

論文の内容の要旨

応用生命工学	専攻
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論文題目 **Phylogenetic studies on nitrogen-fixing bacteria** (窒素固定細菌の系統分類に関する研究)

The purpose of this study

Nitrogen fixation is fundamental biochemical processes making atmospheric nitrogen bioavailable. It has not been found in eukaryotes, but revealing considerable biodiversity among the bacteria and the archaea. The biologically fixed nitrogen is less prone to leaching and volatilization as it is utilized in situ and therefore the biological process contributes an important and sustainable input into agriculture. These systems can provide an ecologically acceptable alternative to the increased application of nitrogen fertilizers. Effort at extending N₂-fixing ability to important nonleguminous crops such as cereals has long been a major goal of workers in the field of biological nitrogen fixation. One approach for achieving this goal has involved the isolation and characterization of N₂-fixing bacteria from a variety of sources. It becomes a major purpose in respect of searching distribution and diversity of the nitrogen-fixing bacteria in this study.

We attempted to isolate strains from legumes, lichen, and soil, and obtained 48 strains from soil, 8 rhizobial strains from leguminous plants, 40 strains from lichen. Some of them were defined as diazotrophs based on the methods (growth on nitrogen-free medium, acetylene reducing activity, and detection of *nifH* gene), whereas some of them are still unknown or having no nitrogen-fixing ability. The phylogenetic study also included some nitrogen-fixing strains isolated from the rice plant by Oyaizu-Masuchi and Komagata (1988) and some diazotrophic bacteria whose phylogenetic position are uncertain such as *Derrxia gummosa*, *Alcaligenes latus*, *Pseudomonas saccharophila*, and genera *Azotobacter*, *Azomonas*, and *Azorhizophilus*.

Symbiotic nitrogen-fixing bacteria

Rhizobia are capable of forming root or stem nodules on leguminous plants. Two non-rhizobial strains Ka9123 and Y103A isolated from *Lotus japonicus* in the seacoast of Chiba, were affiliated with *Aminobacter* lineage based on 16S rRNA and *recA* genes phylogenetic analyses. They could nodulate in *L. japonicus* and *Lotus corniculatus*, the *nodA* and *nifH* genes sequences analyses indicated that the unique genes for nodulation might be transferred from *Mesorhizobium* strains. Phylogenetic studies on the strains isolated from *L. japonicus* in many regions of Japan revealed that the highly divergent strains scattered through the genus *Mesorhizobium* and could be separated at species level. These observations clearly indicate that the legume *L. japonicus* harbor diverse rhizobia distributed among the genus *Mesorhizobium*, and the symbiotic island could be transferred to at genus level in the rhizosphere. Isolates within *Rhizobium* lineage from leguminous plant, and lichen were subjected to investigate the phylogenetic analyses based on 16S rRNA, *nodA*, and *nifH* genes sequences, indicating *nod* and *nif* genes did not co-evolve together and may be acquired from different origins.

Anoxygenic photosynthesis diazotrophs.

Photosynthesis is the physico-chemical process which use light energy to drive the synthesis of organic compounds by plant, algae and photosynthetic bacteria. Aside from performing photosynthesis, some members of the phototrophic bacteria are able to fix nitrogen. Strains R5, R12, R2 (originated from rice plant) and strain O5 (originated from lichen) were found to be the anaerobic photosynthetic bacteria with red pigment, internal photosynthetic membrane and bacteriochlorophyll *a*. The phylogenetic analyses based on 16S rRNA, *gyrB*, *nifH*, and *pufLM* genes sequences as well as phenotypic study revealed that they were two independent members of the genus *Rhodospseudomonas*. The aerobic anoxygenic phototrophic bacterium, strain MCW (originated from lichen), formed pink pigment at aerobic-light condition, and would belong to a novel genus within α -*Proteobacteria* based on the phylogenetic analyses of 16S rRNA, *nifH*, and *pufLM* genes sequences.

Free-living diazotrophic bacteria

Free-living diazotrophic bacteria can fix nitrogen at aerobic condition. The nitrogenase activities of these bacteria were easily detected by acetylene reduction assay

and *nifH* gene sequencing.

(1). The phylogenetic analyses revealed that strain O8 (originated from lichen) and strain KaO3 (isolated from soil) were closely related to genus *Xanthobacter* and genus *Paenibacillus*, respectively.

(2). Fourteen strains isolated from rice plant or its rhizosphere by Oyaizu-Masuchi and Komagata, were included in this study. Taxonomic positions of these strains has been remained uncertain because of the lacking of molecular study. Based both on the newly obtained data in this study such as 16S rRNA, *nifH* and *recA* gene sequence analyses and on the results from previous study, we have assigned the strains to be novel genus or species. *Pleomorphomonas oryzae* gen. nov., sp. nov. (Xie and Yokota, 2005a); *Azospirillum oryzae* sp. nov. (Xie and Yokota, 2005b); *Zoogloea oryzae* sp. nov. (In press); *Sphingomonas oryzae* sp. nov. (In press); and *Amorphomonas oryzae* gen. nov., sp. nov. were proposed for these strains.

(3). The taxonomic positions of some nitrogen-fixing bacteria are still confused, such as *D. gummosa*, *A. latus*, *P. saccharophila*, and the genera *Azotobacter*, *Azomonas*, and *Azorhizophilus* were investigated further and were proposed as new emendation of *D. gummosa* (Xie and Yokota, 2004), reclassification the two strains of *A. latus* as *Azohydromonas lata* gen. nov., comb. nov. and *Azohydromonas australica* sp. nov. (Xie and Yokota, 2005c), and transfer the *Azorhizophilus paspali* to genus *Azotobacter* as *Azotobacter paspali* sp. nov. comb., nov.

Phylogenetic structure of whole diazotrophic bacteria

All recognized nitrogen-fixing bacteria (145 genus/ 270 species arranging 16S rRNA gene phylogeny) were recorded here to understand better their phylogeny and biodiversity. The *nifH* gene phylogeny containing about 100 nearly full sequences was also estimated to elucidate their evolutionary process, a critical part of the emerging evolutionary picture seems to be horizontal gene transfer (HGT) between the nitrogen-fixing bacteria. *Methylococcus capsulatus* Bath was given an example to study *nif* gene HGT. The considerable deviation of G+C content and codon usage between the *nif* genes and the genome sequence clearly revealed that *nif* genes of *M. capsulatus* are exogenous and had different evolution from the genome.

General conclusion

The taxonomy of nitrogen-fixing bacteria was undergoing substantial revisions based on multi-loci phylogenetic analyses. This was proving to unravel the biodiversity of the nitrogen-fixing bacteria within the population from host plant, rice plant, lichen, and soil. Our results maybe beyond the traditional basis for the distribution of nitrogen-fixing bacteria and can provide enormous amounts of information regarding the diversity of the nitrogen-fixing bacteria. Such divergent nitrogen-fixing bacteria would be provided good materials to underpin the field of biological nitrogen fixation and make major contributions to our understanding of fundamental processes in microbiology and plant-microbe interactions in the future study.

References:

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