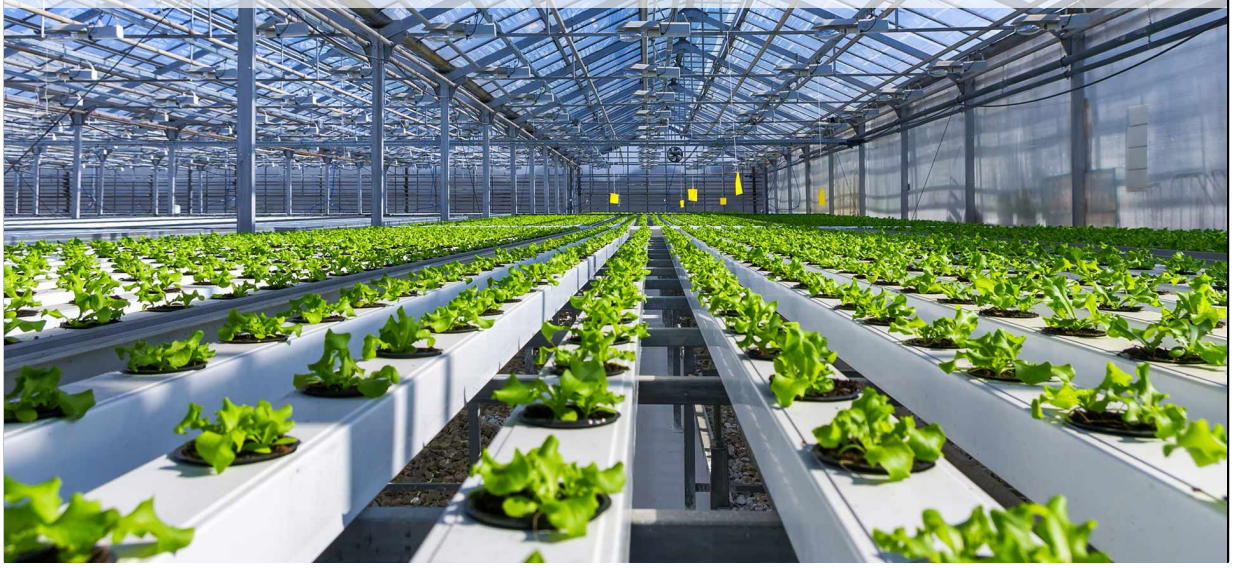
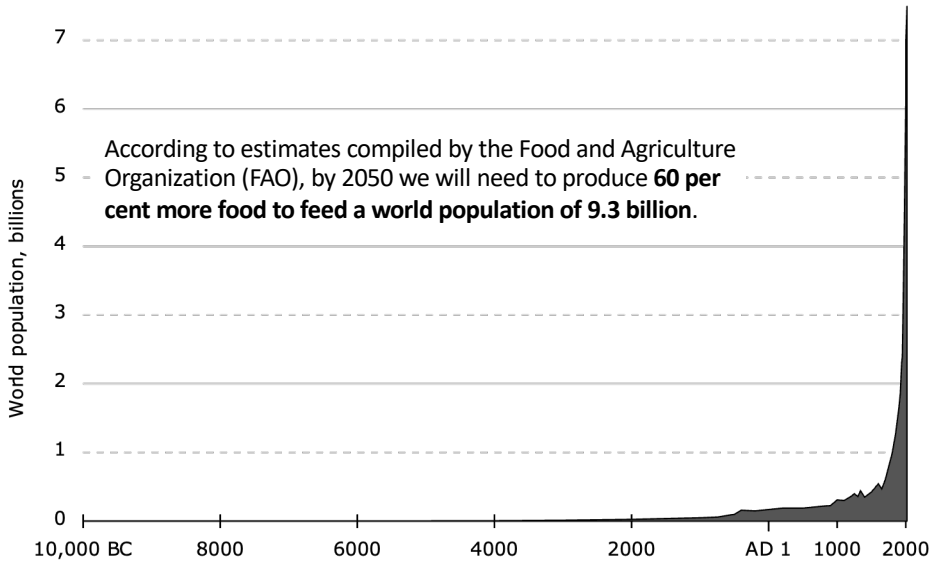
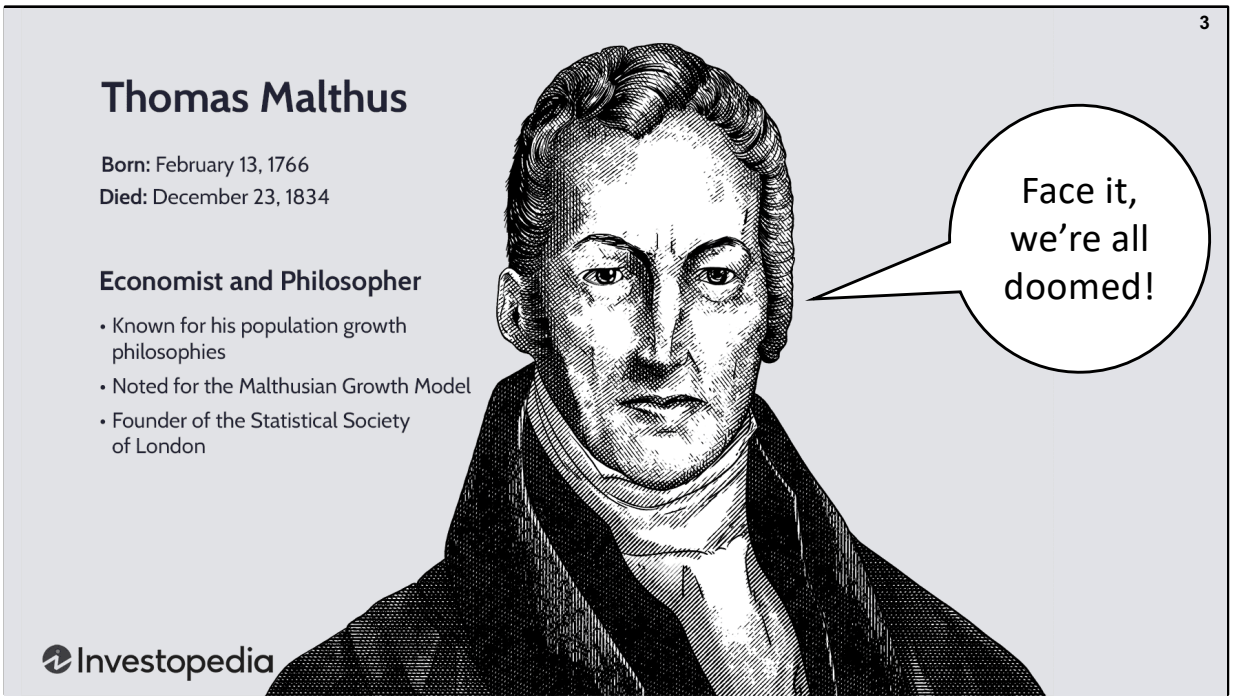
Sustainable agriculture: the future of food



Human population growth over the past 12,000 years

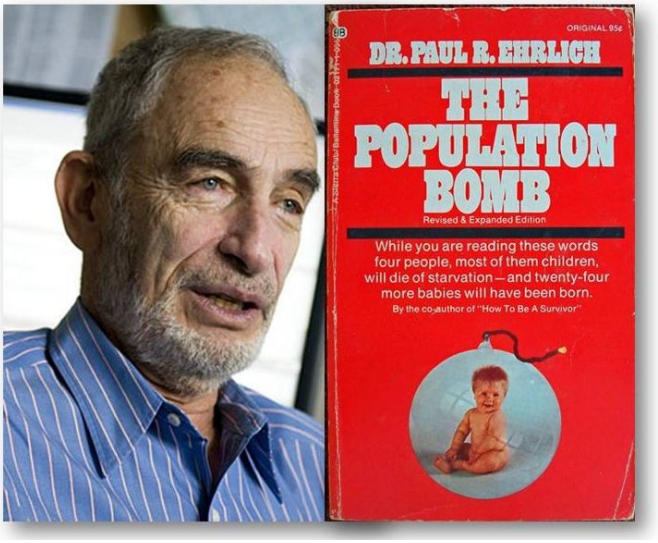


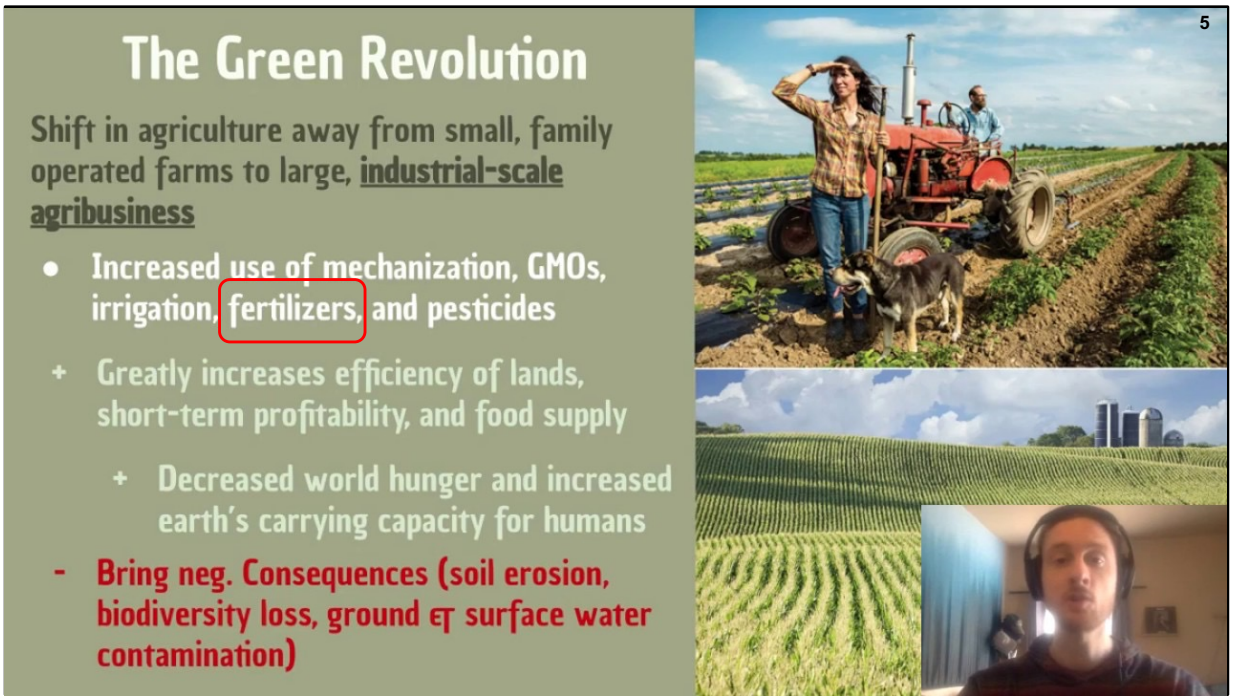
Source: https://www.un.org/en/chronicle/article/feeding-world-sustainably



Source: https://www.investopedia.com/terms/t/thomas-malthus.asp

The “population bomb” (1968) that didn’t go off!





Source: https://www.youtube.com/watch?v=jsrRMYEIxvg

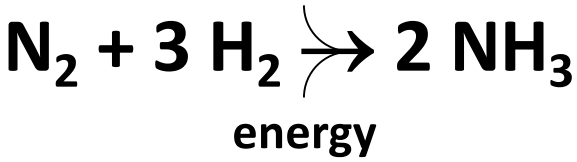
Source: John DA, Babu GR (2021) Lessons from the aftermaths of Green Revolution on food system and health. Front Sustain Food Syst 5: 644559. PMID: 34212131.

Source: https://www.un.org/en/chronicle/article/feeding-world-sustainably

Source: https://theconversation.com/the-green-revolution-is-a-warning-not-a-blueprint-for-feeding-a-hungry-planet-182269

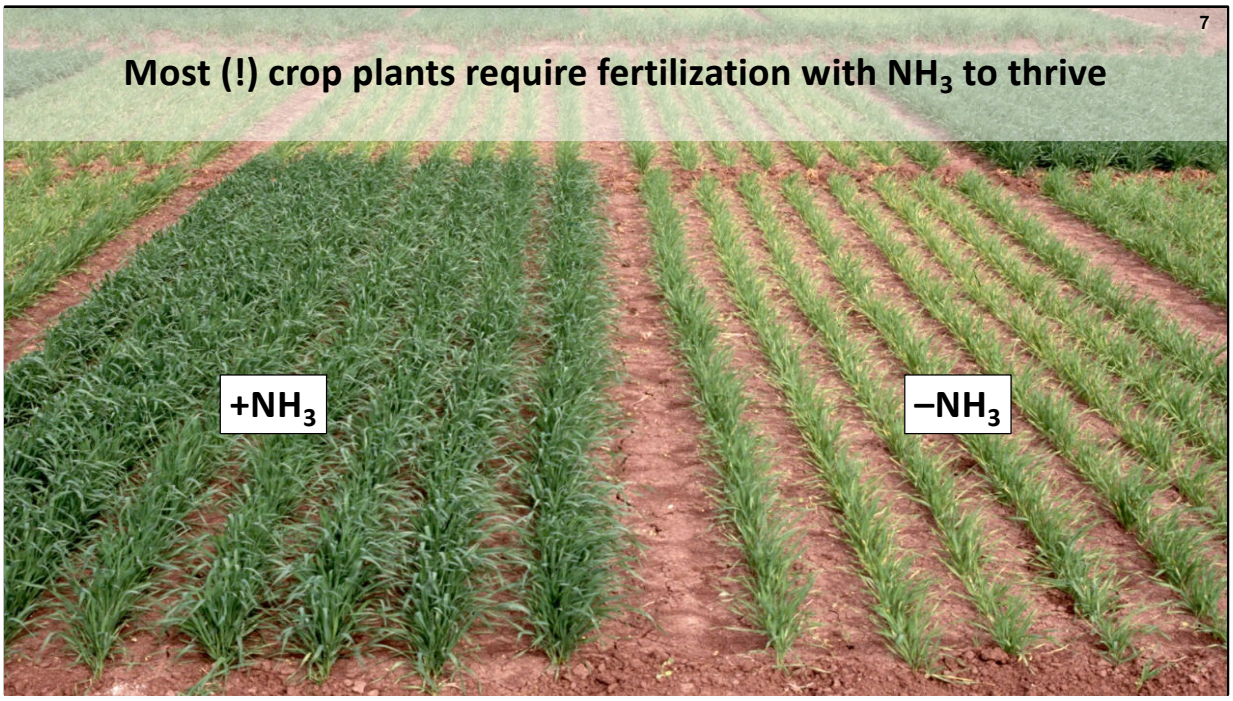
After the green revolution, the production of cereal crops tripled with only a 30% increase in the land area cultivated. This came true all over the world, with a few exceptions. In addition, there were significant impacts on poverty reduction and lower food prices.

Nitrogen fixation: all life on Planet Earth depends on this deceptively simple chemical transformation



Source: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 14: Metabolic Diversity of Microorganisms (pp. 428-486), published by Pearson Education Inc., San Francisco © 2019.

Chapter 14.6: Nitrogen fixation. The biological utilization of dinitrogen (N_2) as cell nitrogen is called "nitrogen fixation". The N_2 is reduced to ammonia (NH_3), a major form of fixed nitrogen, and then assimilated into organic forms, such as amino acids and nucleotides. The ability to fix nitrogen frees an organism from dependence on fixed nitrogen in its environment and confers a significant ecological advantage on it. The process of nitrogen fixation is also of enormous agricultural importance, supporting the nitrogen needs of key crops, such as soybeans, which are able to fix nitrogen thanks to nitrogen-fixing bacterial (rhizobial) endosymbionts living in root nodules.

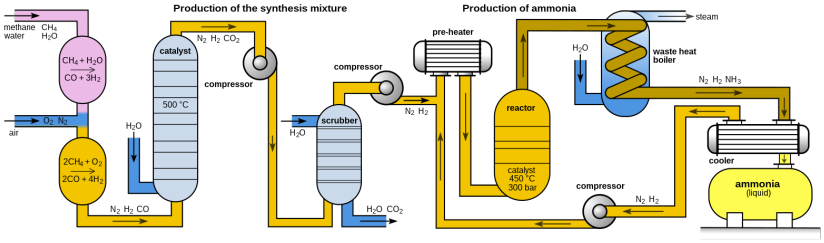


Source: https://www.nue.okstate.edu/Index_NFOA.htm

Industrial nitrogen fixation: the Haber-Bosch process (1910)

$$N_2 + 3\ H_2 \rightarrow 2\ NH_3$$

- High temperature (500ºC)
- High pressure (300 atm)
- Continuous removal of product



Fritz Haber (1918)

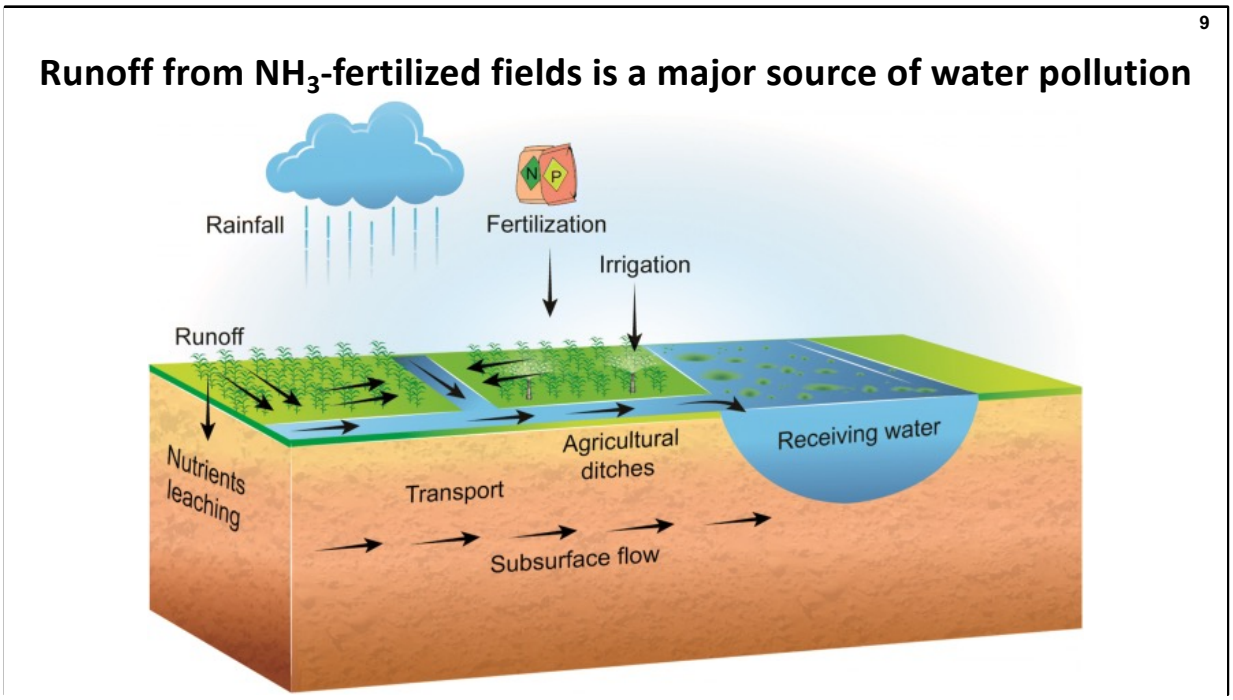
Carl Boch (1927)

Source: https://people.idsia.ch/~juergen/haberbosch.html

Source: https://phys.org/news/2011-11-closer-soluble-solution-haber-bocsh.html

Fritz Haber was awarded the Nobel Prize in 1918 for his work on ammonia synthesis. The process he developed – now known as the Haber process or Haber-Bosch process – fixes nitrogen from the air to make ammonia, which can be used to make synthetic fertilisers.

The Haber-Bosch process has often been called the most important invention of the 20th century (e.g., *V. Smil, Nature 29(415), 1999)* as it "detonated the population explosion," driving the world's population from 1.6 billion in 1900 to more than 8 billion today.



Source: Xia Y, Zhang M, Tsang DCW, Geng N, Lu D, Zhu L, Igalavithana AD, Dissanayake PD, Rinklebe J, Xiao Yang X, Ok YS (2020) Recent advances in control technologies for non-point source pollution with nitrogen and phosphorous from agricultural runoff: current practices and future prospects. *Appl Biol Chem* 63: 8 doi: 10.1186/s13765-020-0493-6.

Figure 2: Schematic diagram of agricultural runoff generation and control.

Source: https://www.nakedcapitalism.com/2018/10/death-by-fertilizer.html

Source: https://www.niehs.nih.gov/health/topics/agents/algal-blooms/index.cfm

Source: https://www.nrdc.org/stories/freshwater-harmful-algal-blooms-101

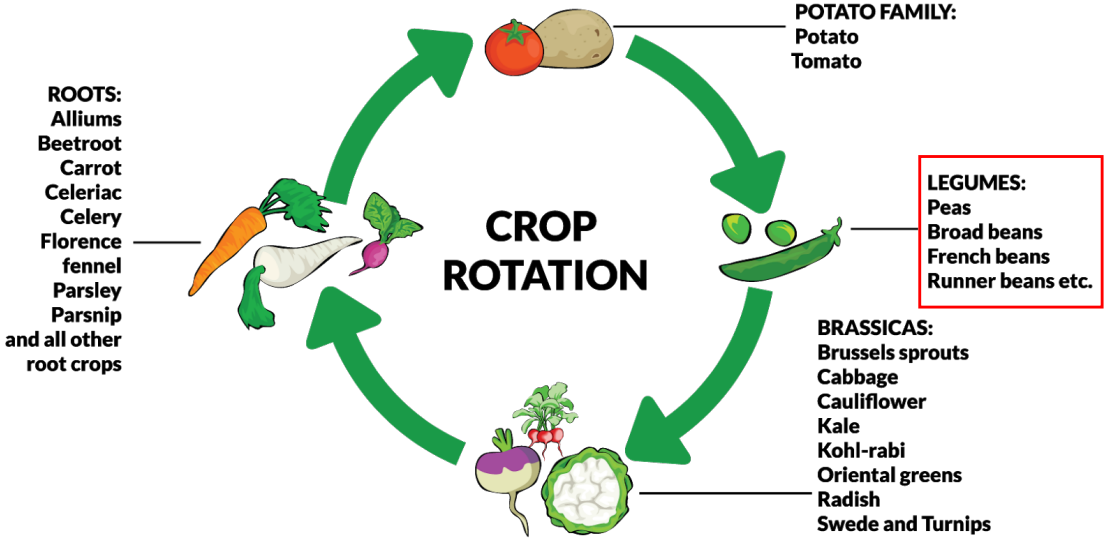
Soyrce: https://www.noaa.gov/what-is-harmful-algal-bloom

What are algae? Algae are microscopic organisms that live in aquatic environments and use photosynthesis to produce energy from sunlight, just like plants. Algae can be found in all types of natural waters, including salt water, fresh water, and brackish water (a mix of salt and fresh water). A few types of algae produce toxins. In these algae, toxin production can be stimulated by environmental factors such as light, temperature, salinity, pH, and nutrient levels. Algal toxins released into the surrounding water or air can seriously harm people, animals, fish, and other parts of the ecosystem.

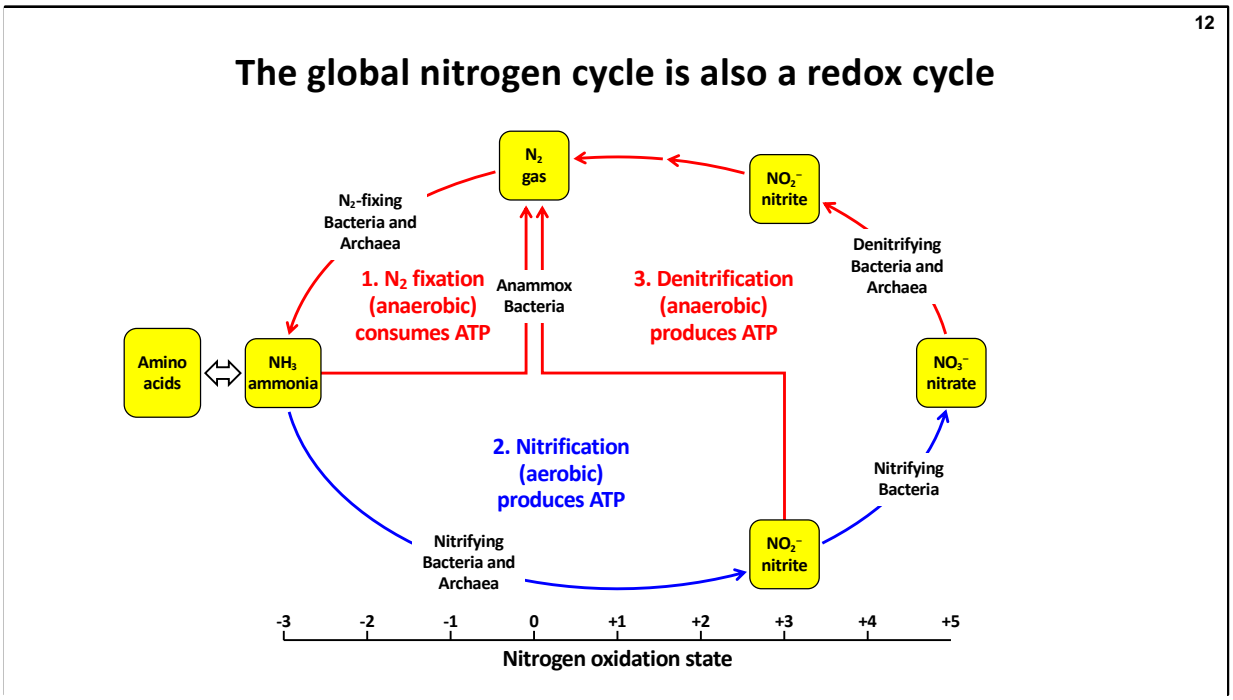
What is a harmful algal bloom? A harmful algal bloom (HAB) occurs when toxin-producing algae grow excessively in a body of water. The excessive algal growth, or algal bloom, becomes visible to the naked eye and can be green, blue-green, red, or brown, depending on the type of algae.
Some blooms are easy to spot, but others are hard to see because they grow near the bottom of water bodies. You cannot tell if a water body has a harmful bloom just by looking at it.

Why do HABs occur? Many factors may contribute to HABs but understanding how these factors come together to create a harmful bloom of algae is a topic of ongoing research. Scientists know that certain environmental conditions, such as warmer water temperatures in the summer and excessive nutrients from fertilizers or sewage waste brought by runoff, trigger HABs, but they are still learning more. As climate change gradually warms the earth’s climate, scientists expect HABs to become more frequent, prolonged, and severe, and in different geographic areas.

Crop rotation: an ancient method for sustainable agriculture

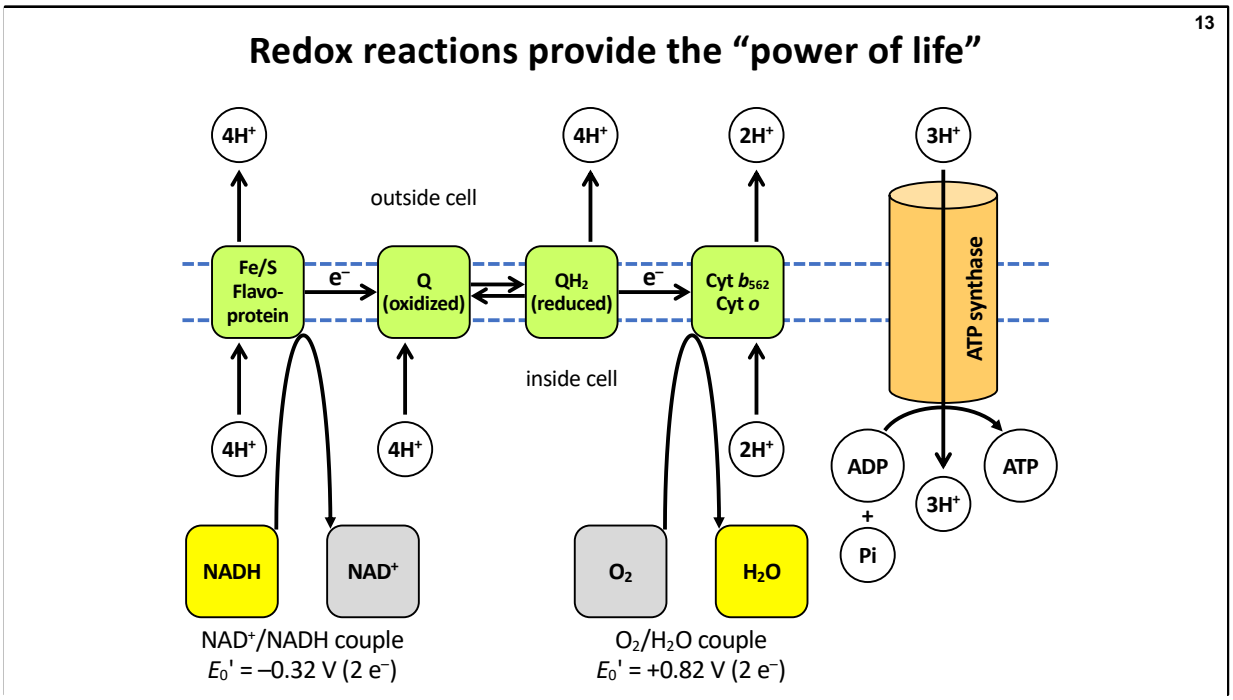


Source: https://psci.princeton.edu/tips/2020/3/21/agriculture-101



Source: *Lehninger Principles of Biochemistry* (p. 852), published by W.H. Freeman & Co., New York, NY © 2008.

Figure 22.1. The nitrogen cycle. The total amount of nitrogen fixed annually is the biosphere exceeds 100,000,000,000 (10^{11}) kg. Reactions with blue arrows occur in aerobic environments. Reactions with red arrows occur in anaerobic environments. The redox states of the various nitrogen species are depicted at the bottom of the figure.



Source: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 3: Microbial Metabolism (pp. 109-137), published by Pearson Education Inc., San Francisco © 2019.

Figure 3.22. Aerobic respiration with oxygen as the terminal electron acceptor. Electron transport processes in the membrane of *Escherichia coli* when molecular oxygen (O_2) is used as the electron acceptor and NADH is the electron donor. $FADH_2$ can also serve as an electron donor to the electron transport chain at the level of the Q cycle (not shown). Transfer of electrons along the electron transport chain, from decreasingly electropositive donors to increasingly electronegative acceptors, generates a proton motive force across the cytoplasmic membrane. This proton motive force can then be used by the membrane-embedded ATP synthase to generate ATP.

The NAD^+/NADH redox couple has a reduction potential of $E_0' = –0.32$ V (2 $e^–$).

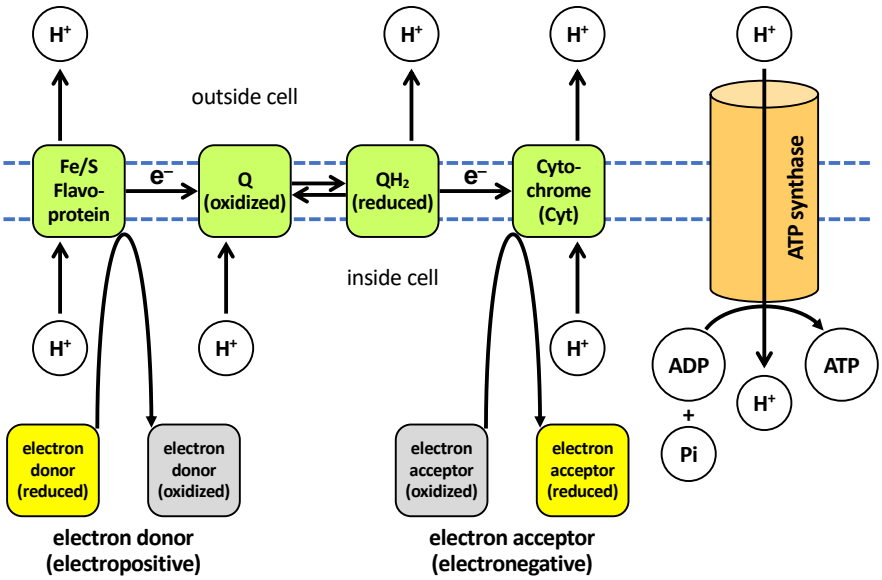
The FAD/$FADH_2$ redox couple has a reduction potential of $E_0' = –0.22$ V (2 $e^–$).

The O_2/H_2O redox couple has a reduction potential of $E_0' = +0.82$ V (2 $e^–$).

Abbreviations: Fp, flavoprotein; Fe/S, iron-sulfur cluster; Q, ubiquinone pool; QH_2 reduced ubiquinone; Cyt b_{556}, cytochrome b_{556}; Cyt *o*, cytochrome *o*.

Components of the electron transport chain are colored green. ATP synthase is colored orange. Reduced forms of electron donors/acceptors are colored red. Reduced forms of electron donors/acceptors are colored grey.

Universal scheme for energy conservation and ATP synthesis



Source : *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 3: Microbial Metabolism (pp. 109-137), published by Pearson Education Inc., San Francisco © 2019.

Components of the electron transport chain (flavoprotein Fe/S; oxidized quinone (Q); reduced quinone (QH_2); cytochrome) are colored green. ATP synthase is colored orange. Reduced forms of electron donors/acceptors are colored red. Oxidized forms of electron donors/acceptors are colored grey.

The "universal scheme" for energy conservation in bacteria, as depicted on this slide, is a "primary concept". You should memorize this general scheme and understand the general principals underlying it. You do not need to memorize the specific components (or their names) of the electron transport chain, which vary from organism to organism. But you should understand that electrons pass from a strong electron donor (more electropositive) to a strong electron acceptor (more electronegative), and some of the energy released in this process is used to move protons across the membrane (from inside to outside). This generates a "proton motive force" that can be used by ATP synthase to make ATP.

15

Calculating the Gibbs free energy ($\Delta G^{o'}$) of redox reactions

Nernst equation: $\Delta G^{o'} = -n * F * \Delta E_0'$

where:

n = number of electrons transferred

F = Faraday's constant (ca. 100 kJ * V^{-1}* $mole^{-1}$)

$\Delta E_0' = (E_0'_{acceptor}) - (E_0'_{donor})$

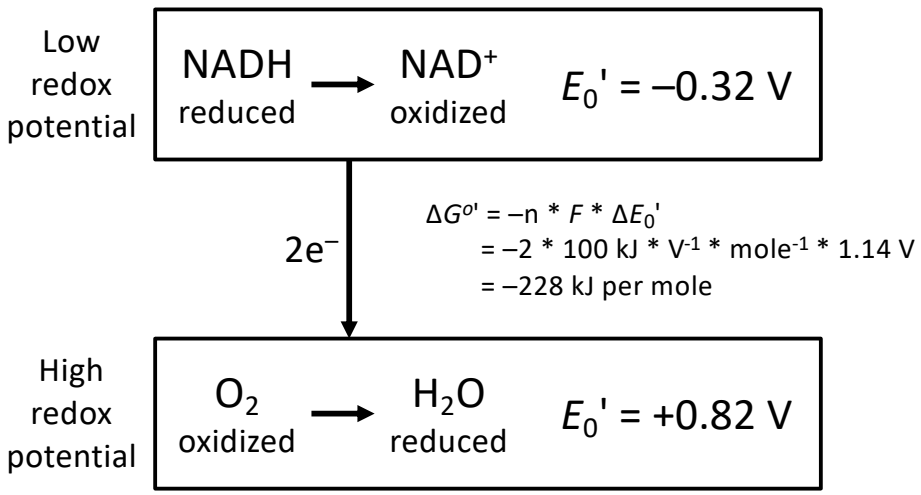
Source: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 3: Microbial Metabolism (pp. 109-137), published by Pearson Education Inc., San Francisco © 2019.

The Nernst equation, which expresses the Gibbs free energy change for reduction-oxidation (redox) reactions, is a "primary concept". You should memorize the Nernst equation and feel comfortable manipulating it. This includes memorizing Faraday's constant: 100 kJ * V^{-1} * $mole^{-1}$ is precise enough for our purposes in this course, but you should also remember that 1 joule = 1 kg * m^2 * s^{-2} in case you need to use these "basic" units in your calculations.

The change in free energy during a reaction is expressed as ΔG°', the free energy change under standard conditions: pH 7.0 (approximate cytoplasmic pH), 25°C, 1 atmosphere of pressure, and all reactants and products at 1 M concentration.

A single **volt** is defined as the difference in electrical potential across a wire when an electric current of one ampere dissipates one watt (joules per second = N * m * s^{-1} = kg * m^2 * s^{-3}) of power. It is also equal to the potential difference between two points 1 meter apart in an electric field of 1 newton per coulomb. Additionally, it is the potential difference between two points that will impart one joule of energy per coulomb of charge that passes through it. It can be expressed in terms of the SI base units m, kg, s, and A as: kg * m^2 * A^{-1} * s^{-3}.

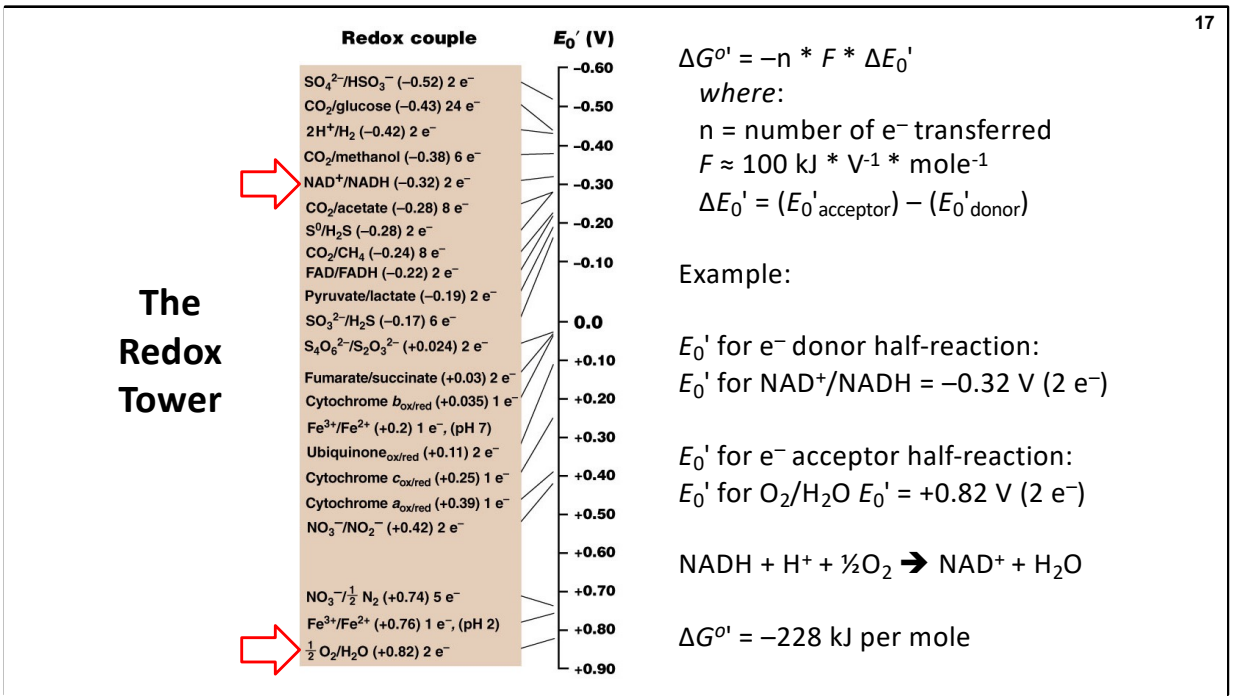
The **ampere** (symbol: A) is the SI unit of electric current (symbol: I) and is one of the seven SI base units. The ampere is a measure of the amount of electric charge passing a point in an electric circuit per unit time with 6.241×10^{18} electrons, or one **coulomb** per second constituting one ampere. The practical definition may lead to confusion with the definition of a coulomb (i.e., 1 ampere-second), but in practical terms this means that measures of a constant current (e.g., the nominal flow of charge per second through a simple circuit) will be defined in amperes (e.g., "a 20 mA circuit") and the flow of charge through a circuit over a period of time will be defined in coulombs (e.g., "a variable-current circuit that flows a total of 10 coulombs over 5 seconds"). In this way, amperes can be viewed as a "rate of flow" and coulombs viewed as an "amount of flow."



The NAD^+/NADH redox couple has a reduction potential of $E_0' = –0.32$ V (2 electrons get transferred).

The O_2/H_2O redox couple has a reduction potential of $E_0' = +0.82$ V (2 electrons get transferred).

Therefore, electrons naturally "transfer" from NADH as the electron donor (which gets oxidized to NAD^+) to O_2 as the electron acceptor (which gets reduced to H_2O).

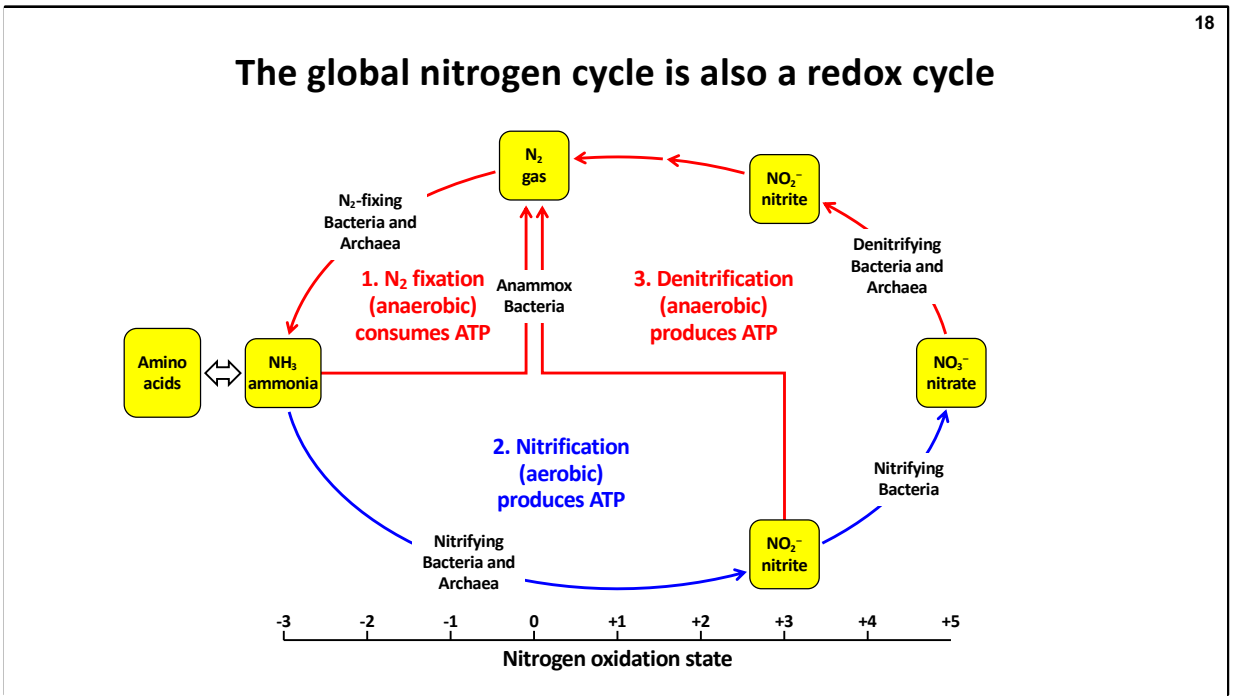


Source: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 3: Microbial Metabolism (pp. 109-137), published by Pearson Education Inc., San Francisco © 2019.

Figure 3.10. The redox tower. Redox couples are arranged from the strongest **electron donors** (most **electropositive**) at the top to the strongest **electron acceptors** (most **electronegative**) at the bottom. Electrons can be "caught" by acceptors at any intermediate level as long as the donor couple has a lower **reduction potential (E_0')** than the acceptor couple. The greater the difference in reduction potential between electron donor and electron acceptor, the more free energy is released when they react.

Substances differ in their tendency to be electron donors or electron acceptors. This tendency is expressed in terms of their reduction potential (E_0', standard conditions), which is measured in volts (V) in reference to a standard redox couple: $2H^+/H_2$ (E_0' = –0.42 V for a 2 electron transfer). By convention, reduction potentials are given for half reactions written as reductions, with reactions at pH 7 because the cytoplasm of most cells is neutral or nearly neutral.

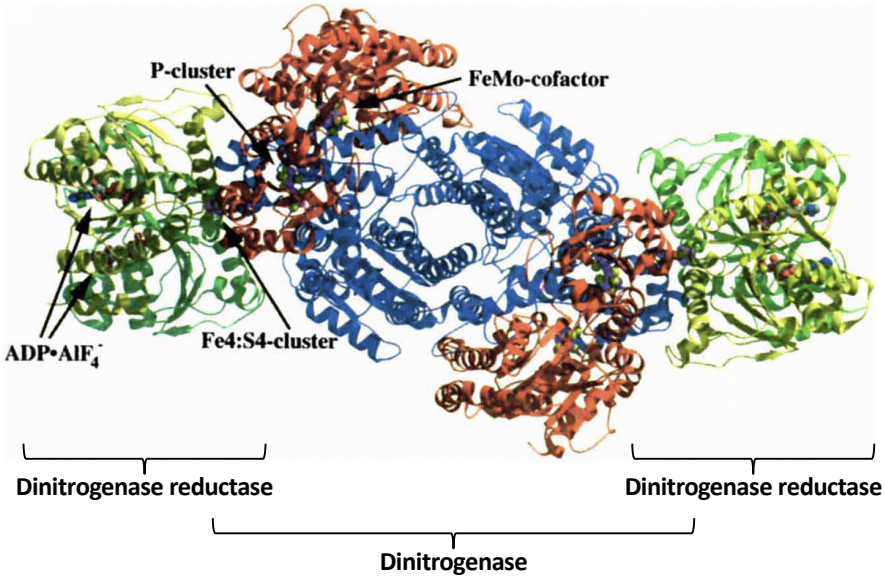
Voltage, also called electromotive force, is a quantitative expression of the potential difference in charge between two points in an electrical field. The greater the voltage, the greater the flow of electrical current (that is, the quantity of charge carriers that pass a fixed point per unit of time) through a conducting or semiconducting medium for a given resistance to the flow. Voltage is symbolized by an uppercase italic letter *V* or *E*. The standard unit is the volt, symbolized by a non-italic uppercase letter V. One volt will drive one coulomb (6.24×10^{18}) of charge carriers, such as electrons, through a resistance of one ohm in one second.



Source: *Lehninger Principles of Biochemistry* (p. 852), published by W.H. Freeman & Co., New York, NY © 2008.

Figure 22.1. The nitrogen cycle. The total amount of nitrogen fixed annually is the biosphere exceeds 100,000,000,000 (10^{11}) kg. Reactions with blue arrows occur in aerobic environments. Reactions with red arrows occur in anaerobic environments. The redox states of the various nitrogen species are depicted at the bottom of the figure.

Nitrogenase is a multisubunit nitrogen-fixing machine

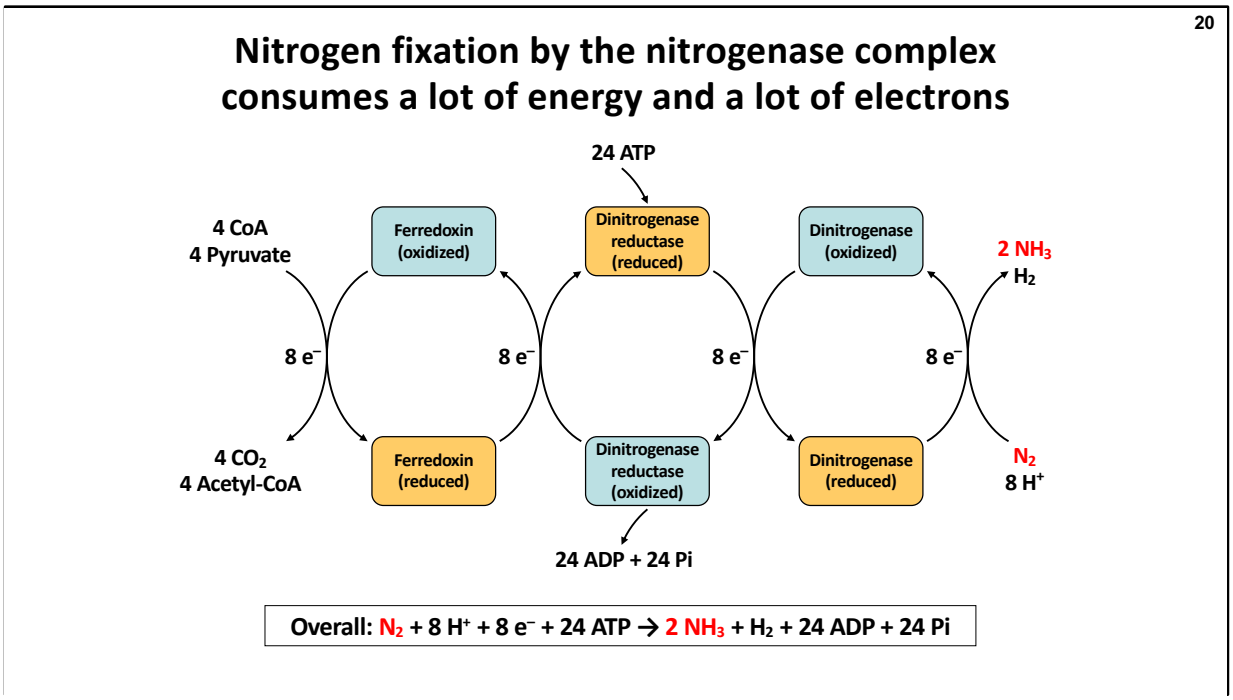


Source: Schindelin H., Kisker C., Schlessman J.L., Howard J.B., Rees D.C. (1997) Structure of ADP x AIF4(-)-stabilized nitrogenase complex and its implications for signal transduction. *Nature* 387(6631): 370-376 PMID:9163420.

Figure 1. Overall structure of the nitrogenase complex from *Azotobacter vinlandyii*. The entire complex with the molybdenum-iron (MoFe) alpha-subunits in red, the beta-subunits in blue, and the individual subunits of each Fe-protein in green and yellow. The cofactors and the bound nucleotides are shown in ball-and-stick representation. Atoms are color-coded, with Al in orange, Mg and Mo in cyan, Fe and P in purple, S in yellow, O in red, N in blue, and C in grey.

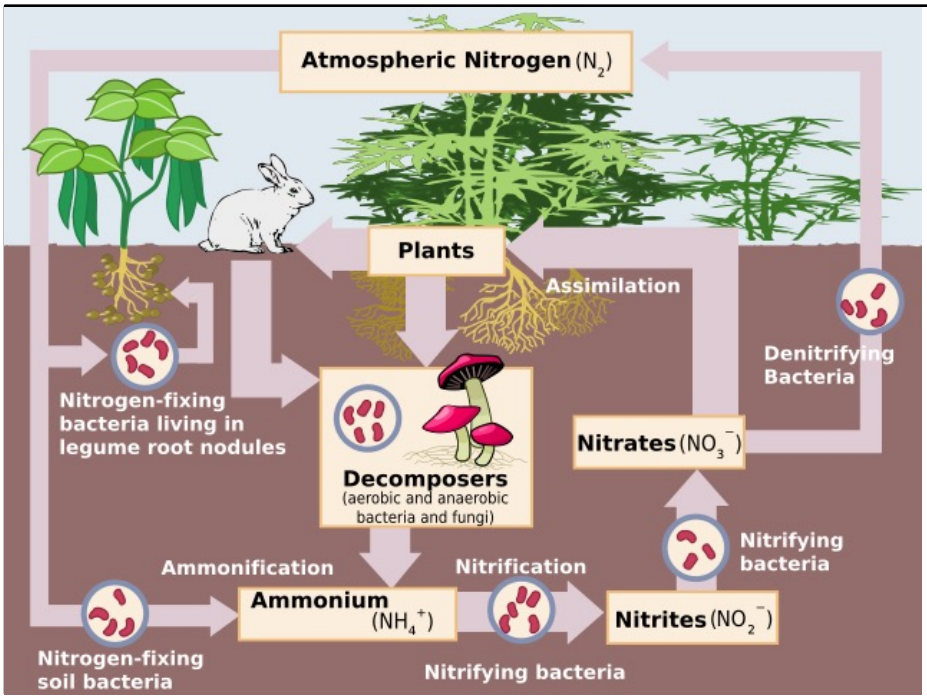
The FeMo (iron-molybdenum) cofactor probably represents the active site for substrate reduction. The P-cluster appears to serve as the intermediate electron transfer center between the Fe-protein donor and the FeMo-cofactor.

$ADP•AlF_4^-$ is ADP stabilized by interaction with aluminum fluoride.



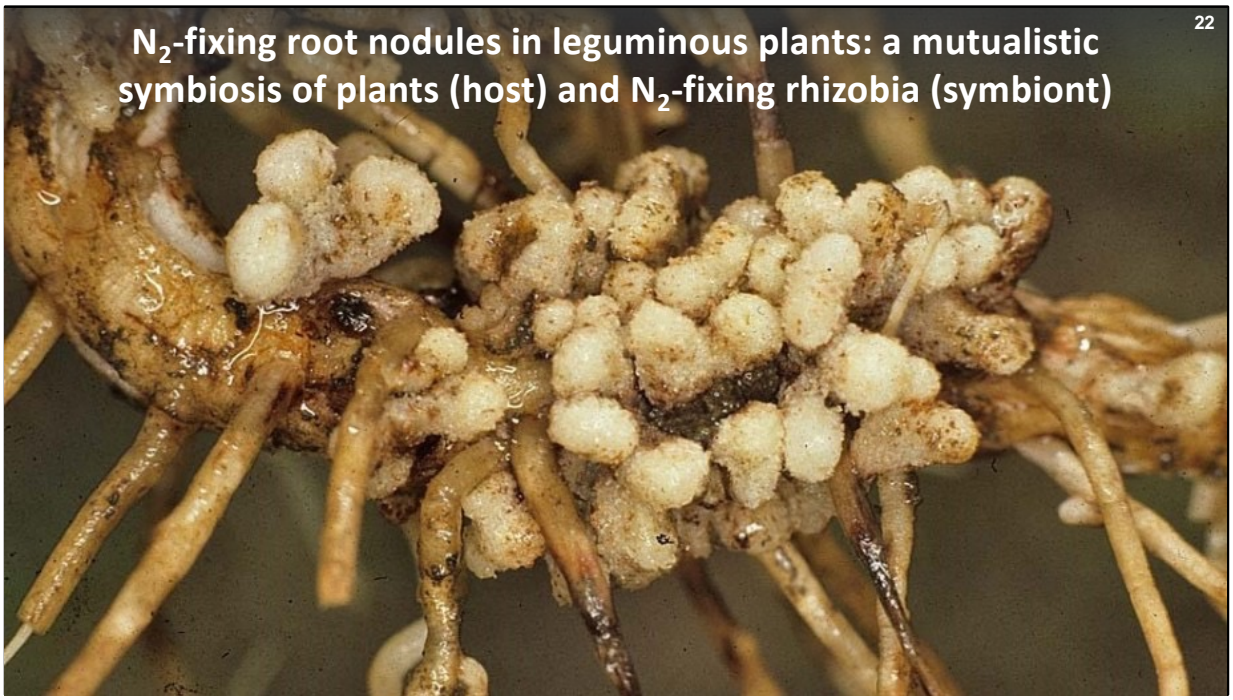
Source: *Lehninger Principles of Biochemistry* (p. 855), published by W.H. Freeman & Co., New York, NY © 2008.

Figure 22.2. Nitrogen fixation by the nitrogenase complex. Electrons are transferred from pyruvate to dinitrogenase via ferredoxin (or flavodoxin) and dinitrogenase reductase. Dinitrogenase reductase reduces dinitrogenase one electron at a time, with at least six electrons required to fix one molecule of N_2. An additional two electrons are used to reduce 2 H^+ to H_2 in a process that obligatorily accompanies nitrogen fixation in anaerobes, making a total of eight electrons required per N_2 molecule reduced.



The global nitrogen cycle

Source: https://byjus.com/biology/nitrogen-cycle/



Source: https://biology.anu.edu.au/news-events/news/nodulation-legumes

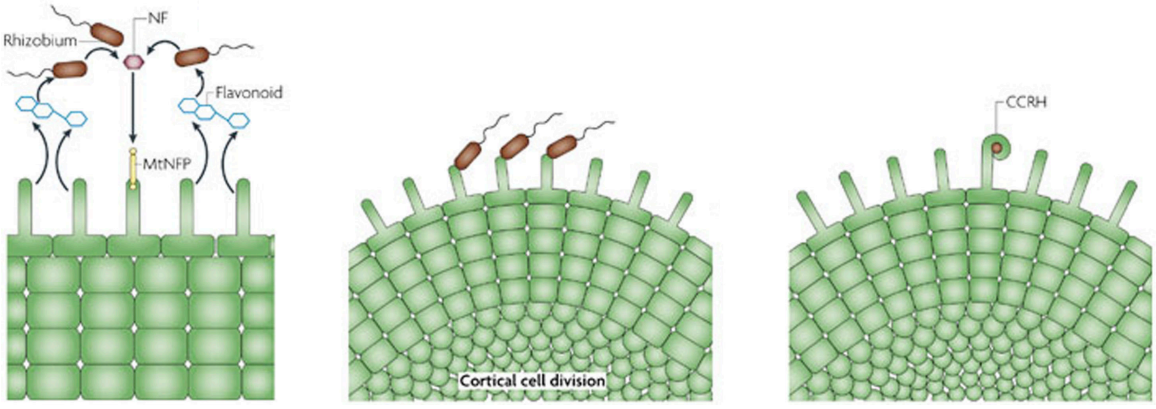
Source: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 23: Microbial Symbioses with Microbes, Plants, and Animals (pp. 732-764), published by Pearson Education Inc., San Francisco © 2019. Figure 23.8.

Effect of nodulation on plant growth. A field of nodulated (left) and non-nodulated (right) soybean plants growing in soil poor in fixed-nitrogen sources.

Source : Gibson K.E., Kobayashi H., Walker G.C. (2008) Molecular determinants of a symbiotic chronic infection. *Annu. Rev. Genet.* 42: 413-441 PMID:18983260.

Rhizobial bacteria colonize legume roots for the purpose of biological nitrogen fixation. The rhizobium-legume symbiosis is established under fixed-nitrogen-limiting soil conditions and is estimated to contribute nearly half of all current biological nitrogen fixation. A complex series of events, coordinated by host and bacterial signal molecules, underlie the development of this symbiotic interaction. Rhizobia elicit *de novo* formation of a novel plant root organ within which they establish a chronic intracellular infection. Legumes permit rhizobia to invade these root tissues while exerting control over the infection process. Once rhizobia gain intracellular access to their host, legumes also strongly influence the process of bacterial differentiation that is required for nitrogen fixation. Symbiotic rhizobia play an active role in promoting their goal of host invasion and chronic persistence by producing a variety of signal molecules that elicit changes in host gene expression. In particular, rhizobia appear to promote their access to the host by producing a variety of signal molecules capable of suppressing a general pathogen defense response by the plant.

Nodulation begins with mutual recognition between the plant root "hair cells" and the rhizobial symbiont



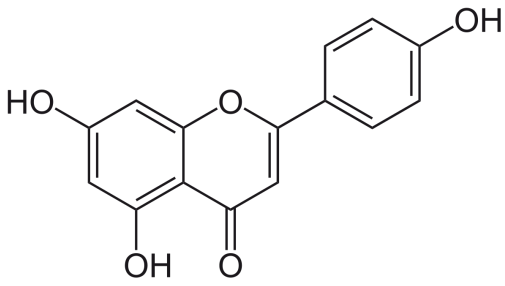
Bidirectional signaling: Flavonoids (plant host) Nod factors (rhizobia) → **Attachment** of rhizobia to "hair cells" and stimulation of "cortical cell" divisions → **Curling** of root "hairs" in preparation for invasion of "hair cells" by rhizobia

Source : Jones KM, Kobayashi H, Davies BW, Taga ME, Walker GC (2007) How rhizobial symbionts invade plants: the *Sinorhizobium-Medicago* model. *Nature Reviews Microbiology* 5(8): 619-633 PMID:17632573. Figure 1.

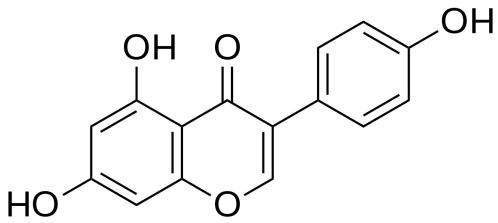
The initial signaling dialogue between *Sinorhizobium meliloti* (rhizobial symbiont) and *Medicago truncatula* (plant host).
(A) Initial Nod factor (NF) signalling. The induction of rhizobial *nod* genes requires plant **flavonoids**. The *nod* gene products produce Nod factor (NF), which is initially perceived by the *M. truncatula* MtNFP receptor.
(B,C) Cortical cell divisions (B) and root hair curling (C) require many *M. truncatula* gene products; MtNFP; *MtDMI1*; *MtDMI2*; *MtDMI3*; *MtNSP1*; *MtNSP2*; *MtCRE1*; MtNIN. *MtLYK3/HCL* is required for colonized curled root hair (CCRH) formation, but not for the induction of cortical cell divisions.
The required rhizobial genes are boxed in brown. The required plant genes are boxed in light green.

25

Flavonoids stimulate rhizobial symbiont responses



Luteolin (5,7,3',4'-Tetrahydroxyflavone)
induces *nod* gene expression in rhizobia
(specifically, *Rhizobium leguminosarum*)

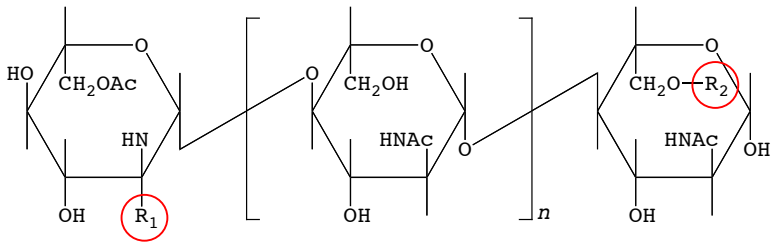


Genistein (5,7,4'-Trihydroxyisoflavone)
inhibits *nod* gene expression in rhizobia
(specifically, *Rhizobium leguminosarum*)

Source: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 23: Microbial Symbioses with Microbes, Plants, and Animals (pp. 732-764), published by Pearson Education Inc., San Francisco © 2019. Figure 23.14.

Plant flavonoids and nodulation. Structures of flavonoid molecules that are **(A)** an inducer of *nod* gene expression and **(B)** an inhibitor of *nod* gene expression in *Rhizobium leguminosarum* biovar *viciae,* the species that nodulates peas. Note the similarities in the structures of the two molecules. The common name of the structure shown in **(A)** is *luteolin*, and it is a flavone derivative. The structure in **(B)** is called *genistein*, and it is an isoflavone derivative.

Rhizobial Nod factors stimulate plant host responses

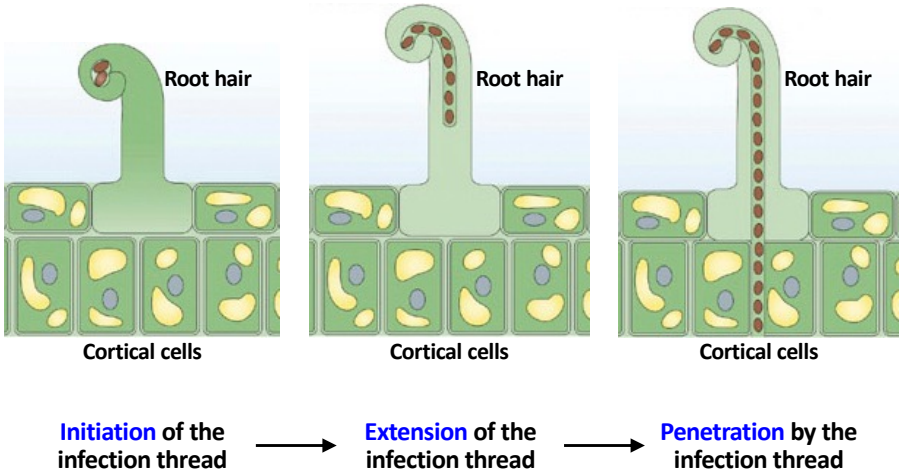


Species	R_1	R_2
Rhizobium leguminosarum	-C18:1 or -C18:4	-H or -Ac
Sinorhizobium meliloti	-C16:2 or -C16:3	$-SO_4^{2-}$

Source: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 23: Microbial Symbioses with Microbes, Plants, and Animals (pp. 732-764), published by Pearson Education Inc., San Francisco © 2019. Figure 23.12.

Nodulation (Nod) factors. General structure of the Nod factors produced by *Sinorhizobium meliloti* and *Rhizobium leguminosarum* biovar *viciae*, and the structural differences (R_1, R_2) that define the precise Nod factor of each species. The central hexose unit can repeat up to three times (*n* = 1-3). C16:2, palmitic acid with two double bonds; C16:3, palmitic acid with three double bonds; C18:1, oleic acid with one double bond; C18:4, oleic acid with four double bonds; Ac, acetyl.

The rhizobial “infection thread” first invades root “hair cells” then penetrates into the underlying root “cortical cell” layers



Source: Jones KM, Kobayashi H, Davies BW, Taga ME, Walker GC (2007) How rhizobial symbionts invade plants: the *Sinorhizobium-Medicago* model. *Nature Reviews Microbiology* 5(8): 619-633 PMID:17632573. Figure 3.

Root hair invasion by *Sinorhizobium meliloti*.

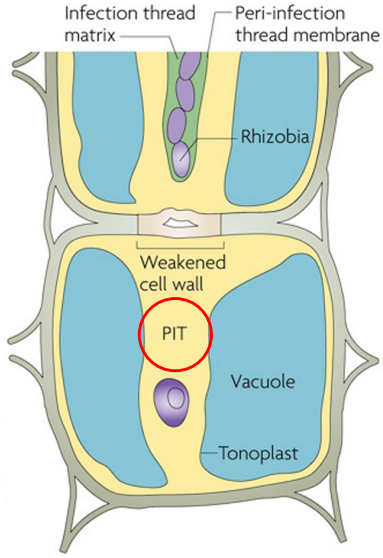
(A) Initiation of the infection thread. *S. meliloti* gene *exoY* gene is required. *Medicago trunculata* genes *MtLIN* and *MtNIN* are required.

(B) Extension of the infection thread to the base of the root hair cell. *S. meliloti* gene *exoH* gene is required. *M. trunculata* genes *MtNFP*, *MtLYK3/HCL*, *MtBIT1/ERN*, *MtNIN*, and *MtCRE1* are required.

(C) Penetration of the infection thread into the underlying cell layers. *M. trunculata* genes *MtCRE1*, *MtBIT1/ERN*, *MtRIT1*, and *MtSLI* are required.

28

The rhizobial “preinfection thread” (PIT) forms ahead of the “infection thread” to prepare plants cells for invasion

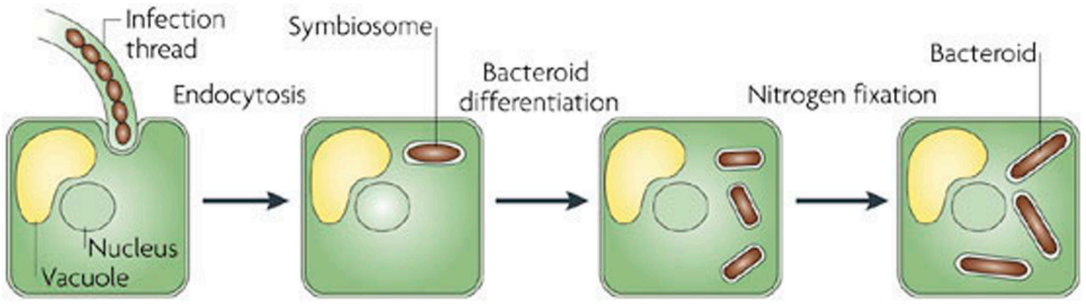


Source: Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbiosis. *Nature Reviews Microbiology* 6(10): 763-775 PMID:18794914. Figure 5.

Intracellular accommodation structure in plant root nodule endosymbiosis involving rhizobia symbionts. A preinfection thread (PIT) forms ahead of the bacteria-filled infection thread. The PIT can be induced by bacterial signals alone and contains an array of microtubules that resemble the arrangement within the pre-penetration apparatus of arbuscular mycorrhiza (fungal symbionts). The PIT is unique to the nodulating clade of rhizobia and is likely to have evolved from the pre-penetration apparatus of arbuscular mycorrhiza. A plant-derived perimicrobial membrane encloses the bacteria-filled infection thread and prevents microbial contact with the plant cytoplasm. This membrane synthesizes cell wall material, which contributes to the composition of the apoplastic interface between the symbiotic organisms.

29

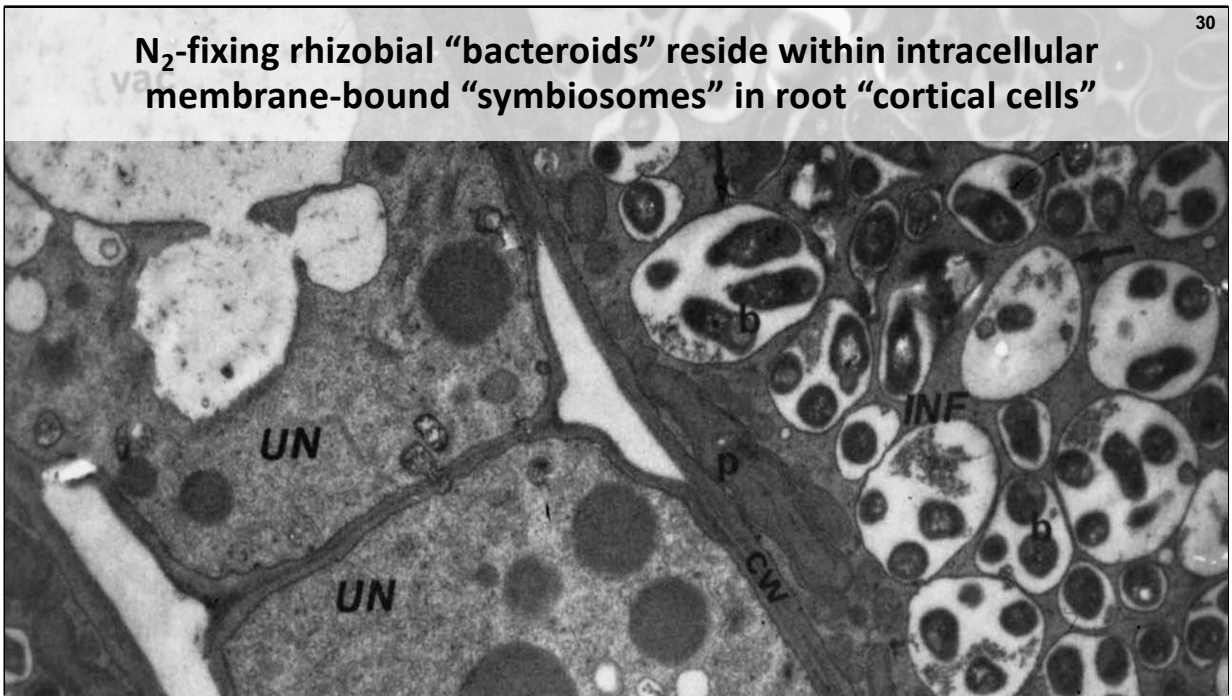
The rhizobial “infection thread” first invades root “hair cells” then penetrates into the underlying root “cortical cell” layers



Source: Jones KM, Kobayashi H, Davies BW, Taga ME, Walker GC (2007) How rhizobial symbionts invade plants: the *Sinorhizobium-Medicago* model. *Nature Reviews Microbiology* 5(8): 619-633 PMID:17632573. Figure 5.

Endocytosis of bacteria and bacteroid differentiation. Bacterial endocytosis requires the *Sinorhizobium meliloti hemA* gene, the *Medicago truncatula NIP* gene, and wild-type expression levels of the *M. truncatula MtDMI2* and *MtHAP2-1* genes. *S. meliloti lpsB* and *bacA* are required for bacterial survival within the symbiosome membrane. *S. meliloti fixJ*, *M. truncatula MtSYM1*, *MtDNF1,-4,-5,* and *-7*, and pea (*Pisum sativum) PsSYM13* are required for bacteroid differentiation. The *S. meliloti nifHDK* genes encode nitrogenase, which is the key enzyme for nitrogen fixation. The pea *PsRUG4* gene encodes sucrose synthase, which is required to support bacteroid nitrogen fixation. The *M. truncatula MtDNF3* and *-6* genes are required for the maintenance of nitrogen fixation.

The required rhizobial genes are boxed in brown. The required plant genes are boxed in light green.



Source: https://alchetron.com/Bradyrhizobium

Source: https://www.fao.org/3/cc0520en/cc0520en.pdf

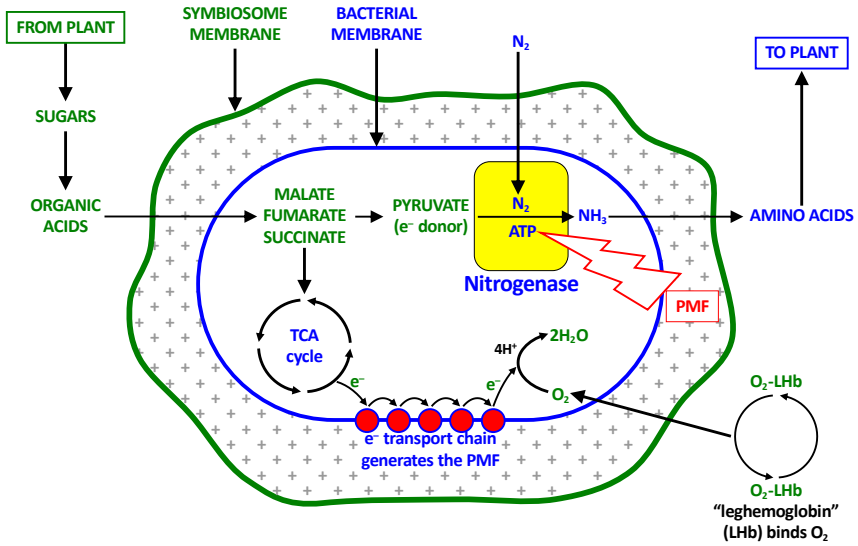
Bradyrhizobium japonicum is a Gram-negative, rod-shaped, nitrogen-fixing bacterium that develops a symbiosis with the soybean plant *Glycine max*. *B. japonicum* belongs to the family *Rhizobiaceae*, which includes other nitrogen-fixing bacteria that develop symbiosis with legumes. The bacterium "communicates" with the host plant and begins a process of plant and bacterial development that leads to a symbiotic partnership. The bacterium will attach to root hairs and release compounds that cause the root hairs to curl. Coordination of bacterial multiplication and inward-directed growth of the root hair results in formation of an infection thread (a tube derived from plant membranes). It is through this infection thread that the bacteria enter the cortical cells of the root and begin to colonize the developing root nodule.

Within the developing root nodule, bacteria divide and begin to differentiate into a bacteroid that is capable of fixing nitrogen. The term “bacteroid” refers to the bacterium existing in a symbiotic relationship to distinguish it from the free-living bacterium. As shown in the electron micrograph shown on this slide, the bacteroids are located inside a structure referred to as a symbiosome, which is derived from plant membrane. One to several bacteroids can be found in a single symbiosome. Therefore, nutrients must traverse multiple membranes to reach the bacteroids and fixed nitrogen must follow a similar complex path to reach the plant tissue. The nodule that results from this process is a highly specialized structure. It provides a physical barrier that keeps the free oxygen concentration low.

The plant cells within the nodule produce leghemoglobin, which serves as an oxygen carrier to the bacteria within the nodule. This enables the bacteria to obtain enough oxygen for respiration but ensures that the oxygen is in a bound form so that it cannot harm nitrogen-fixing enzymes inside the bacteria. Cutting open a nodule reveals the deep red color typical of leghemoglobin when it binds oxygen.

What do the individual partners in this symbiosis get from each other? The plant provides the bacterium with a "safe" environment and a steady supply of fixed carbon for energy and growth. This carbon source is referred to as “photosynthate”, as it is derived from the product of photosynthesis. In most rhizobium/legume symbiosis, photosynthate refers to the dicarboxylic acids succinate, fumarate, and malate. In return, the bacteria provide the plant with fixed nitrogen, i.e., dinitrogen (N_2) gas that has been reduced and converted into a form (amino acids) readily utilized by the plant. The result of this symbiosis is a dramatic increase in plant production without the need for adding external fertilizer.

Intracellular symbiosomes function like N_2-fixing organelles



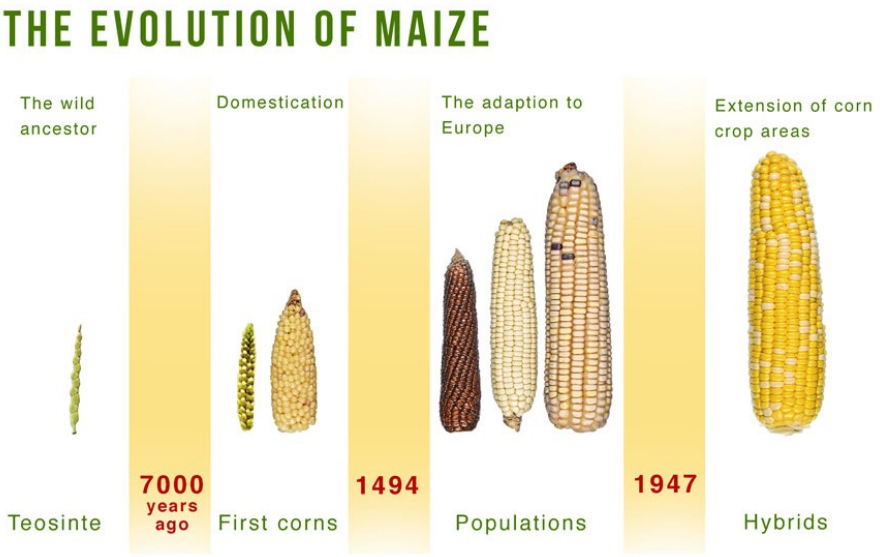
Source: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 23: Microbial Symbioses with Microbes, Plants, and Animals (pp. 732-764), published by Pearson Education Inc., San Francisco © 2019. Figure 23.15.

The root nodule bacteroid. Schematic diagram of major metabolic reactions and nutrient exchanges in the bacteroid. The symbiosome is a collection of bacteroids surrounded by a single membrane originating from the plant.

Nitrogen fixation requires the enzyme nitrogenase. Nitrogenase from bacteroids shows the same biochemical properties as the enzyme from free-living N_2-fixing bacteria, including O_2 sensitivity and the ability to reduce acetylene as well as N_2. Bacteroids are dependent on the plant to provide the electron donor for N_2 fixation. The major organic compounds transported across the symbiosome membrane and into the bacteroid proper are citric acid cycle intermediates - in particular, the C_4 organic acids succinate, malate, and fumarate. These molecules are used as electron donors for ATP production and, following conversion to pyruvate, as the source of electrons for reduction of N_2 to ammonia (NH_3).

The product of N_2 fixation is ammonia (NH_3)., and the plant assimilates most of this ammonia by forming organic nitrogen compounds, such as amino acids. The ammonia-assimilating enzyme glutamine synthetase is present in high levels in the plant cell cytoplasm and converts glutamate and ammonia into glutamine. Glutamine and a few other organic nitrogen compounds transport fixed nitrogen throughout the plant.

All modern crop plants have been genetically engineered!



Source: https://thetranscriptome.com/gmos-next-revolution-or-hazard-to-be-avoided/

GMOs are one of the most controversial subjects of recent years. So much, that not a month goes by without some kind of a scandal appearing in your local newspaper. For example, a moratorium on the use of GMOs has been in force since 2005 in Switzerland and continues to be extended by the government [1]. Yet one might imagine, that with so much discussion about it the general public will be well informed, and aware of its possibilities and its risks. However, it would appear that the population remains very concerned about the possible negative effects of GMOs: only 3 out of 10 Europeans consider that an apple that has integrated a gene from another species does not present a risk [2].

Chihuahu: an example of “human unnatural guided selection” (HUGS)

Source: Traub A (2022) Mr. Happy Face, a Chihuahua Mix With a Mohawk, Crowned World’s Ugliest Dog *New York Times* June 25, 2022.

34

Genetic engineering of N_2 fixation in non-leguminous plants (NLPs)

✗ 1. Engineer NLPs to recreate the rhizobial root nodule symbiosis (RNS)

✗ 2. Engineer NLPs to express bacterial nitrogenase from N_2-fixing bacteria

✓ 3. Engineer NLPs to promote biofilms of N_2-fixing soil bacteria on roots

Main target: non-leguminous grain crop plants like rice, wheat, barley…

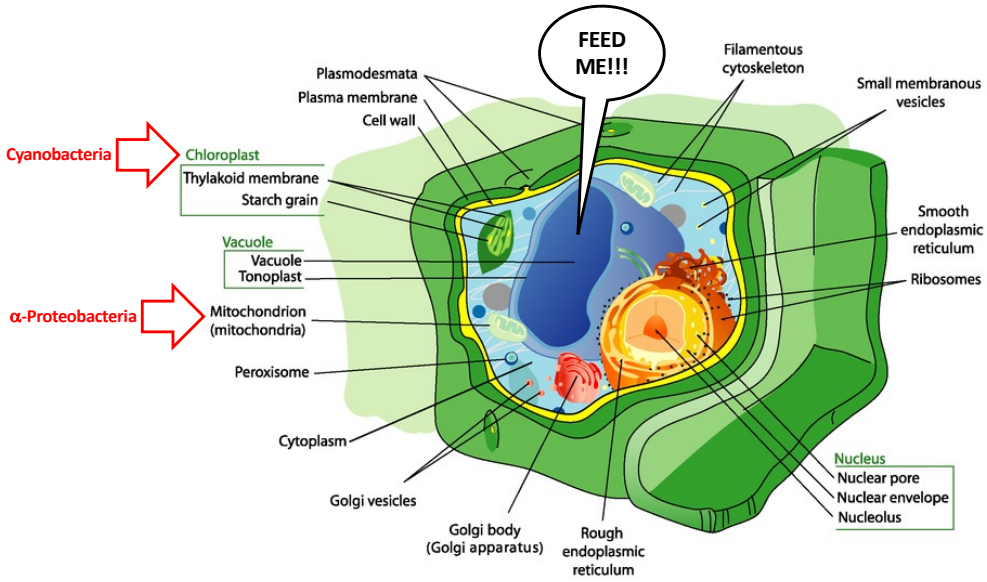
Source: Ahmed N, Ishfaq M, Ali G (2023) Genetic engineering for enhanced biological nitrogen fixation in cereal crops. *Trends Biotechnol* 41(4): 473-475 PMID: 36344382. Review.

Source: Rosenblueth M, Ormeño-Orrillo E, López-López A, Rogel MA, Reyes-Hernández BJ, Martínez-Romero JC, Reddy PM, Esperanza Martínez-Romero E (2018) Nitrogen fixation in cereals. *Front Microbiol* 9: 1794 PMID: 30140262. Review.

Source: Mus F, Crook MB, Garcia K, Garcia Costas A, Geddes BA, Kouri ED, Paramasivan P, Ryu MH, Oldroyd GED, Poole PS, Udvardi MK, Voigt CA, Ané JM, Peters JW (2016) Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. *Appl Environ Microbiol* 82(13): 3698-3710 PMID: 27084023. Review.

35

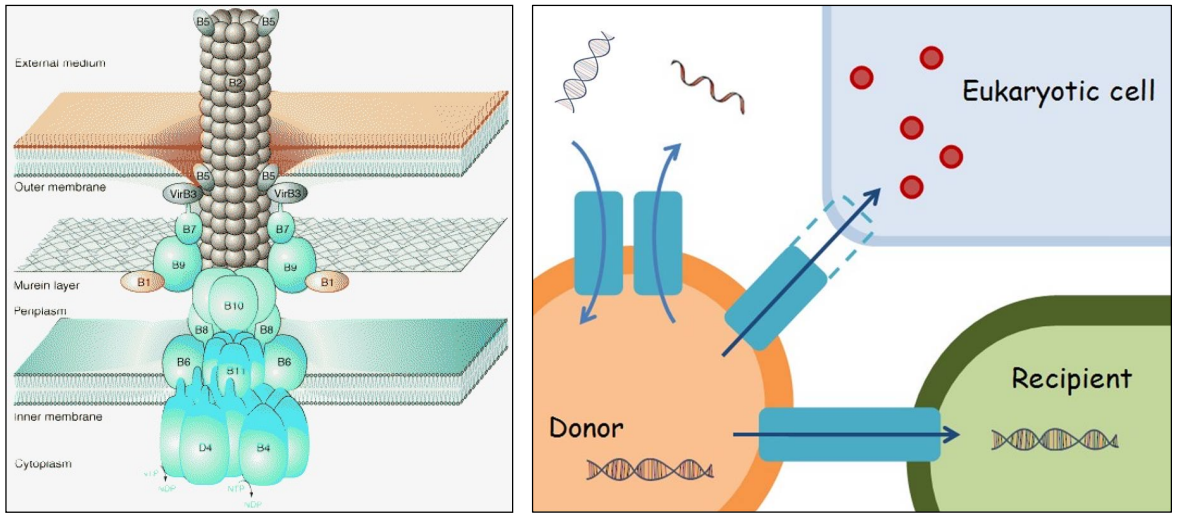
Genetic transformation of plant cells is extremely difficult!



Source: https://courses.lumenlearning.com/wm-biology2/chapter/plant-cells/

Chloroplasts evolved from endosymbiotic cyanobacteria. Mitochondria evolved from endosymbiotic alpha-proteobacteria.

Bacteria use type IV secretion systems (T4SS) to secrete DNA



Source: Backert S, Meyer TF (2006) Type IV secretion systems and their effectors in bacterial pathogenesis. *Curr Opin Microbiol* 9(2): 207-217 PMID: 16529981.

Source: https://bioinfo-mml.sjtu.edu.cn/SecReT4/index.php

Figure 2(B). Model of the assembly process of the prototypical *Agrobacterium tumefaciens* VirB/VirD4 type IV secretion system "injectisome". The type IV secretion system is a multicomponent protein complex spanning the inner and outer membranes of *A. tumefaciens* and other Gram-negative bacteria. Current knowledge on type IV secretion system functions and the cellular localization of type IV secretion system components is depicted in a simplified scheme. The coupling protein VirD4 and structural components (VirB1-VirB11) are typically required for secretion and are presented according to their proposed functions. This transporter enables secretion of substrates from the bacterial cytoplasm directly into the cytoplasm of infected host cells or into the extracellular milieu. The assembly and structure of type IV secretion systems and the secretion route of substrates has been analyzed for numerous pathogens.

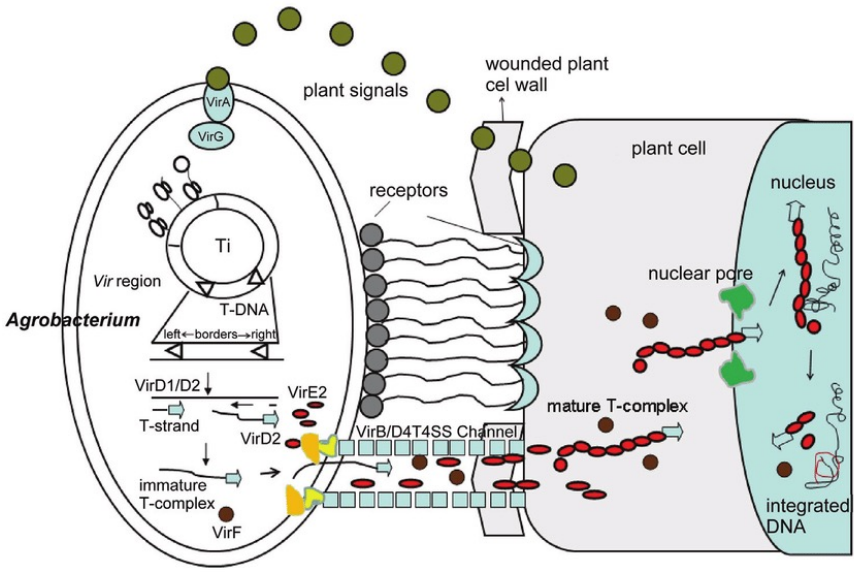
Source: Lybarger SR, Sandkvist M (2004) Microbiology: a hitchhiker's guide to type IV secretion. *Science* 304(5674): 1122-1123 PMID: 15155939.

Source: http://microbewiki.kenyon.edu/index.php/Agrobacterium_tumefaciens

Photo of a crown gall tumor on the stem of a tree, caused by *Agrobacterium tumefaciens*. The bacterial type IV secretion system is localized predominantly at one pole of bacterial cells. In *A. tumefaciens*, this machinery mediates the translocation of bacterial "transforming DNA" (T-DNA) across both the inner membrane and outer membrane of the bacterial cell and injects the T-DNA into the target plant cell, where it becomes integrated into the host cell genome. Virulence factors encoded by the T-DNA induce tumor-like growths (crown gall disease) in host plants and force production of highly unusual amino acids (called opines) that only the bacteria can eat.

Source: https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/agrobacterium

Agrobacterium tumefaciens transfers "T-DNA" to the plant nucleus



SOURCE: Zechner EL, Lang S, Schildbach JF (2012) Assembly and mechanisms of bacterial type IV secretion machines. *Philos Trans R Soc Lond B Biol Sci* 367(1592): 1073-1087 PMID: 22411979.

Figure 2. Stages of *Agrobacterium tumefaciens* "transforming DNA" (T-DNA) delivery to plant cells.

(1) The VirA/VirG system induces Ti plasmid *vir* gene expression in response to host-produced signal molecules at wound sites.

(2) VirD2 relaxase and relaxosome components excise T-DNA at *oriT*-like border sequences.

(3) Recruitment of the relaxase T-DNA intermediate to the type IV secretion system (T4SS) involves virulence binding proteins (Vbp) and spatial determinant VirC1.

(4) T4CP VirD4 (yellow) recognizes protein and nucleoprotein secretion substrates and initiates transfer. Pilin VirB2 contacts plant receptor proteins (BTI).

(5-7) Delivery of bacterial effectors to plant cytosol is followed by **(5)** VirE2 T-complex maturation and nuclear import, **(6)** integration of T-DNA into the plant chromosome, and **(7)** bacterial transgene expression in the host cell nucleus.

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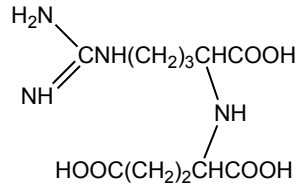
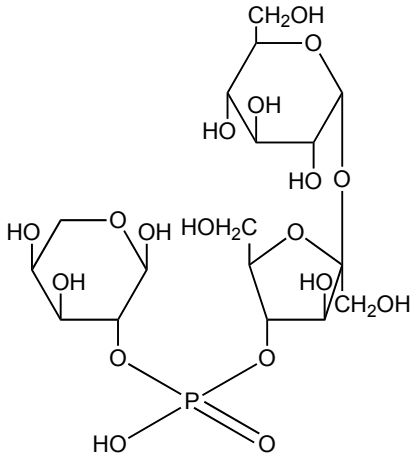
Agrobacterium tumefaciens induces production of nutrients ("opines") by the transformed plant cells

Two examples of "opines":



Nopaline



Agrocinapine

SOURCE: http://microbewiki.kenyon.edu/index.php/Agrobacterium_tumefaciens

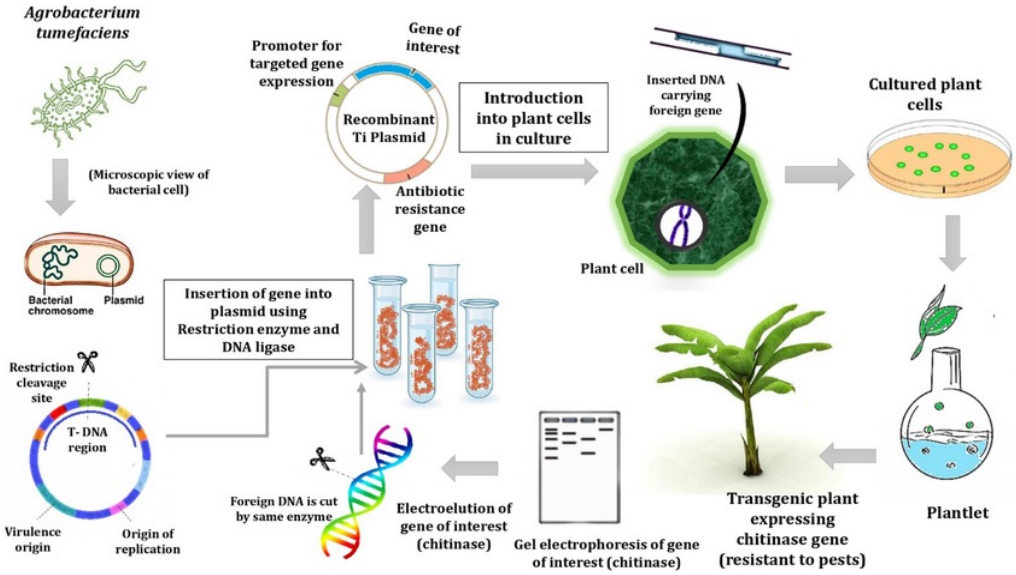
Genes encoded on *Agrobacterium tumefaciens* virulence plasmids and "transforming DNA" (T-DNA).

(1) Hormones. In order to cause crown gall formation the T-DNA encodes genes for the production of **auxin** (**indole-3-acetic acid**) via the indole-3-acetamide (IAM) pathway. Plants do not use the IAM biosynthetic pathway to produce auxin; consequently, the plant has no molecular means of regulating it and auxin will be produced constitutively. Genes for the production of **cytokinins** are also expressed. This stimulates plant cell proliferation and Crown Gall formation.

(2) Opines. The T-DNA contains genes encoding enzymes that cause the plant to create **opines**, specialized amino acids that the bacteria can metabolize as a food source. Opines are a class of chemicals that serve as a source of carbon and energy for *A. tumefaciens*, but not for most other organisms. The specific type of opine produced byplants infected with *A. tumefaciens* strain C58 plants is **nopaline**. Two nopaline type Ti plasmids, pTi-SAKURA and pTiC58, have been fully sequenced.

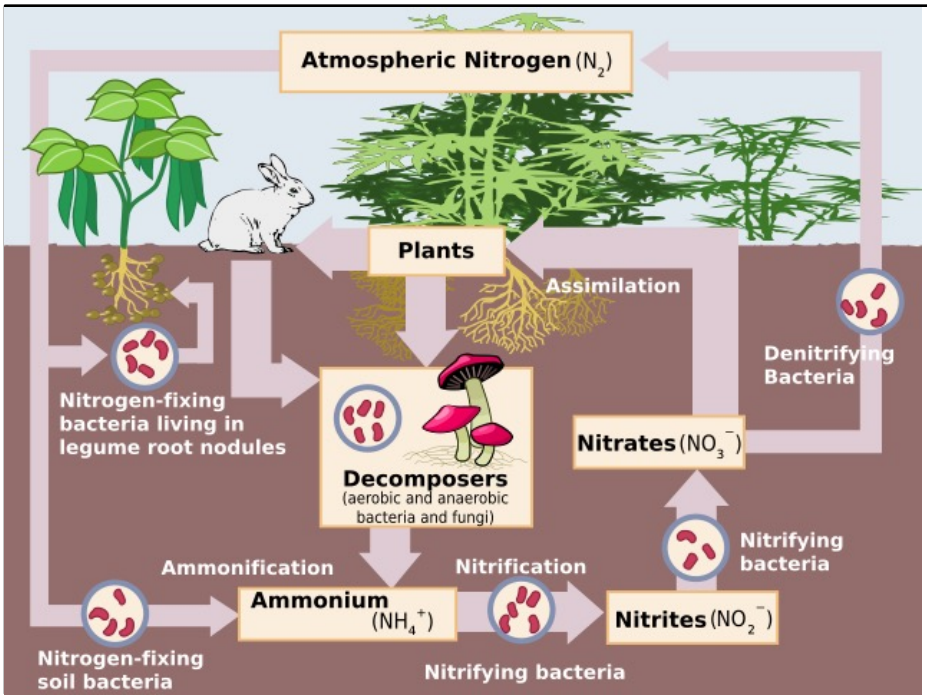
(3) Plasmids. *A. tumefaciens* strain C58, the first fully sequenced pathogenic strain of *A. tumefaciens*, was originally isolated from a cherry tree crown gall. The genome of *A. tumefaciens* C58 consists of a circular chromosome, two plasmids, and a linear chromosome. The presence of a covalently bonded circular chromosome is common to bacteria, with few exceptions. However, the presence of both a single circular chromosome and single linear chromosome is unique to a group in the genus *Agrobacterium*. The two plasmids are **pTiC58**, which is responsible for the processes involved in virulence (crown gall disease), and **pAtC58**, which is involved in the metabolism of opines and conjugation with other bacteria in the absence of the pTiC58 plasmid. The pTi plasmid is essential for crown gall disease; if it is removed the tumor growth that is the means of classifying this species of *Agrobacterium* does not occur.

Construction of genetically modified plants with *A. tumefaciens*



Source: Akram F, Jabbar Z, Aqeel A, Haq IU, Tariq S, Malik K (2022) A Contemporary Appraisal on Impending Industrial and Agricultural Applications of Thermophilic-Recombinant Chitinolytic Enzymes from Microbial Sources. *Mol Biotechnol* 64(10): 1055-1075 PMID: 35397055. Review.

Source: Rahmna SU, Khan MO, Ullah R, Ahmad F, Raza G (2023) *Agrobacterium*-mediated transformation for the development of transgenic crops; present and future prospects. *Mol Biotechnol* doi: 10.1007/s12033-023-00826-8 PMID: 37573566.

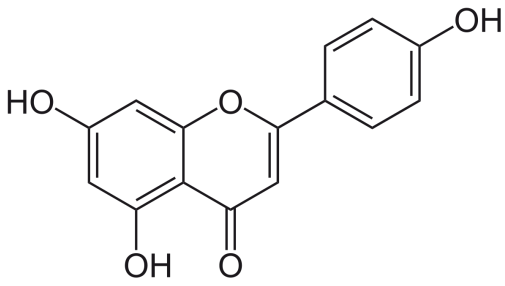


The global nitrogen cycle

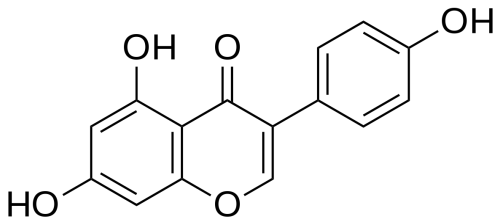
Source: https://byjus.com/biology/nitrogen-cycle/

42

Reminder: Flavonoids stimulate rhizobial symbiont responses…



Luteolin (5,7,3',4'-Tetrahydroxyflavone)
induces *nod* gene expression in rhizobia
(specifically, *Rhizobium leguminosarum*)

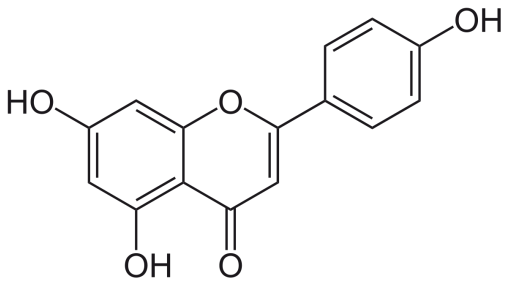


Genistein (5,7,4'-Trihydroxyisoflavone)
inhibits *nod* gene expression in rhizobia
(specifically, *Rhizobium leguminosarum*)

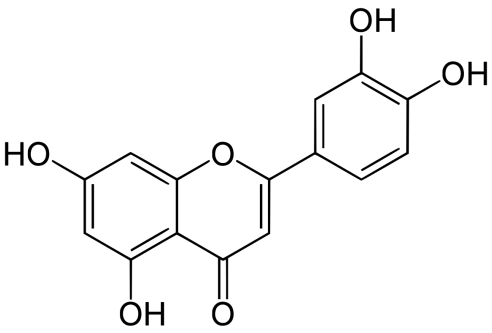
Source: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 23: Microbial Symbioses with Microbes, Plants, and Animals (pp. 732-764), published by Pearson Education Inc., San Francisco © 2019. Figure 23.14.

Plant flavonoids and nodulation. Structures of flavonoid molecules that are **(A)** an inducer of *nod* gene expression and **(B)** an inhibitor of *nod* gene expression in *Rhizobium leguminosarum* biovar *viciae,* the species that nodulates peas. Note the similarities in the structures of the two molecules. The common name of the structure shown in **(A)** is *luteolin*, and it is a flavone derivative. The structure in **(B)** is called *genistein*, and it is an isoflavone derivative.

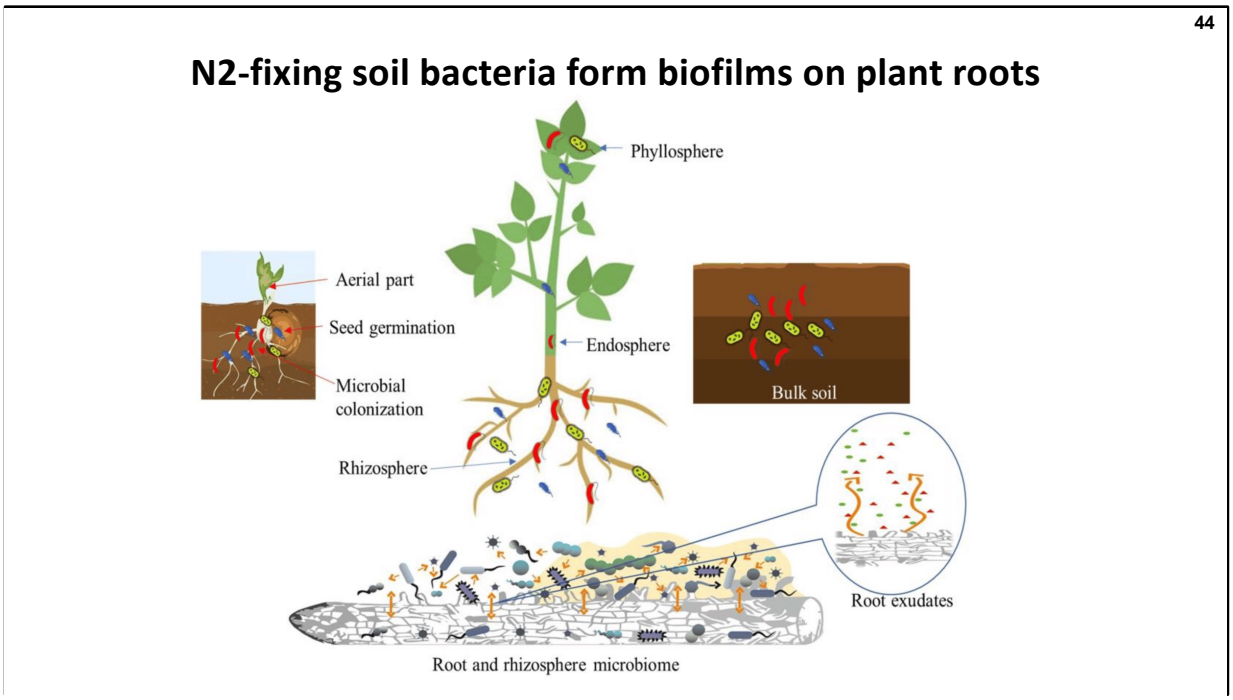
…and flavonoids stimulate biofilm formation by N_2-fixing soil bacteria



Luteolin (5,7,3',4'-Tetrahydroxyflavone)



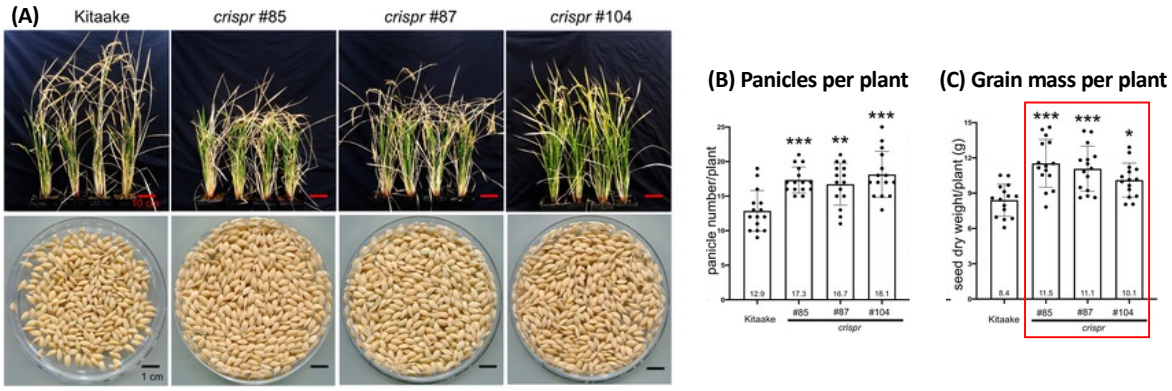
Apigenin (4',5,7-Trihydroxyflavone)



Source: Ajijah N, Fiodor A, Pandey AK , Rana A, Pranaw K (2023) Plant growth-promoting bacteria (PGPB) with biofilm-forming ability: a multifaceted agent for sustainable agriculture. *Diversity* 15(1): 112 doi: 10.3390/d15010112. R

Figure 1. Plant–microbe interaction: regions involved in the development of plant microbiome.

Genetic engineering of rice plants to secrete flavonoids, which enhances biofilm formation by N_2-fixing bacteria on plant roots



Source: Yan D, Tajima H, Cline LC, Fong RY, Ottaviani JI, Shapiro HY, Blumwald E (2022) Genetic modification of flavone biosynthesis in rice enhances biofilm formation of soil diazotrophic bacteria and biological nitrogen fixation. *Plant Biotechnol J* 20(11): 2135-2148 PMID: 35869808.