Purinyl-cobamide is a native prosthetic group of reductive dehalogenases

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Cobamides such as vitamin B12 are structurally conserved, cobalt-containing tetrapyrrole biomolecules that have essential biochemical functions in all domains of life. In organohalide respiration, a vital biological process for the global cycling of natural and anthropogenic organohalogen, cobamides are the requisite prosthetic groups for carbon–halogen bond-cleaving reductive dehalogenases. This study reports the biosynthesis of a new cobamide with unsubstituted purine as the lower base and assigns unsubstituted purine a biological function by demonstrating that Cooc-purinyl-cobamide (purinyl-Cba) is the native prosthetic group in catalytically active tetrachloroethene reductive dehalogenases of Desulfitobacterium hafniense. Cobamides featuring different lower bases are not functionally equivalent, and purinyl-Cba elicits different physiological responses in corrinoid-auxotropic, organohalide-respiring bacteria. Given that cobamide-dependent enzymes catalyze key steps in essential metabolic pathways, the discovery of a novel cobamide equivalent and the realization that lower bases can effectively modulate enzyme activities generate opportunities to manipulate functionalities of microbiomes.

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PCE-dehalogenating *Dsf* pure cultures grow in defined medium without exogenous corrinoids, indicating that these *Dsf* strains are capable of de novo corrinoid biosynthesis\(^{19}\). Genome sequence analysis corroborates that *Dsf* strains possess the complete set of genes required for cobamide biosynthesis and lower base activation and attachment, but the identity of the lower base of native *Dsf* cobamide(s) remains elusive\(^{18}\).

Here we report the discovery and characterization of purinyl-Cba (1) as the native cobamide produced in axenic PCE-dechlorinating *Dsf* cultures and demonstrate its distinct functionality in different corrinoid-auxotrophic organohalide-respiring bacteria. Direct detection of purinyl-Cba was achieved in catalytically active PCE RDase following electrophoretic separation, illustrating an innovative approach for rapid characterization of corrinoid prosthetic groups. The results obtained expand the diversity of naturally occurring cobamide structures and assign a biological function to unsubstituted purine.

**RESULTS**

**Discovery and structure of a novel cobamide**

HPLC analysis of the cyanated form (for example, a cyano group as the upper β ligand) of corrinoid extracts from PCE-grown *Dsf* pure cultures (i.e., *Dsf* strains Y51, JH1, Viet1 and PCE1) revealed the presence of three candidate corrinoids with retention times of 11.79 min (1), 15.74 min (2) and 15.95 min (3) (Fig. 2a and Supplementary Results, Supplementary Fig. 1a). Integrated peak areas at 361 nm of the total fractions eluting between 10 and 18 min (i.e., the range of retention times for seven Cba standards, 4–10; Fig. 2a) showed that the predominant corrinoid produced in the *Dsf* cultures was associated with the 11.79 min fraction. This retention time was different from that of cobamide (Cbi), which eluted at 8.77 min as monocyano-Cbi and 15.63 min as dicyano-Cbi\(^{16}\); Supplementary Fig. 1b) and any of the cobamide standards, indicating that the *Dsf* strains produced a distinct corrinoid. The UV-visible (Vis) spectrum of the predominant *D. hafniense* strain Y51 corrinoid (1) substantially overlapped with that of vitamin B\(_12\) (10), including the 361-nm absorption maximum, but was distinct from the Cbi spectrum with a 355 nm absorption maximum (Fig. 2b). Two minor fractions that eluted after the predominant *Dsf* corrinoid showed absorption spectra and retention times (15.74 and 15.95 min) similar to those of Cbi, suggesting that these minor fractions may represent Cbi precursors (Supplementary Fig. 2).

Mass spectra of the *D. hafniense* strain Y51 corrinoid and of vitamin B\(_12\) standard obtained using LC–MS displayed strong masses of 1,351.52 and 1,377.56, respectively (Supplementary Fig. 3a). The base peaks with m/z values of 1,351.52 and 1,377.56 represented the [M+Na]\(^{+}\) ion form of the strain Y51 corrinoid and vitamin B\(_12\), respectively. The mass spectra of the predominant corrinoid from *Dsf* strains JH1, Viet1 and PCE1 matched that of *D. hafniense* strain Y51, showing base peaks with m/z values of 1,329.54 and 1,351.52 corresponding to their [M+H]\(^{+}\) and [M+Na]\(^{+}\) ion forms, respectively (Supplementary Fig. 3c–e). As the molecular mass of the α-ribazole-5′-phosphate (α-RP) moiety in natural cobamides ranges from 306.2 (with phenol as the lower base) to 425.31 Da (with 2-methylsulfonyladenine as the lower base), a mass difference of 312.42 Da between the measured molecular mass of the *Dsf* corrinoid and the calculated molecular mass of Cbi (1,016.12 Da in the monocyano form) indicated that the predominant *Dsf* corrinoid was a cobamide carrying an α-RP moiety with a yet-undefined lower base. Comparison to the calculated masses of naturally occurring cobamides in their cyanated form suggested that the organohalide-respiring *Dsf* strains produce a unique cobamide that is distinct from any currently known natural cobamide.

Based on mass information, a possible molecular formula for the *Dsf* cobamide was deduced as C\(_{24}\)H\(_{31}\)CoN\(_7\)O\(_6\)P.

### Figure 1 | Cobamide general structure and the 16 known lower bases found in naturally occurring cobamides

Variations in cobamide structures are found in the CoG upper ligand, the side chain at position C176, and the CoX lower base. 5-OhBza, 5-hydroxybenzimidazole; 5-OhMeBza, 5-methoxybenzimidazole; 5-OhMe-6-MeBza, 5-methoxy-6-methylbenzimidazole; DMB, 5,6-dimethylbenzimidazole; Bza, benzimidazole; Bz, benzaldehyde; MeBza, 1-methylbenzimidazole; MeAd, 2-methyladenine; MeSMeAde, 2-methylmercaptoadenine; 2-SMeMeAde, 2-methylsulfonyladenine; 2-SO\(_2\)MeAd, 2-methylsulfonyl adenine.

[Diagram showing cobamide general structure and the 16 known lower bases found in naturally occurring cobamides.]
of the $^{15}$N-labeled cobamide shifted from 1,329.54 to 1,344.48 and 1,351.52 to 1,366.48, respectively, corresponding to a 14.94 Da mass increment. The $^{13}$C and/or $^{15}$N isotope labeling spectra differed in the aromatic region. The lower base aromatic protons in the Dsf cobamide lacked a correlation with other protons in the molecule, suggesting that the aromatic moiety does not have any adjacent protons. Hydrogen atoms A, B, and C in Figure 2f directly correlate with the three hydrogen atoms of purine. In comparison, the vitamin B$_2$ COSY spectrum demonstrated direct correlations between the benzyl protons (7.3 and 6.5 p.p.m.) and the adjacent methyl group (2.2 p.p.m.) of the DMB moiety. These experiments support the assignment of unsubstituted purine as the lower base of native Dsf cobamide. Purine-containing compounds can exist as four regioisomers through covalent bonding at each of the N heteroatoms, but the limited amount of purinyl-Cba extractable from Dsf cultures did not allow stereoisomer confirmation by $^{13}$C and/or $^{15}$N NMR spectroscopic analyses. Prior research has

Figure 2 | Spectrophotometric and structural features of Desulfobulbusi native corrinoids. (a) HPLC chromatograms of the native corrinoids produced by D. halodisc strain YS1 eluted at 11.79 min (1), 15.74 min (2), and 15.94 min (3). The predominant corrinoid peak at 11.79 min accounted for 86% of the total corrinoids produced by strain YS1 based on 361 nm peak area integration. Cobamide standards (5 mg/L each) were norpsuedo vitamin B$_2$ (A), 11.23 min; B2-cba (B), 15.34 min; 5-0MEBza-Cba (C), 13.88 min; p-cresol-Cba (D), 17.23 min and 17.64 min; 5-OHBza-Cba (E), 13.13 min; vitamin B$_2$ (F), 15.02 min. mAU, milli-absorbance units. (b) UV-Vis spectra (250–600 nm) of cobamide (cb), vitamin B$_2$, and the 11.79-min native Dsf corrinoid. (c) HPLC chromatogram and (d) UV-Vis spectrum of the non-native cobamide produced in strain YS1 cultures with 25 μM DMB amendment. Dotted lines in b and d indicate the maximum absorbance wavelengths ($\lambda_{max}$) of a cobamide or a cobamide, respectively. (e) Mass spectra of $^{15}$N-labeled and unlabeled native Dsf corrinoid. Mass shifts in [M+H]+ and [M+Na]+ values (as shown by red arrows) confirmed that the Dsf cobamide contains four N atoms in the lower base structure. (f) The superimposed $^1$H NMR spectra of the Dsf corrinoid (black line) and the vitamin B$_2$, standard (blue line). The letters A, B and C in red correlate the protons on the structure to the corresponding $^1$H NMR signals.

Figure 3 | Identification of purinyl-Cba as the native prosthetic group in Dsf PCE Rdase following non-denaturing, gel-electrophoretic separation of Dsf crude protein extracts using BN-PAGE. (a) TCE-to-DCE dechlorination activity of different BN-PAGE gel slices measured using dehalogenation enzyme assays. (b,c) UHPLC separation and MS analysis of the corrinoid recovered from BN-PAGE gel slice #4 showing the highest dechlorination activity (b) and the purinyl-Cba standard (0.25 mg/L) (c).
shown that the 9H and 7H purine tautomers of purine are favored over 3H and 1H purine because of increased aromaticity (tautomer stability; 9H > 7H > 3H > 1H)\(^9\). Based on these observations and on precedence for purines to form bonds with an imidazole nitrogen\(^7\), the N-glycosidic bond was inferred to be analogous to that observed in other biomolecules. This combined suite of analytical techniques offers a practical approach for lower base characterization and can aid in the discovery of new corrinoids.

**Purinyl-Cba is the prosthetic group of Dsf PceA RDases**

Mature Dsf PceA RDases are in the 30–60 kDa mass range\(^9\), but maximum reductive dechlorination activity is generally associated with bands excised from blue native PAGE (BN–PAGE) gels in the 242–480 kDa region, presumably because of complexation with other proteins\(^9\). Following the non-denaturing separation of *D. hafniense* strain H1 crude extracts (Supplementary Fig. 6), the highest dechlorination activity was found to be associated with gel slice #4, whereas the other five gel slices exhibited no detectable or negligible (<8%) cDCE production from TCE (Fig. 3a). Subsequent in-gel extraction recovered corrinoid from gel slice #4, but not from slices #3 and #5, and ultra-high-performance liquid chromatography–high-resolution mass spectrometry (UHPLC–HRMS) revealed that the corrinoid associated with gel slice #4 and the purinyl-Cba standard (Fig. 3b,c) had matching retention times and m/z values. The proteomic analysis of gel slices #3, #4, and #5 demonstrated that the Dsf PceA RDase (WP_011460641.1) was enriched in gel slice #4 along with carbon monoxide dehydrogenase (CODH) and flavin adenine dinucleotide (FAD)-dependent fumarate reductase, which are both corrinoid-independent enzymes (Supplementary Table 1)\(^9\). CODH may occur in complexes with corrinoid-dependent, Wood–Ljungdahl pathway enzymes (for example, 5-methyltetrahydrofolate-homocysteine methyltransferase), but no such proteins were detected in gel slice #4. Purinyl-Cba was the only corrinoid detected in this gel slice, confirming that the Dsf PceA RDase uses purinyl-Cba as the native prosthetic group.

**Dsf CobT substrate specificity and phylogenetic analysis**

The nicotinate-nucleotide-dimethylbenzimidazole phosphoribosyltransferase (CobT) activates lower bases to their respective α-ribozole-5′-phosphate (α-RP) forms. NaMN, nicotinic acid mononucleotide. (b) Phylogenetic relationship of 40 CobT and homologous proteins from phylogenetically diverse corrinoid-auxotrophic and prototrophic bacteria. Solid black dots indicate CobT enzymes with biochemically determined substrate specificities. CobT enzymes of other members of the Peptococcaceae may share catalytic features with the Dsf CobT and activate purine as a lower base for cobamide biosynthesis (indicated by the dashed line–enclosed pink area). (c) HPLC analysis demonstrating purified CobT activity with NaMN and a lower base as the substrates. The small peak to the left of nicotinic acid in the bottom trace (Dhc CobT assay with purine) has a retention time similar to that of α-RP [purine] shown in the panel above, but spectral analysis revealed distinct absorbance features. (d) The formation of α-RP [DMB] or α-RP [purine] in CobT assays with DMB or purine provided as a lower base substrate. The α-RP concentrations were calculated on the basis of the concentration decreases of the respective lower base substrates. Data are averages of measurements from duplicate assays. The (‘) indicates that no α-RP [purine] production was observed in Dhc CobT assays with purine as the lower base. (e) MS analysis of α-RP [DMB] and α-RP [purine] formed in CobT enzyme assays.
clustered bacterial CobT sequences into distinct clades reflecting the substrate specificities (i.e., lower base type activated) of characterized CobT, and provided clues about the native cobamides produced by the respective host organisms (Fig. 4b and Supplementary Table 2). This analysis further revealed that the Dsf CobT clade was distinct (<52% amino acid identity) from any other CobT implicated in phenol-p-cresol, adenine, guanine–hypoxanthine or Bta-type lower base activation. The most closely related sequences to the Dsf CobT clade (except for D. metallireducens CobT) were found in Dehalobacter (Dhb) and Desulfosporosinus (e.g., Desulfosporosinus youngiae strain DSM 17734, CM001441.1; Desulfosporosinus meridiei strain DSM 13257, CP003629.1; Desulfosporosinus orientis strain DSM 765, CP003108.1) genomes, with 57.8–59.3% and 56.6–61.7% amino acid identity, respectively (Fig. 4b); however, the native corrinoids produced in either Dhb or Desulfosporosinus spp. strains have not yet been identified. The high CobT sequence identities among these members of the Peptococcaceae suggested that purine activation to the α-RP derivative is a shared feature of this family (Fig. 4b).

Cultivation work indicates that Dhc CobT utilizes a variety of benzimidazole-type lower bases with a preference for DMB,[11] In vitro enzyme assays with purified Dsf CobT and Dhc CobT (Supplementary Fig. 7a) with nicotinic acid mononucleotide (NaMN) and purine or DMB as substrates verified that Dsf CobT and Dhc CobT both activated DMB to the α-RP form of DMB (α-RP [DMB]; Fig. 4c), consistent with the observation that exogenous DMB guided Dsf to produce cobalamin as a non-native prosthetic group of the PceA RDase. The Dsf and Dhc CobT enzymes activated DMB (250 µM), and 166.9 ± 2.70 and 159.4 ± 1.13 µM α-RP [DMB], respectively, were produced following a 30-min incubation period (Fig. 4d). In Dsf CobT assays in which purine (250 µM) replaced DMB, the conversion yield to α-RP [purine] exceeded 99% after a 30-min incubation period (Fig. 4c,d). In assays with Dhc CobT, purine was not consumed, α-RP [purine] was not produced, and only small amounts of nicotinate were observed, indicating that Dhc CobT was unable to activate unsubstituted purine (Fig. 4c,d). No α-RP formation occurred in controls lacking NaMN, a lower base, or CobT, corroborating that Dsf CobT catalyzed the activation of unsubstituted purine to α-RP [purine] (Supplementary Fig. 7b).

MS analysis performed on fractions containing CobT enzyme assay products showed strong adducts with m/z ([M+H]+) values of 359.10 and 333.06 matching the calculated molecular mass of α-RP [DMB] and α-RP [purine], respectively (Fig. 4c). These findings demonstrated an unprecedented Dsf CobT specificity for unsubstituted purine, consistent with the discovery of purinyl-Cba as the native prosthetic group of Dsf PceA.

**Effects of purinyl-Cba on dechlorinating activity**

To test whether purinyl-Cba supports corrinoid-dependent reductive dechlorination in corrinoid-auxotrophic organohalide-respiring bacteria, we grew axenic Dehalobacter restrictus strain PER-K23 and Dhc cultures with purinyl-Cba. Strain PER-K23 harbors a PceA RDase with 98.7% amino acid identity to the PceA of D. hafniense strain Y51. Strain PER-K23 cultures that received 36.9 nM of vitamin B_{12} (positive control) or purinyl-Cba completely dechlorinated the initial amount of 64.4 ± 2.3 µmol of PCE to cDCE within 6 d at statistically indifferent rates of 427 ± 25 and 471 ± 39 µmol Cl\textsuperscript{−} released per day, respectively (Fig. 5a). Much lower dechlorination rates of 23.7 ± 9.3 µmol Cl\textsuperscript{−} released per day, resulting in the formation of small amounts of trichloroethene (TCE; 8.2 ± 2.5 µmol) and cDCE (5.4 ± 2.5 µmol), occurred in control incubations without exogenous corrinoid (Fig. 5a). Strain PER-K23 cell numbers increased about 50-fold in purinyl-Cba- and vitamin B_{12}-amended cultures, whereas growth was negligible in control cultures without corrinoid addition (Supplementary Table 3). Biomass from purinyl-Cba- and vitamin B_{12}-fed Dhc cultures exclusively contained purinyl-Cba and vitamin B_{12}, respectively, indicating that cobamide or lower base modifications did not occur (Fig. 5b). These findings demonstrated that both D. restrictus strain PER-K23 and D. hafniense strain Y51 PceA RDases were fully functional with either purinyl-Cba or cobalamin as the prosthetic group.

Corrinoid-auxotrophic Dhc pure cultures demonstrated a different response to purinyl-Cba. Dhc strains BAV1 and GT express the BvcA and VcrA RDases, respectively, which share less than 20% amino acid identity with the Dsf and Dhb PceA RDases. Complete reductive dechlorination of cDCE to ethene occurred in vitamin B_{12}-amended Dhc strain BAV1 and strain GT cultures at rates of

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**Figure 5 | Effects of purinyl-Cba substitution for vitamin B_{12} on the activity of corrinoid-auxotrophic, organohalide-respiring bacteria.**

(a) Reductive dechlorination activity in D. restrictus cultures supplied with PCE as the electron acceptor and 36.9 nM vitamin B_{12} (positive control), 36.9 nM purinyl-Cba, or no corrinoid addition (negative control).

(b) HPLC chromatograms of the total intracellular corrinoids extracted from vitamin B_{12}- or purinyl-Cba-amended Dhc cultures. Purinyl-Cba was the only cobamide recovered from purinyl-Cba-amended Dhc cultures.

(c) Reductive dehalogenation of cDCE to ethene in Dhc strain BAV1 cultures amended with 36.9 nM vitamin B_{12} (positive control), 36.9 nM purinyl-Cba, or no corrinoid addition (negative control). Unfilled squares, PCE; filled diamonds, TCE; filled triangles, cDCE; inverted unfilled triangles, vinyl chloride (VC); filled circles, ethene. Error bars represent mean values ± s.d. from three independent cultures.
201 ± 11 and 197 ± 10 μM Cl− released per day, respectively (Fig. 5c and Supplementary Fig. 8). Strain BAV1 and strain GT cultures with purinyl-Cba as the sole corrinoid amendment exhibited much slower cDCE dechlorination rates of 17.6 ± 1.2 and 11.7 ± 0.7 μM Cl− released per day, respectively (Fig. 5c and Supplementary Fig. 8). After a 32-d incubation period, the cultures dechlorinated about half of the initially supplied cDCE (83 μmol) but produced no ethene. In control cultures without exogenous corrinoid, strain BAV1 and strain GT dechlorinated no more than 22% and 7.3%, respectively, of the initial cDCE to vinyl chloride (presumably enabled by corrinoid carryover with the inoculum), and formed no ethene (Fig. 5c and Supplementary Fig. 8). Consistent with the observed reductive dechlorination activity, 16S rRNA gene enumeration indicated robust Dhc growth in cultures amended with vitamin B12, but not in cultures that received purinyl-Cba (Supplementary Table 3). In contrast to the PceA RDases, the Dhc RDases BveA and VcrA had a pronounced preference for DMB over unsubstituted purine as the lower base, emphasizing that the lower base can modulate the activity of corrinoid-dependent RDases.

DISCUSSION

The purine structure is the most widely distributed nitrogen-containing heterocycle in nature, and purine derivatives fulfill many essential functions in biological systems22. Obvious examples of purine derivatives include adenine and guanine, which are essential building blocks of nucleic acids, and ubiquitous molecules such as adenosine 5’ triphosphate (ATP), nicotinamide adenine dinucleotide (NAD+ and NADH), and flavin adenine dinucleotide (FAD and FADH2). Remarkably, compounds containing an unsubstituted purine moiety are rare, and a specific biological function has never been assigned to purine itself. To date, nebularine (purine nucleoside) is the only known biological compound that contains unsubstituted purine. Nebularine was first isolated from the fungus Clitocybe nebularis and is also produced by Streptomyces yokosukakensis and Microbispora isolates. Nebularine is a potent antibiotic against various Mycobacterium species, exhibits cytotoxic effects in cell cultures and plants23,24, and is toxic to the schistosomiasis (bilharzia) parasite25; however, its biological functions in the hosts are unclear. In S. yokosukakensis, nebularine biosynthesis involves catalysis of the reductive deamination of adenosine by a single enzyme26. Apparently, at least some members of the domains Bacteria and Eukaryota synthesize unsubstituted purine, indicating that purine is not a xenobiotic, a finding that has potential human health implications. Purinergic membrane receptors, which include the adenosine-responsive P1 purinoceptors, are found in almost all mammalian tissues, wherein they fulfill crucial functions that can affect disease progression26–30. Although the occurrence of unsubstituted purine in mammals is not established, the simple enzymatic conversion of adenine to nebularine has been demonstrated31, and enzymatic hydrolysis of the N-glycosidic bond catalyzed by common nucleoside hydrolases (i.e., purine nucleosides) releases the corresponding free bases32. Thus, the formation of unsubstituted purine (or purine nucleoside) could affect the regulation of cellular functions (purinergic signaling), potentially enabling new disease therapies. Purinyl-Cba synthesis using Dsf CobT will facilitate detailed investigations of its therapeutic potentials.

Corrinoid-dependent enzyme systems fulfill essential metabolic functions for organisms in all branches of life, but only some members of Bacteria and Archaea have the machinery for de novo corrinoid biosynthesis.21,29–40. The discovery of a new member of the corrinoid family of molecules, purinyl-Cba in organohalide-respiring members of the Peptococcaceae, expands the number of naturally occurring, functional lower base structures and assigns a function to unsubstituted purine in biological systems. From this study and from recent reports, a concept emerges that lower base structures are modulators of corrinoid-dependent enzyme function41,42,43. This concept applies to the Dhc RDases, but may possibly expand to many other corrinoid-dependent enzyme systems, emphasizing that enzyme-specific corrinoid cofactor requirements must be understood to predict, and possibly manipulate, catalytic activity (for example, Dhc reductive dechlorination rates and extents)19. The exact role(s) of the lower base in RDase function is not resolved, but recent findings reveal a base-off configuration during catalysis16. If not directly involved in catalysis, the lower base structures may affect holoenzyme maturation, or, in the case of periplasmic enzymes such as respiratory RDases, export through the cytoplasmic membrane. Cobamides fulfill essential metabolic functions for the majority of organisms, including mammals, and the principle of lower base-controlled activity of key corrinoid-dependent enzyme systems may provide new avenues to manipulate environmental (for example, bioremediation) and biotechnological (for example, anaerobic digestion, biogas production) processes and to expand treatment options to affect progression of relevant human diseases.

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METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

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Author contributions

F.E.L., J.Y. and S.R.C. conceptualized the research and designed experiments. J.Y., M.B., B.S., Y. Yang and Y. Yin performed cultivation work, corrinoid extraction and purification, and phylogenetic analyses. A.K.B. and A.T.F. performed LC–MS and structural analyses. F.W., O.M. and A.T.Q. performed BN–PAGE, enzyme assays, and proteomic analysis. N.J. generated colE expression clones. All authors contributed to data analysis and interpretation, and J.Y., S.R.C., E.A.E., and F.E.L. wrote the manuscript.

Competing financial interests

The authors declare no competing financial interests.

Additional Information

Any supplementary information, chemical compound information and source data are available in the online version of the paper. Reprints and permissions information is available at http://www.nature.com/reprints/index.html. Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations. Correspondence and requests for materials should be addressed to J.Y. or F.E.L.
Corrinoid analysis. Intracellular corrinoids were analyzed by HPLC, UV-Vis spectroscopy, and UPLC-HRMS. HPLC analysis was performed with an Eclipse XDB-C18 column (Agilent Technologies, 5 μm pore size, 4.6 mm inner diameter × 250 mm length) operated at a flow rate of 1 mL per min at 30 °C using 0.1% (v/v) formic acid (2888, w/v; Thermo Fisher, Waltham, MA, USA) in water as eluent A and 0.1% (v/v) formic acid in methanol as eluent B. The initial mobile phase composition was 82% eluent A and 18% eluent B, before the fraction of eluent B increased linearly to 25% over a 12-min and further to 75% over an additional 3-min time period, held at 75% B for 5 min before being restored to initial column conditions. UV-Vis spectra between 250 to 600 nm were collected using a diode array detector. The upper ligand, cobalt oxidation state, and lower base coordination influence the corrinoid absorbance spectrum in the UV region. All corrinoid samples and standards in this study carried the cobalt atom in the fully oxidized +3 state and a cyano group as the upper ligand. Cbl lacks the entire tr-ribazole-5'-phosphate (tr-RP) moiety and exhibited an UV-Vis spectrum with an absorption peak at 355 nm, distinct from the 361 nm absorption peak of vitamin B12 (Fig. 2b). The corrinoids extracted from BN–PAGE gels were analyzed using an UltiMate 3000 UHPLC system in tandem with a high-resolution Exactive Plus Orbitrap mass spectrometer (Thermo Fisher) and a UV-Vis detector set to 361 nm as described. Corrinoids were separated on a Hypersil Gold C18 column (Thermo Fisher; 1.9 mm pore size, 2.1 mm inner diameter × 50 mm length) at a flow rate of 0.2 mL min−1 at 30 °C using 2.5 mM ammonium acetate in water as eluent A and 100% methanol as eluent B. The mobile phase was 100% eluent A and 0% eluent B for 0.46 min, followed by linear increases to 15% eluent B after 0.81 min, 50% eluent B after 3.32 min, and 90% eluent B after 5.56 min with a 0.3-min hold before equilibration to initial column conditions. Corrinoid mass spectra were collected in positive ionization mode using a HESI II electrospray ionization source (Thermo Fisher) with a m/z scan ranging from 1,000–1,500 and a resolution of 140,000. The detection limit for this LC–MS method was 2.5 ng of corrinoid.

NMR spectroscopy. Growth of D. hafniense strain Y51 in twelve 2.2-L vessels yielded approximately 22 L of cell culture suspension. Each vessel received neat TCE (400 μL; 2.3 mM aqueous concentration), yeast extract (2 g/L) and Cbl (0.5 μM) to enhance corrinoid production. Cells were harvested after three TCE amendments (a total of 1,200 μL) were completely dechlorinated to dCDE. Total intracellular corrinoids were extracted, and the predominant corrinoid-containing fraction was obtained following HPLC separation and manualcollection from the detector outlet informed by the detector response at 361 nm. Approximately 0.7 mg of purified corrinoid was dissolved in methanol-d4 (250 μL) to conduct 1H and COSY NMR experiments using an INOVA 600 MHz NMR spectroscopy system (Varian). The ‘1H NMR experiments consisted of 1,024 scans, using PRESAT solvent suppression multiple peak selection and a total measuring time of 1 h. The gradient-selected COSY NMR experiments consisted of 256 scans per increment at 96 increments. The delay time between scans was 1.5 s, and the total measuring time was 1 h.

Blue native PAGE (BN–PAGE), enzyme assays and proteomic analysis. D. hafniense strain JH1 cells were harvested from 100 mL cultures and the crude protein extracts were prepared as described. BN–PAGE using pre-cast 4 to 16% gradient Bis–Tris gel (Thermo Fisher) stained with Coomassie Blue native PAGE (BN–PAGE), enzyme assays and proteomic analysis. D. hafniense strain JH1 cells were harvested from 100 mL cultures and the crude protein extracts were prepared as described. BN–PAGE using pre-cast 4 to 16% gradient Bis–Tris gel (Thermo Fisher) stained with Coomassie Blue dye and gel residuals.
Laboratory) as described46. The assays (1 mL) were conducted in 2-mL sealed glass vials containing 100 mM Tris–HCl buffer (pH 7.4) amended with 2 mM titanium citrate, 2 mM methyl viologen, and 2 mM TCE. Each assay mixture was incubated in an anoxic chamber for 24 h and analyzed for dCE formation using gas chromatography69. In-gel digestion and MS analysis were performed at the BioZone Mass Spectrometry Facility (University of Toronto, Toronto, Canada) using X! Tandem for peptide/protein identification (The GPM, http://www.thegpm.org/; version X! Tandem Vengeance (2015.12.21)6). X! Tandem was set up to search for tryptic peptides based on the D. hafniense strain Y51 genome, all characterized RDases, other Dsf proteins in the NCBI database, as well as common contaminants such as human keratins and trypsin (total of 33,950 proteins) using a fragment ion mass tolerance of 0.40 Da and a parent ion tolerance of 2.5 Da. Deamidation of asparagine and glutamine, oxidation of methionine and tryptophan, N-terminal amino acid loss, or cyclization of glutamine or glutamic acid to pyroglutamine or pyroglutamic acid were allowed in X! Tandem as possible peptide modifications. Further validation and refinement was performed using Scaffold software version 4.5.3 (Proteome Software, Inc.) with a reverse decoy database to establish a false discovery rate. Peptide identifications were accepted as valid at a Peptide Prophet probability of greater than 99%62, which were filtered to include those that had at least two unique peptide identifications.

**Primer and probe design.** Primers and TaqMan probe targeting the pceA gene in D. hafniense strain Y51 (locus # DSY2839), D. restrictus strain PER-K25 (locus # DEHRE_12145), and pceA homologs (>95% sequence identity) identified in other Dsf and Dhh strains (GenBank accession numbers CAD28792.1, CDX02974.1, AAO6101.1 and AIA38680.1) were designed using Primer Express software (Applied Biosystems). A consensus region of the Dsf and Dhh pceA gene sequences was selected to meet the quantitative PCR (qPCR) design criteria of (i) an amplicon size between 50–150 bp, (ii) a primer and end products, TCE/C=O and vinyl chloride/ethylene, respectively. Each dechlorination step is associated with the release of one chloride ion and the PCE--dCE--and dCE--ethene dechlorination rates were reported as micromolar Cl− released per day. The list of potential molecular formulas for the lower base of the Dsf cobamide was generated using the web-based platform ChemCalc44. To obtain genomic DNA, cells from 1 mL culture suspension were harvested via vacuum filtration onto a 0.22 μm polycarbonate membrane filter (Merck Millipore Ltd.) and processed using the MO BIO Soil DNA Isolation kit (MO BIO) as described44. Dhc 16S rRNA gene-targeted qPCR was performed following established protocols using primer set Dhc1200F/Dhc1271R and TaqMan probe Dhc1240probe43. The qPCR assay targeting the Dhb pceA gene with the newly designed primers and probe used identical qPCR conditions. Standard curves were generated using a serial dilution of plasmid pMK-RQ (Life Technologies) with a D. hafniense strain Y51 pceA fragment (1,650 bp) insertion. The pceA qPCR assay standard curve had a slope of −3.785, a y-intercept of 40.41, a R2 of 0.999, and a PCR amplification efficiency of 83.8%. The assay spanned a linear range from 4.64 × 10−1 to 4.64 × 105 pceA gene copies per reaction, with 4.6 pceA gene copies per reaction as the detection limit. All qPCR assays used template DNA samples extracted from triplicate cultures, and standard curves were generated from three independently prepared serial dilutions of the respective plasmid DNA standards.

**Statistical analysis.** The PCE-to-dCDE dechlorination rates from three independent Dhh cultures grown with purinyl-Cba or vitamin B12 were statistically compared. The two-sample t-test was carried out using the data analysis tool provided in Microsoft Excel 2016.

**A Life Sciences Reporting Summary** for this paper is available.

**Bioinformatics and phylogenetic analyses.** Rdase sequence alignments and identity comparisons were performed with Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo)45. Reciprocal BLAST analysis was used to query the sequenced Dsf genomes and search for orthologous bza genes implicated in anaerobic DMB biosynthesis47. Twenty CobT amino acid sequences from the genomes of nine Dsf strains, five Dhh strains and six Desulfoalpinis strains, along with 20 CobT and homologous protein (i.e., ArsA and ArsB) sequences from other bacteria (Supplementary Table 2) were retrieved from GenBank, and aligned using the MUSCLE plug-in in Geneious 8.1.7 with 10 iterations. The phylogenetic tree of CobT and homologous proteins was constructed using the PHYLML maximum likelihood tree builder plug-in in Geneious 8.1.7 with 100 bootstraps and the Le and Gascuel substitution model48.

**Heterologous expression and purification of CobT.** The pET-28a(+) vector (EMD Millipore) was used to clone and express cobT carrying an N-terminal hexa-histidine tag. Dsf cobT (locus # DSY2114) and Dhc cobT (locus # DehAV1_0626) were amplified using Phusion Flash High-Fidelity PCR Master Mix (Thermo Fisher) and primer sets NH45-9460 and NH161-9462 (Supplementary Table 4), respectively, using genomic DNA from Dsf strain Y51 and Dhc strain BAV1 axenic cultures. PCR products were cleaned using the UltraClean PCR Clean-Up Kit (MO BIO). The pET-28a(+) vector backbone was digested with EcoRI and NdeI, dephosphorylated with rSAP, and gel extracted to remove remaining supercoiled constructs. The prepared vector and cobT inserts were then co-transformed into BW25113 electrocompetent cells with pre-induced A. Red recombinase from plasmid pKD46 for homologous recombination49. Following sequence verification, the recombinant vectors pET50 (carrying DSY2114) and pET49 (carrying DehAV1_0626) (Supplementary Table 5) were introduced into E. coli strain BL21(DE3) (New England Biolabs) for overexpression and purification.

Enzyme expression and purification were performed as described with modifications43. In brief, E. coli strain BL21(DE3) carrying a cobT expression plasmid was grown in 1 L of Terrific Broth (Thermo Fisher) with 50 μg/mL kanamycin at 37 °C and 180 r.p.m. to an optical density at 600 nm (OD600) of 0.8. Cultures were induced with 0.4 mM isopropyl β-D-1-thiogalactopyranoside (Thermo Fisher) and incubated overnight at 16 °C and 180 r.p.m. Cells were collected by centrifugation at 9,000 × g for 20 min, suspended in HEPES buffer (30 mM HEPES, 300 mM NaCl and 10 mM imidazole, pH 7.5), and sonicated in an ice bath for 45 min (3-s on and 4-s off). The lysate was centrifuged at 38,000 × g for 20 min and the supernatant was passed through a 25-mL glass column with 0.5 mL Ni2+-NTA resin (Qiagen). After washing with HEPES buffer (20 mM imidazole), proteins were eluted with 5–7 M of HEPES buffer containing 250 mM imidazole and 5% (w/v) glycerol. Protein concentrations were estimated using the Bradford assay47 and protein purity was examined by SDS–PAGE and Coomassie Blue staining. The protein stock solutions (Dsf Y51 CobT, 15 mg/mL; Dhc BAV1 CobT, 10 mg/mL) were frozen in liquid nitrogen and stored at −80 °C. The N-terminal hexa-histidine tag was retained for all experiments.

**In vitro CobT activity.** CobT assays were performed as described45. Briefly, each reaction (300 μL) contained 30 μg CobT (corresponding to a final enzyme concentration of ~2 μM), 2 mM Na2MoO4, 10 mM MgCl2, and 0.25 mM of either pure or DMB in 50 mM pH 7.5 Tris–HCl buffer. The assay vials were incubated at 30 °C for 30 min before the reactions were terminated by the addition of 15 μL formic acid (288% w/v) and then transferred to a boiling water bath for 1 min. Following neutralizing the pH with 5 M NaOH, precipitated protein was removed by centrifugation at 13,000 × g for 5 min. Lower bases and their respective α-RPs were analyzed by injecting 10 μL samples into an Agilent Technologies 1200 series HPLC system equipped with a diode array detector set to 262 nm. Separation was performed with an Eclipse XDB-C18 column (5 μm pore size; 4.6 mm inner diameter × 250 mm length) at a flow
rate of 1 mL per min at 30 °C using 0.1% (v/v) formic acid in water as eluent A and 0.1% (v/v) formic acid in methanol as eluent B. The initial mobile phase and time parameters for the gradient elution were as follows: t = 0, 15% eluent B, 85% eluent A; t = 5 min, 25% eluent B, 75% eluent A; t = 11 min, 85% eluent B, 15% eluent A, t = 15 min, end of run. To obtain material for subsequent MS analysis, semi-preparative HPLC was performed using the method outlined above with minor modification as follows. The injection volume was increased to 100 μL, and a 1-mL fraction containing α-RP [DMB] or α-RP [purine] was manually collected from the detector outlet once the target peak was detected. Three such injections were performed and the α-RP fractions from each were combined, and then concentrated tenfold from 3 mL to 300 μL using a Savant ISS110 vacuum dryer (Thermo Fisher). Aliquots (10 μL) of the concentrated α-RP solutions were introduced into an Exactive Plus Orbitrap MS via direct injection. The analytes were ionized using electrospray ionization operated in positive mode and detected via high-resolution MS with a mass range of 200 to 400 m/z.

Data availability. All data generated or analyzed in this study are included in this published article and its supplementary information files.

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## Experimental design

1. **Sample size**
   - Describe how sample size was determined.
   - Triplicate measurements with independent experiment verification. Information was provided in the Method section.

2. **Data exclusions**
   - Describe any data exclusions.
   - No data was excluded.

3. **Replication**
   - Describe whether the experimental findings were reliably reproduced.
   - The experimental findings were reliably reproduced.

4. **Randomization**
   - Describe how samples/organisms/participants were allocated into experimental groups.
   - Culture samples were randomly allocated into experimental groups.

5. **Blinding**
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - The investigators were blinded to group allocation during data collection and/or analysis.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. **Statistical parameters**
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   | n/a | Confirmed |
   |-----|-----------|
   |     | ✔️        |
   |     | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
   |     | ✔️        |
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   |     | ✔️        |
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   |     | ✔️        |
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   |     | ✔️        |
   |     | Clearly defined error bars |

See the web collection on statistics for biologists for further resources and guidance.
Software

Describe the software used to analyze the data in this study.

The two-sample t-test was carried out using the data analysis tool provided in Microsoft Excel 2016.

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Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

Not applicable

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

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Provide details on animals and/or animal-derived materials used in the study.

No animal studies were conducted.

Describe the covariate-relevant population characteristics of the human research participants.

No human subjects were used in this study.