ABSTRACTS (MASTER THESIS)

Analysis of dynamics and function of daidzein in the soybean rhizosphere

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Rhizosphere is defined as the “area affected by the root of the plant in soil” and previous works have shown that the rhizosphere has the higher microbial activity mainly due to the influence of root exudates. In the rhizosphere of soybean, it has been reported that isoflavones such as daidzein and genistein secreted by soybean root functions as symbiotic signals with rhizobium and also phytoalexins. These are also shown to be involved in the regulation of rhizosphere microbial communities. Rhizosphere is closely related to the plant growth. It is thought that the metabolites from root play important roles in the formation and maintenance of the rhizosphere; however, its dynamics in the rhizosphere is currently unknown. It is difficult to understand the rhizosphere area precisely. The aim of this study is to define the rhizosphere by creating soybean root zone model. The dynamics of daidzein, the major root root-secreted isoflavone, is analyzed both biochemically and soil physically. I also investigated daidzein concentrations that potentially affect rhizosphere microbial community.

First, to know the tendency of daidzein secretion in soybean fields, daidzein secreted from the root of field-grown soybean was quantified using HPLC. The method is as follows: daidzein secreted from root was quantified directly sandwiching the lateral root with a cellulose acetate membrane which is capable of adsorbing isoflavone effectively. As a result, the secretion of daidzein under field conditions was the highest during the vegetative stages and decreased as it shifted to the reproductive stages, which is consistent with the hydroponic conditions.

Based on the simulation of the dynamics of daidzein calculated from the daidzein distribution coefficient and degradation coefficients, it was shown that in soybean field soil daidzein transfer from roots was limited within about 1 mm in 7 days. Daidzein dynamics was also analyzed using Toyoura sands. The field soil contains higher humic substances and clay minerals. They have daidzein adsorption sites, while Toyoura sand is composed of mainly quart, which is characterized to have no organic matter and uniform particle size. In Toyoura sand, daidzein was not degraded. The distribution coefficient was calculated to be about one twentieth of that of the soybean field soil. Based on these parameters it was simulated that the migration of daidzein from the root surface was about 3 mm for 7 days.

Finally, in order to clarify the extent of the influence of daidzein on the rhizosphere microbial communities, the daidzein concentration that affects bacteria in field soil is analyzed. The effect of daidzein was measured by comparing the bacterial community in which daidzein was added with different concentrations. The content of daidzein in each sample was calculated to be 0.8-28.0 nmol / g soil, which corresponds to the rhizosphere concentration at vegetative growth (10-20 nmol/ g soil). Soil DNA was then extracted from each sample and 16S rRNA amplicon sequence was performed using MiSEQ. The sequences were classified into OTU. PCoA analysis indicated that it forms a significantly different bacterial communities.