Supplementary Information

Materials and methods

Bacterial strains

Alicycliphilus denitrificans DSMZ 14773, *Geobacillus kaustophilus* DSM 7263, *Gordonia cholesterolivorans* DSMZ 45229, *Hydrogenophaga pseudoflava* DSM 1034, *Steroidobacter* (*Sdo.*) *denitrificans* DSMZ18526, *Sterolibacterium* (*Stl.*) *denitrificans* DSMZ 13999, and *Thauera terpenica* 58Eu (DSMZ 12139) were purchased from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). *Comamonas testosteroni* ATCC 11996 was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). *Stl. denitrificans* was anaerobically grown on testosterone (Wang *et al*., 2014), whereas *A. denitrificans* was grown with cyclohexanol under denitrifying condition as described in Jin *et al*. (2011). *T. terpenica* was anaerobically grown on acetate (16 mM) and testosterone (1 mM) in the minimal medium DSMZ Medium 1315. *G. kaustophilus* and *H. pseudoflava* were aerobically grown on nutrient agar (DSMZ Medium 1) at 55 ℃ and on trypticase soy broth agar (DSMZ Medium 535) at 30 ℃, respectively. The other aerobic bacteria were aerobically cultured in Luria-Bertani medium.

Denitrifying growth of Sdo. denitrificans with testosterone

Sdo. denitrificans was grown with nitrate and testosterone (4 mM) at 28 ℃ in anoxic fed-batch cultures (2 l) according to published procedures (Wang *et al*., 2013). The amounts of residual testosterone in the anoxic culture were monitored using HPLC (Wang *et al*., 2014).

Molecular biological methods

Genomic DNA was extracted from bacterial cells by using Easy Tissue & Cell Genomic DNA Purification Kit (GeneMark). PCR reaction mixtures (50 μl) contained nuclease-free H_2O, 25 μl of 2 × PCR buffer, 2 mM dNTPs, 1.2 U *Taq* polymerase (BioTherm; NatuTec GmbH), 10 pmol of each primer, and 30 ng of template DNA. The PCR products were checked using standard gel electrophoresis with the SafeView DNA Stain (GeneMark), and the amplicons were purified using GenepHlow Gel/PCR Kit (Geneaid).

Assembly of Sdo. denitrificans genome

The PCR-free paired-end and mate-pair reads of the *Sdo. denitrificans* genome were obtained using a whole-genome shotgun strategy, and the two libraries were sequenced on the IlluminaHiSeq 2000 system (Illumina). Overall, 13 336 548 reads (read length 101-bp) and 7 381 106 reads (read length 101-bp) were respectively obtained in the PCR-free paired-end and mate-pair libraries, providing a 500-fold coverage of the genome. The bacterial genome was *de novo* assembled *in silico* by using ALLPATHS-LG (Gnerre *et al*., 2011), which resulted in 32 contigs (>1000 bp) with an N_{50} length of 3 469 203 bp. The gaps were filled using Sanger sequencing of the PCR products and the bioinformatics tool GapCloser module (Luo *et al*., 2012). The gap filling results were verified in two steps. All trimmed DNA reads were realigned to the complete genome to obtain consensus sequences. The resulting consensus sequences were confirmed using optical mapping. The genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline and was manually revised. Functional profile was predicted according to the COG database (updated: 2014). Metabolic pathways were reconstructed using the BlastKOALA tool (http://www.kegg.jp/blastkoala) of KEGG.

RNA extraction and RNA-Seq sequencing of Sdo. denitrificans mRNA

Total RNA was extracted from *Sdo. denitrificans* grown under different conditions (aerobic growth with testosterone, denitrifying growth with testosterone, and denitrifying growth with heptanoic acid). rRNA was removed from total RNA samples by using the Ribo-Zero rRNA Removal Kit (Epicentre Biotechnologies). The quality of the resulting library was assessed using the Experion RNA Analysis Kit (Bio-Rad), and only RNA samples with an integrity number between 8 and 10 were selected for further analysis. First-strand cDNA was synthesized using the purified RNA fragments as reverse-transcription templates. Second-strand

cDNA was synthesized in a reaction mixture containing Second-Strand Synthesis Buffer (New England Biolabs), dNTPs with dUTP replaced by dTTP, RNaseH, and DNA polymerase I. cDNA was purified using the Qiagen purification kit followed by performing end repair. Subsequently, the DNA samples were treated with the Uracil-Specific Excision Reagent Enzyme (New England Biolabs) to digest the antisense strand DNA, followed by a PCR reaction. The constructed sequencing libraries were sequenced as single-end reads (with a read length of 101-bp) on the IlluminaHiSeq 2000 system (Illumina). The transcriptome libraries were mapped to the coding DNA sequences of *Sdo. denitrificans* using Tophat v2.1.0 (Trapnell *et al*., 2009). The reads per kilobase per million (RPKM) values were calculated using Cufflinks v2.2.1 (Roberts *et al*., 2011; Trapnell *et al*., 2010) and the expression differences between samples were calculated using Cuffdiff v2.2.1 (Trapnell *et al*., 2013) with default settings.

Purification of AtcABC from denitrifying Sdo. denitrificans

For preparing cell extracts, 20 g of frozen *Sdo. denitrificans* cells was suspended in two volumes of 20 mM Tris-HCl buffer (pH 7.8). The cells were broken by passing the cell suspension through a French pressure cell (Thermo Fisher Scientific, USA) twice at 140 MPa. The cell lysate was fractionated by two steps of centrifugation: the first step involved a centrifugation for 20 min at 20 000 × *g* to remove cell debris, unbroken cells, and residual testosterone. The supernatant was then centrifuged at 100 000 × *g* for 1.5 h to separate soluble proteins from membrane-bound proteins. All steps for column chromatography were performed using an FPLC system (Ä KTA start, GE Healthcare Life Sciences) at 4 ℃. The soluble proteins of *Sdo. denitrificans* were applied to a DEAE Sepharose Fast Flow column (70-ml; GE Healthcare) equilibrated with buffer A (20 mM Tris–HCl, pH 7.8). Elution was performed using buffer B (20 mM Tris–HCl and 500 mM NaCl, pH 7.8) with a gradient from 0% to 100% at a flow rate of 3.5 ml min^{-1}. Fractions showing hydratase/dehydrogenase activity were pooled and mixed with an equal volume of Tris–HCl buffer (20 mM, pH 7.8) containing 1 M ammonium sulfate. The resulting protein solution (50 mL) was applied to a Butyl Sepharose Fast Flow column (25-ml; GE Healthcare) equilibrated with buffer C (20 mM Tris–HCl and 500 mM ammonium sulfate, pH 7.8). The bound 1-testosterone hydratase/dehydrogenase was eluted by a linear gradient from 0% to 100% of buffer A at a flow rate of 2 ml min^{-1}. Active fractions were pooled and concentrated through ultrafiltration (10-kDa cutoff membrane; Vivaspin; GE Healthcare). After desalting with a PD-10 column (GE Healthcare), the active protein fraction (2 ml) was applied to a HiPrep 16/60 Sephacryl S-300 HR column (120-ml; GE Healthcare) equilibrated with buffer A containing 150 mM NaCl. Elution was performed at a flow rate of 0.5 ml min^{-1}. The dominant protein peak showing activity was concentrated to 2 ml through ultrafiltration (10-kDa cutoff membrane). Protein content was determined using a Pierce BCA protein assay kit.

Enzyme assays and the characterization of AtcABC

To determine the substrate preference of purified enzyme, assay mixtures (0.9 ml) contained 20 mM Tris-HCl (pH 7.5), 5 μg purified enzyme, 50 μM DCPIP, 125 μM substrates, and 1.25% 2-propanol. Buffers used to determine the pH optimum were 100 mM Na^+-phosphate (pH 5.0, 5.5, and 6.0), 100 mM MOPS-KOH (pH 6.5 and 7.0), 100 mM Tris-HCl (pH 7.5 and 8.0), and 100 mM glycine-NaOH (pH 8.5, 9.0, 9.5 and 10.0). The apparent K_m and V_{max} values of 1-testosterone were determined by varying the substrate concentration (2~250 μM; serial 2-fold dilution) and maintaining fixed concentrations of DCPIP (100 μM) and purified enzyme (5 μg ml^{-1}). The apparent K_m and V_{max} values were obtained by fitting Michaelis-Mention curve to the data.

Molecular weight determination of AtcABC through gel filtration chromatography

The native molecular weight of the purified enzyme was determined using gel filtration on a TSKgel G3000SWxl HPLC column (7.8 × 300 mm; TOSHO Bioscience) at a flow rate of 0.25 ml min^{-1}. The column was equilibrated with 20 mM Tris–HCl (pH 7.0) and 150 mM NaCl. For calibration, the Gel Filtration Markers Kit (Sigma) containing thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), and carbonic anhydrase (29 kDa) was

used.

SDS-PAGE, MS/MS analysis, and protein identification

SDS-PAGE was performed in Bolt 4%–12% Bis-Tris Plus gels (Novex). SeeBlue Plus2 Prestained Protein Standard (3–198 kDa; Novex) was used for estimating the molecular weight of the separated proteins. The gels were stained with SimplyBlu SafeStain (Invitrogen). In-gel digestion and LC-MS/MS analysis of protein spots were performed as described in Lin *et al*. (2011). MASCOT MS/MS data searches were performed using the protein database derived from the genome sequences of *Sdo. denitrificans*.

Measurement of protein content and nitrate concentration

The protein content was determined by performing a bicinchoninic acid protein assay (Pierce BCA protein assay kit; Thermo Fisher Scientific) according to manufacturer's instructions using bovine serum albumin as the standard. The nitrate content in sewage samples was determined using cadmium reduction according to manufacturer's instructions (Nitrate Reagent Kit HI93728-01, Hanna Instruments).

Preparation of water samples

DHSTP water samples were centrifuged at 1000 × *g* at room temperature for 10 min to remove sludge and debris. The supernatants were then vacuum-filtered through a cellulose acetate membrane filter (pore size = 0.45 μm) before solid-phase extraction. Oasis HLB LP Extraction Cartridges (6 ml, Waters) with 0.5 g of sorbent were used to extract androgens from 5 l of water samples. Cartridges were pre-conditioned sequentially with methanol (20 ml) and deionized water (10 ml). Water samples were loaded into the cartridges at a flow rate of approximately 5 ml min^{-1}. The cartridges were washed with deionized water (10 ml) to remove residual formaldehyde and dried under vacuum. Subsequently, androgens were eluted with 12 ml of methanol, followed by 7 ml of methanol-acetone (6:1, v/v). The extracts were collected, dried, and re-dissolved in 500 μl of methanol. The samples were centrifuged at 13 500 × *g* for 5 min and the resulting supernatants were analyzed through LC-MS.

LC-MS analysis and steroid quantification

A Shimadzu LC system (Shimadzu) coupled with an ABI triple quadrupole mass spectrometer was used for steroid identification and quantification as described in Sun *et al*. (2014) with a slight modification. A Kinetex C18 column (100 mm × 4.6 mm, 2.6 μm; Phenomenex) and a binary gradient were employed to perform chromatographic separation. The flow rate was 0.8 ml min^{-1} with the mobile phase A containing 0.1% formic acid in water, whereas the mobile phase B was 100% methanol. The sample injection volume was 10 μl. All analytes were analyzed through positive electrospray ionization in the multiple reaction monitoring (MRM) mode. Declustering potentials, entrance potentials, collision energies, and collision cell exit potentials were optimized.

UPLC-MS/MS identification of androgenic metabolites in sewage

Anoxic sewage samples (1 ml) were extracted with the same volume of ethyl acetate for three times. The extracts were pooled, solvent was evaporated, and residues were re-dissolved in 100 μl of methanol. The ethyl acetate extractable samples were analyzed using UPLC-APCI-MS/MS as described in Wang *et al*. (2013).

lacZ-based yeast androgen bioassay

Anoxic sewage samples (0.5 ml) were extracted with ethyl acetate for three times. After evaporating the solvent, the extracts were re-dissolved in the same volume of dimethyl sulfoxide (DMSO) and androgenic activity in the sewage samples was determined using a *lacZ*-based yeast androgen assay as described in Wang *et al*. (2014).

Illumina MiSeq sequencing of bacterial 16S rRNA amplicons

DNA was extracted from the frozen sewage samples by using the PowerSoil DNA isolation kit (MO BIO Laboratories). The 16S amplicon library was prepared according to the Illumina 16S Metagenomic Sequencing Library Preparation Guide (http://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/

16s-metagenomic-library-prep-guide-15044223-b.pdf) with slight modifications. Genomic sections flanking the V3-V4 region of the bacterial 16rRNA gene were amplified from 36 sewage treatments by using HiFi HotStart ReadyMix (KAPA Biosystems) through PCR (95 ℃ for 3 min; 25 cycles: 95 ℃ for 30 s, 55 ℃ for 30 s, 72 ℃ for 30 s, and 72 ℃ for 5 min). A primer pair flanked by the Illumina Nextera linker sequence (Forward primer: 5′–TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG–3′; and Reverse primer: 5′–GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC–3′) was used. The PCR products were first separated on agarose gel and those with an expected size (approximately 445 bp) were excised from the gel and purified using GenepHlow Gel/PCR kit (Geneaid). Next, Illumina Nextera XT Index (Illumina) sequencing adapters were integrated to the ends of the amplicons through PCR (95 ℃ for 3 min; 8 cycles: 95 ℃ for 30 s, 55 ℃ for 30 s, 72 ℃ for 30 s; and 72 ℃ for 5 min). The final libraries were purified using AMPure XP beads (Beckman Coulter) and quantified using the Qubit dsDNA HS Assay Kit (Life Technologies). The library profiles were randomly analyzed by Agilent High Sensitivity DNA Kit on BioAnalyzer. To ensure the evenness of pooling, all 36 libraries were subjected to qPCR normalization by using KAPA Library Quantification Kits to derive molar concentration, and the final library mixture was verified through qPCR. For sequencing, the library pool was run on Illumina MiSeq V2 sequencer by using MiSeq Reagent Kit V3 for paired-end (2 × 300 bp).

16S rRNA gene-based taxonomic analysis

We analyzed the sequencing data in the Illumina BaseSpace cloud service by using the BaseSpace 16S Metagenomics app (Illumina) (http://support.illumina.com/content/dam/illumina-support/documents/documentation/software_documentation/basespace/16s-metagenomics-user-guide-15055860-a.pdf). The reads were classified against the Illumina-curated version of May 2013 Greengenes taxonomy database by using the Ribosomal Database Project (RDP) naïve Bayesian algorithm (http://rdp.cme.msu.edu/classifier/).

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Table S3 Molybdopterin-containing subunits of bacterial proteins used for constructing the phylogenetic tree in Figure 5. The most similar sequences were extracted from the NCBI database by performing a standard BLASTP search with the molybdopterin-containing subunit (AtcA) of 1-testosterone hydratase/dehydrogenase from *Sdo. denitrificans* DSMZ 18526 as a query. Bacterial proteins with experimentally confirmed functions were obtained from the UniProtKB/Swiss-Prot database by performing a standard BLASTP search with the *Sdo. denitrificans* AtcA sequence as a query.

Designation in the tree	Organism	Group	Protein / Sequence identity (%)	Database	Accession
Sdo. denitrificans AtcA	*Steroidobacter denitrificans* DSMZ 18526	γ-proteobacteria	1-testosterone hydratase/dehydrogenase (100%)	NCBI	CP011971
Stl. denitrificans 1-t hydratase/DH	*Sterolibacterium denitrificans* DSMZ 13999	β-proteobacteria	putative 1-testosterone hydratase/dehydrogenase (59%)	NCBI	LFZK00000000
Azoarcus uncharacterized	*Azoarcus toluclasticus*	β-proteobacteria	uncharacterized protein (58%)	NCBI	WP_040395723
Thauera uncharacterized	*Thauera terpenica* 58Eu	β-proteobacteria	uncharacterized protein (57%)	NCBI	EPZ16113
Alicycliphilus Michael hydratase	*Alicycliphilus denitrificans* DSMZ 14773	β-proteobacteria	Michael hydratase/alcohol dehydrogenase (3-hydroxycyclohexanone dehydrogenase) (38%)	NCBI	ADV16271.1
Thermomicrobium CO DH	*Thermomicrobium roseum* DSM 5159	chloroflexi	carbon monoxide dehydrogenase (36%)	NCBI	ACM04667
Pseudomonas caffeine DH	*Pseudomonas* sp. CBB1	γ-proteobacteria	caffeine dehydrogenase (34.4%)	UniProtKB/ Swiss-Prot	D7REY3
Oligotropha CO DH	*Oligotropha carboxidovorans* DSM 1227	α-proteobacteria	carbon monoxide dehydrogenase (33.1%)	UniProtKB/ Swiss-Prot	P19919
Hydrogenophaga CO DH	*Hydrogenophaga pseudoflava* DSM 1034	β-proteobacteria	carbon monoxide dehydrogenase (32.7%)	UniProtKB/ Swiss-Prot	P19913
Arthrobacter nicotine DH	*Arthrobacter nicotinovorans*	actinobacteria	6-hydroxypseudooxynicotine dehydrogenase (30.2%)	UniProtKB/ Swiss-Prot	Q933N0
Thauera 4-OH-benzoyl-CoA reductase	*Thauera aromatica*	β-proteobacteria	4-hydroxybenzoyl-CoA reductase (29.0%)	UniProtKB/ Swiss-Prot	O33819
Eubacterium nicotine DH	*Eubacterium barkeri*	firmicutes	nicotinate dehydrogenase (28.4%)	UniProtKB/ Swiss-Prot	Q0QLF2
E. coli K12 xanthine DH	*Escherichia coli* K12	γ-proteobacteria	xanthine dehydrogenase (27.9%)	UniProtKB/ Swiss-Prot	Q46799
Bacillus xanthine DH	*Bacillus subtilis* 168	firmicutes	xanthine dehydrogenase (27.8%)	UniProtKB/ Swiss-Prot	O32144
Desulfovibrio CHO oxidase	*Desulfovibrio gigas*	δ-proteobacteria	aldehyde oxidoreductase (25.9%)	UniProtKB/ Swiss-Prot	Q46509
E. coli hypoxanthine oxidase	*Escherichia coli* O157:H7	γ-proteobacteria	hypoxanthine oxidase (24.8%)	UniProtKB/ Swiss-Prot	Q8XD64
E. coli O157:H17 xanthine DH	*Escherichia coli* O157:H7	γ-proteobacteria	xanthine dehydrogenase (23.9%)	UniProtKB/ Swiss-Prot	P77489
Brevundimonas quinoline oxidase	*Brevundimonas diminuta*	α-proteobacteria	isoquinoline 1-oxidoreductase (23.2%)	UniProtKB/ Swiss-Prot	Q51698
Gluconacetobacter CHO DH	*Gluconacetobacter polyoxogenes*	α-proteobacteria	aldehyde dehydrogenase (21.4%)	UniProtKB/ Swiss-Prot	P17201
Pseudomonas nicotine DH	*Pseudomonas putida*	γ-proteobacteria	nicotinate dehydrogenase (21.0%)	UniProtKB/ Swiss-Prot	Q88FX8

Table S4 Physical and chemical characteristics of the DHSTP influent and effluent waters sampled in June 2014. Nitrogen content and basic parameters, including temperature, pH, dissolved oxygen, BOD, and COD, were measured every two weeks. Androgen concentrations shown here are those of water samples collected on June 9, 2014.

	Influent			**Effluent**		
Physical and chemical properties	**Mean**	**Max.**	**Min.**	**Mean**	**Max.**	**Min.**
Temperature (℃)	24.9	25.0	24.8	26.3	26.7	25.9
pH value	7.4	7.5	7.3	6.9	7.0	6.8
Dissolved oxygen (mg l^{-1})	4.0	4.9	3.0	6.8	7.2	6.4
BOD (mg l^{-1})	87	89	84	11	12	10
COD (mg l^{-1})	166	168	164	22	24	21
Nitrogen content						
Ammonia nitrogen (mg l^{-1})	14.4	17.0	11.1	4.4	5.8	1.5
Nitrate nitrogen (mg l^{-1})	0.2	0.4	0.0	2.3	5.0	0.1
Nitrite nitrogen (mg l^{-1})	0.1	0.2	0.0	0.4	0.5	0.3
Organic nitrogen (mg l^{-1})	14.4	16.2	11.7	3.8	4.1	3.5
Androgen content						
Testosterone (ng l^{-1})	9738			31		
Androsta-1,4-diene-3,17-dione (ng l^{-1})	142			234		
Androsta-4-en-3,17-dione (ng l^{-1})	0			0		
Epiandrosterone (ng l^{-1})	0			0		

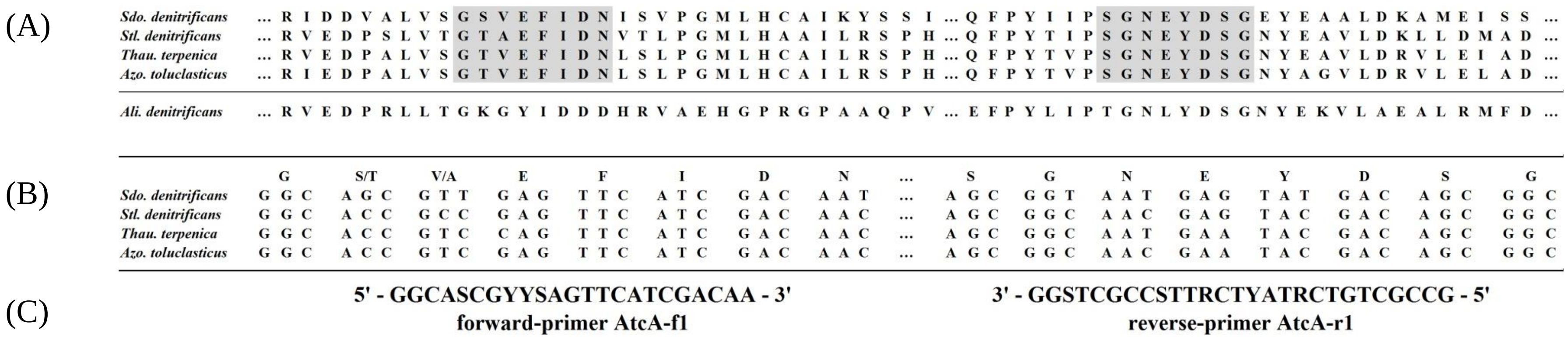
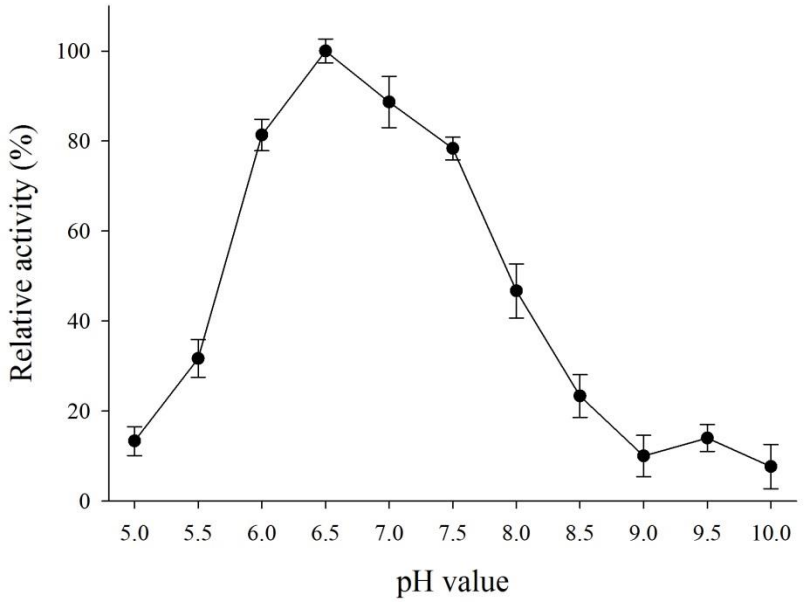


Figure S1 Conserved regions in the amino acid sequences of AtcA-like proteins and the deduction of the *atcA* gene probe. (A) Comparison of the amino acid sequences of the molybdenum-binding subunit (AtcA) of 1-testosterone hydratase/dehydrogenase from *Sdo*. *denitrificans* DSMZ 18526 with highly similar sequences (around 60% identity) from betaproteobacteria: *Stl*., *Sterolibacterium*; *Thau*., *Thauera*; and *Azo*., *Azoarcus*. Bottom row: the sequence of the molybdenum-containing subunit of the 3-hydroxycyclohexanone dehydrogenase from *Alicycliphilus* (*Ali*.) *denitrificans* DSMZ 14773. (B) Highly conserved nucleotide sequence regions of the *atcA* genes used to deduce the *atcA*-specific degenerate primers are shown in (C). Degenerate primer pair for detecting *atcA* genes. R = A + G, S = G + C, and Y = C + T.

(A)



(B)

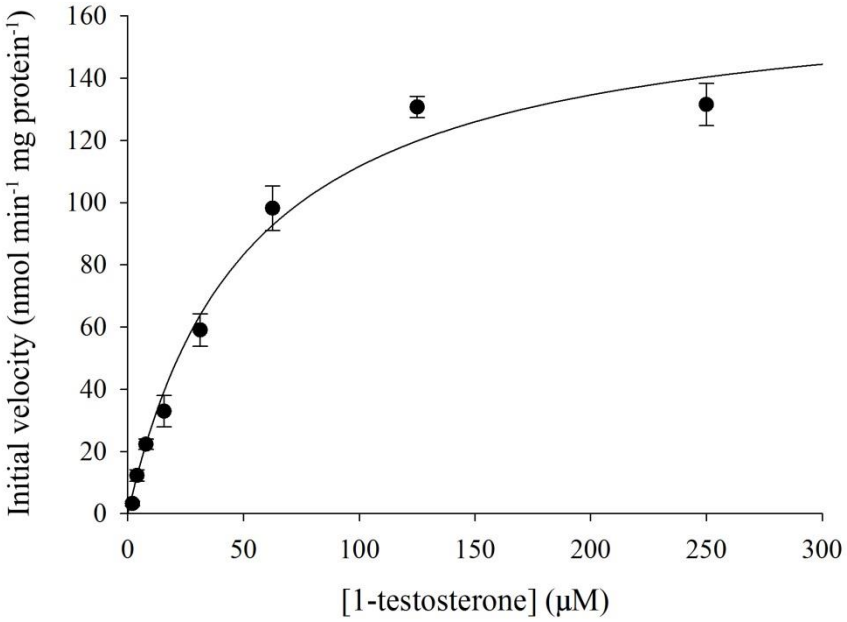


Figure S2 (A) Effect of pH on the activity of AtcABC. The values are shown as percentages of the optimal activity at pH 6.5, taken as 100% (= 158 nmol min^{-1} mg $protein^{-1}$). Data are means ± S.E. of three experimental measurements. (B) Steady-state kinetic analysis of AtcABC-catalyzed hydratation/dehydrogenation. The solid line represents a best fit of the Michaelis-Menten equation to the data. The fit parameters are *Km* = 52 ± 9 μM and V_{max} = 169 ± 12 nmol min^{-1} mg $protein^{-1}$. The data shown are the means ± S.E. of three experimental measurements.

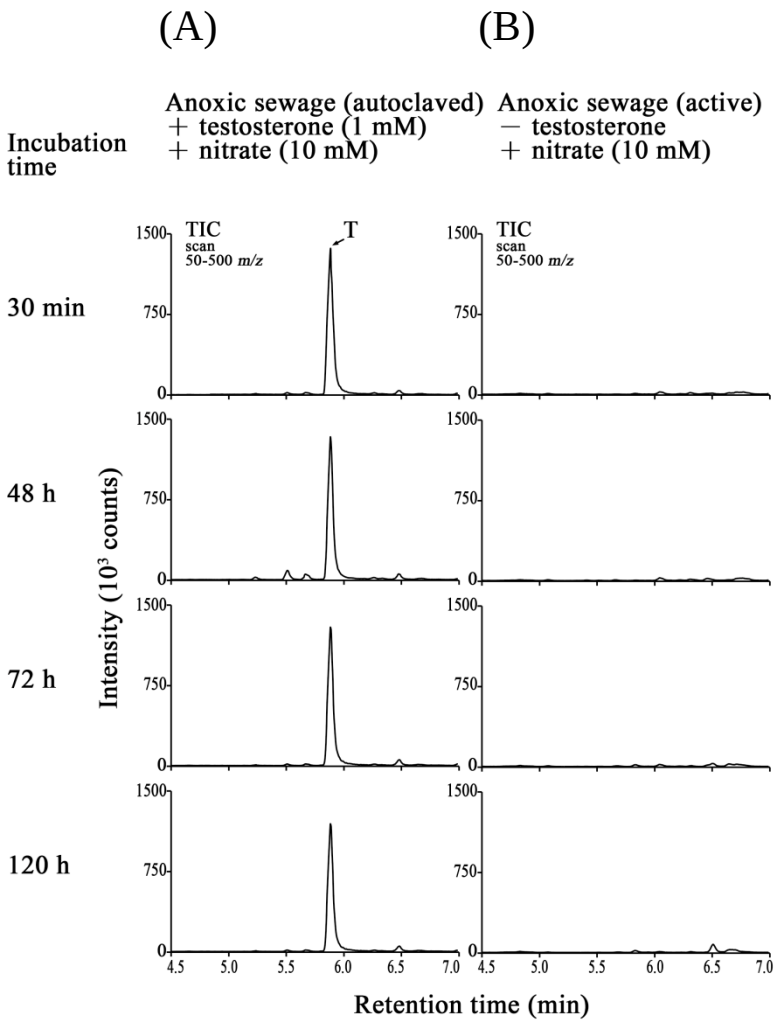


Figure S3 UPLC-APCI-MS/MS analysis of the ethyl acetate extracts of anoxic DHSTP sewage incubated under different conditions. (A) Total ion chromatograms of autoclaved sewage incubated with testosterone and nitrate. (B) Total ion chromatograms of active sewage incubated with nitrate but without testosterone.

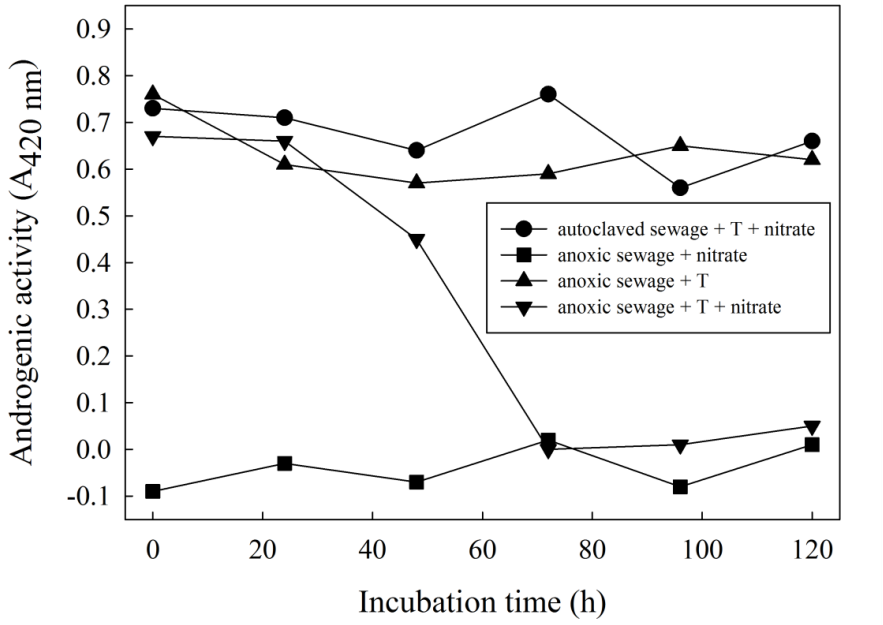


Figure S4 Time course of androgenic activities in the negative control (autoclaved sewage incubated with testosterone and nitrate) and three treatments of anoxic DHSTP sewage. The A_{420} of the solvent, DMSO (1% v/v), was set to zero.

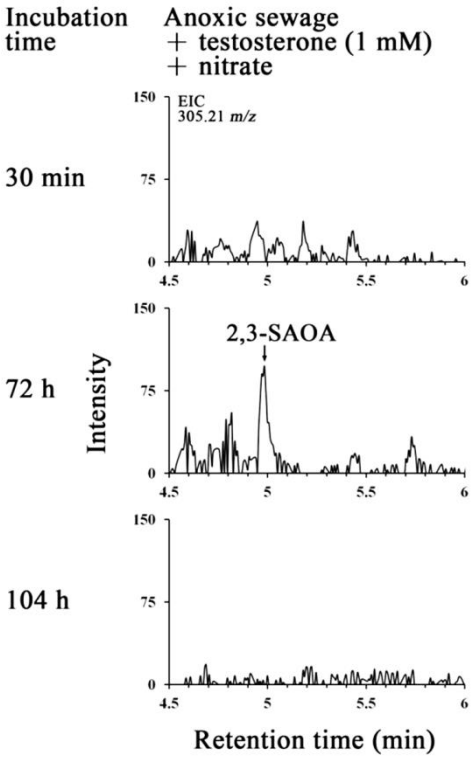


Figure S5 The extracted ion chromatograms (*m/z* 305.21 for 2,3-SAOA) revealed the presence of the signature metabolite in the testosterone-incubated sewage. Anoxic DHSTP sewage samples collected in April 2014 were incubated with testosterone (1 mM) and nitrate (10 mM), extracted with ethyl acetate, and analyzed through UPLC-APCI-MS/MS.

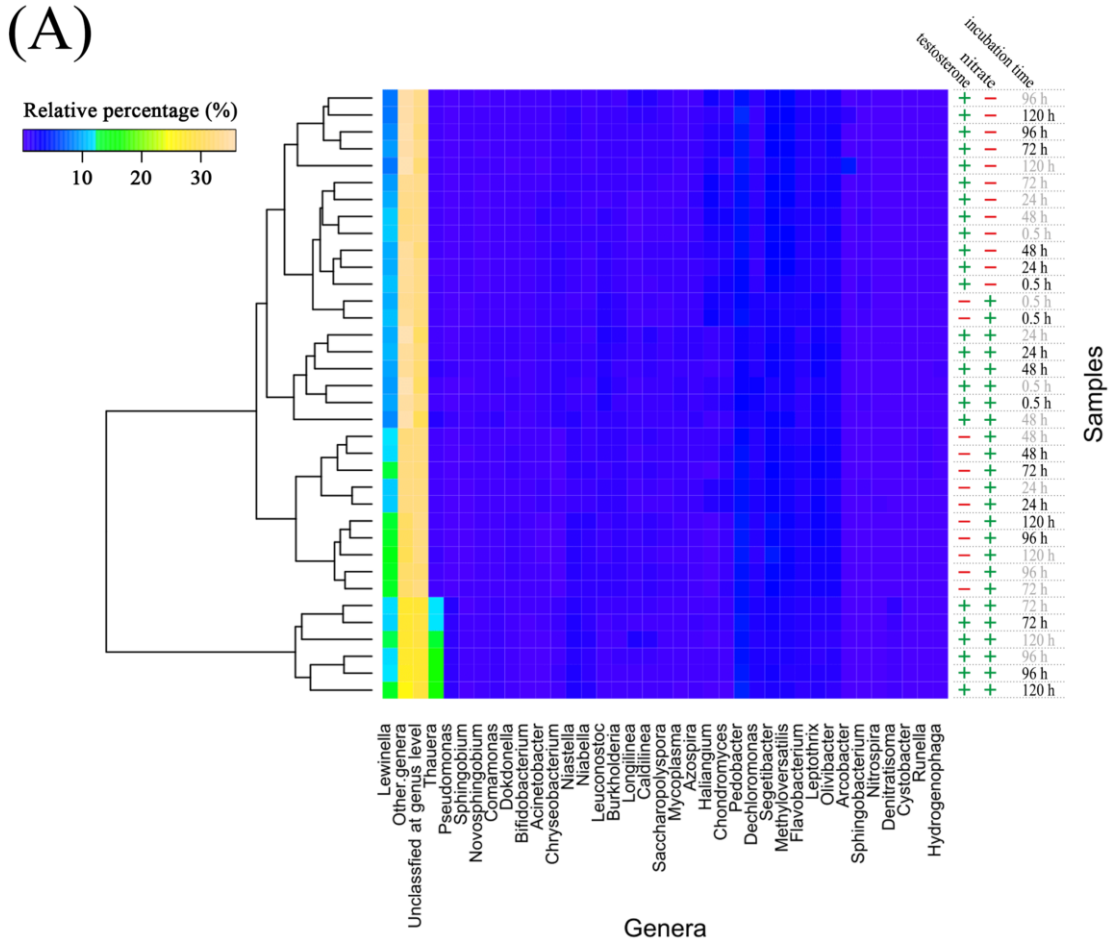
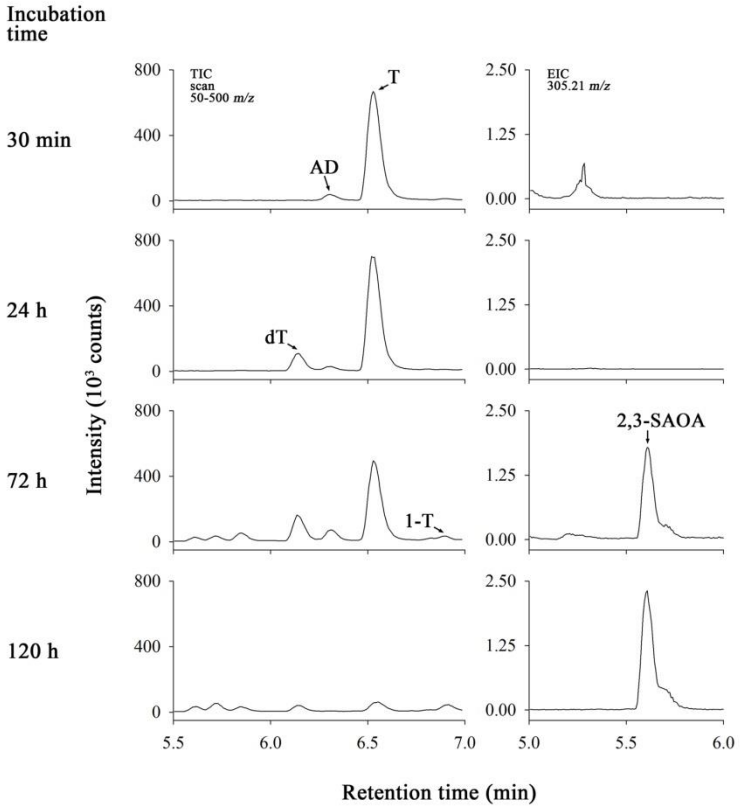


Figure S6 The heatmap represents the relative abundances of the indicated genera within microbiota sampled from individual sewage treatments at a single time point. Sewage treatments were performed in duplicate and the individual incubation time labeled black and grey represents different replicates. Individual genera with a relative percentage of <1% are grouped as "other genera." The dendrogram represents the average linkage hierarchical clustering of sewage samples based on the Bray-Curtis dissimilarity matrix.

(A)



(B)

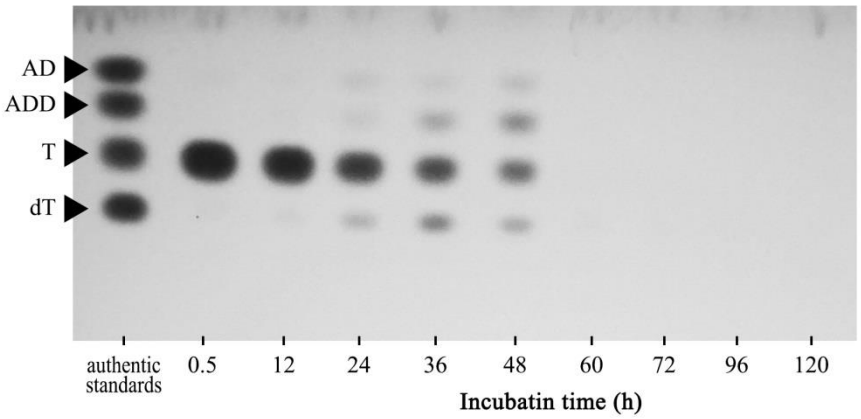


Figure S7 The anaerobic degradation of testosterone by *Thauera terpenica* 58Eu. *T. terpenica* (200 ml) was anaerobically grown on acetate (16 mM) and testosterone (1 mM) in the minimal medium. Two independent experiments were performed. The bacterial cultures were incubated at 25 ℃ with stirring at 160 rpm for 6 days. Cultural samples (1 ml) were withdrawn from the bottles every 12 hours. The samples were extracted with ethyl acetate and the extracts were analyzed using UPLC-APCI-MS/MS and thin-layer chromatography (TLC). (A) Total ion chromatograms (left panel) and extracted ion chromatograms (right panel; *m/z* 305.21 for 2,3-SAOA) revealed the anaerobic transformation of testosterone to 2,3-SAOA in a denitrifying *T. terpenica* culture. (B) TLC chromatogram indicated anaerobic degradation of testosterone in another *T. terpenica* culture. Androgen metabolites were visualized under UV light at 254 nm. See Figure 1 for the abbreviations of androgen metabolites.

Figure S8 Twenty *atcA* sequences amplified from anoxic DHSTP sewage (incubated with testosterone and nitrate for 120 hours) by using *atcA*-specific primers AtcA-f1/AtcA-r1.

>Anoxic DHSTP *atcA*_1
TGGCAGCGTCCAGTTCATCGACAACCTGTCCCTGCCCGGCATGCTGCACTGCGCCATCC
TGCGCAGCCCGCACCCGCACGCCCGCATCGTGTCGGTGGACGTCAGCGCCGCGCTGGC
CGCCGAAGGTGTGGTGGCGGTGATCAGCGGCGAAGACGTCAAGCGCTGGTGCGCGCCC
GGCTTCACCGCACCCGAAGGCTGGGGCAATTACTGCATGGCGGTGGACAAGGTGCGCT
TCGTCGGCGAACCGGTCGCCGCCGTCGCCGCCACCAGCCGCTACCTGGCCGAGGACGC
GCTCGAGCTGCTGCACATCGAGTACGAAACCCTGACCCCGGTGTGCAACCCGGAACAG
GCCATGGCACCCGACGCCCCGGTGGTGTTCGAGGAGCGCGGCACCAACGTCATGCTCC
ACCGCGACTACACCTGGGGCGAGGTCGATCGCGTGTTCGCCGAGGCCGACCACATCATC
AGCCGCCGCTTTCGCTGGAACCGCGTCGGCGCCAACCCGACCGAAACCTTCGGCTGCA
TCTGCCAGTGGGACCTCGCCGACAACAGCCTGACCTGCCACGGCGCCTATCAGACGCC
GCGTTTCATGGCCATCGGCCGCGCCGCCTCGCTCCAACTGCCGGCCAACCGCGTGCGCA
TCGTCACCCATCCGCAAGGCGGTGGCTTCGGTGGCAAGGGCGGCCCGCGTGGCACGGA
CATCGCCGCCTTGCTCTCGCGCAAGTCGAACGGGCGGCCGGTGAAGTACATCGAAGAC
CGCATGGAATACCTCCTCGCCGGAGGTGGGCAGTCCTGGGACCGCTATTACGATGCCGC
GCTGGCGGTGAAGGCGGACGGCACCGTGACCGGCTTTCGTGTCCATCTGGTCGATGACC
AGGGCGCGGGCGCCGAGGGCTACGGCACCATCTCCGCGGCCAAGCCGCTGGCTGCCTT
CACTGGCAACTACCGCATCGAGGCGGCGTCCTACGACCTCACTCTGGTCGCCACCAACC
GTGCGCCCACCTATCCCTACCGCGGCTATGGCCCCCCACCGCACAACCTGGTGCTCGAG
TCGCTGATGGACTGCACCGCGCGCGAGCTGGGTATGGACCCGGCCGAGTTGCGCCGCC
AAAACTTCATCCGCCCCGAGCAGTTCCCCTACACCGTGCCGAGCGGGAACGAATATGAC

>Anoxic DHSTP *atcA*_2
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GCCGAAGGTGTGGTGGCGGTGATCAGCGGCGAAGACGTCAAGCGCTGGTGCGCGCCCG
GCTTCACCGCACCCGAAGGCTGGGGCAATTACTGCATGGCGGTGGACAAGGTGCGCTTC
GTCGGCGAACCGGTCGCCGCCGTCGCCGCCACCAGCCGCTACCTGGCCGAGGACGCGC
TCGAGCTGCTGCACATCGAGTACGAAACCCTGACCCCGGTGTGCAACCCGGAACAGGC
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CGCGACTACACCTGGGGCGAGGTCGATCGCGTGTTCGCCGAGGCCGACCACATCATCAG
CCGCCGCTTTCGCTGGAACCGCGTCGGCGCCAACCCGACCGAAACCTTCGGCTGCATCT
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TTTCATGGCCATCGGCCGCGCCGCCTCGCTCCAACTGCCGGCCAACCGCGTGCGCATCG
TCACCCATCCGCAAGGCGGTGGCTTCGGTGGCAAGGGCGGCCCGCGTGGCACGGACAT
CGCCGCCTTGCTCTCGCGCAAGTCGAACGGGCGGCCGGTGAAGTACATCGAAGACCGC
ATGGAATACCTCCTCGCCGGAGGTGGGCAGTCCTGGGACCGCCATTACGATGCCGCGCT
GGCGGTGAAGGCGGACGGCACCGTGACCGGCTTTCGTGTCCATCTGGTCGATGACCAG
GGCGCGGGCGCCGAGGGCTACGGCACCATCTCCGCGGCCAAGCCGCTGGCTGCCTTCA
CTGGCAACTACCGCATCGAGGCGGCGTCCTACGACCTCACTCTAGTCGCCACCAACCGT
GCGCCCACCTATCCCTACCGCGGCTATGGCCCCCCACCGCACAACCTGGTGCTCGAGTC
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>Anoxic DHSTP *atcA*_3
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CCCCGGTGGTGTTCGAGGAGCGCGGCACCAACGTCATGCTCCACCGCGACTACACCTG
GGGCGAGGTCGATCGCGTGTTCGCCGAGGCCGACCACATCATCAGCCGCCGCTTTCGCT
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GCCGCGCCGCCTCGCTCCAACTGCCGGCCAACCGCGTGCGCATCGTCACCCATCCGCAA
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CGCCGGAGGTGGGCAGTCCTGGGACCGCTATTACGATGCCGCGCTGGCGGTGAAGGCG
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ATCGAGGCGGCGTCCTACGACCTCACCCTGGTCGCCACCAACCGTGCGCCCACCTATCC
CTACCGCGGCTATGGCCCCCCACCGCACAACCTGGTGCTCGAGTCGCTGATGGACTGCA
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>Anoxic DHSTP *atcA*_4
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GGCGGTGAAGGCGGACGGCACCGTGACCGGCTTTCGTGTCCATCTGGTCGATGACCAG
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CTGGCAACTACCGCATCGAGGCGGCGTCCTACGACCTCACTCTGGTCGCCACCAACCGT
GCGCCCACCTATCCTTACCGCGGCTATGGCCCCCCACCGCACAACCTGGTGCTCGAGTC
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>Anoxic DHSTP *atcA*_5
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CGCCGAAGGCGTGGTAGCGGTGATCAGCGGCGAAGACGTCAAGCGCTGGTGCGTGCCC
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TGCGCCCACCTATCCCTACCGTGGCTATGGCCCCCCACCGCACAACTTGGTGCTCGAGTC
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>Anoxic DHSTP *atcA*_6
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GCTTCACCGCGCCCGAAGGCTGGGGCAATTACTGCATGGCGGTGGACAAGGTGCGCTTC
GTCGGCGAACCGGTCGCCGCCGTCGCCGCCACCAGCCGCTACCTGGCCGAGGACGCGC
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>Anoxic DHSTP *atcA*_7
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GCGCCCACCTATCCCTACCGCGGCTATGGCCCCCCACCGCACAACCTGGTGCTCGAGTC
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>Anoxic DHSTP *atcA*_8
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>Anoxic DHSTP *atcA*_9
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GCGCCCACCTATCCCTACCGCGGCTATGGCCCCCCACCGCACAACCTGGTGCTCGAGTC
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>Anoxic DHSTP *atcA*_10
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>Anoxic DHSTP *atcA*_11
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CCGCCGCTTTCGCTGGAACCGCGTCGGCGCCAACCCGACCGAAACCTTCGGCTGCATCT
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CGCCGCCTTGCTCTCGCGCAAGTCTGGCGGGCGGCCGGTGAAGTACATCGAAGACCGC
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GGCGGTGAAGGCGGACGGCACCGTGACCGGCTTTCGTGTCCATCTGGTCGATGACCAG
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>Anoxic DHSTP *atcA*_12
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>Anoxic DHSTP *atcA*_13
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GCCCACCTATCCCTACCGTGGCTATGGCCCCCCACCGCACAACTTGGTGCTCGAGTCGCT
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>Anoxic DHSTP *atcA*_14
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>Anoxic DHSTP *atcA*_15
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