Chapter III

Conclusions

The production of vitamin B_6 was studied in about 1,590 bacterial isolates from soil, and an isolate, 28-21, identified as *Rhizobium leguminosarum* was obtained as a vitamin B_6 high producer. Then, the production of vitamin B_6 by commercially available *Rhizobium* strains was examined, and many of the tested strains excreted large amounts of vitamin B_6 into the culture broth. The best producer of vitamin B_6 was *R. meliloti* IFO 14782, which produced 51 mg per liter. Media study for the vitamin B_6 production was done with *R. meliloti* IFO 14782; the strain was able to excrete 84 mg of vitamin B_6 per liter, 79mg per liter of which was pyridoxol.

The biosynthetic pathway of pyridoxol (vitamin B_6) in *Rhizobium* was clarified by studies on the incorporation of ^{13}C- or ^{15}N-labeled precursors into pyridoxol or its biosynthetic intermediates. Pyridoxol was formed by ring closure of two compounds, 1-deoxy-D-xylulose and 4-hydroxy-L-threonine. The former was formed from D-glyceraldehyde and pyruvate through decarboxylation of pyruvate, and the latter from glycine and glycolaldehyde.

In vitro system for pyridoxol formation from 1-deoxy-D-xylulose and 4-hydroxy-L-threonine was established in *R. meliloti*. The enzyme reaction system required NAD^+, $NADP^+$, and ATP as coenzymes, and differed from the *E. coli* enzyme reaction system comprising PdxA and PdxJ proteins, which requires only NAD^+ for formation of pyridoxol 5′-phosphate from 1-deoxy-D-xylulose 5-phosphate and 4-(phosphohydroxy)-L-threonine.