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Nitrite, an Electron Donor for Anoxygenic Photosynthesis

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Although compounds of the sulfur cycle, and more recently the iron cycle, are well studied electron donors for anoxygenic photosynthesis, no analogous oxidations in the nitrogen cycle are known. We report a previously unknown process in which anoxygenic phototrophic bacteria use nitrite as an electron donor for photosynthesis, providing a microbial mechanism for the stoichiometric oxidation of nitrite to nitrate in the absence of oxygen. To examine nitrite as a possible electron donor for anoxygenic phototrophs, we established enrichment cultures derived from local sewage sludge and several freshwater sediments in anoxic, bicarbonate buffered mineral medium (*1*). Low amounts of nitrite (1 to 2 mM) were fed repeatedly to avoid toxicity, and the cultures were incubated continuously in the light.

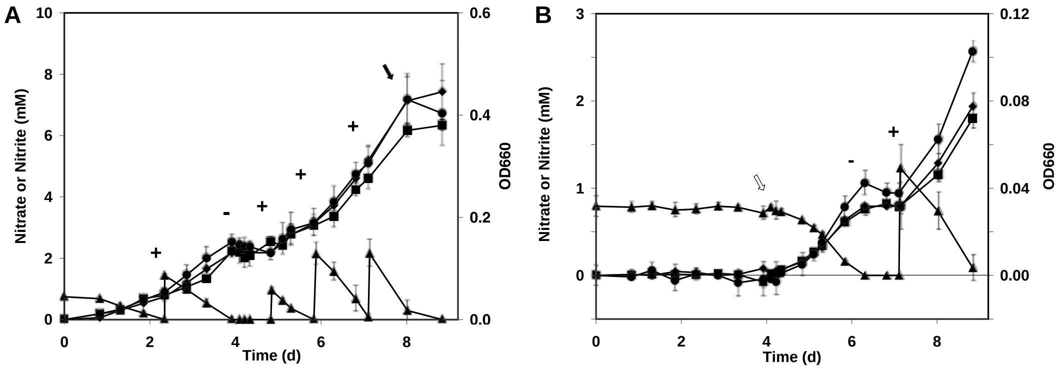
After incubating in the light for several weeks, enrichment cultures from 10 out of 14 sampling sites oxidized nitrite to nitrate and developed pink coloration, as typical of anoxygenic phototrophs. Absorption spectra of intact cells revealed maxima at 799 nm and 854 nm, which are characteristic of bacteriochlorophyll a (*2*). No chlorophyll a or oxygen was observed in nitrite oxidizing cultures, suggesting that nitrate did not form because of a combination of oxygenic photosynthesis and aerobic nitrification. No growth or nitrite oxidation occurred in cultures incubated in the dark or in uninoculated bottles, thereby ruling out the possibilities that nitrate was produced by anaerobic ammonia oxidation (anammox) or abiotic, photochemical processes.

Light dark shift experiments performed over several days with enrichment cultures transferred five times showed that growth and nitrate production depended on both light and nitrite (Fig. 1). The rate of nitrite consumption increased on multiple feedings and approached 2 mM per day after 1 week in the light. As expected for a photoautotrophic process, nitrite consumed, nitrate produced, and biomass formed were all tightly correlated; nitrate was formed from nitrite near stoichiometrically.

We isolated the numerically dominant coccus (2 to 3 μm in diameter) from the most active enrichment culture derived from Konstanz sewage sludge by dilution to extinction in liquid medium (Fig. 1C) (*1*). Analysis of the 16*S* ribosomal RNA gene sequence revealed that the strain, designated KS, is most closely related to *Thiocapsa roseopersicina* (98% identical). *Thiocapsa* species are widely distributed purple sulfur bacteria of the order Chromatiales and are metabolic generalists capable of photoautotrophic growth on a variety of common inorganic electron donors, in addition to aerobic chemolithoautotrophic growth (*3*).

Although phototrophs are known to directly influence the nitrogen cycle through reductive processes such as nitrogen fixation, assimilation, and respiration (*4*), this is the only example of a photosynthetically driven oxidation in the nitrogen cycle. In principle, this photosynthetic process could compete for nitrite in the environment with other key nitrogen cycle processes such as denitrification, aerobic nitrification, or anammox.

In 1970, Olson proposed in detail how the water oxidizing activity of oxygenic photosynthesis may have evolved from anoxygenic photosynthesis through a series of inorganic nitrogen electron donors with increasing midpoint potentials (*5*). The nitrite nitrate couple, with a standard redox potential of +0.43 V, could theoretically donate electrons to the quinone type reaction center in purple sulfur bacteria, where the bacteriochlorophyll primary donor has a midpoint potential as high as +0.49 V (*6*). This work demonstrates nitrite as the highest potential electron donor for anoxygenic photosynthesis known so far and provides a modern example of an electron donor once implicated in the evolution of oxygenic photosynthesis.



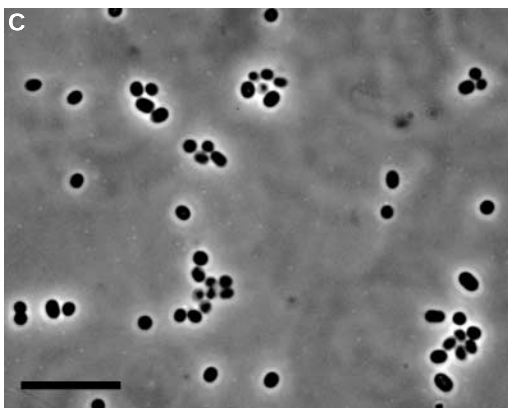


Fig. 1. Time courses for nitrite consumed (▲), nitrate produced (■), cumulative nitrite consumed (◆), and growth as the change in optical density (ΔOD_{660}) (●) for triplicate enrichment cultures ($N = 3$). Data are mean ± SD. (**A**) Initially incubated in the light. (**B**) Initially incubated in the dark. The plus signs indicate nitrite feedings, and arrows denote a switch from the initial light condition. The minus signs indicate when the cultures were starved of nitrite to assess nitrite dependence of growth. (**C**) Phase-contrast micrograph of strain KS. The scale bar represents 10 μm.

References and Notes

1. Materials and methods are available on *Science* Online.
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Supporting Online Material
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Materials and Methods
References

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