganisms to produce indole compounds. The four tested microorganisms exhibited a pink to red color with a little variation in intensity.
IAA analysis using HPLC on four isolates showed that there was a peak chromatogram with a retention time of 7.8 minutes, the peak of the chromatogram was the peak from IAA (Figure 3). The chromatogram peak retention time was compared with pure IAA. Based on the standard curve, the concentration of IAA produced by the isolates was 28 ppm. The IAA hormone is synthesized as a secondary metabolite that is produced under conditions of growth of suboptimal bacteria or when available amino acid tryptophan precursors. IAA biosynthesis by bacteria can be enhanced by the addition of L-tryptophan as a precursor to the bacterial growth medium. Indole acetic acid produced by more than 80% of bacteria, which have ability to regulate plant growth [15]. However, phytohormones produced by microbes are more effective in plant growth due to their continuous and slow release [16].

Siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation [17]. Eight isolates have ability to production of siderophore by the formation of halo zone. Hence, bacterial siderophores help to alleviate the stresses imposed on plants by high soil levels of heavy metals. Siderophores contributes to provide nutrition to plant and microorganism but also in other environmental applications such as soil mineral weathering, biogeochemical cycling of Fe in oceans, and biotechnological applications such as enhancing growth and pathogen biocontrol of plants, biocontrol of fish pathogens, microbial ecology and taxonomy, bioremediation of environmental pollutants, petroleum hydrocarbons, nuclear fuel reprocessing, optical biosensor, bio-bleaching of pulp [18].

Two isolates have ability to degrade lignin (CP.1ALG and CP.1BLG) and two isolate have ability to degrade cellulose (CP.2ACC and CP.2ECC).

C/N ratio of coconut coir dust decreased from 58 to 27 after composting process. The result showed that composting of coconut coir dust increased number of lignin and cellulose degrading bacteria. Composting of organic materials requires a C/N ratio of 30 :1 or less [19], the development of composting technologies for coir with high C/N ratio and lignin content involved fertilizer nitrogen supplements and lignin degrading microbial cultures. These technologies have enabled the utilization of composted coconut coir as a valuable plant growth medium, plant nutrient source and soil conditioner [20].

4. Conclusion

The present study clearly indicate the potential activities of plant growth promoting bacteria from coconut coir dust as phosphate solubilizing, IAA hormone producing, siderophore producing, lignin
and cellulose degrading bacteria and proteolytic enzyme production. Composted coconut coir as valuable plant growth medium with the C/N ratio 27.

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6. References
[1] Arenas C S, Vavrina J A, Cornell E A, Hanlon and Hochmuth 2002 *Hortscience* 37(2):309–312
[2] Asiah A, Mohd R I, Mohd K Y, Marziah M and Shaharuddin M 2004 *Pertanika J. Trop. Agric. Sci.* 27(2):121-133
[3] Tejano E A 1984 *State of the Art of Coconut Coir Dust and Husk Utilization* (General Overview) Paper presented during the National Workshop on Waste Utilization Coconut Husk held on November 12 at the Philippine Coconut Authority Diliman Quezon City Phillipines
[4] Meerow A W 1997 *Greenhouse Product News* January17–21
[5] Abad M, Noguera P, Puchades R, Maquieira A and Noguera V 2002 *Bioresource Technology* 82(3) 241–245
[6] Glick B R 1995 *Canadian Journal of Microbiology* 41(2): 109–117
[7] Santoyo G, Moreno H G, del C O M and Glick B R 2016 *Microbiol* 183 : 92– 99
[8] Pikovskaya 1948 *Mikrobiologiya* 17 : 362-370
[9] Milagres A M, Machuca A and Napoléao D 1999 *J. of Microbiol. Methods* 37 (1): 1-6
[10] Mehnaz S and Lazarovits G 2006 *Microb Ecol* 51: 326–335
[11] Vessey J K 2003 *Plant and Soil* 255(2): 571–586
[12] Harley J P and Prescott L M 2002 *Laboratory Exercises in Microbiology* 5th Edition The McGraw-Hill Companies
[13] Nadeem S M, Naveed M, Zahir Z A and Asghar H N 2013 *Springer* 51-103.
[14] Upadayay S K, Maurya S K, Singh D P 2012 *Ind J Sci Res* 3: 73-78.
[15] Jha C K, Saraf M 2015 *Journal of Agricultural Research and Development* 5(2): 0108-0119
[16] Gupta G, Parihar S S, Ahirwar N K, Snehi S K, Singh V 2015 *J Microb Biochem Technol* 7(2): 96-102
[17] Indiragandhi P, Anandham R, Madhaiyan M and Sa T M 2008 *Curr. Microbiol.* 56 327–333
[18] Ahmed E, Holmström S J M 2014 *Microbial Biotechnology* 7:196-208
[19] Hammouda G H H and Adams W A 1987 *Elsevier Applied Science* 245–253
[20] Prabhu S R and Thomas G V 2002 *J. Plantation Crops* 30 1–17