Acta Bot. Neerl. 27 (3/4), August 1978, p. 239–242.

MEETING OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

SYMPOSIUM: SYMBIOTIC NITROGEN FIXATION, ON NOVEMBER 18, 1977

A. QUISPEL *(Botanisch Laboratorium, Leiden)*

Symbiotic nitrogen fixation: developments and perspectives

The increased demand for nitrogen for world food production and the problems imposed by the decrease of fossil energy reserves stimulate research on biological nitrogen fixation. These practical demands on research can be answered by important successes of fundamental research. The introduction of the $^{15}N_2$ methods and more recently the C_2H_2 method has made it possible to demonstrate N_2 fixation in a rapid and reliable way. Since the enzyme system, nitrogenase, was obtained in a cell-free condition rapid progress has been made in elucidating the biochemistry of N_2 fixation and the regulation of its activity.

The successful application of the methods of microbial genetics has made it possible to locate the nif genes on the bacterial chromosome and to transfer these nif genes to originally non-N_2 fixing bacteria. Stimulating experiments were performed on the regulation of enzyme synthesis and the role of the NH_3-binding enzyme glutamin synthetase. Special attention was given to the protection against O_2.

In symbiotic nitrogen fixation, as it occurs in the root nodules of leguminous plants, it was generally believed that symbiosis was a prerequisite for N_2-fixation as cultivated Rhizobia could not fix N_2. In 1975, however, a number of publications appeared in which it was shown that some strains of *Rhizobium* could fix N_2 in laboratory cultures under well-defined conditions of which a very low pO_2 is very important. It is doubtful whether the bacteria really use the fixed Nitrogen for their own protein synthesis, as most of the fixed Nitrogen is excreted as NH_4^+. One of the important problems is whether the regulation of N_2 fixation in the symbiotic bacteroids is related to the regulation of N_2-fixation in cultivated Rhizobium. This makes it necessary to obtain a good insight into the bacteroid cells and their formation by the symbiotic bacteroids is related to the regulation of N_2-fixation in cultivated *Rhizobium*. These interactions lead to the formation of cells in which the bacteria are transformed into a type of N_2 fixing "organelles", providing an internal NH_4^+ source for the plant. This NH_4^+ source is made possible as well as limited by the supply of photosynthates.

In non-leguminous root nodules progress has been considerably delayed by the impossibility to cultivate the endophytes. There is good hope that at least one strain of the endophyte of *Alnus glutinosa* now has been obtained in pure, infectuous laboratory cultures.

A. VAN KAMMEN *(Laboratorium voor Moleculaire Biologie, Wageningen)*

Molecular biology of nitrogen fixation

J. W. KIJNE *(Botanisch Laboratorium, Leiden)*

On the role of lipopolysaccharide and lectins in legume-*Rhizobium* symbiosis.

Root nodule symbiosis involves body-contact of both partners. Contact of bacterial and plant surface structures may function in two ways: a) by the contact itself (e.g. recognition), or b) by the consequences of this contact (e.g. mutual cell wall or membrane changes as a result of the contact). Bacterial lipopolysaccharide (LPS) is known to be involved in both aspects: recognition of phages, specific interactions in the vertebrate immune response towards gram-negative bacteria, mitogenic activity towards B-lymphocytes, triggering of B-lymphocyte differentiation.

ad a) Binding of Rhizobial LPS to carbohydrate-binding proteins (lectins) in legume root hair slime is the main element of a promising hypothesis, clarifying Rhizobial host-specificity.

Pea seed lectins (specifically interacting with glucose and mannose) bind to a glucose-rich LPS-component (PS_1) of *Rh. leguminosarum* (PLANQUÉ & KIJNE 1977). A glucose-binding protein fraction has recently been isolated from pea root slime in our laboratory. PA gel electrophoresis indicates the presence of two proteins comparable with both seed lectins.

Clover seed lectin specifically interacts with deoxyclucose (DOG) (DAZZO & HUBBELL 1975). *Rh. trifolii*-LPS contains DOG, the bacterial lectin receptor probably is a PS_1-like polysaccharide (Dazzo, pers. comm.), and a protein fraction specifically agglutinating *Rh. trifolii* can be eluted from clover roots with DOG. It is hypothesized that every *Rhizobium*-species has its specific sugar constituents in the LPS-complex which recognize the corresponding host lectins (WOLPERT & ALBERSHEIM 1976), resulting in specific binding to the host root hairs. These findings do not exclude the need of "aspecific" factors for a successful root hair infection.

ad b) Nodule cell infection by *Rhizobium* triggers remarkable changes in host cell ultrastructure (KIJNE 1975) especially concerning the host endomembrane system. These changes only occur after *Rhizobium*- host plasma membrane contact, and are thought to be a feature of redifferentiation. A role of hormones in redifferentiation of pea root cells has been established (LIBBENGA 1970). Pea root explants growing with auxins and kinetin show a particular pattern of cortical cell proliferation which also can be observed after growth with auxins and a drop of Rhizobial LPS (KIJNE, ADHIN & PLANQUÉ 1977). Lipid A turned out to be the active LPS-component. This effect is not *Rhizobium*-specific, in view of the comparable activity of *E. coli*- and *Salmonella*-LPS in the same experimental system. Bacterial LPS possibly interferes with hormonal balance.

Cell division stop, and LPS-reduction are characteristic steps in the turnover of *Rhizobium leguminosarum* into the bacteroid form (VAN BRUSSEL 1973). Lectin-immobilized *Pseudomonas* shows a cell division stop (SING & SCHROTH 1977); leguminous lectins may degrade the corresponding Rhizobial LPS (ALBERSHEIM & WOLPERT 1977). LPS-lectin interactions inside the host cells could play a role in the development of the symbiosis.

The study of changes in plant- and Rhizobial membrane properties after mutual contact will provide necessary information about the events leading to the N_2- fixing root nodule cell.

ALBERSHEIM, P. & J. WOLPERT (1977): In: *Cell Wall Biochemistry related to specificity in host-plant pathogen interactions*, ed. SOLHEIM & RAA, pp. 373–376.

BRUSSEL, A. A. N. VAN (1973): Ph. D. Thesis, University of Leiden.

DAZZO, F. & D. HUBBELL (1975): *Appl. Microbiol.* **30:** 1017–1033.

HOWARD, J., L. SHANNON, L. OKI & T. MURASHIGE (1977): *Exp. Cell Res.* **107:** 448.

KIJNE, J. W. (1975): *Physiol. Plant Pathol.* **5:** 75–79.

KIJNE, J. W., S. D. ADHIN & K. PLANQUÉ (1977), in: *Cell Wall Biochemistry related to specificity in host-plant pathogen interactions*, ed. SOLHEIM & RAA, pp. 415–416.

LIBBENGA, K. R. (1970): Ph. D. Thesis, University of Leiden.

PLANQUÉ, K. & J. W. KIJNE (1977): *FEBS Letters* **73:** 64–66.

SING, V. O. & M. N. SCHROTH (1977); *Science* **197:** 759–761.

WOLPERT, J. & P. ALBERSHEIM (1976): *Biochem. Biophys. Res. Comm.* **70:** 729–737.

A. D. L. AKKERMANS *(Laboratorium voor Microbiologie, Landbouwhogeschool, Wageningen)*

Nitrogen fixation in association with non-legumes

A description was given of the symbiotic relationships between non-legumes and 3 types of nitrogen-fixing prokaryotes: cyanobacteria, *Rhizobium* and actinomycetes. The processes involved in symbiotic nitrogen fixation with non-legumes were discussed and were compared with these processes in the *Rhizobium*-legume system. Attention was paid to the role of nitrogen-fixing non-legumes in their ecosystems.

a. *Cyanobacteria-non-legume symbioses.* Cyanobacteria which can grow and fix nitrogen symbiotically all belong to the group of heterocystous, filamentous organisms, e.g. *Nostoc* and *Anabaena* spp. Usually these organisms grow as ectosymbionts with or within the hosts: fungi ("lichens"), liverworts (*Anthoceros* and *Blasia* spp.), water ferns (*Azolla* spp.) and *Cycads*. Occasionally the cyanobacterium is an endosymbiont: within one phycomycete (*Geosyphon* sp.), certain diatoms (*Rhizosolenia* spp.) and within specialized glands of few angiosperms (*Gunnera* spp.). In all these different types of symbioses a significant part of the fixed nitrogen is transported from the bacteria to the host. This specific property of nitrogen-fixing symbionts can also be induced in free-living cyanobacteria by addition of inhibitors of glutamine synthetase to the medium.

The importance of spacial separation of photosynthesis and nitrogen fixation in cyanobacteria, and the function of the heterocysts were discussed.

b. *Rhizobium-non-legume symbiosis.* A general discussion was given of the recently discovered symbiosis between a *Rhizobium* species and the non-legume *Parasponia* spp. (Ulmaceae). Attention was paid to the apparent lack of leghaemoglobin and the aberrent structure of *Rhizobium* sp. in the root nodules of *Parasponia* spp. as compared with the nodules of legumes. It was shown that nodule formation on Ulmaceae was known already since 1909 in Java, however, neglected by biologists until recently.

c. *Actionomycete-non-legume symbioses.* Some actinomycetes are able to grow and fix nitrogen obligate-symbiotically within root nodules of certain angiosperms. A review was given of the structure of the nodules. It was shown that the vesicle clusters of the actinomycete are the main sites of nitrogen fixation.

The three types of symbioses mentioned before showed an increased grade of complexity, which is correlated to an increased specificity between the partners in the symbiosis. A comparative study of these different kinds of symbioses will increase the insight in the fundamental processes of symbiotic nitrogen fixation.

A. W. S. M. VAN EGERAAT *(Laboratorium voor Microbiologie, Landbouwhogeschool, Wageningen)*

Agricultural importance of the symbiotic nitrogen fixation

Research on biological nitrogen fixation has increased considerably during the last 4 to 5 years. This is caused by the rapid increase of nitrogen-fertilizer costs, by the energy crisis (enormous amounts of fossil energy are needed for the production of nitrogen fertilizer) and by the awareness of a very rapid increase of the world population, implicating the need of a doubling in food production in the year 2000.

The total biological nitrogen fixation amounts to 175.10^6 T annually, of which the *Rhizobium-* legume symbiosis accounts for approximately 80.10^6 T. However, these amounts have not noticeably increased during the last 25 years.

When comparing the "grain legumes" (very high protein content of the seed, up to 45%) with the "cereal grains" during this period, a constant increase in production of cereal grains has taken place mainly because of the enormous response on fertilizer nitrogen, but with the exception of soybeans, hardly any increase in the production of grain legumes occurred.

Nevertheless, the *Rhizobium-* legume combination is most promising to increase the amount of biologically fixed nitrogen.

Several environmental factors affect the nitrogen fixation: temperature, light, soil humidity, nutrient status and pH of the soil. Furthermore the so-called biological factors which include fungal and bacterial pathogens of plants, nematodes, plant viruses, and insects, all of which can affect nodule formation and/or function.

The role of combined nitrogen on nitrogen fixation is still unclear. A high concentration of combined nitrogen completely inhibits nitrogen fixation, but a certain amount of combined nitrogen is needed to achieve optimal production.

The role of CO_2 is of the utmost importance. When increasing the concentration of this gas, the amount of nitrogen fixed dramatically increases.

Selection of *Rhizobium* strains for more efficient nitrogen fixation, together with good competitive abilities is promising. The same is true for strains fixing nitrogen at lower pH or at higher concentrations of soil nitrogen.