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Soil mineralization as effects of plant growth promoting bacteria isolated from microalgae in wastewater and rice straw application in a long-term paddy rice in Central Viet Nam

Nguyen-Sy Toan [a], Thi Dong Phuong Nguyen [a], Tran Thi Ngoc Thu [a], Duong Thi Lim [b], Pham Duy Dong [c], Nguyen Thanh Gia [d], Kuan Shiong Khoo [e], Kit Wayne Chew [f, g], Pau Loke Show [e]

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Soil mineralization as effects of plant growth promoting bacteria isolated from microalgae in wastewater and rice straw application in a long-term paddy rice in Central Viet Nam

Nguyen-Sy Toan, Thi Dong Phuong Nguyen, Tran Thi Ngoc Thu, Duong Thi Lim, ... Pau Loke Show

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Abstract

This study investigates the effects of rice straw and potential nitrogen fixing *Bacillus subtilis* (*Bacillus* sp.) isolated from seafood wastewater on carbohydrate- and nitrogen mineralization from a long-term rice paddy soil by four weeks anaerobic incubation. Soil collected at the depth of 0–15 cm from a long-term rice paddy cultivation, and set up as following: (1) Control (10 g air dry soil), (2) Rice straw (control + 0.2% rice straw w/v), (3) *Bacillus* sp., and (4) Combine (rice straw 0.2% w/v and *Bacillus* sp.) was subjected to an incubator in dark at room temperature/ submerge condition for 4 weeks anaerobic incubation. As showed in results, content of decomposed carbohydrate ranged from 83–447 mg kg $^{-1}$ soil. Content of extracted carbohydrate was not affected by rice straw application, but significant decreased with bacillus or rice straw-bacillus combine inoculation (2.0–2.2 times decreased) compared to Control treatment, which is closely to the initial soil extraction carbohydrate. In contrary, mineralization nitrogen increased 120% in *Bacillus* sp. inoculation compared to Control treatment, but Rice straw and Combine treatments

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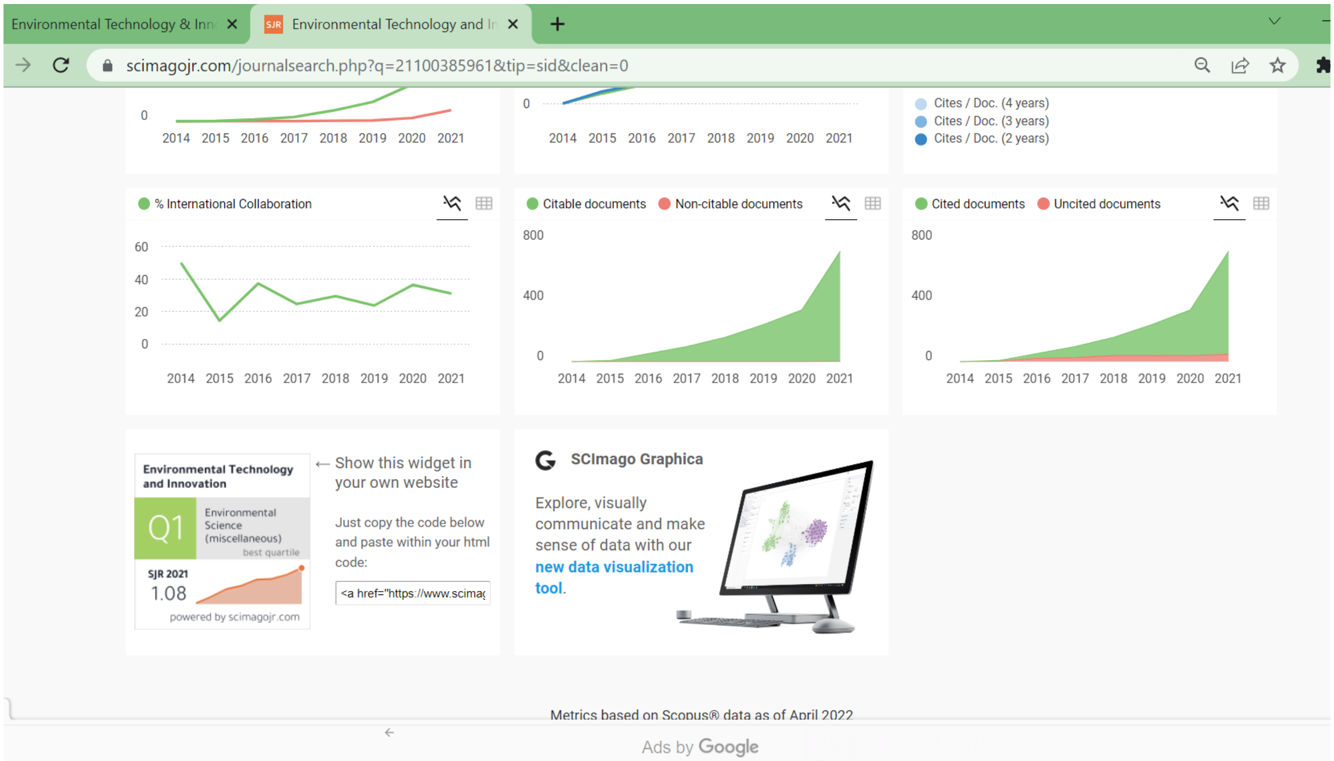
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Soil mineralization as effects of plant growth promoting bacteria isolated from microalgae in wastewater and rice straw application in a long-term paddy rice in Central Viet Nam

Nguyen-Sy Toan [a,*], Thi Dong Phuong Nguyen [a,*], Tran Thi Ngoc Thu [a], Duong Thi Lim [b], Pham Duy Dong [c], Nguyen Thanh Gia [d], Kuan Shiong Khoo [e], Kit Wayne Chew [f,g,*], Pau Loke Show [e,*]

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A B S T R A C T

This study investigates the effects of rice straw and potential nitrogen fixing *Bacillus subtilis* (*Bacillus* sp.) isolated from seafood wastewater on carbohydrate- and nitrogen mineralization from a long-term rice paddy soil by four weeks anaerobic incubation. Soil collected at the depth of 0–15 cm from a long-term rice paddy cultivation, and set up as following: (1) Control (10 g air dry soil), (2) Rice straw (control + 0.2% rice straw w/v), (3) *Bacillus* sp., and (4) Combine (rice straw 0.2% w/v and *Bacillus* sp.) was subjected to an incubator in dark at room temperature/ submerge condition for 4 weeks anaerobic incubation. As showed in results, content of decomposed carbohydrate ranged from 83–447 mg kg^{-1} soil. Content of extracted carbohydrate was not affected by rice straw application, but significant decreased with bacillus or rice straw-bacillus combine inoculation (2.0–2.2 times decreased) compared to Control treatment, which is closely to the initial soil extraction carbohydrate. In contrary, mineralization nitrogen increased 120% in *Bacillus* sp. inoculation compared to Control treatment, but Rice straw and Combine treatments resulted in immobilization more than nitrogen mineralization. Inoculation of *Bacillus subtilis* is recommended as a good environment friendly method to enhance the soil fertility and reduce nitrogen immobilization. Further research within rice plant is therefore needed to consolidate the preliminary results.

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1. Introduction

Rice is one of the world's important staple foods, with the production increased by 3.5 times and land used area increased by 1.5 times from 1961 to 2019 (FAO, 2021). The increase of world population requires us to improve the rice production. However, the process of growing rice incurs two main problems; (i) how to deal with the rice plant residue, especially rice straw after harvesting and, (ii) how to reduce chemical fertilizers but improve rice production. It has been reported that growing rice and treating rice residue contribute a considerable amount of greenhouse gas emission (FAO, 2021). Secondly, the application of large amount of chemical fertilizer also contribute to the environmental pollution.

To deal with the rice straw, the waste materials could be removed from the field, burned *in situ*, piled or spread in the field, incorporated in the soil, or used as mulch for the next crop. Rice straw is one important source of organic matter for agriculture, and in the long-term, rice straw could be used as inorganic material which can improve soil organic matter, as well as crop productivity (Cheng et al., 2016; Kautsar et al., 2020; Tang et al., 2016). Rice straw as a lignocellulosic waste material has been widely applied as organic fertilizer as it has beneficial properties as a soil amendment (Cheng et al., 2016; Chia et al., 2020; Kautsar et al., 2020; Nguyen-Sy et al., 2020). Applying rice straw or rice straw compost increased significantly the soil organic carbon, nitrogen content as well as their labile pools (which regards to hot water and cold water extracted carbon and nitrogen). It is interesting that soil organic carbon in bulk soil creates a slow-release environment as compared to those changes in the labile part, for example, in water extracted organic carbon (Nguyen-Sy et al., 2020). The increase of carbon and nitrogen stock in rice paddy affected by rice straw amendment are also reported elsewhere (Cheng et al., 2016; Kautsar et al., 2020; Tang et al., 2016). The effect of organic matter addition are observed in the soil organic matter stock, and also in their isotope values. A recent study reported that rice straw incorporated with soil changed the stable isotope carbon after long term additions (Nguyen-Sy et al., 2020), however, the change in soil stock and labile fraction were completely different. The impact of rice straw on soil dynamics is therefore not fully understood.

To prevent the increasing usage of chemical fertilizers in the paddy field, plant growth-promoting bacterial (PGPB) has been considered as a promising method, which is proven for its beneficial impacts in agriculture (Bashan, 1998; Chew et al., 2019; Hashem et al., 2019). PGPB has been considering as the green technology in term of reducing chemical fertilizer input, resulted in improving soil health (Ramakrishna et al., 2019). The benefits of PGPB includes serving as supporting nutrient for plant (Meena et al., 2016; Nanjundappa et al., 2019), helping plants to growth well under environment stresses (Hashem et al., 2019; Saravanakumar et al., 2011), and to protect plants from diseases (Rekha et al., 2018). It is clear that the impact of PGPB not only supports soil fertility enhancement, but also aids in the plant health aspects.

Researches on rice straw application has been conducted and beneficial bacterial inoculation was performed to improve soil quality, however, there are very few reports combining rice straw and bacterial application. It is important to understand the interaction between paddy field residues such as rice straw and PGPB. An *in situ* experiment to evaluate potential of combining rice straw and PGPB could be a potential idea for further pilot scale solution. In this work, two available forms of carbon (decomposable carbohydrate) and nitrogen (mineralized nitrogen) were suggested to be main parameters for this experiment.

In this study, isolated microbial from wastewater make it as the main novelty of this work since the other researches are mostly focused on isolating soil bacterial. Therefore, the objective of this study was to investigate the effects of rice straw and wastewater *Bacillus* sp. on improving soil quality for its ability of generating decomposed carbohydrate and nitrogen mineralization.

2. Material and methods

2.1. Site description and treatment design

Soil samples were collected the paddy field in Hoa Vang District, Da Nang City (15°58'29.0"N 108°11'31.3"E). Soil was classified as alluvial soil type, with 32% sand, 38% silt and 30% clay. More detail information of soil properties in the study site showed in Table 1. The site belongs in central region of Vietnam that runs a long-term paddy cultivation twice a year. The field size is 20 m × 30 m, and has been used for rice cultivation for more than 100 years. The mean annual temperature in the area was 26.2 °C, mean annual precipitation was 2488 mm in the duration of 2013–2018 in Da Nang city. Surface soil layer was collected at the depth of 0–15 cm that is considered as ploughed layer.

2.2. Bacterial strain and growth media

In this study, *Bacillus subtilis* MT300403 (*Bacillus* sp.), which was chosen from the microbial isolation at a seafood wastewater treatment factory in Da Nang, Viet Nam. The LBB (Luria-Bertani broth) ingredients (% w/v) including 1.0% NaCl, 0.5% yeast extract, 1.0% trypton was utilized as the control culture of bacteria. Bacterial was inoculated in LBB in an incubator at 37 °C until its OD 600 reached 1.0 (measured by a UV–Vis UVD-300, Labomed. Inc, USA). Bacterial solution was centrifuged at 4300RPM/10 mins to get the pellet, and then diluted in distilled water for anaerobic incubation experiment in Section 2.3.