学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Establishment of artificial symbiosis between *Lemna minor* and the diazotrophic bacterium *Azotobacter vinelandii*, and elucidation of the mechanisms of bacterial plant growth promotion

（ウキクサ*Lemna minor*と窒素固定細菌*Azotobacter vinelandii*の人工共生系構築と植物成長促進機構の解析）

Duckweed, family Lemnaceae, is recently being highlighted as a cash crop owing to its various biotechnological applications. Not only limited to toxicological studies, this ubiquitous aquatic plant is being considered as a valuable biomass for animal feed, human food and biofuel feedstock. Duckweed found in natural lakes and ponds may have broader biotechnological applications if cultivated in controlled environment. Duckweed usually prefer ammonium as nitrogen source. After the depletion of ammonium, it absorbs nitrate from the medium. Although nitrogen is a limiting nutrient for growth, it is not well known how the aquatic macrophytes such as duckweed may be benefitted from the nitrogen fixing environmental bacterial communities. In this study, an artificial symbiosis between *Lemna minor* and a nitrogen fixing soil bacteria *Azotobacter vinelandii* ATCC 12837= NBRC 13581 denoted as A81 was established in order to study the plant-bacteria interaction and the bacterial mechanisms of plant growth promotion.

Although *L. minor* and *A. vinelandii* does not have a history of association in nature, the artificial symbiosis was stable for over a month of incubation in both nitrogen free and nitrogen containing hydroponic medium. During this time the number of A81 cells on the duckweed frond increased several folds while promoting the growth of the plant even in extremely nitrogen limited condition. Nitrogen fixation, along with other plant growth promoting compounds produced by the A81 was identified as the bacterial growth promoting mechanisms. The phenomena of nitrogen fixation by A81 attached to the plant surface was only found in *L. minor* and not on the giant duckweed *Spirodela polyrhiza*. This indicated a differential metabolic interaction of duckweed hosts with their nitrogen fixing symbionts. *L. minor* also prompted nitrogen fixation of A81 cell suspension. These data provided sufficient proof that the artificial symbiosis between A81 and *L. minor* is mutually beneficial.

The stable symbiotic *L. minor*/A81 was then applied to wastewater containing high level of Na^+ where *L. minor*/A81 had significantly higher growth rate than that of the no bacteria control. The simultaneous reduction of the Na^+ from the wastewater indicated adsorption of Na^+ into the bacterial biofilm led to stress alleviation on the plant which could be a possible growth promotion mechanism in the wastewater along with nitrogen fixation. Finally, A81 showed species specific syntrophic and antagonistic interactions when co-inoculated other plant growth-promoting bacteria isolated previously from duckweed microbial community.

I further wondered if extracellular substances (EPS) secreted by *A. vinelandii* may have the ability to promote the growth of *L. minor*. A81 is an abundant producer of alginates. When applied to the *L. minor*, A81 alginate along with alginate of algal origin failed to show a growth promotion activity on *L. minor*. However, in our screening experiments, we found that EPS of a different strain, *A. vinelandii* ATCC 13705 denoted as CA had significant growth promotion activity on *L. minor*.

From this study, new insights on how aquatic plants interact with soil borne nitrogen fixing bacteria was elucidated. A stable and mutually beneficial plant bacterium symbiosis in hydroponic culture can open new biotechnological applications of growing duckweed biomass in nutrient stress conditions.