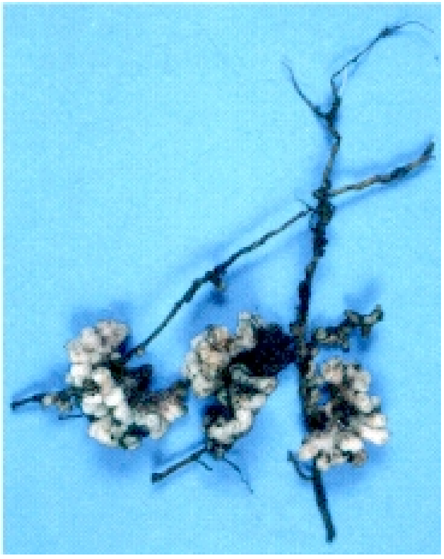
Rhizobium, Root Nodules & Nitrogen Fixation

to find out more about these mini 'fertilizer factories' read on…



Nodules on leguminous plant roots

Nitrogen in the air

Nitrogen is required by all living organisms for the synthesis of proteins, nucleic acids and other nitrogen-containing compounds. The earth's atmosphere contains almost 80% nitrogen gas. It cannot be used in this form by most living organisms until it has been fixed, that is reduced (combined with hydrogen), to ammonia. Green plants, the main producers of organic matter, use this supply of fixed nitrogen to make proteins that enter and pass through the food chain. Micro-organisms (the decomposers) break down the proteins in excretions and dead organisms, releasing ammonium ions. These two processes form part of the nitrogen cycle.

The nitrogen cycle

The nitrogen cycle is a series of processes that converts nitrogen gas to organic substances and back to nitrogen in nature. It is a continuous cycle that is maintained by the decomposers and nitrogen bacteria. The nitrogen cycle can be broken down into four types of reaction and micro-organisms play roles in all of these as the table below shows.

Reaction	Micro-organism	Condition	Process
Nitrogen fixation	Nitrogen-fixing bacteria eg *Rhizobium*	aerobic/anaerobic	The first step in the synthesis of virtually all nitrogenous compounds. Nitrogen gas is fixed into forms other organisms can use.
Ammonification (decay)	Ammonifying bacteria (decomposers)	aerobic/anaerobic	The decomposers, certain soil bacteria and fungi, break down proteins in dead organisms and animal wastes releasing ammonium ions which can be converted to other nitrogen compounds.
Nitrification	Nitrifying bacteria eg *Nitrosomonas* & *Nitrobacter*	aerobic	Nitrification is a two-step process. Ammonia or ammonium ions are oxidized first to nitrites and then to nitrates, which is the form most usable by plants.
Denitrification	Denitrifying bacteria	anaerobic	Nitrates are reduced to nitrogen gas, returning nitrogen to the air and completing the cycle.

Nitrogen fixation

Nitrogen can be fixed in three ways

1. **Atmospheric fixation -** this occurs spontaneously due to lightning; a small amount only is fixed this way.
2. **Industrial fixation** - the Haber process, which is very energy inefficient, is used to make nitrogen fertilizers.
3. **Biological fixation** - nitrogen-fixing bacteria fix 60% of nitrogen gas.

Biological fixation

The reduction of nitrogen gas to ammonia is energy intensive. It requires 16 molecules of ATP and a complex set of enzymes to break the nitrogen bonds so that it can combine with hydrogen. Its reduction can be written as:

$$N_2 + 3H_2 \xrightarrow{\text{energy}} 2NH_3$$

Fixed nitrogen is made available to plants by the death and lysis of free living nitrogen-fixing bacteria or from the symbiotic association of some nitrogen-fixing bacteria with plants.

Examples of nitrogen-fixing bacteria are shown in the table below.

Nitrogen-fixing bacteria			
Free living		Symbiotic association with plants	
Aerobic	Anaerobic	Legumes e.g. peas, beans	Non-legumes e.g. alder tree
Azotobacter	*Clostridium*	*Rhizobium*	*Frankia*

Rhizobium

Rhizobium is the most well known species of a group of bacteria that acts as the primary symbiotic fixer of nitrogen. These bacteria can infect the roots of leguminous plants, leading to the formation of lumps or nodules where the nitrogen fixation takes place. The bacterium's enzyme system supplies a constant source of reduced nitrogen to the host plant and the plant furnishes nutrients and energy for the activities of the bacterium. About 90% of legumes can become nodulated.

In the soil the bacteria are free living and motile, feeding on the remains of dead organisms. Free living rhizobia cannot fix nitrogen and they have a different shape from the bacteria found in root nodules. They are regular in structure, appearing as straight rods; in root nodules the nitrogen-fixing form exists as irregular cells called bacteroids which are often club and Y-shaped.

Root nodule formation

Sets of genes in the bacteria control different aspects of the nodulation process. One *Rhizobium* strain can infect certain species of legumes but not others e.g. the pea is the host plant to *Rhizobium leguminosarum* biovar *viciae*, whereas clover acts as host to *R. leguminosarum* biovar *trifolii*. **Specificity genes** determine which *Rhizobium* strain infects which legume. Even if a strain is able to infect a legume, the nodules formed may not be able to fix nitrogen. Such rhizobia are termed **ineffective**. **Effective** strains induce nitrogen-fixing nodules. Effectiveness is governed by a different set of genes in the bacteria from the specificity genes. **Nod genes** direct the various stages of nodulation.

The initial interaction between the host plant and free-living rhizobia is the release of a variety of chemicals by the root cells into the soil. Some of these encourage the growth of the bacterial population in the area around the roots (the rhizosphere). Reactions between certain compounds in the bacterial cell wall and the root surface are responsible for the rhizobia recognizing their correct host plant and attaching to the root hairs. Flavonoids secreted by the root cells activate the nod genes in the bacteria which then induce nodule formation. The whole nodulation process is regulated by highly complex chemical communications between the plant and the bacteria.

Once bound to the root hair, the bacteria excrete **nod factors**. These stimulate the hair to curl. Rhizobia then invade the root through the hair tip where they induce the formation of an infection thread. This thread is constructed by the root cells and not the bacteria and is formed only in response to infection. The infection thread grows through the root hair cells and penetrates other root cells nearby often with branching of the thread. The bacteria multiply within the expanding network of tubes, continuing to produce nod factors which stimulate the root cells to proliferate, eventually forming a root nodule. Within a week of infection small nodules are visible to the naked eye. Each root nodule is packed with thousands of living *Rhizobium* bacteria, most of which are in the misshapen form known as bacteroids.

Portions of plant cell membrane surround the bacteroids. These structures, known as symbiosomes, which may contain several bacteroids or just one, are where the nitrogen fixation takes place.

Nitrogenase

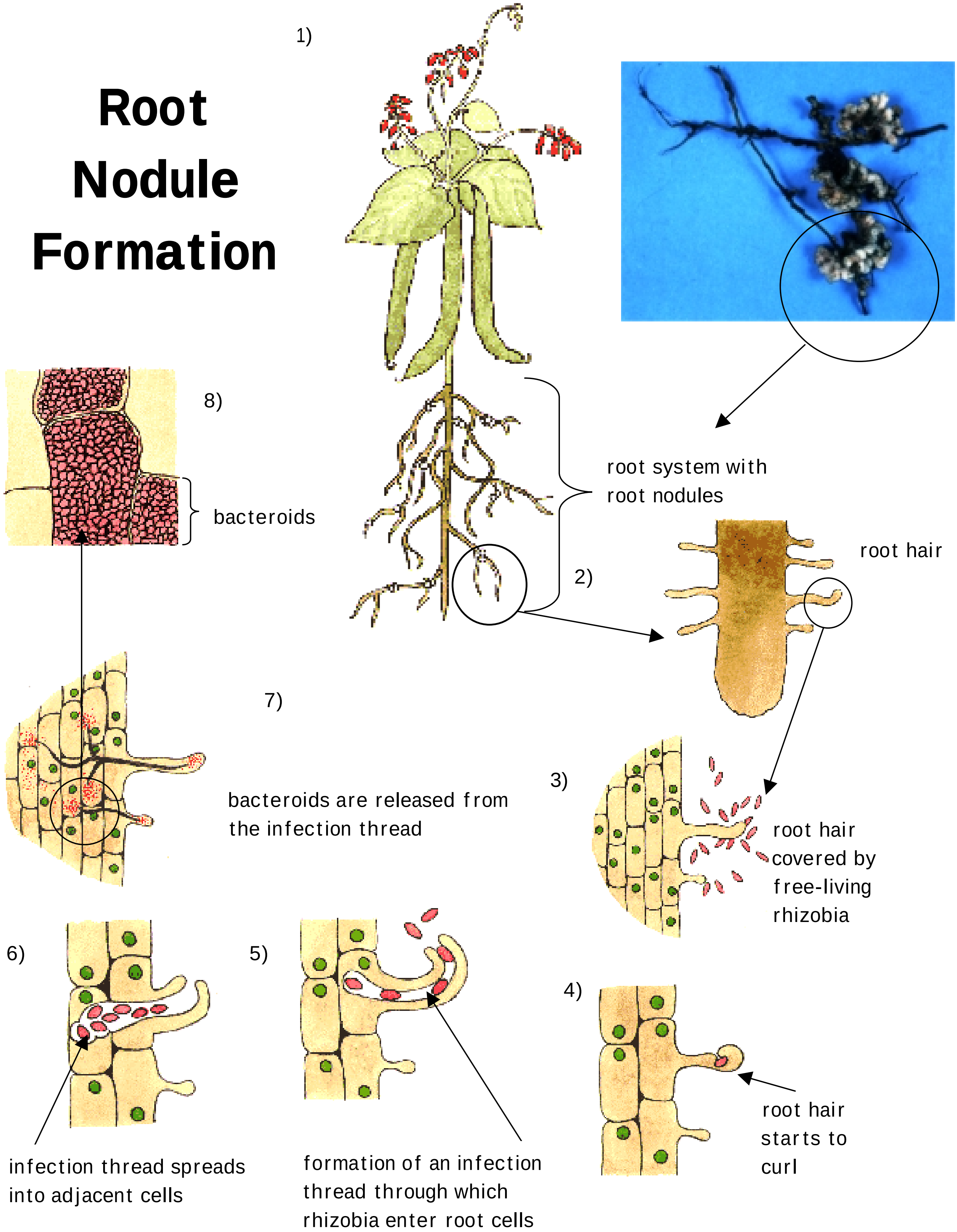
An enzyme called nitrogenase catalyses the conversion of nitrogen gas to ammonia in nitrogen-fixing organisms. In legumes it only occurs within the bacteroids. The reaction requires hydrogen as well as energy from ATP. The nitrogenase complex is sensitive to oxygen, becoming inactivated when exposed to it. This is not a problem with free living, **anaerobic** nitrogen-fixing bacteria such as *Clostridium*. Free living **aerobic** bacteria have a variety of different mechanisms for protecting the nitrogenase complex, including high rates of metabolism and physical barriers. *Azotobacter* overcomes this problem by having the highest rate of respiration of any organism, thus maintaining a low level of oxygen in its cells.

Rhizobium controls oxygen levels in the nodule with leghaemoglobin. This red, iron-containing protein has a similar function to that of haemoglobin; binding to oxygen. This provides sufficient oxygen for the metabolic functions of the bacteroids but prevents the accumulation of free oxygen that would destroy the activity of nitrogenase. It is believed that leghaemoglobin is formed through the interaction of the plant and the rhizobia as neither can produce it alone.

Observation of a cut root nodule

If a root nodule is cut open and the inside is pink/red the nodule is active and fixing lots of nitrogen for the plant. The colour is due to the presence of plenty of leghaemoglobin. The redder the nodule, the more active it is. When nodules are young and not yet fixing nitrogen they are white or grey inside. Legume nodules that are no longer fixing nitrogen turn green and may be discarded by the plant. This may be the result of an inefficient *Rhizobium* strain or poor plant nutrition.

Root Nodule Formation



Nitrogen fixation and agriculture: the way forward

Nitrogen fixation by rhizobia is of great importance in agriculture in several ways. Legumes such as peas, beans, lentils, soybeans, alfalfa and clover help to feed the meat-producing animals of the world as well as humans. Crop yields are greatly improved in nodulated plants; legumes can also grow well in poor soils where there is not enough fixed nitrogen to support other types of plants. After harvest legume roots left in the soil decay, releasing organic nitrogen compounds for uptake by the next generation of plants. Farmers take advantage of this natural fertilization by rotating a leguminous crop with a non leguminous one.

Nitrogen fixation by natural means cuts down on the use of artificial fertilizers. This not only saves money but helps to prevent the many problems brought about by excessive use of commercial nitrogen and ammonia fertilizers such as eutrophication of rivers and lakes, generation of acid rain, and overgrowth of agricultural land by non-food crops.

Research

Fixed nitrogen is often the limiting factor for plant growth in all environments where there is a suitable climate and availability of water to support life. Research is being carried out to find ways of improving the amounts available to plants. This includes not only enhancing the efficiency of rhizobia as nitrogen fixers in legumes, but also using genetic engineering to bring about nitrogen fixation in other crops.

Projects include:

1. Inoculating the seeds of legumes with pure cultures of rhizobia.
2. Improving the ability of the more efficient strains of rhizobia to compete with less efficient strains in the soil to ensure the most effective nodulation of legume plants.
3. Researchers are trying to insert the genes that code for the enzymes involved in nitrogen fixation from bacteria to crop plants, in particular cereals such as wheat, maize and rice. This would decrease the need for nitrogen fertilizers and increase the protein content of the crop. One of the problems encountered is that there are 17 genes responsible for the synthesis of nitrogenase!

Further information

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