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Identification of a Perchlorate Reduction Genomic Island with Novel Regulatory and Metabolic Genes▽

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A comparative analysis of the genomes of four dissimilatory (per)chlorate-reducing bacteria has revealed a genomic island associated with perchlorate reduction. In addition to the characterized metabolic genes for perchlorate reductase and chlorite dismutase, the island contains multiple conserved uncharacterized genes possibly involved in electron transport and regulation.

Perchlorate is a stable, soluble, and toxic anion that is deposited in the environment by both natural and anthropogenic means (18). It represents a potential health risk to humans, as it accumulates in food sources (17, 24), where it can interfere with thyroid function upon ingestion (20). As a result of its persistence in the environment, certain bacteria have evolved a mechanism to reduce perchlorate and chlorate [collectively (per)chlorate] that is coupled to energy metabolism (8). The two key enzymes in the perchlorate reduction pathway are perchlorate reductase and chlorite dismutase, which are encoded by the *pcrABCD* and *cld* genes, respectively (3, 4, 8, 27).

The first (per)chlorate-reducing organism whose genome was sequenced was *Dechloromonas aromatica* strain RCB (9). Recently, we have acquired draft genome sequences for several other perchlorate reducers, including *Azospira suillum* strain PS (2), *Magnetospirillum bellicus* strain VDY^T (26), and *Dechloromonas agitata* strain CKB (2, 6) (DOE Joint Genome Institute and Eureka Genomics). Additionally, the only known non-(per)chlorate-reducing member of the *Dechloromonas* genus, *Dechloromonas* sp. strain JJ (9), was also sequenced as part of this study (Eureka Genomics).

The genera *Dechloromonas* and *Azospira* are both located within the *Rhodocyclaceae* family of the *Betaproteobacteria*, from which perchlorate-reducing organisms are frequently isolated (10). In contrast, *M. bellicus* strain VDY^T is a member of the *Alphaproteobacteria* and shares 96% 16S rRNA gene sequence identity with the magnetotactic species *Magnetospirillum magnetotacticum* and *Magnetospirillum gryphiswaldense*, despite its apparent inability to form magnetosomes (26) (Fig. 1).

In our initial analysis of the *A. suillum* genome, we identified 11 genes adjacent to the genes encoding both perchlorate reductase and chlorite dismutase that were also present in *D. aromatica*, which was unsurprising, given their close phylogenetic relationship (Fig. 1). To examine the complete extent of the synteny surrounding *pcr* and *cld* across all four perchlorate reducers, we used a simple protein similarity search (phmmer) (12) by querying the translations of the genes surrounding *pcrA* from *D. aromatica* against a database of 1,109 curated and finished genomes from HAMAP (21), in addition to the draft genomes of *A. suillum*, *M. bellicus*, *D. agitata*, and *Dechloromonas* strain JJ. We found 17 pairs of similar genes with conserved synteny between *A. suillum* and *D. aromatica*, in addition to subsets of these genes in *D. agitata* (10 genes) and *M. bellicus* (12 genes) (Fig. 2). While in most cases the best-scoring hits from the *D. aromatica* queries were from the other (per)chlorate reducers, there were a few aberrant instances. For example, the gene encoding the molybdopterin biosynthesis protein A (*moaA*) homolog in *M. bellicus* had only a moderate degree of similarity to the closely related *moaA* genes of *D. aromatica*, *A. suillum*, and *D. agitata*, despite its similar genomic location (Fig. 2). *Dechloromonas* strain JJ did not have high-scoring hits to most of the 17 genes shared by *A. suillum* and *D. aromatica*, despite its close phylogenetic affiliation, and any homologs were noncontiguous, suggesting that there is no comparable genomic region in that organism.

While it has been proposed that *cld* has a phylogenetic history indicative of lateral gene transfer (5), the nature and extent of such a transfer event were unknown. However, the conservation of 10 or more genes among four (per)chlorate reducers leads us to propose that this 10- to 25-kb region constitutes the core of a horizontally transferred (per)chlorate reduction-associated genomic island (PRI).

We justify the definition of this region as a genomic island on the basis of the criteria presented by Juhas et al., some of which we will summarize here (16). Like the GC content of most genomic islands, that of the PRI core is different from the background GC content of the surrounding chromosome (Table 1). Additionally, the region that we have delineated as the PRI core in *D. aromatica* was identified as a genomic island by the Web-based IslandViewer software, which integrates multiple methods for identifying genomic islands (19). While we have not identified any flanking direct repeats indicative of site-specific integration or homologous recombination, there is a Pro tRNA gene within 3 kb of the PRI in *A. suillum* and 35 kb of the PRI core in *D. aromatica*. This was previously identified as a chromosomal integration site for a phage of *Rhizobium meliloti* (25). In the genome of *M. bellicus*, there is no Pro tRNA in the vicinity of the PRI core, but a Glu tRNA is

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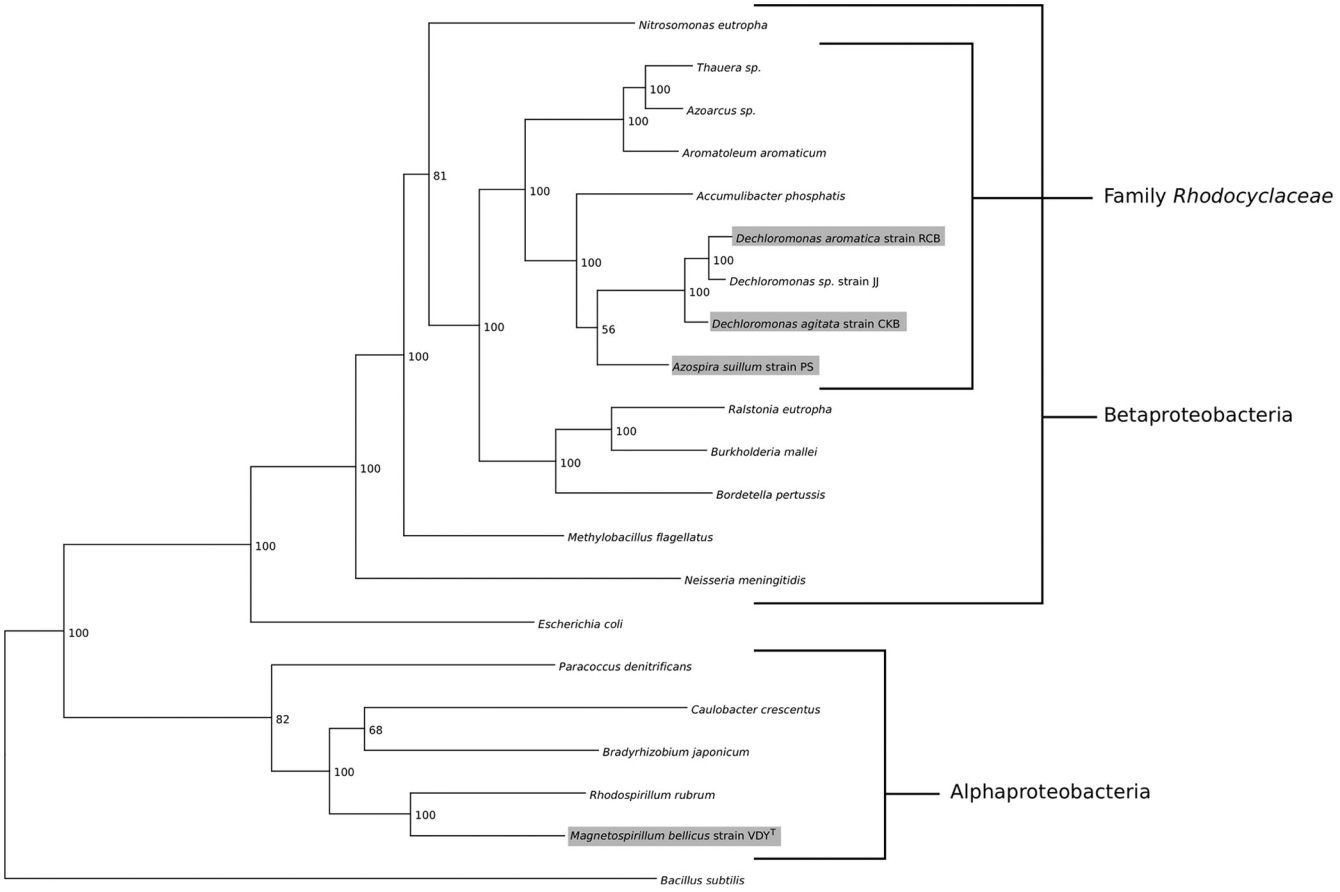

FIG. 1. Phylogenetic distribution of genomes of perchlorate reducers. This tree is the result of multilocus sequence analysis using a concatenated alignment of nine conserved housekeeping proteins (AtpD, DnaA, DnaK, FtsZ, GyrB, RecA, RpoB, SecA, and Srp54). The parameters for this alignment were optimized using ProtTest, and the tree was calculated with bootstrap values by phyml (1, 14). The names of perchlorate reducers are identified by a gray background.

located 7 kb upstream of the *moaA* gene of the PRI. In the ~30-kb region downstream of the histidine kinase of the PRI in *M. bellicus*, there are two genes from the XerCD site-specific recombinase family, in addition to a restriction endonuclease/restriction site modification operon. The core of the PRI of *D. agitata* is located on a relatively small contig (19 kb), but we were still able to identify a transposase 2 kb away from the PRI core which was not homologous to any gene from

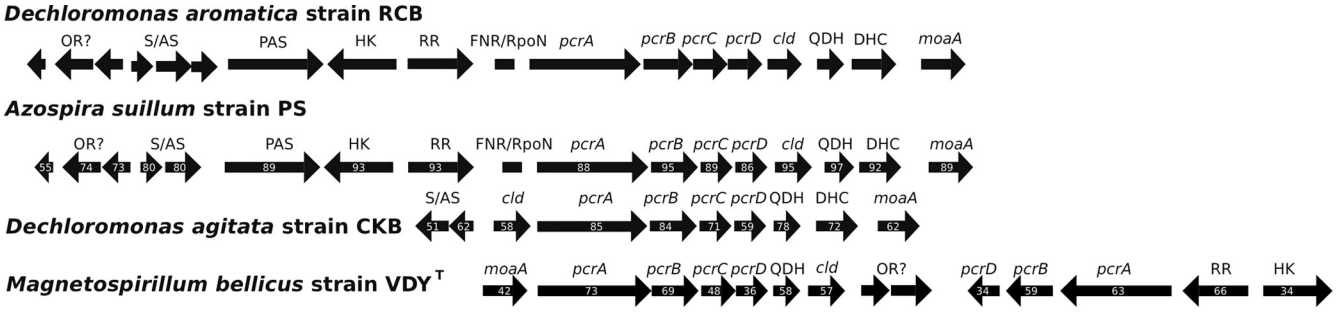

FIG. 2. Structure of the conserved "core" of the PRI. The conserved genes from four examples of the PRI are shown here, using the conserved *pcrABCD* operon structure to generally align the four islands. Each gene is identified by either its given name or an abbreviation/acronym for the predicted function of the gene product. The white numbers represent amino acid similarity to the homologous genes in the *D. aromatica* PRI. *pcrABCD* are components/accessory genes of perchlorate reductase, *cld* is chlorite dismutase, and *moaA* is a part of the molybdenum cofactor biosynthesis pathway. QDH denotes a gene predicted to encode a membrane-associated tetraheme *c*-type cytochrome with quinol dehydrogenase activity, while DHC denotes a diheme *c*-type cytochrome. HK, RR, and PAS represent the histidine kinase, response regulator, and PAS domain sensor of a putative two-component system, while S/AS is a predicted sigma factor/anti-sigma factor system. OR? indicates genes annotated as various types of oxidoreductase components, but the substrate and function of their gene products within the context of perchlorate reduction are unknown. FNR/RpoN represents a conserved promoter for *pcrA* that contains consensus binding sites for FNR and RpoN.

TABLE 1. Characteristics of the PRI from the four sequenced perchlorate reducers

Parameter	*Dechloromonas aromatica* RCB	*Azospira suillum* PS	*Dechloromonas agitata* CKB	*Magnetospirillum bellicus* VDY
Genome status (no. of contigs)	Finished	Draft (22)	Draft (120)	Draft (324)
Taxonomy				
Class	*Betaproteobacteria*	*Betaproteobacteria*	*Betaproteobacteria*	*Alphaproteobacteria*
Order	*Rhodocyclales*	*Rhodocyclales*	*Rhodocyclales*	*Rhodospirillales*
Family	*Rhodocyclaceae*	*Rhodocyclaceae*	*Rhodocyclaceae*	*Rhodospirillaceae*
Genome size (Mb)	4.5	3.8	4.0	8.4
No. of predicted protein-coding genes	4,171	3,454	3,957	8,040
Size of PRI core (kb)	19.1	19.5	10.3	17.6
GC content (%) of:				
Chromosome	59.2	65.3	62.9	61.5
PRI core	50.9	51.2	43.4	63.2
Chlorite dismutase (*cld*)	Yes	Yes	Yes	Yes
Perchlorate reductase (*pcrABCD*)	Yes	Yes	Yes	Yes (*pcrABD* duplicated)
Mo cofactor biosynthesis gene (*moaA*)	Yes	Yes	Yes	Yes
Two-component system	Yes	Yes	No	Yes (missing PAS domain protein)
Putative sigma factor/anti-sigma factor pair	Yes	Yes	Yes	No
Unknown oxidoreductases	Yes	Yes	No	Yes (unrelated to RCB/PS genes)
Unknown *c*-type cytochromes	Yes	Yes	Yes	Yes
FNR/RpoN *pcrA* promoter	Yes	Yes	No	No

the PRIs of the other three genomes. In the ~35-kb region between the PRI core and the Pro tRNA in *D. aromatica*, there are homologs of the F plasmid conjugative transfer genes *traIDLEBVFWUNH* (15). None of these gene families are present in the PRI of *A. suillum*, but there are multiple genes in its flanking regions annotated as "phage associated." This genomic evidence suggests that the PRI may have been historically mobilized by conjugation, transformation, and transduction with subsequent integration into host chromosomes via a variety of mechanisms. The fact that there is such heterogeneity among the non-core PRI genes of the four isolates, including three closely related strains from the family *Rhodocyclaceae*, suggests that mobile elements containing the PRI core genes are extremely diverse and we have sampled only a fraction of that genetic diversity. It is worth noting that we have never observed a spontaneous mutant incapable of (per)chlorate reduction, unlike in other organisms, where genomic island integration and excision can be directly observed (11, 13). As a result, the exact boundaries delineating the PRI from the host chromosome may be ambiguous, particularly if there has been fixation of the PRI via erosion of site-specific excision loci or loss of a flanking direct repeat following a homologous recombination event.

The conservation of PRI genes outside of *pcrABCD* and *cld* suggests that there are additional regulatory and metabolic mechanisms involved in (per)chlorate reduction that are undescribed. The presence of the molybdenum cofactor biosynthesis protein homolog *moaA* is unsurprising, as perchlorate reduction is dependent on molybdenum, presumably due to the use of molybdopterin as a cofactor of PcrA (3, 7). Additionally, there are three distinct families of *c*-type cytochromes in the PRI, none of which have an experimentally validated function but which we predict are involved in electron transport to perchlorate.

Furthermore, there are two separate modules in the PRI that are predicted to regulate the transcription of (per)chlorate metabolism genes, including *pcrABCD* and *cld*. These two modules consist of a putative σ factor/anti-σ factor pair and a two-component system. The two-component system is of particular interest, as the response regulator contains a σ^{54} interaction/activation domain and we have identified a promoter upstream of *pcrA* in *A. suillum* and *D. agitata* with binding sites for the σ^{54} factor RpoN and the regulator of anaerobic metabolism FNR (23).

All perchlorate-reducing organisms utilize oxygen as an electron acceptor, and all but one (*D. agitata*) can sustain growth via denitrification. Both of these electron acceptors are preferred to perchlorate by model perchlorate reducers. Because σ^{54}-interacting response regulators generally act as transcriptional activators, we propose that the two-component system is active in the absence of nitrate and FNR is activated anaerobically. When both of these systems are active, transcription of *pcrABCD* would be induced. The absence of this regulatory system in *D. agitata* supports such a hypothesis, as this organism is unable to denitrify and would not need to repress the transcription of *pcrABCD* when nitrate is available.

This is the first identification of conserved gene families linked with the perchlorate reductase and chlorite dismutase genes in (per)chlorate-reducing organisms. The architecture of the PRI and surrounding chromosomal regions suggests that

these genes have been cotransferred and are completely absent in investigated organisms closely related to (per)chlorate reducers (e.g., *Dechloromonas* strain JJ and *M. magnetotacticum*). These findings imply that the PRI represents a modular metabolism in that it packages necessary regulatory elements with metabolic genes in order to facilitate the integration of the horizontally transferred PRI into the host metabolism. To the best of our knowledge, this represents only the second example of a genomic island that encodes a respiratory process in addition to the recently identified genomic island involved in organohalide reduction (22). However, the PRI is the first respiratory genomic island known that crosses phylogenetic boundaries (class, order, and family), as the organohalide genomic island has been observed only in the genus *Dehalococcoides*.

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