Dechlorination of Lindane by the Cyanobacterium *Anabaena* sp. Strain PCC7120 Depends on the Function of the *nir* Operon

TANYA KURITZ,* LAURA V. BOCANERA,† AND NILDA S. RIVERA‡

*Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830*

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Nitrate is essential for lindane dechlorination by the cyanobacteria *Anabaena* sp. strain PCC7120 and *Nostoc ellipsosporum*, as it is for dechlorination of other organic compounds by heterotrophic microorganisms. Based on analyses of mutants and effects of environmental factors, we conclude that lindane dechlorination by *Anabaena* sp. requires a functional *nir* operon that encodes the enzymes for nitrate utilization.

Cyanobacteria are photoautotrophic microorganisms common to a variety of environments including polluted ones. Earlier, we reported that two filamentous nitrogen-fixing cyanobacteria, *Anabaena* sp. strain PCC7120 and *Nostoc ellipsosporum* transformed lindane (14) first to γ-pentachlorocyclohexene and then to a mixture of chlorobenzenes (Fig. 1). This process was cometabolic and depended on the presence of nitrate (14).

Nitrate-dependent dehalogenation of organic compounds by different heterotrophic bacteria has been described in the past (1, 9, 21), but no mechanism for this process or link to genetic systems has been proposed. For some microorganisms dehalogenation was coupled with denitrification (9). In both cyanobacteria and anaerobic denitrifying microorganisms, nitrate uptake and reduction are initial processes of nitrate utilization, and the genes for these processes are organized in similar operons (4, 17, 19, 24, 26). At the level of nitrite reduction, metabolic pathways diverge and lead to the assimilatory chain of nitrate (14).

Wild-type cyanobacteria screened by us degraded lindane. Earlier, we reported that two filamentous nitrogen-fixing cyanobacteria, *Anabaena* sp. strain PCC7120, *Nostoc ellipsosporum*, and the genes for these processes are organized in similar operons (6, 8, 15, 16, 19), these results also suggest a role for the products of the *nir* operon in this process. Since lindane was associated with the cells in all mutants, it is unlikely that the products of the operon are involved in lindane uptake, though we know of nothing in the literature that suggests lindane uptake mechanisms. The presence of lindane did not affect the growth rate of the wild type or mutant cultures.

Effect of darkness or ammonium on lindane degradation. Wild-type cultures supplemented with 15 ng of lindane/liter dechlorinated the lindane in the presence of 5 mM nitrate only when illuminated; addition of 1 mM (NH₄)₂SO₄ to nitrate-supplemented wild-type cultures greatly inhibited dechlorination (Fig. 3). Since ammonium and darkness are environmental inhibitors of the function(s) encoded by the *nir* operon (6, 8, 15, 16, 19), these results also suggest a role for the products of the operon in lindane dechlorination.

Only the production of pentachlorocyclohexene is nitrate dependent. When supplied with the *linA* gene, the product of which dechlorinates lindane to γ-pentachlorocyclohexene (11), *Anabaena* dechlorinated lindane to trichlorobenzenes in the...
absence of nitrate (14). We found no sequence homology to linA upon Southern hybridization of Anabaena DNA with an excess of this gene. Cyanobacteria and Pseudomonas paucimobilis may employ heterologous systems for this transformation.

**Nitrate uptake and nitrite reductase activities do not correlate with lindane degradation.** Nitrate reductase activity was measured as described by Herrero et al. (10) with modifications that included use of cell suspensions with chlorophyll content of ca. 10 to 15 μg ml⁻¹ and incubation at 28°C. Nitrate reductase activity was found in the wild-type cultures (21.3 ± 9.9 pmol of NO₃⁻ · μg of chlorophyll⁻¹ · min⁻¹) and in nrtC (13.0 ± 3.8 pmol of NO₃⁻ · μg of chlorophyll⁻¹ · min⁻¹) and nrtD (11.4 ± 2.3 pmol of NO₃⁻ · μg of chlorophyll⁻¹ · min⁻¹) mutant cultures. Nitrate reductase activity was present in the nirA mutant cultures, but its values were inconsistent through an independent series of experiments, possibly due to differences in the physiological state of the cultures that were sick on nitrate. Only the narB mutant had no nitrate reductase activity. An absence of the polar effect of the nirA, nrtC, and nrtD mutations (all of which are transpositional) on nitrate reductase activity may be due to either the presence of the weak promoter at the IS₅₀₀R of the transposon (Cai and Wolk [2]) or the existence of a nitrate-regulated promoter upstream to the narB gene, or both.

Nitrate uptake experiments were carried out as described by Flores et al. (6). The specific rate of nitrate uptake was 35.7 ± 12.0 nmol of NO₃⁻ · g of chlorophyll⁻¹ · min⁻¹ for the wild type and 30.6 ± 13.5 nmol of NO₃⁻ · g of chlorophyll⁻¹ · min⁻¹ for the nirA mutant. The nrtC, nrtD, and narB mutant cultures did not evince nitrate uptake. Nitrate uptake inhibition by the mutation in the narB gene may be explained if nitrate is a transcriptional regulator of the nir operon, as was suggested by Kikuchi et al. (13). Cyanobacterial cultures supplemented with ammonium did not take up nitrate and had no nitrate reductase activity, which results agree with the results presented earlier by Flores et al. (5) and Omata et al. (18).

The amount of nitrite accumulated in nitrate-supplemented medium under CO₂-limited conditions as reported by Suzuki et al. (25) served as a measure of the difference between the activities of nitrate and nitrite reductases. Concentrations of nitrite were determined as described in Snell and Snell (22) after the transfer of the cultures into fresh medium containing 100 μM nitrate. The values for specific accumulation of nitrite (Fig. 4) were much higher for the TLN10 mutant (202.0 ± 34.9 pmol of NO₂⁻ · mol of chlorophyll⁻¹ · min⁻¹), which took up nitrate and reduced nitrate but was deficient in nitrate reductase, than for the wild-type cultures (12.7 ± 0.5 pmol of NO₂⁻ · μg of chlorophyll⁻¹ · min⁻¹). TLN12 did not accumu-
Anabaena sp. PCC7120
Anabaena sp. P30
Calothrix sp. ATCC29112
Fischeraella muscicola UT1829
Fischeraella sp. CALU926
Nostoc ellipsosporum
Nostoc muscorum UT387
Nostoc palleoides UT1627
Nostoc sp. GSV39
Nostoc sp. GSV40
Nostoc sp. GSV236
Phormidium uncinatum
Plectonema boryanum
Plectonema sp.
Synechococcus PCC7942

tions show that cyanobacteria represent a largely unstudied different efficiencies (Fig. 5). In the past, the search for bio-
fortuitous system for lindane degradation. One can speculate essential, although not sufficient, enzyme in what may be a
dinated regulation of the genes in the lindane transformation. Our results illustrate the lack of coor-
ded pathways does not allow us to reach conclusions as to
which suggests that either the product of this gene is less

cultivation (1.3 ± 0.6 pmol of NO2-·μg of chlorophyll·min-1), which suggests that either the product of this gene is less

tane probably due to its inability to take up nitrate. TLN21 accumulated nitrite at a lower rate than the wild-type

late nitrite probably due to its inability to take up nitrate. DR796 did not accumulate nitrite. We saw no direct correlation of lindane degradation with

Lindane degradation by other cyanobacteria. The 15 strains of cyanobacteria attributed to three taxonomic groups by Rippka et al. (20) were able to degrade lindane although with different efficiencies (Fig. 5). In the past, the search for biologival agents that degrade lindane and other organic xenobi-

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REFERENCES
1. Aftring, R. P., and B. F. Taylor. 1981. Aerobic and anaerobic catabolism of phthalic acid by a nitrate-respiring bacterium. Arch. Microbiol. 130:101–104.
2. Cai, Y., and C. P. Wolk. 1997. Nitrogen deprivation of Anabaena sp. strain PCC7120 elicits rapid activation of a gene cluster that is essential for uptake and utilization of nitrate. J. Bacteriol. 179:258–266.
3. Cannons, A. L. C., Pendleton, and L. P. Solomonson. 1995. Characterization of functional domains of nitrate reductase. FASEB J. 9:Al358.
4. Cole, J. 1995. Nitrate reductase to ammonia by cyanobacteria. Frontiers in redoxology, or a strategy for survival during oxygen starvation. FEMS Microbiol Lett. 136:1–11.
5. Flores, E., M. G. Guerrero, and M. Losada. 1980. Short-term ammonia inhibition of nitrate utilization by Anacystis nidulans and other cyanobacteria. Arch. Microbiol. 128:137–144.
6. Flores, E., M. G. Guerrero, and M. Losada. 1983. Photosynthetic nature of nitrite uptake and reduction in the cyanobacterium Anacystis nidulans. Biochim. Biophys. Acta 722:408–416.
7. Frias, J. E., E. Flores, and A. Herrero. 1997. Nitrate assimilation gene cluster from the heterocyst-forming cyanobacterium Anabaena sp. strain PCC 7120. J. Bacteriol. 179:477–486.
8. Guerrero, M. G., and C. Lara. 1987. Assimilation of inorganic nitrogen, p. 163–186. In P. Fay and C. Van Baalen (ed.), The cyanobacterium. Elsevier, Amsterdam, The Netherlands.
9. Hӓggblom, M. M., M. D. Rivera, and L. Y. Young. 1993. Influence of alternative electron acceptors on the anaerobic biodegradability of chlorinated phenols and benzoic acids. Appl. Environ. Microbiol. 59:1162–1167.
10. Hernández, E. Flores, and M. G. Guerrero. 1981. Regulation of nitrate reductase levels in the cyanobacteria Anacystis nidulans, Anabaena sp. strain 7119, and Nostoc sp. strain 6719. J. Bacteriol. 145:175–180.
11. Imai, R., Y. Nagata, M. Fukuda, M. Takagi, and K. Yano. 1991. Molecular cloning of a Pseudomonas paucimobilis gene encoding a 17-kilodalton polypeptide that eliminates HCl molecules from γ-hexachlorocyclohexane. J. Bacteriol. 173:6811–6819.
12. Kalhoff, M., W. Zimmer, and H. Bothe. 1994. Genetic evidence for the occurrence of assimilatory nitrate reductase in arbuscular mycorrhizal and other fungi. Mycorrhiza 2:23–28.
13. Kikuchi, H., M. Aichi, I. Suzuki, and T. Omata. 1996. Positive regulation by nitrate of the nitrate assimilation operon in the cyanobacterium Synechococcus sp. PCC7942 and Plectonema boryanum. J. Bacteriol. 178:5822–5825.
14. Kuritz, T., and C. P. Wolk. 1995. Use of filamentous cyanobacteria for biodegradation of organic pollutants. Appl. Environ. Microbiol. 61:234–238.
15. Manzano, A., P. Caudau, C. Gomez-Moreno, A. M. Relimpio, and M. Losada. 1976. Ferredoxin-dependent photosynthetic reduction of nitrite and nitrite by particles of Anacystis nidulans. Mol. Cell. Biochem. 10:161–169.
16. Omata, T., X. Andriesse, and A. Hirano. 1993. Identification and characterization of a gene cluster involved in nitrate transport in the cyanobacterium Synechococcus sp. PCC7942. Mol. Gen. Genet. 236:193–202.
17. Omata, T. 1995. Structure, function and regulation of the nitrate transport system of the cyanobacterium Synechococcus sp. PCC7942. Plant Cell Physiol. 36:207–213.
18. Omata, T., M. Kobayashi, R. Rodriguez, M. G. Guerrero, and C. Lara. 1995. Molecular studies on the regulation of the nitrate/nitrite transporter of Synechococcus sp. PCC7942, p. 487–490. In P. Mathis (ed.), Photosynthesis: from light to biosphere. Kluwer Academic Publishers, Dordrecht, The Nether-
lands.
19. Reyes, F., M. D. Roldan, W. Klipp, F. Castillo, and C. Moreno-Vivian. 1996. Isolation of periplasmic nitrate reductase genes from Rhodobacter sphaeroides DSM 158: structural and functional differences among prokaryotic nitrate reductases. Mol. Microbiol. 19:1307–1318.
20. Rippka, R., J. Deruelles, J. E. Waterbury, M. Herdman, and R. Y. Stanier. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111:1–61.
21. Schellen, U., K. Braun, and H.-J. Knackmuss. 1985. Anaerobic degradation of 2-fluorobenzene by benzoate-degrading, denitrifying bacteria. J. Bacte-
riol. 161:321–325.
22. Snell, F. D., and C. T. Snell. 1949. Colorimetric methods of analysis, 3rd Edition, Vol. II. D. Van Nostrand Co., Inc., New York, N.Y.
23. Suzuki, I., T. Sugiyama, and T. Omata. 1993. Primary structure and transcrip-
tional regulation of the gene for nitrate reductase from the cyanobac-
terium Synechococcus PCC7942. Plant Cell Physiol. 34:1311–1320.
24. Suzuki, I., H. Kikuchi, S. Nakashishi, Y. Fujita, T. Sugiyama, and T. Omata. 1995. A novel nitrate reductase gene from the cyanobacterium Plectonema boryanum. J. Bacteriol. 177:6137–6145.
25. Suzuki, I., T. Sugiyama, and T. Omata. 1995. Regulation of nitrate reductase activity under CO2 limitation in the cyanobacterium Synechococcus sp. PCC7942. Plant Physiol. 107:791–796.
26. Suzuki, I., N. Horie, T. Sugiyama, and T. Omata. 1995. Identification and characterization of two nitrogen-regulated genes of the cyanobacterium Syn-
ecochoccus sp. strain PCC7942 required for maximum efficiency of nitrogen assimilation. J. Bacteriol. 177:293–296.
27. Vaucheret, H., and M. Coboche. 1995. Induction of nitrate reductase host gene expression has a negative effect on the expression of transgenes driven by the nitrate reductase promoter. Plant Sci. 107:95–104.
28. Ye, R., A. McQuil, and J. M. Tiedje. 1994. Denitrification: production and consumption of nitric oxide. Appl. Environ. Microbiol. 60:1053–1058.