CHAPTER IV

DISCUSSION

4.1 Effect of *Klebsiella* inoculation on ultra-structure of the root and leaf

Inoculation of rice seedling with *K. oxytoca* R15 were shown to produce root hair curling, branching and root hair proliferation which were similar to previous report (Boonjawat *et al.*, 1991). In addition, association between *Azospirillum brasilense* and wheat seedling (Patriquin *et al.*, 1983) resulted in the same phenomenon. The previous studies lead me to propose that the morphological changing was an important step in an association of *Azospirillum* or *Klebsiella* inoculation, as more nutrients can be absorbed, and increasing root mass should also stabilize the plant. As reviewed by Limpananont, (1987), that *Azospirillum* inoculation enhanced wheat growth during early stages through effects other than nitrogen fixation, such as by root elongation, increases in root's surface area, and density of root hairs, which lead to more mineral uptake into root segments of *Zea maizes* and *Sorghum.* In conclusion, *Klebsiella* inoculation induce

morphological change, and the mechanism was unclear.

4.2 Effect of association on nitrogen fixing-activity and number of *K. oxytoca* R15

Study of an association effect on nitrogen-fixing activity, showed that inoculation of *K. oxytoca* R15 on RD7 rice plants induced the nitrogenase activity of the *K. oxytoca* R15 about 20 fold compared with non-inoculated on day 8 after inoculation. The previous reports in the same condition, Boonjawat *et al.*, 1991, showed that the nitrogen-fixing activity increased 400-500 fold on day 7 after inoculation. Pitaksuteepong, 1992, showed that the nitrogen-fixing activity of *K. oxytoca* R15 increased about 2000 fold on day 8 after inoculation. All the previous studies supported that the rice-*Klebsiella* association induced nitrogen-fixing activity but the degree of the increasing nitrogen-fixing activity was quite different. The difference may be from the different of stock of bacteria and rice seedlings and environmental effect e.g. temperature and light.

In the other system, as reviewed by Chong-Biao and Hui-Xian, 1990, that in the associative bacteria, *Alcaligenes faecalis*-rice association, the bacteria showed higher nitrogenase activity than the non-inoculated. Malik *et al.*,1991, studied the associative

nitrogen-fixing bacteria, *Enterobactor*, *Citobacter*, and *Azospirillum* in the rhizophere of 10 species of *Graminae* growing in saline sodic soils showed that 9 species of these *Graminae* could grow quite well in saline sodic soils, a very low nitrogen and practically non fertile, because the rhizophere fixed N_2 effectively in this condition, 100-500 nmol/g dry weight of roots/h. The previous studies indicated that bacteria can fix N_2 more effectively in associative condition. The mechanism was not clear.

This study has shown that the roots of rice excreted more mucigel in the present of bacteria providing suitable niche for attachment of bacteria, and some of the bacteria penetrated to the epidermal surface of roots in associative condition. The result lead me to propose that this mucigel may protect the nitrogenase enzyme from the O_2 (the nitrogenase enzyme irreversibly switched off by O_2, Veeger et al., 1980), then the nitrogen-fixing activity increased in associative condition.

The study of effect of association on the number of bacteria showed that in non-inoculated condition the number of bacteria was slightly decreased or increased to day 10 after inoculation but the number of bacteria significantly increased in associative condition. Under plant-microbe interaction, the excretion of rice

roots should enhance bacterial growth. An increase in nitrogen-fixing activity and an increase of number of bacteria were in the same order 50 and 60 fold respectively, indicating good correlation between number of rhizopheric bacteria and N_2-fixation.

4.3 Effect of *K.oxytoca* R15 inoculation on GS specific activity and total proteins of rice leaves and roots

Inoculation of rice seedling with *K. oxytoca* R15 resulted in an increase in leaf GS specific activity but not that in root. The GS specific activity that determined by transferase hydroxamate assay was 30% higher than non-inoculated leaf. These results were supported by the activity stain of GS protein on native gel electrophoresis and Western blot analysis by antibody to cytosolic GS of maize's leaf after an SDS-PAGE.

A few studies about GS in associative condition was reported. As reviewed by Chong-Biao and Hui-Xian, 1990, that the bacteria associated with roots and submerged portion of shoot can fix N_2 and supply at last part it to rice plant. Saengduan, 1991, found that in *Klebsiella*-rice association ,the GS activity of inoculated rice roots slightly decreased.

There were various studies on GS enzyme in symbiotic

conditions. Orr and Haselkorn, 1982, reported that in *Anabaena-Nostoc* symbiosis, the GS antigen of *Anabaena* was about 5% of the level in non-inoculated condition. Meek *et al.*, 1985, studied in *Anthoceros-Nostoc* symbiosis reported that *Nostoc* assimilated about 10% of the fixed-N_2 and the rest was exported and assimilated by GS/GOGAT pathways in *Anthoceros* tissue. As reviewed by Linblad *et al.*, 1987, in *Cycas revoluta-Nostoc* symbiosis, in symbiotic condition the fixed-N_2 is rapidly transported from the cyanobiont to the *Cycas*.

In leguminous plant-*Rhizobium* symbiosis as reviewed by Cullimore, 1983, in *Phaseolus* root nodule it was reported that the amount of GS activity in bacteria represents only 2% of the total GS activity of the nodule, then the fixed-N_2 from the *Rhizobium* assimilated by GS enzyme in the plant fraction. Gebhard *et al.*, 1986, studied in the same system, reported that the end product of bacterial N_2 fixation was ammonia which exported from the bacteroid and assimilated in cytoplasm of the host plant through the activity of GS. The previous reports indicated that in symbiotic condition the GS activity of bacteria was inhibited, and the fixed-N_2 was exported to plant and was assimilated in the plant fraction. In this research on *Klebsiella*-rice association, the GS activity of the *Klebsiella* was not inhibited in the

associative condition, on contrary the GS activity in rice root slightly decreased and only the GS activity in rice leaf that specifically increased about 30% in response to associative N_2-fixation. Based on different patterns of GS specific activity and GS proteins in Klebsiella-rice association, increasing GS specificity in bacteria indicated that ammonium should assimilated in bacterial cells resulting in glutamine, glutamate and other amino acids, which could be excreted in to the surrounding. If the hypothesis were true the amino acids in the growth medium should be increased.

Conclusion

In associative condition *K. oxytoca* R15 can fix N_2 more effectively. The association induces morphological changes in the inoculated rice root such as, root curling, root hair proliferation and branching, but no morphological change in the rice leaf. In this experimental condition the GS activity of rice leaf increased 30% on day 7-21 after inoculation. The results indicate that the cytosolic GS in the rice leaf indirectly assimilated part of the fixed-N_2 previously assimilated by GS/GOGAT pathway in *K. oxytoca* R 15 that were excreted and adsorbed by the rice root, then transferred to the rice leaf and enter the amino acids pool in the plant by the transferase activity of cytosolic leaf GS.