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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation

Study on Improvement of agricultural productivity using biotic and abiotic factors
（生物的･非生物的要素による農業生産性の向上に関する研究）

学位論文要約：
Dissertation Summary

Agricultural productivity is influenced by many factors, biotic and abiotic. Major biotics include weeds (plants), pests (animals), and diseases (fungi and bacteria). The most important abiotic are water, sun, oxygen, soil, and temperature. In this study, I tried to improve productivity with two biotic factors, seaweed polysaccharide ulvan and bacteria utilizing Yuzu waste, and one abiotic material, slightly acidic electrolyzed water (SAEW).

Draft genome sequences of marine bacteria, degraders of sulfated polysaccharide ulvan extracted from a green algae *Ulva meridionalis*

In **chapter 1**, I present the draft genome sequences of uIvan-degrading strains belonging to the genera *Alteromonas* and *Tenacibaculum* were determined. *U. meridionalis* cultivated in Uranouchi bay (Kochi, Japan). The harvested green algae were used to enrich ulvan-utilizing bacteria by incubating at 25 ºC for two days in the artificial seawater (Tetra Marin Salt Pro, Tetra Japan) with 0.2 % (w/v) ulvan extracted from *U. meridionalis*. Enriched cells were spread on the artificial seawater agar media with 0.2 % (w/v) ulvan as a sole carbon source and incubated at 25 ºC for two days. Isolated bacteria were used for 16S rDNA sequence. Nucleotide sequences of the amplified fragments were determined using a 3130 genetic analyzer (Applied Biosystems). Seven strains have been identified. To identify PULs and elucidate the evolution of ulvan degradation enzymes, I determined the draft genome sequences of the strains. The first enzyme in the degradation pathway of ulvan, a ulvan lyase, was detected in the polysaccharide utilizing loci (PULs) in all ulvan-degrading strains.

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Isolation and characterization of useful bacteria for citrus waste utilization

In **chapter 2**, I studied isolating bacteria, which use sugars in Yuzu waste as sole carbon sources, and characterize them for future application to Yuzu waste composting. Yuzu is one of the important fruits in Kochi. Large amounts of waste are produced in the process of squeezing Yuzu. The post-squeezing Yuzu waste is thought to be unused biomass resources. One of the useful applications of Yuzu waste is decomposing by bacteria. Soils were collected from Yuzu farms at Mihara village in Kochi (soils A and B) and the farm of Education and Research Center for Subtropical Field Science at Monobe campus, Kochi University (soil C). The squeezed Yuzu waste was homogenized and used as a sole carbon source for bacterial enrichment at neutral and acidic conditions. Nitrogen, phosphorus, and potassium were supplied by using an M63 minimal medium (100 mM KH_2PO_4, 15 mM $(NH4)_2SO_4$, 1.7 μM $FeSO_4$, and 1 mM $MgSO_4$). Standard M63 was adjusted pH to 7.0 with KOH (M63pH7) and acidic M63 was not adjusted pH (M63pH4). Enriched and isolated bacteria at neutral conditions belonged to the genus *Bacillus*. Isolated bacteria at acidic conditions belonged to the genus *Burkholderia*. Species in the genus *Burkholderia* are known to be acid-tolerant. For making compost with Yuzu waste, the acidic-tolerant feature of *Burkholderia* is highly advantageous. Phylogenetic analysis was conducted with MEGA-X. Seven *Burkholderia* strains, YK2014, YK2015, YK2022, YK2025, YK2051, YK2052, and YK2054 were used. Besides YK2051, which was classified into group A including several plant-associated and saprophytic species, 6 other strains belonged to group B, which includes human, animal, and plant pathogens. Cell growth was automatically monitored with a biophotorecorder (TVS062CA, Advantec Toyo). Although all 7 strains grew at pH 4.0, acidic tolerance was different among strains. YK2014, YK2022, YK2025, and YK2052 stopped growing earlier at pH 4.0 than at higher pHs. YK2015 and YK2051 grew well even at pH4.0. Interestingly, YK2054 grew slower at pH 7.0 than at other pHs.

The sugar content was analyzed with COSMOSIL Sugar-D (4.6mmI.D.-250mm, NACALAI TESQUE) using a CLASS-LC10 HPLC system (Shimadzu). The analysis condition was as follows: mobile phase, acetonitrile/H2O = 75/25; flow rate, 1.0 ml/min; temperature, 30°C; detection, RI. The Yuzu waste contained three carbon sources, fructose, glucose, and sucrose. The *Bacillus* strain YK1110 isolated at neutral conditions could not consume any sugars, which is consistent with no growth at acidic conditions. When homogenized Yuzu waste was added at different levels, 10%, 20%, and 50%, to the minimal media, the pH of the growth media became 4.00, 3.83, and 3.77, respectively. The 7 *Burkholderia* strains consumed all three sugars at a 10% level. Only glucose was consumed by 7 strains at a 20% level. At a 50% level, only YK2054 consumed glucose. It signified the examined bacterial strains are unable to grow at 50% waste treatment since the pH of the higher waste is very much lower which inhibits the growth of bacteria. The results concluded that the *Burkholderia* sp.YK2054 was most tolerant to acidic pH and could be used for composting.

Microbial Control in Greenhous e by Spraying Slightly Acidic Electrolyzed water

In **chapter 3**, I discussed how SAEW mist was used to control airborne bacteria in greenhouses. In addition, this study combined integrated pest management (IPM) and humidity using SAEW spraying. We also investigated the effects of SAEW spraying on the environment in the greenhouse, such as plant growth and bacterial community structures in the soil and on plant leaf surfaces. Slightly acidic electrolyzed water (SAEW) is now considered as such an alternative. SAEW has been increasingly used to prevent and control microorganisms in various agricultural fields. SAEW has strong bactericidal activity and is relatively safe compared to other disinfectants. SAEW has no adverse effects, such as corrosion of equipment, skin irritation, or phytotoxicity in plants, and no safety problems caused by Cl2 waste gas. SAEW has received more attention in agriculture and has been shown to prevent and control bacterial infection. In this study, SAEW was freshly prepared by electrolysis of an aqueous dilute solution of HCl and tap water using a KC-5000 generator (Kowatech, Kochi, Japan). The available chlorine concentration was measured immediately after preparation using an AQUAB AQ-202P chlorine tester (Sibata Scientific Technology, Saitama, Japan). Different chlorine concentrations were used in this study: 5-25 mg/L for in vitro experiments and 25-40 mg/L for greenhouse spraying. In vitro experiments were performed with Gram-negative *Escherichia coli* (NBRC 3972) and Gram-positive *Bacillus subtilis* (NBRC 3134). The result suggested that *Escherichia coli* and *Bacillus subtilis* to SAEW at more than 25 mg/L chlorine concentration for 3 min completely killed bacterial cells. As part of this study, SAEW mist was sprayed in the greenhouse to control airborne microorganisms. The greenhouse (11 m × 12 m × 3 m) is located on the Monobe campus of Kochi University, Japan. The mist was generated with a MUM602 Universal Mist Generator (Maruyama, Tokyo, Japan) using spray nozzle type CKBC 045 (H. Ikeuchi,

Osaka, Japan). The mean droplet diameter was 10-30 µm. SAEW or tap water was sprayed for 2 hours at a flow rate of 12.5 L/h at intervals of 1 min spraying and 30 s stop. The viability of microorganisms was analyzed using 4, 6-diamino-2-phenylindole dihydrochloride (DAPI) at 15 µg/mL or propidium iodide under microscope. When SAEW was sprayed in the greenhouse at around 30 mg/L chlorine concentration, the viability of airborne microorganisms was significantly reduced. On the other hand, SAEW spray did not affect the growth of eggplant and cucumber plants in the greenhouse. I investigated the activity of SAEW on soil organisms and microorganisms on plant leaves. Metagenomic DNA was extracted from the soil samples and plant leaf surface using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. SAEW spray did not influence microorganisms in the soil or the plant leaf surface. SAEW could be used as a substitute for tap water to increase the relative humidity during the daytime, which is expected to increase photosynthesis. SAEW spraying reduces airborne microorganisms and improves the environmental conditions in the greenhouse.