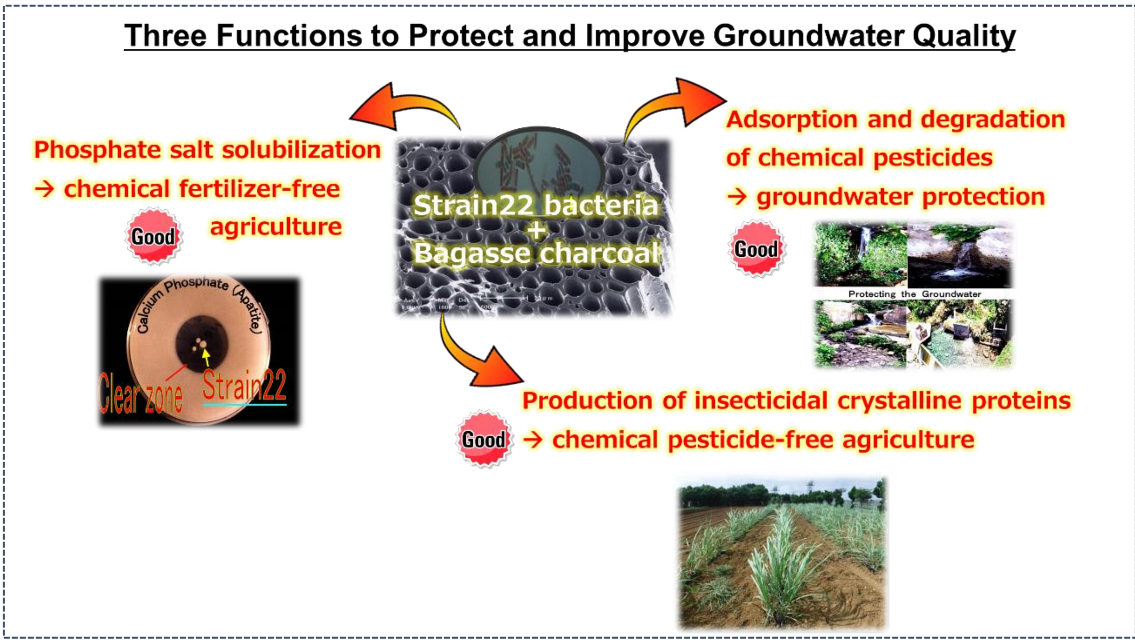
Protecting and Improving Our Groundwater Quality

：A Sustainable Solution from Miyako Island



For the Entry to the Stockholm Junior Water Prize (2025)

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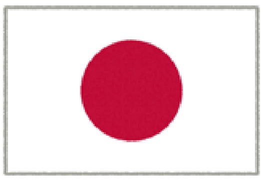

Abstract

Miyako Island relies entirely on groundwater for its drinking water. Agriculture is the island's main industry, and about 60% of the land is used as farmland. However, we confirmed that tap water and groundwater contain chemical pesticides that are regulated in EU countries.

We conducted this research to develop farming methods that do not use chemical pesticides. To do this, we used a phosphate-dissolving bacteria called strain 22, identified as *Bacillus thuringiensis*, along with bagasse charcoal (charcoal made from sugarcane waste) which helps the bacteria grow. Our results showed that bagasse charcoal can strongly absorb pesticides, and *B. thuringiensis* can break them down. This means that using both together can help stop pesticides from entering the groundwater. However, our ultimate objective is not just to prevent leaching, but to stop using chemical pesticides. One important characteristic of *B. thuringiensis* is that the bacteria produce insecticidal crystal proteins. In our field experiment, the area without treatment had a germination rate of 43.3%, while the area treated with *B. thuringiensis* and bagasse charcoal had a higher rate of 55.8%—even higher than the area treated with chemical pesticides. These results suggest that it is possible to grow crops without chemical pesticides by using the insecticidal proteins produced by *B. thuringiensis*. We are now working to spread this sustainable technology in Miyako Island and around the world.

Key words: Groundwater, Chemical pesticides, Bagasse charcoal, Phosphate-dissolving bacteria, Sugarcane, Insecticidal protein

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1. Introduction

Miyako Island is located 2,040 km from Tokyo, 290 km from the main island of Okinawa, and 380 km from Taiwan (Fig. 1). It lies in the southwestern part of the Ryukyu Islands and consists of a flat, elevated coral reef plateau made of Ryukyu limestone. The island has an area of 158.6 km^2, a circumference of 114.6 km, and an average elevation of 60 meters.

Miyako Island is a small island with a population of about 55,000. There are no rivers or lakes, so the island relies entirely on groundwater from rainfall not only for drinking water but also for all domestic and industrial use. About 60% of the land is used for farming (Fig. 2), and since the 1980s, large amounts of chemical pesticides have been used. In recent years, several types of pesticides, including those regulated in the European Union (EU) (Fig. 3), have been widely applied. The island's geology is made up of porous coral limestone, which allows water to pass through very easily. As a result, chemical pesticides have polluted the groundwater, making groundwater protection an urgent issue.

On the other hand, Miyako Island has dark red soil rich in calcium, formed from coral-derived limestone. According to previous studies conducted by our seniors, more than 90% of the total phosphate in the soil exists as insoluble inorganic

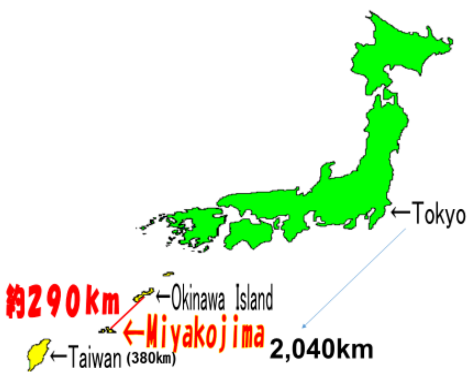

Fig.1 Location of Miyako Island

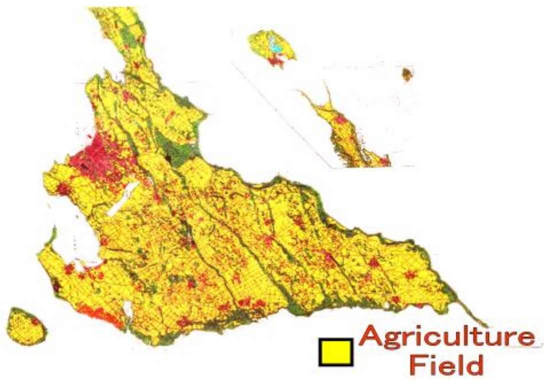

Fig.2 Land use of Miyako Island

calcium phosphate, which is difficult for crops to absorb. To solubilize poorly soluble inorganic phosphate, they selected and identified bacteria (strain22) that produces organic acids. In addition, to help the bacteria survive in the soil, they used bagasse charcoal — made by carbonizing sugarcane residue — as a carrier material to support and protect the bacteria.

	EU Water quality management standards(ng/L)	Global pesticide regulations situation
Clotianidine	<100	Germany, US, Italia, Canada, Brazil, South Korea, etc
Dinotefuran	<100	Germany, US, Italia, Canada, Brazil, South Korea, etc
Fibronil	<100	Pesticide(Fibronil) registrations expired in the EU in 2017.
Chlorantranylprole	<100	

Fig.3 EU water quality management standards for pesticides investigated in our study.

Building on our seniors' research, we hypothesized that if we could use this phosphate-recycling technology to reduce or eliminate the usage of chemical pesticides, we could help establish sustainable farming without chemical fertilizer and pesticides (Fig. 4). To test this idea, we carried out the following three experiments:

Test 1) We measured the concentrations of neonicotinoid, phenylpyrazole, and diamide pesticide components in tap water and groundwater to assess pollution levels in Miyako Island.

Test 2) We evaluated the capability of bagasse charcoal and phosphate-dissolving bacteria strain22 (fixed on bagasse charcoal) to adsorb and degrade pesticides, as a way to prevent pesticide runoff into groundwater.

Test 3) We performed field experiments to evaluate the effect of insecticidal crystalline proteins produced by strain22 on sugarcane growth.

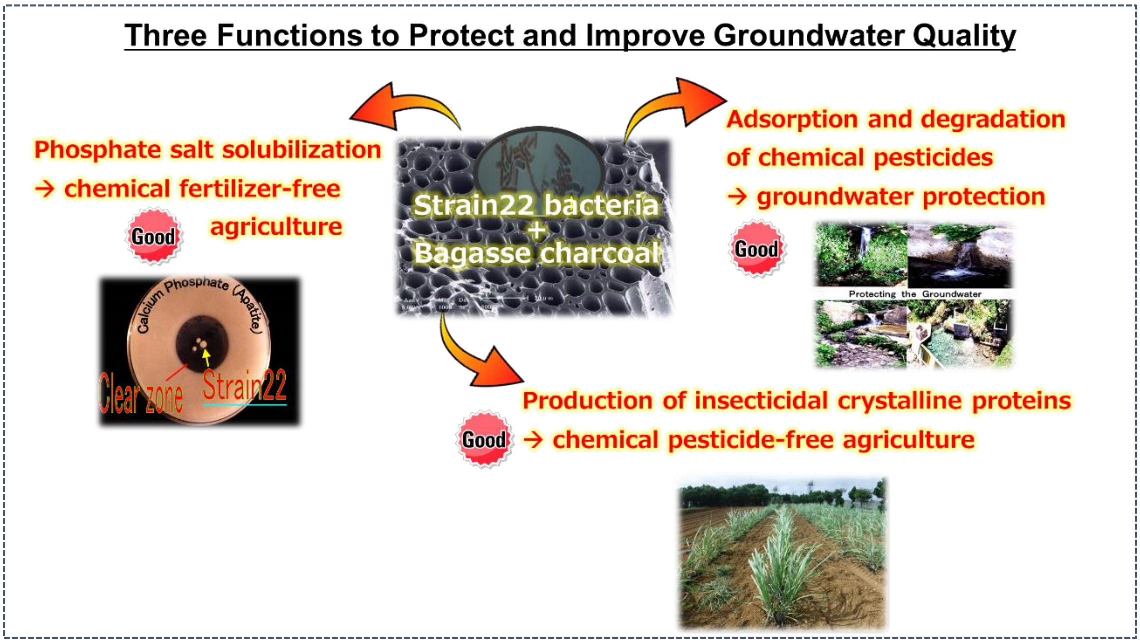

Fig.4 Concept of our research

2. Methods

2-1 Chemical pesticide concentrations in tap water and groundwater

We measured the concentrations of chemical pesticides in tap water and groundwater collected near our school (Photo 1). The quantitative analysis was carried out by Hiyoshi Co., Ltd. using LC/MS/MS. The target pesticides included two neonicotinoid compounds (clothianidin and dinotefuran), one phenylpyrazole compound (fipronil), and one diamide compound (chlorantraniliprole).

Photo1 Sampling of tap water and groundwater

2-2 Isolation and identification of phosphate-dissolving bacteria strain 22

Among 100 strains of phosphate-dissolving bacteria isolated from the dark red soil of Miyako Island, strain 22, which showed excellent organic acid production (phosphate-solubilizing ability), was used in the experiment (Photo 2).

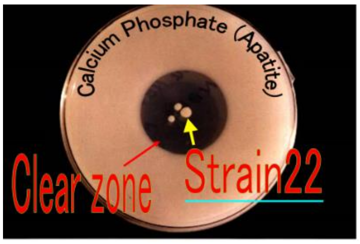

Photo2 Dissolution of calcium phosphate by strain 22

The molecular identification of strain 22 and a homology search were conducted by the Japan Food Analysis Center Foundation. Crystal protein was confirmed by staining the bacteria with malachite green, washing with water, staining with 0.5% safranine solution, and observing them using an optical microscope (×1000 magnification).

For the molecular identification, DNA was extracted from strain 22, and the 16S rDNA region was amplified by PCR to determine the base sequence. The obtained sequence was then compared with sequences registered in the international nucleotide databases (DDBJ/EMBL/GenBank) and the MicroSeq ID Analysis Software Version 2.1 (Applied Biosystems).

2-3 Morphological and physical properties of bagasse charcoal

Cross-sections of bagasse charcoal were observed using a scanning electron microscope (JSM-6510LA, JEOL). To evaluate the water retention capacity of the bagasse charcoal, a pF test was conducted. The samples were prepared by mixing bagasse charcoal (sieved through a 2 mm mesh) with soil collected from the field. Water content was calculated using the method proposed by Yamane (1986).

2-4 Fixation of strain 22 using bagasse charcoal as a carrier

To examine whether bagasse charcoal, a carbonized material, could function as a carrier for strain 22, the bacterium was added to bagasse charcoal that had been sterilized at 105°C for 6 hours, so that the final concentration was approximately 1×10^{3} CFU/g. The number of bacteria was measured using a medium containing synthetic hydroxyapatite, and colonies that formed clear zones were counted using the soil dilution plate method (Photo 3).

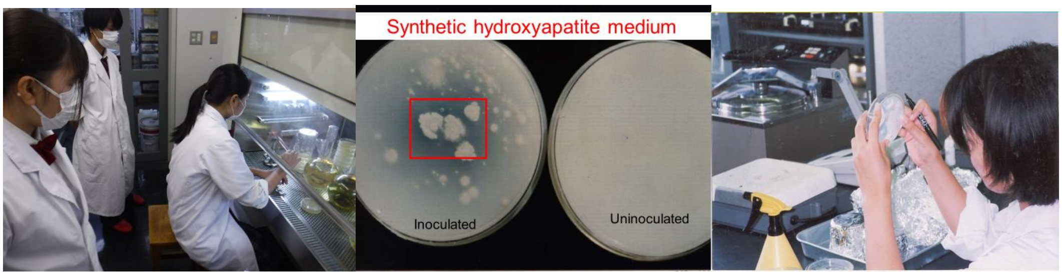

Photo3. Counting the number of colonies using the soil dilution plate method

2-5 Adsorption and degradation tests of pesticides by bagasse charcoal and strain 22

In order to evaluate the effectiveness of bagasse charcoal and strain 22 in preventing pesticide runoff into groundwater, we examined their adsorption and degradation performance under four experimental conditions.

In test condition #1 (Photo 4), 1/2NB medium (half-strength nutrient broth) was sterilized in an autoclave, then pesticides (16 mg clothianidin, 1 mg dinotefuran, 0.5 mg fipronil, and 0.5 mg chlorantraniliprole) were added. After incubation at room temperature for 30 days, the mixture was dispensed into designated containers and used as analysis samples.

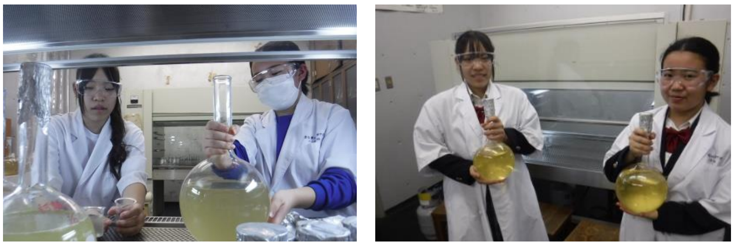

Photo4. Experiments (test condition #1) within a clean bench.

In test condition #2, the pesticides were added to 1 kg of bagasse charcoal, followed by 500 mL of distilled water. The mixture was stirred and kept at room temperature for 30 days. Filtrate was collected using 3 L of distilled water and dispensed into containers for analysis. In test condition #3, 500 mL of liquid culture of strain 22 (1×10^7 cells/mL) was added to 1 kg of bagasse charcoal, mixed well, and kept at room temperature for 30 days. Filtrate was collected with 3 L of distilled water and dispensed into containers for analysis. In test condition #4, 500 mL of liquid culture of strain 22 (1×10^7 cells/mL) was added to a mixture of 950 g of soil and 50 g of bagasse charcoal. After mixing, it was incubated at room temperature for 30 days. Filtrate was then collected using 3 L of distilled water and transferred into containers for analysis.

In addition to the pesticide concentrations in the filtrates, pesticide residues remaining in the soil or bagasse charcoal after filtration were also measured by extraction using acetonitrile.

2-6 Field experiments to demonstrate the possibility of pesticide-free agriculture by utilizing the crystalline insecticidal protein produced by strain 22

To confirm the growth-inhibitory effect of the crystalline proteins produced by strain 22, chemical pesticide-free sugarcane cultivation experiments were conducted (Photo 5). To control wireworms, which feed on the buds of two-node seedlings at the time of planting, 120 two-node sugarcane seedlings were treated either with a phenylpyrazole-based chemical pesticide (Prince Bait, containing 0.5% fipronil) or with bagasse charcoal inoculated with strain 22. Germination rates were measured after 100 days (Photo 6). Quality and yield surveys were conducted after 1 year and 4 months by measuring sugar content, plant height, stalk length, and stalk fresh weight. To evaluate the phosphorus supply capacity of the soil, samples were collected near the base of the plants, and both available phosphorus

Photo 5. Field experiments to confirm growth inhibition effect of the crystalline proteins produced by phosphate-solubilizing bacteria strain 22

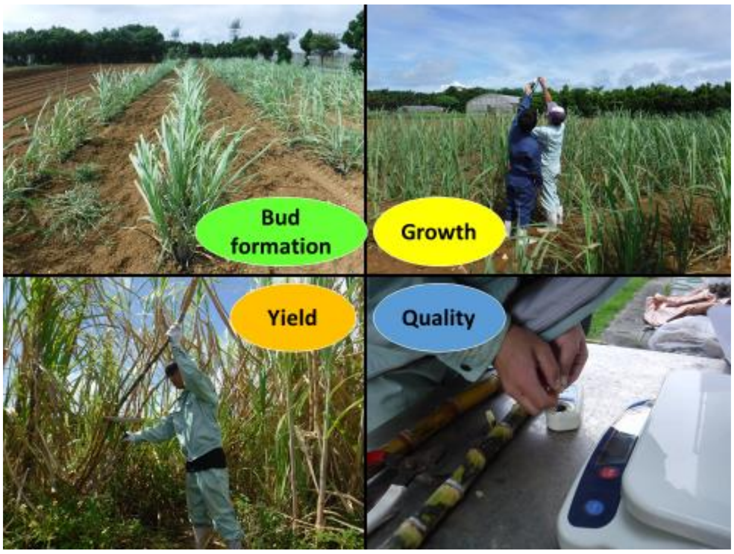

Photo 6. Measurement of germination rate, growth, sugar content, and yield during field experiments.

(Truog method) and biomass phosphorus (toluene treatment method) were measured (Kimura and Nishio, 1992).

3. Results and discussion

3-1 Chemical pesticide concentrations in tap water and groundwater

As a result of the quantitative analysis of chemical pesticide components in tap water and groundwater, clothianidin (42.4 ng/L) and dinotefuran (40.6 ng/L) were detected in tap water. In groundwater from the tap water source area, clothianidin (94.7 ng/L), dinotefuran (55.1 ng/L), chlorantraniliprole (18.0 ng/L), and fipronil (3.2 ng/L) were detected (Fig. 5). The concentrations of these pesticides are approaching the EU's target values for tap water quality management (Fig. 3), raising concerns about potential health risks for the residents of Miyako Island.

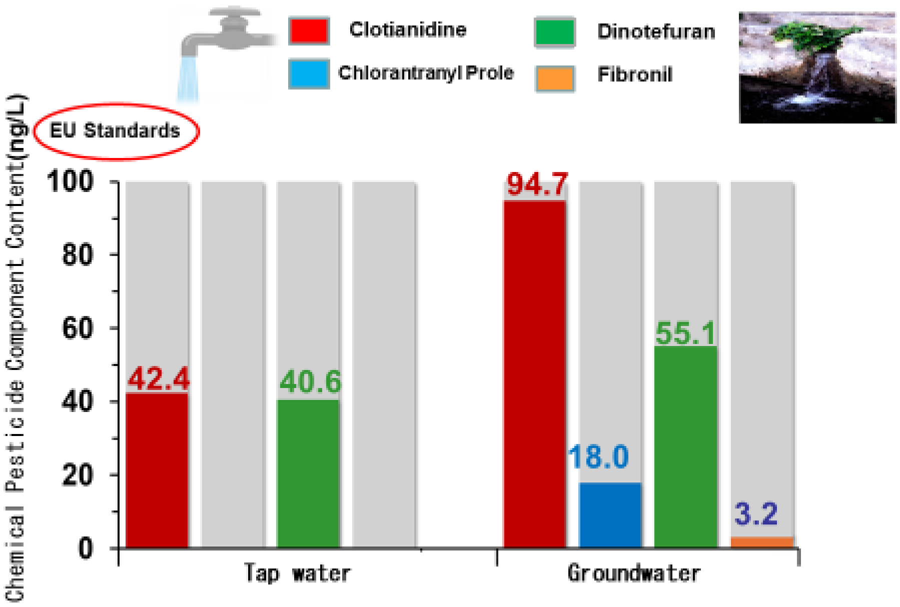

Fig. 5 Chemical pesticide concentrations in tap water and groundwater

3-2 Isolation and identification of phosphate-dissolving bacteria strain 22

Optical microscopic observation revealed that the cells were approximately 3 μm in size and the spores about 2 μm (Photo 7). Additionally, the characteristics of the isolated strain 22 (Table 2) showed that it is an anaerobic, gram-positive bacterium that produces crystal proteins.

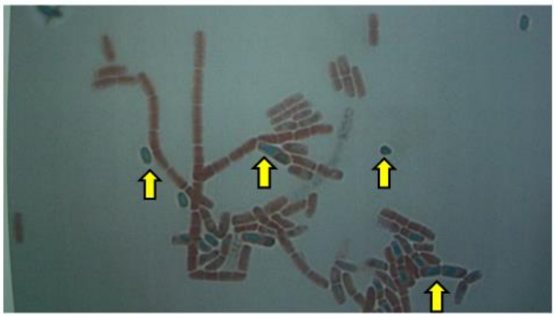

Photo7. Micrograph of strain 22 cells (red) and spores(blue)

Table 2. Characteristics of strain

Test items	Test results
Morphology	
Gram staining	+
Spores	+ *
Shape	
Position	
	Subarachnoid
Motility	+
Attitude towards oxygen	Non-swelling
catalase	+
Crystal protein	+ *

A sequence analysis of the 16S rDNA region showed 99.85% homology with *Bacillus thuringiensis* ATCC 10792 (Table 3). *B. thuringiensis* is a soil bacterium widely distributed in various soils. Watanabe (1988) reported that the crystal proteins produced by soil microorganisms have insecticidal effects against pests, and thus have potential for practical use as environmentally friendly microbial pesticides in many agricultural fields. Since crystal proteins were also found in strain 22, there is growing interest not only in its ability to solubilize poorly soluble inorganic phosphate but also in its insecticidal properties against pests.

Table 3. Result of 16S rDNS homology test of strain22

Strain	Homology(%)
Bacillus thuringiensis ATCC=10792	99.85
Bacillus thuringiensis DSM=6091	99.72
Bacillus thuringiensis ATCC=33679	99.44
Bacillus thuringiensis DSM=6025	99.44
Bacillus anthracis	99.42
Bacillus cereus	99.42
Bacillus thuringiensis DSM=6110	99.33
Bacillus thuringiensis DSM=6099	99.18
Bacillus thuringiensis DSM=6054	99.13
Bacillus pseudomycoides	98.74
Bacillus mycoides	98.58
Bacillus weihenstephanensis	98.58
Bacillus herbersteinensis	92.95
Bacillus acidicola	92.38
Bacillus niabensis	92.20
Bacillus idriensis	92.18
Bacillus amyloliquefaciens	92.04
Bacillus altitudinis	91.88
Bacillus aerophilus	91.88
Bacillus shackletonii	91.38

3-3 Morphological and physical properties of bagasse charcoal

The surface structure of bagasse charcoal used as a carrier for strain 22 was observed by SEM, revealing a honeycomb structure with regularly arranged voids of

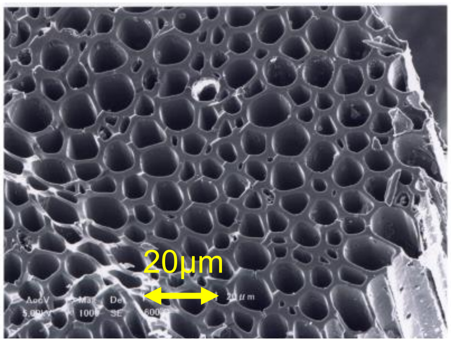

Photo8. SEM image of bagasse charcoal

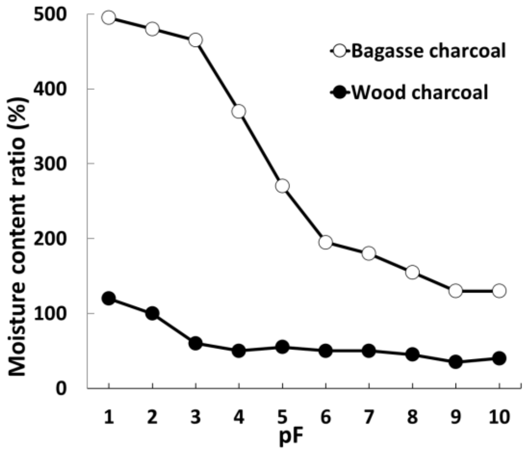

Fig. 6 Water retention curve of bagasse and wood charcoals

10-20 μm in diameter (Photo8). This shape is larger than that of strain 22 (Photo7) and is presumed to be suitable for carrying the bacteria. To compare the moisture content of the charcoal, the pF-moisture curves of bagasse charcoal and wood charcoal are shown in Fig. 6. Bagasse charcoal showed a moisture content of 480% at pF=1.5, which is the available moisture for crops, while wood charcoal showed a moisture content of 100%. Furthermore, bagasse charcoal showed a moisture content of 270% at pF=3 that inhibits crop growth, while wood charcoal showed a moisture content of 60%. These results indicate that bagasse charcoal has a higher water retention capacity than wood charcoal and supports the growth of *B. thuringiensis*.

3-4 Fixation of strain 22 using bagasse charcoal as a carrier

The fixation (survival rate) of strain 22 (*B. thuringiensis*) in soil when attached to bagasse, wood charcoal, and bagasse charcoal was examined (Fig. 7). After about six months of adding bagasse (not bagasse charcoal) containing strain 22, the number of bacteria decreased slightly, and by eight months, it had decreased significantly. This was likely due to the decomposition of the bagasse and the resulting death of strain 22 from various causes. Strain 22 fixed to wood charcoal showed a slight decline after about eight months. In contrast, strain 22 fixed to bagasse charcoal maintained a high survival rate even after 12 months. This high fixation rate is attributed to the physical properties of bagasse charcoal, which has a honeycomb-like structure with many fine pores. These pores help protect strain 22 from environmental stresses that would otherwise reduce its viability.

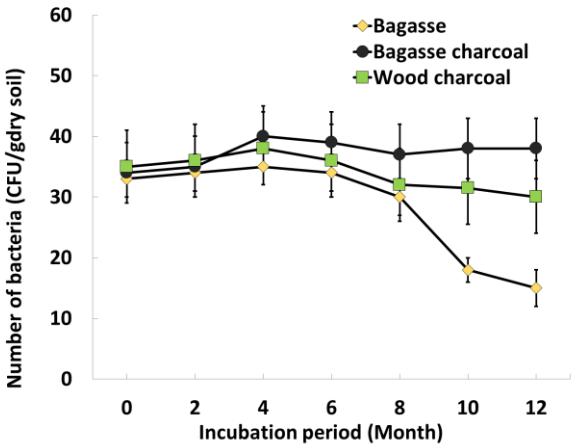

Fig. 7 The fixation of strain 22 in soils mixed with bagasse, wood charcoal, and bagasse charcoal

3-5 Adsorption and degradation tests of pesticides by bagasse charcoal and strain 22

The concentrations of pesticide components in the filtrate are shown in Table 4. A comparison between test conditions #1 and #2 revealed that the concentrations of pesticides were drastically reduced by the addition of bagasse charcoal, demonstrating its high adsorption capacity. Furthermore, a comparison between test conditions #2 and #3 showed that the concentrations of chlorantraniliprole and fipronil were significantly reduced by the addition of strain 22, although the levels of dinotefuran and clothianidin remained unchanged. The high adsorption capacity of bagasse charcoal and the degradation ability of strain 22 were also observed under test condition #4, in which the amount of bagasse charcoal was reduced to 5%. Based on these experimental results, it was found that the leaching of dinotefuran and clothianidin into groundwater is effectively suppressed mainly through

Table4 Leached concentrations of pesticide components in the filtrate (mg/L)

	Dinotefuran	Clotianidine	Chlorantraniliprole	Fibronil
Test #1 a)	0.039	1.0	0.020	0.028
Test #2 b)	0.00002	0.000045	0.00001	0.000048
Test #3 c)	0.00003	0.000052	0.000002	0.000002
Test #4 d)	0.00008	0.001	0.0000008	0.000001

a) Without bagasse charcoal
b) Bagasse charcoal
c) Bagasse charcoal + strain 22
d) Soil (95%) + Bagasse charcoal (5%) + strain 22

Table5 total pesticide content in the soil and bagasse charcoal after leaching test (mg/kgdrysoil)

	Test #2 Bagasse charcoal (100%)	Test #4 Soil (95%) Bagasse charcoal (5%) strain 22
Dinotefuran	1.8	0.63
Clotianidine	18	14
Chlorantraniliprole	0.38	0.41
Fibronil	0.83	0.17

adsorption, while the leaching of chlorantraniliprole and fipronil is effectively suppressed through a combination of adsorption and microbial degradation, including that by strain 22.

The degradation of pesticides by soil microorganisms, including strain 22, was also supported by the results of measuring the total pesticide content extracted with acetonitrile from the soil and bagasse charcoal after the leaching test. As shown in Table 5, the amounts of pesticides except chlorantraniliprole were reduced by the addition of soil and strain 22. Although further studies are needed to clarify why chlorantraniliprole was not degraded, these results demonstrate that our system is capable of retaining and degrading certain pesticides, thereby preventing their movement into groundwater.

3-6 Field experiments to demonstrate the possibility of chemical pesticide-free agriculture by utilizing the crystalline insecticidal protein produced by strain 22

Before presenting the effects of chemical pesticide and microbial pesticide (strain 22 and bagasse charcoal) on sugarcane growth, we will first show the results regarding the phosphate-solubilizing ability of strain 22, which is one of its key characteristics. As shown in Table 6, the levels of soil biomass phosphorus and available phosphorus at the time of harvest were higher in the microbial pesticide area compared to the untreated and chemical pesticide areas. These findings reconfirm that applying microbial pesticide containing strain 22 can reduce the need for chemical fertilizers, as strain 22 releases organic acids that solubilize insoluble inorganic phosphate, converting it into a form that plants can absorb.

Table 6. Effects of phosphate-dissolving strain22 on the increase of biomass phosphorus and available phosphorus in sugarcane soil.

	Biomass P (P_2O_5mg/100gsoil)	Available P (P_2O_5mg/100gsoil)	Accumulated P in the soil (P_2O_5mg/100gsoil)
Untreated	3.56	13.52	792.6
Chemical pesticide	5.13	20.25	785.9
Microbial pesticide (Phosphate-dissolving strain22+Bagasse charcoal)	21.68	32.86	773.2

The results of the germination rate measurements (Table 7) showed that the untreated area had a germination rate of 43.3%, while the chemical pesticide area (using fipronil) had a rate of 55.0%, and the microbial pesticide area (strain 22 + bagasse charcoal) had an average rate of 55.8%.

Table 7 The effect of chemical and microbial pesticides on the growth of sugarcane[a)]

	Sprouting rate[b)] (%)	Sugar content (Brix)	Plant height (cm)	Stem live weight (g)
Untreated[c)]	43.3	20.6	291.3	798.5
Chemical pesticide[d)]	55.0	21.9	303.5	844.3
Microbial pesticide (Phosphate-dissolving strain22+Bagasse charcoal)	55.8	23.3	300.3	832.1

a) Average of 10 plants
b) Measured after 100 days
c) Not treated with chemical or microbial pesticides
d) Treated with fipronil

The sugar content in the microbial pesticide area was 23.3% and higher than in the untreated and chemical pesticide areas. Regarding plant height, stalk length, and stalk live weight, the microbial pesticide area showed higher values than the untreated area and similar values to the chemical pesticide area, suggesting the possibility of growing sugarcane without chemical pesticides. Again, the key mechanism behind this chemical pesticide-free cultivation is the ability of strain 22 to produce intracellular crystalline insecticidal proteins during spore formation (Table 2). When pest larvae consume this protein, it suppresses their activity through biological control, reducing the need for chemical pesticides.

3-7 Activities for social implementation of our technology

Through our experiments, we found that sugarcane can be cultivated without pest damage even in the absence of chemical pesticides by combining bagasse charcoal with strain 22. However, many farmers in Miyako Island still rely heavily on chemical pesticides. Therefore, to promote our technology, encourage pesticide-free farming, and prevent groundwater contamination, we have carried out various social activities:

1) To raise awareness among Miyako Island residents about the presence of neonicotinoid-based pesticide residues in tap water and to help them understand the potential health risks these substances pose to humans, we have been assisting in organizing public study sessions. These sessions are led by Dr. Naoki Tomori, a medical doctor and co-representative of the Miyako Island Groundwater Research Association (Photo 9).

Photo9

2) As part of our environmental education efforts, we worked with elementary and junior high school students in Miyako Island to teach them about groundwater pollution on the island. Through water quality measurements (Photo 10), we explained the current situation and learned together with the students about the importance of protecting groundwater.

Photo10

3) Through our school's training program, we have produced approximately 6,000 to 9,000 bags of microbial pesticide annually (containing strain 22 fixed to bagasse charcoal), and promoted its use among local farmers to support agriculture without chemical pesticides (Photo 11).

Photo11

4. Conclusions

In this study, we demonstrated the potential of using bagasse charcoal and phosphate-solubilizing bacterium strain 22 (identified as *Bacillus thuringiensis*) as a sustainable alternative to chemical pesticides in sugarcane cultivation on Miyako Island. Our findings revealed that:

- Tap and groundwater on Miyako Island contain pesticide components including neonicotinoids and other components regulated in the EU, raising significant health and environmental concerns.
- **Long-term survival of strain 22 was enhanced by bagasse charcoal**, which provided physical protection and stability under field conditions.
- **Bagasse charcoal showed a high capacity for pesticide adsorption**, and when combined with strain 22, further degradation of certain pesticide components was achieved.
- **Strain 22 demonstrated both phosphate-solubilizing ability and insecticidal activity** through the production of organic acids and crystalline proteins, enabling pesticide-free sugarcane cultivation with less amount of chemical fertilizers without compromising germination rates, crop yield, or sugar content.
- **Social outreach and education activities**, including public seminars and environmental programs with local students, helped raise awareness of groundwater pollution and promoted the adoption of pesticide-free farming practices among local farmers.

These results suggest that the integration of microbial and biochar-based technologies can provide a practical and eco-friendly solution for sustainable agriculture in regions vulnerable to groundwater contamination. Moving forward, further research and broader implementation are needed to expand this approach and encourage a shift away from chemical pesticide dependence in similar agricultural settings worldwide.

Acknowledgements

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