Rice husk biochar with beneficial microbes

A promising agricultural inoculant and soil ameliorant

The research of Shohei Ebe and Takashi Ano from Kindai University into the relationship between a microbe and rice husk biochar (RHB) suggest the latter is an activator of beneficial microbes that can be used to combat phytopathogenic microorganisms. Having isolated a novel lipopeptide producing Bacillus sp. that benefits from RHB presence in the soil, these are promising insights into how agriculture can benefit from biotechnology and how unavoidable agricultural waste can be used to create sustainable and environmentally friendly farming methods.

According to the UK's Royal Horticultural Society, the use of waste such as the ash resulting from wood fires can be beneficial to the soil when added to compost heaps or directly to the ground. The benefits of this stem from the potassium and trace elements it contains and its action as a liming agent which can help to remedy acidic soils. Moving away from the use of chemical fertilisers and pesticides has been promoted in efforts to switch to environmentally friendly farming methods. Organisms that are harmful to plants are known as phytopathogens. These microorganisms in the soil make up part of its existing microbial community known as the microbiome. They are causative agents of plant diseases and it is estimated that the presence of these phytopathogens reduces the harvest of major crops by up to 10% across the world. In order to minimise this loss and reduce the use of agrochemicals, crops can be protected through the application of beneficial microorganisms.

RICE HUSK BIOCHAR

The rice plant is one of the most common food crops cultivated in many countries. As a result, over 150 million tons of rice hulls are produced around the world as unavoidable agricultural waste material when the rice is separated from the paddy. The term paddy is used to refer to rice still containing hull and is derived from the Malay word padi, meaning "rice plant". Effective utilisation of rice hulls is important for both solving the problem of agricultural waste and creating sustainable agriculture.

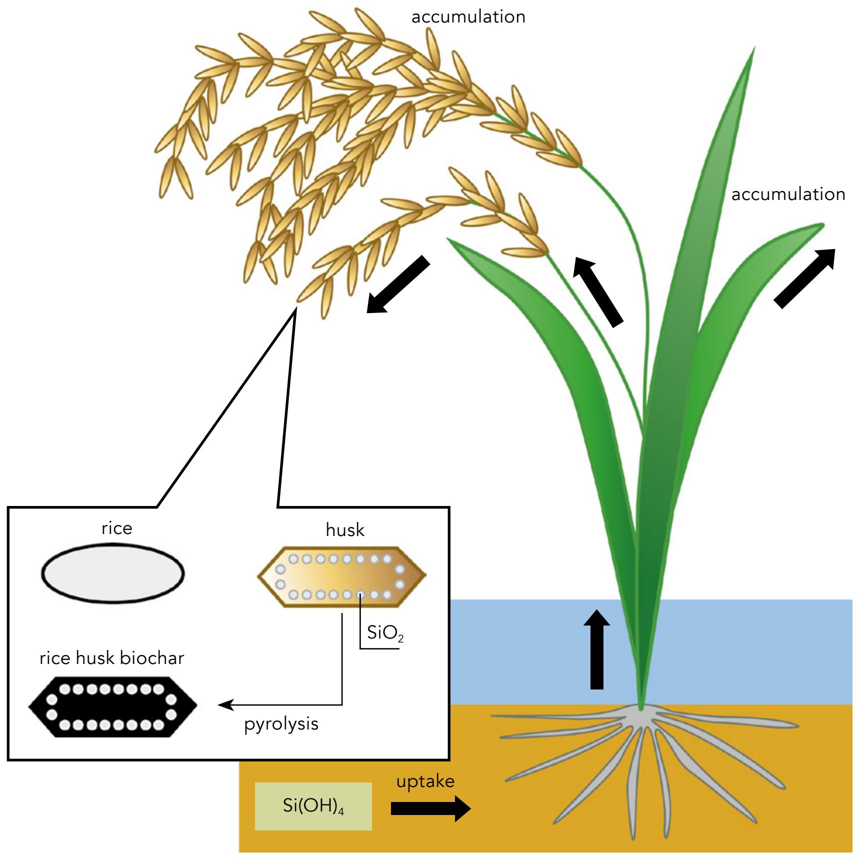
Rice husk biochar (RHB) is produced by low temperature pyrolysis of these rice hulls and has been traditionally used in Japan as a soil ameliorant. Pyrolysis of biomass is a thermal degradation process in the absence of oxygen and produces gas, tar, and char. The product distribution depends on pyrolysis conditions which range from heating rate and peak temperature to particle size. RHB had been thought to increase crop productivity by amending soil structure and improving nutrient adsorption. These effects had been explained only through physical and chemical properties.

Recently, researchers found that biochar also affects the biological properties in agricultural soil. For example, they reported that biochar was effective in the formation of asymbiotic association between a mycorrhizal fungi and a plant and an increased number of root-associated yeasts and *Trichoderma* spp. In addition, biochar application enhanced the abundance and altered the composition of ammonia-oxidising microorganisms. However, the direct effect of biochar on a pure culture of a beneficial microorganism had not been uncovered. Shohei Ebe and Takashi Ano's study aimed to clarify the effect of biochar on beneficial microorganisms using RHB.

BACILLUS STRAIN IA

In an attempt to isolate microorganisms whose growth is promoted by RHB, strain IA was isolated as its growth area increased on an agar plate supplemented with RHB when compared to its growth on the plate without RHB. Based on the sequence of the 16S rRNA gene, strain IA belongs to the genus *Bacillus*. *Bacillus* species are well known to produce antifungal lipopeptides, such as surfactin, iturin, and fengycin. Lipopeptides are lipids attached to peptides and many of them are produced by bacteria, functioning as either antibiotics or cell receptors that facilitate the movement of molecules in and out of the bacterial cell. When tested for antifungal activity, strain IA strongly inhibited the growth of a phytopathogenic fungus *Rhizoctonia solani* K1 which suggested to the researchers that strain IA might produce antifungal lipopeptides. The antifungal activity of strain IA suggests it could protect plants from phytopathogenic fungi in soil. One of the *Bacillus* species, *Bacillus amyloliquefaciens*, has been used in agriculture, aquaculture, and hydroponics to combat a variety of root pathogens. Strain IA can be used as a bacterium for a biocontrol in agriculture.

To examine the effect of RHB, the strain IA was cultured in the liquid medium supplemented with RHB. As a result, it showed that the cell number and spore number of strain IA increased more than those in the medium without RHB. The *Bacillus* species are well known to form spores which are induced under low levels of nutrients in the environment. These spores can survive for extended periods under little or no nutrient conditions and return to life if nutrients become available. For these reasons, strain IA with a spore-forming ability will be expected to increase in cell number and to survive until needed in soil to which RHB has been applied. Along with the increases in cell number and spore number, an antibiotic produced by strain IA was promoted in the liquid culture medium with RHB and strongly inhibited the growth of *R. solani* K1. According to the analysis of the active compounds, strain IA produced a strong antifungal lipopeptide iturin A, which inhibited the growth of a broad species of plant pathogenic fungi. When RHB was added to the medium, the yield of iturin A by strain IA was up to eight times higher than that in the medium without RHB.



Schematic diagram of uptake and accumulation of silicon (Si) in rice plant.

Effective utilisation of rice hulls is important for both solving the problem of agricultural waste and creating sustainable agriculture

MAIN COMPONENTS OF RHB FOR THE PROMOTION OF THE METABOLISM OF STRAIN IA

The researchers decided to investigate which components of RHB were involved in the promotion of strain IA metabolism. The main components of RHB are carbon (C) and silicon dioxide (SiO_2). For this, strain IA was cultured in a medium containing activated charcoal (AC) or scaly silica (SS) – an artificially synthesised silica – as a standard material of C and SiO_2, respectively. As a result, the growth and sporulation were not promoted in both media. However, iturin A production was promoted only in the medium with SS. This result indicated that silicon dioxide was one of the factors to promote strain IA metabolism. Next, the researchers focused on the minor components such as Al, Fe, Mn, Mg, Ca, Na, K, Ti, and P which RHB contains. To test the promotion effects of these elements, each one was added to a medium and strain IA was cultured in these media. Only the medium which contained

Rice husk biochar could help improve agricultural soil.

Application of RHB to agricultural soil may reduce levels of plant disease.

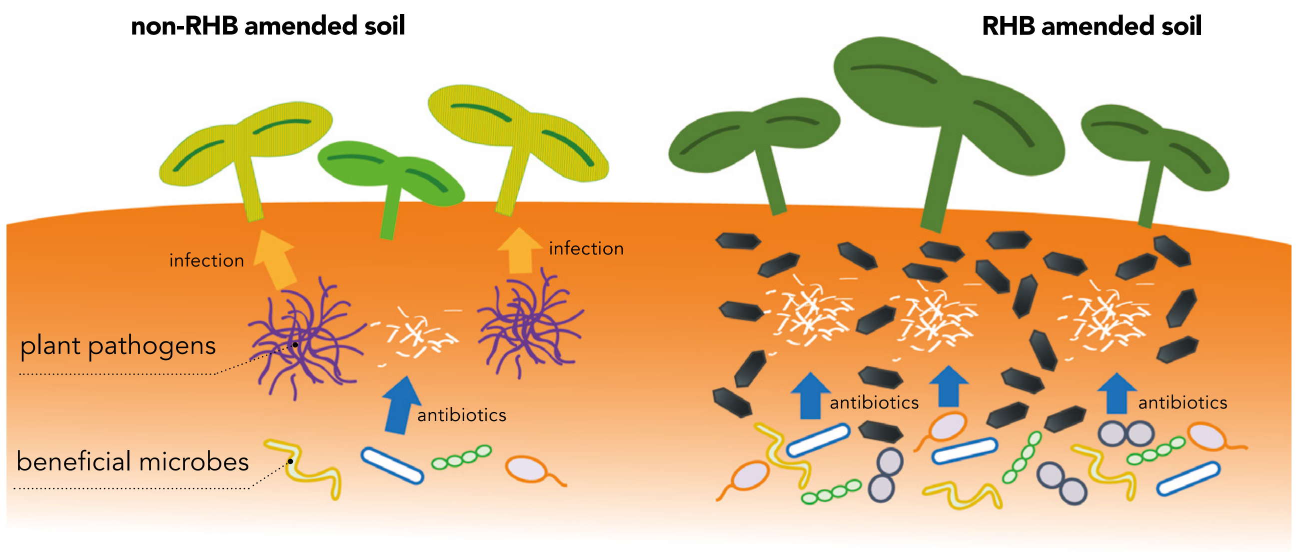
a manganese ion (Mn^{2+}) showed the promotion effects of sporulation and iturin A production. The other factor on the promotion of strain IA metabolism was therefore shown to be Mn^{2+}. Strain IA was cultured in a medium which combined scaly silica (SS) and manganese ion (Mn^{2+}). The growth, sporulation and iturin A production were promoted to the same levels of those in the medium with RHB. According to these results, silicon dioxide (SiO_2) and manganese ion (Mn^{2+}) are the compounds thought to be involved in the promotion of metabolism, which would contribute to the growth, spore formation and antibiotic iturin A production of strain IA. Further experiments clarified that the silicon dioxide contained in RHB, absorbed the metabolic inhibitor(s) and promoted the iturin A production of strain IA. The adsorption of the metabolic inhibitor(s) suggests that an application of RHB to soil makes it a comfortable place to live for microbes.

POTENTIAL OF RHB IN AGRICULTURE

Ebe and Ano successfully discovered the bacterial strain IA whose microbial activities such as growth, spore formation and antibiotic iturin A production are promoted in the presence of RHB and clarified the RHB components which are involved in the promotion of strain IA's activities. The finding that RHB promoted an antibiotic production may be applicable to the various microbial species which produce antibiotics. Because beneficial microbes, such as *Pseudomonas*, *Streptomyces* and *Trichoderma*, exist in soil and are involved in protecting plants from phytopathogens, application of RHB to agricultural soil may reduce levels of plant disease. RHB has been used as a soil ameliorant to improve drainage properties and air permeability. Ebe and Ano's study supports the enhancement of agricultural productivity and discovers new horizons in the relationship between soil microbes and RHB.

CONCLUSION

The looming threat of climate change and food insecurity have had a global impact on farming. As those in the agricultural field struggle to find alternatives to harmful chemicals and adapt to changes in soil microbiomes, the solution to these problems is essentially right under our noses. The ubiquity of rice farming across the world and the presence of rice hull present an opportunity to capitalise on agricultural waste. This research must not be limited to the discovery of *Bacillus* sp. strain IA whose antibiotic production is promoted by RHB, but must also propose sustainable solutions for present-day agriculture problems.



Schematic diagram of the mechanism of protection from plant pathogens in RHB amended soil.

Behind the Research

Dr Shohei Ebe

Dr Takashi Ano

E: ebe.microbiotech@gmail.com **T:** +81 90 3671 1361 **W:** https://www.researchgate.net/profile/Shohei_Ebe

Detail

Division of Biotechnological Science
Graduate School of Biology-Oriented Science and Technology, Kindai University,
Wakayama, Japan

Bio

Shohei Ebe

Shohei Ebe received a PhD in engineering from Kindai University in 2019. He conducts research at Kindai University as a researcher from Towada Green-tuff Agro-Science Co., Ltd. His current research is about development of the agricultural inoculant combined with RHB and strain IA and elucidation of the relationship between Towada green tuff and microorganisms in plant growth.

Takashi Ano

Takashi Ano is a professor of Kindai University. His doctoral degree was awarded by Osaka University. His major fields are biocontrol agents, and microbial fuel cells for a sustainable society. He has previously worked at Tokyo Institute of Technology for 20 years as a research associate, and an associate professor.

Research Objectives

Drs Ebe and Ano investigate the effect of rice husk biochar on soil microorganisms.

Personal Response

How do you hope your research will be applied to agriculture?

" Our laboratory has studied bioconversion of waste material using microbes to realise a sustainable society. It is important to utilise beneficial microorganisms and crop residues after crop harvest for environmentally friendly farming. To minimise harvest reduction and also consumption of agrochemicals, it is necessary to protect plants against phytopathogens by applying beneficial microorganisms. We consider that application of RHB to agricultural soil increases crop productivity, because it has potential to promote a growth of soil beneficial microbes involved in plant protection. We hope that farmers think about soil microbes and add RHB to their fields. "

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