Effect of oxygen on nitrogenase activity

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Presence of oxygen & Nitrogen fixation

- Diazotrophs are scattered across bacterial taxonomic groups (mostly in the Bacteria but also a couple of Archaea).
- Even within a species that can fix nitrogen there may be strains that do not fix nitrogen.
- Fixation is shut off when other sources of nitrogen are available, and, for many species, when oxygen is at high partial pressure.
- Nitrogenase enzyme synthesis is regulated at transcriptional level by oxygen and other sources of nitrogen.
- Nif genes are the genes encode for nitrogenase enzyme.
- Regulation of nif genes transcription is done by the nitrogen sensitive NifA protein.
- If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein NifL is activated.
- NifL inhibits NifA activity resulting in the inhibition of nitrogenase formation.
- The Fe protein is irreversibly damaged by oxygen, while the MoFe protein is relatively insensitive.

Bacteria have different ways of dealing with the debilitating effects of oxygen on nitrogenases, listed below:

Free-living diazotrophs

- **Anaerobes**—these are obligate anaerobes that cannot tolerate oxygen even if they are not fixing nitrogen.
- They live in habitats low in oxygen, such as soils and decaying vegetable matter eg. *Clostridium*.
- Sulphate-reducing bacteria are important in ocean sediments (e.g. *Desulfovibrio*), and some Archean methanogens fix nitrogen in muds and animal intestines.
- **Facultative anaerobes**—these species can grow either with or without oxygen, but they only fix nitrogen anaerobically.
- Often, they respire oxygen as rapidly as it is supplied, keeping the amount of free oxygen low.
- Examples include *Klebsiella pneumoniae*, *Bacillus polymyxa, Bacillus macerans*, and *Escherichia intermedia.*
- **Aerobes**—these species require oxygen to grow, yet their nitrogenase is still debilitated if exposed to oxygen.
- *Azotobacter vinelandii* is the most studied of these organisms.
- It uses very high respiration rates, and protective compounds (secondary Fe-protein or schithna protein-binds nitrogenase enzyme at high O_2 levels, then remove when O_2 level is low) to prevent oxygen damage.
- Many other species also reduce the oxygen levels in this way, but with lower respiration rates and lower oxygen tolerance.

Free-living diazotrophs

- **Oxygenic photosynthetic bacteria** generate oxygen as a by-product of photosynthesis, yet some are able to fix nitrogen as well.
- These are colonial bacteria that have specialized cells (heterocysts) that lack the oxygen generating steps of photosynthesis.
- Examples are *Anabaena cylindrica* and *Nostoc commune*.
- Other cyanobacteria lack heterocysts and can fix nitrogen only in low light and oxygen levels (e.g. *Plectonema*).
- **Anoxygenic photosynthetic bacteria** do not generate oxygen during photosynthesis, having only a single photosystem which cannot split water.
- Nitrogenase is expressed under nitrogen limitation.
- Normally, the expression is regulated via negative feedback from the produced ammonium ion but in the absence of N_2, the product is not formed, and the by-product H_2 continues unabated [Biohydrogen].
- Example species: *Rhodobacter sphaeroides, Rhodopseudomonas palustris, Rhodobacter capsulatus.*
- **Microaerophilic bacteria** *Azospirillum* uses high respiration rates to prevent oxygen damage.

Symbiotic diazotrophs

- **Rhizobia --** these are the species that associate with legumes, plants of the Fabaceae family.
- Oxygen is bound to leghemoglobin in the root nodules that house the bacterial symbionts, and supplied at a rate that will not harm the nitrogenase.
- **Frankia --** much less is known about these 'actinorhizal' nitrogen fixers.
- The bacteria also infect the roots leading to the formation of nodules.
- Actinorhizal nodules consist of several lobes, each lobe has a similar structure as a lateral root.
- Frankia is able to colonize the cortical tissue of nodules where it fixes nitrogen.
- Actinorhizal plants and Frankias also produce hemoglobins, but their role is less well established than for rhizobia.
- Although at first it appeared that they inhabit sets of unrelated plants (alders, Australian pines, California lilac, bog myrtle, bitterbrush, Dryas), revisions to the phylogeny of angiosperms show a close relatedness of these species and the legumes.
- These footnotes suggest the ontogeny of these replicates rather than the phylogeny. In other words, an ancient gene (from before the angiosperms and gymnosperms diverged) that is unused in most species was reawakened and reused in these species.

Symbiotic diazotrophs

- **Cyanobacteria --** there are also symbiotic cyanobacteria.
- Some associate with fungi as lichens, with liverworts, with a fern, and with a cycad.
- These do not form nodules (indeed most of the plants do not have roots).
- Heterocysts exclude the oxygen, as discussed above.
- The fern association is important agriculturally: the water fern *Azolla* harbouring *Anabaena* is an important green manure for rice culture.
- **Association with animals—**although diazotrophs have been found in many animal guts, there is usually sufficient ammonia present to suppress nitrogen fixation.
- Termites on a low nitrogen diet allow for some fixation, but the contribution to the termite's nitrogen supply is negligible.
- Shipworms may be the only species that derive significant benefit from their gut symbionts.

Questions

- Explain effect of oxygen on dinitrogenase enzyme complex activity.
- How different diazotrophs protect their dinitrogenase enzyme complex from oxygen?