学　位　論　文　要　旨

Application of Biofertilizer Containing Spores of *Bacillus pumilus* TUAT1 to Agricultural Rice Cultivation and Exploration for Substances Inducing Plant Growth Promoting Effect

バチルス芽胞バイオ肥料のイネ実用栽培技術への応用と植物生長促進物質の探索

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Bacteria belonging to *Bacillus* genus form spores that are tolerant to chemicals, ultraviolet lights, and heat stresses. Due to the property, *Bacillus* can be characterized as one of the most suitable bacteria for biofertilizers among manufacturing, transportation, and storage for sustainable agriculture. A biofertilizer named “Kikuichi / Yume-bio” has been developed, containing 10^7 CFU g^{-1} of spores of strain *B. pumilus* TUAT isolated from farming fields of Tokyo University of Agriculture and Technology. The biofertilizer and the strain were reported that they promoted the growth and yield of rice plants. Also, this strain has been reported that the plant response of rice differed between spore inoculation or vegetative cell inoculation; the former promoted the biomass higher than the latter. Similar spore-specific plant growth promotion was reported, such as autoclaved dead spores of several *Bacillus* spp. showed the improvement of rice root development.

At first of this study, the experiments were conducted on *Setaria viridis*, a C4 model plant to find the spore-specific plant growth promoting mechanism if it can apply to other plants in part 2. The plant assays using viable spores and autoclaved, dead spores (ADS) were performed. Viable spore inoculation at 10^7 CFU ml^{-1} improved the plant growth greatly. The growth of *Setaria* was also enhanced by ADS at 10^8 (ADS8) or 10^9 CFU ml^{-1} (ADS9). The plant response to ADS application was the differentiation of crown roots leading to increases in total lateral roots length, resulting in improvement of nitrogen uptake and the biomass. This part indicated

the spore-specific plant growth promotion could be applied to other C4 plants.

For further application of this strain as a biofertilizer, the evaluation of the effects on the growth, yield, and lodging resistance of forage rice in transplanting cultivation was conducted in part 3. At first, each concentration of spore suspensions (from 10^1 to 10^9 CFU ml^{-1}) inoculated to forage rice of a cultivar 'Fukuhibiki' and two lines LTAT-29 and TAT-26. 10^7 CFU ml^{-1} was evaluated as the most beneficial. The biofertilizer was applied to the forage rice on nursery box in greenhouse, showing the promoting effect on the shoots of seedlings. The seedlings were transplanted into the field designed as two fertilizations (2 N kg or 4 N kg) and two transplanting distances (15 by 30 cm as a conventional, or 30 by 30 cm as a sparse condition). The SPAD values of the leaf of inoculated seedlings at the early vegetative growth stage in the field were higher than those not inoculated, especially in sparsely transplanted LTAT-29 with 2 N. The shoot heights and tiller numbers of inoculated plants at the late vegetative growth stage were improved provided by increased SPAD values, especially in 'Fukuhibiki' and LTAT-29. Biofertilizer improved the feeding yields of 'Fukuhibiki' and LTAT-29 that it was ascribed to increases of those total panicle numbers while the biofertilizer application was correlated with total spikelets number of LTAT-29 only. In the yield of TAT-26 as a whole crop silage (WCS), only the shoot dry weight was promoted by biofertilization, especially in sparse transplanting with 4 N. Biofertilization decreased the lodging indexes provided by the improvement of pushing resistance per culm in three genotypes.

In addition, the seed coating system for direct sowing using this biofertilizer on forage rice, LTAT-29 was also developed in part 4. Three chemical materials for seed coating (e.g., CALPER®, Iron, Benmoly) were fused with the biofertilizer as a double coated. Only the Benmoly was promoted the effect of biofertilizer on the seedlings. Five times increased amount of coated biofertilizer indicated higher promotion. The best timing of coating seeds was before the coleoptile emerged.

Lastly, the analyses of siderophores coded in the genome as a spore-specific substance were conducted using dead spores in part 5. The supernatants and residues of ADS9 were collected separately to apply on *Setaria*. Surprisingly, both promoted the plant growth. To seek the spore-specific substances inducing the plant response, chrome azurol sulphonate (CAS) reagent which detects siderophores coded in the genome of *B. pumilus* TUAT1 as an aerobactin biosynthesis protein was reacted with the ADS supernatants of seven strains including *B. pumilus*, *B. altitudinis* and *B. megaterium*. Siderophores were detected from supernatants of ADS but were not from autoclaved dead vegetative cells. The gene expressions of the siderophore biosynthesis were higher in vegetative cells of *B. pumilus* TUAT1 than those in spores, suggesting the siderophores accumulated by spores could be exuded during the germination.