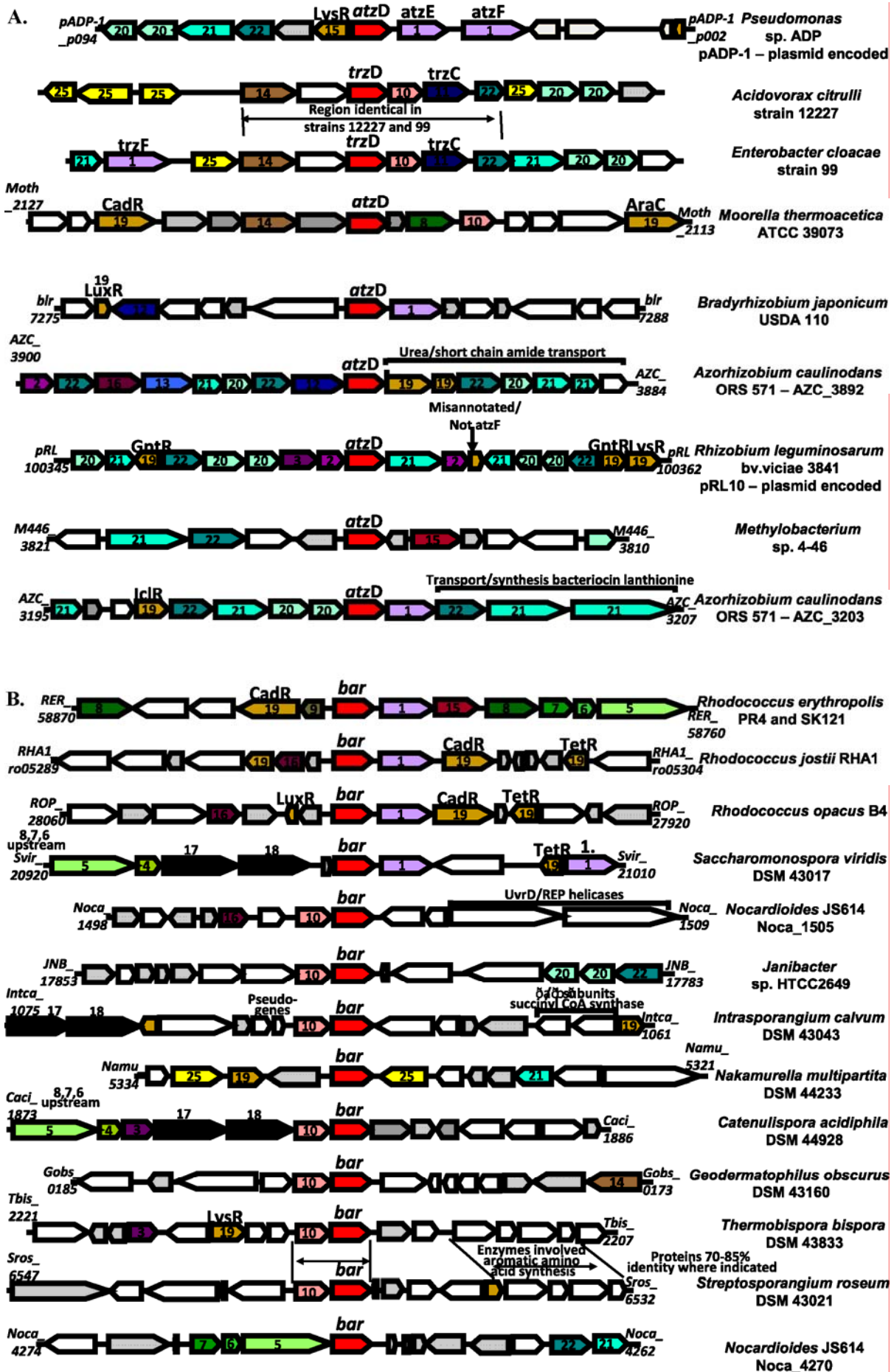


Fig. S1

Gene context diagram for representative organisms in (A) AtzD/TrzD clustered homologs and (B) barbiturase cluster. The numbering represents proteins belonging to these various groups: 1. COG0154, Asp-tRNA Asn/Glu-tRNA Gln amidotransferase belonging to the amidase signature superfamily; 2. COG1335, amidase related to nicotinamidase and isochorismatase that belong to the cysteine hydrolase superfamily; 3. COG0388, Predicted amidohydrolase that belong to the nitrilase superfamily like nitrilase and cyanide hydratase; 4-7. COG3427, COG1529, COG2080, and COG1319, uracil/thiaine dehydrogenase – precursor to barbiturase; 8. COG2233, Xanthine/uracil permeases; 9. COG0035, Uracil phosphoribosyltransferase; 10. COG0549, Carbamate kinase which belongs to the amino acid kinase superfamily, including uridylate, carbamate, asparto-, and acetylglutamate kinases; 11. trzC, ammelide amidohydrolase. 12. OG0402, Cytosine deaminase and related metal-dependent hydrolases; 13. COG0044, Dihydroorotase and related cyclic amidohydrolases, likely L-Hydantoinases or Allantoinase; 14. FdrA like protein; 15. COG0161, Adenosylmethionine-8-amino-7-oxononoate aminotransferase that belongs to acetyl ornithine aminotransferase family of the pyridoxal phosphate (PLP)-dependent aspartate aminotransferase superfamily, including ornithine aminotransferase, acetylornithine aminotransferase, alanine-glyoxylate aminotransferase, dialkylglycine decarboxylase, 4-aminobutyrate aminotransferase, beta-alanine-pyruvate aminotransferase, adenosylmethionine-8-amino-7-oxononoate aminotransferase, and glutamate-1-semialdehyde 2,1-aminomutase; 16. COG1028, Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases); 17. COG0145, N-methylhydantoinase A/acetone carboxylase, beta subunit; 18. COG0146, N-methylhydantoinase B/acetone carboxylase, alpha subunit; 19. regulator; 20. ABC transporter permease – representing various COGs; 21. ABC transporter ATP binding domain – representing various COGs; 22. ABC transporter periplasmic component; 23. hypothetical; 24. DUF – domain of unknown function; and 25. transposase.



Gene context of AtzD/barbiturase family members. Despite the diverse origins of the source organisms and the highly divergent protein sequences, the characterized proteins throughout Clade 1 had only cyanuric acid hydrolase activity, while the only functional member of Clade 2 had barbiturase activity. To determine if additional functional information could be identified by genomic context, the flanking regions of the genes were compared (Fig. S2).

Gene context – Clade 1. Group 1 proteins were comprised of proteins found in the phylum Firmicutes. *Moorella thermoacetica* ATCC 39073 was shown previously to exhibit cyanuric acid hydrolase activity (1). Although the *Paenibacillus* and *Bacillus* proteins are also members of the Firmicutes, the tree location of these proteins was method-specific, clustering in either Group 1 or in an adjacent cluster. For this reason they were not included specifically in Group 1. The Firmicutes are diverse in sequence and organismal origins. *Clostridium asparagiforme* DSM 15981 was isolated from human feces, *Paenibacillus* sp. JDR-2 was isolated from sweet gum stem wood buried in surface soil and was shown to degrade hemicellulose, and *Bacillus cellulosilyticus* DSM 2522 was isolated from soil and is known to degrade cellulose. The AtzD homologs in these organisms have only 65, 58, and 41% sequence identity to the *Moorella* cyanuric acid hydrolase, respectively. *Clostridium* and *Bacillus* strains share a common metal-dependent amidohydrolase gene similar to cytosine deaminase (COG0402) located downstream of the *atzD* homolog genes. Members of this amidohydrolase superfamily are responsible for the atrazine degradation pathway upstream of cyanuric acid. It has yet to be determined whether the amidohydrolase protein encoded adjacent to the *atzD* gene in these organisms is producing cyanuric acid.

Gene context information is not available for all organisms in Group 2 since individual genes were sequenced, and the genome sequence is unknown. An operon was found to be present in *P. ADP*, encoding *atzD*, *atzE*, and *atzF* (2). The AtzE and AtzF proteins are members of the amidase signature superfamily and catalyze consecutive reactions, resulting in complete mineralization of cyanuric acid to ammonia and carbon dioxide (Fig. 1). From the sequence information available, an identical transposon associated region, containing *trzD* and its precursor gene *trzC*, was located in both *Acidovorax citrulli* strain 12227 and *Enterobacter cloacae* strain 99 (Fig. S2A). These two genes encoded enzymes that convert ammelide to cyanuric acid and catalyze the subsequent ring-opening reaction. TrzF, a homologous protein to AtzE and AtzF, located upstream of the TrzD protein in *Enterobacter cloacae* strain 99, was shown to catalyze the allophonate hydrolase reaction in that strain (24). A similar homolog is adjacent to the *atzD* gene in *Bradyrhizobium japonicum* USDA 110. This enzyme shares 27% identity to AtzE and 25% identity to AtzF from *P. ADP*, and could possibly be involved in deamination of the products generated by the cyanuric acid hydrolase.

Group 2 contains the only protein found in a eukaryote, the common photosynthetic marine alga *Micromonas* sp. RCC299. Along with the USDA 110 protein, this is the only other Group 2 protein from an organism not isolated for its *s*-triazine degradation ability. The *atzD* homolog gene is located on chromosome 7 and is surrounded by mainly predicted proteins that have no close function to proteins of known function. Unlike the USDA100 protein, there is no evidence of potential *s*-triazine degradation pathway genes located proximal to the *atzD* homolog. This protein has 45, 41, 42, and 32 % identity to AtzD (*Pseudomonas* sp. ADP), TrzD (*Acidovorax citrulli* strain 12227), the *Moorella* cyanuric acid hydrolase, and barbiturase (*Rhodococcus erythropolis* JCM 3132), respectively. Because of its sequence divergence and unique origin, this protein is a good candidate for future functional studies.

Group 3 enzymes were found solely in α -proteobacteria (Fig. 2). The proteins had 37-55% identity to AtzD, TrzD, the *Moorella* cyanuric acid hydrolase, or barbiturase (Table 1). No functionality was determined among this group prior to this study. It should be noted that although all of these proteins originate in α -proteobacteria, they are not found in a large range of bacteria and are not present in all organisms in that genera. For instance, *Methylobacterium radiotolerans* JCM 2831 and sp. 4-46 contain AtzD homologs, while the genomes for *Methylobacterium extorquens* AM1 and *M. nodulans* ORS 2060 do not. All of the Group 3 organisms, with the exception of *Oceanicola granulosus* HTCC2516, are involved in nitrogen fixation or are plant pathogens. The relevance of this observation has yet to be determined. The two *Rhizobium* enzymes are encoded on large plasmids and have similar gene context, which include nitrilase and cysteine hydrolase superfamily members directly upstream of *atzD* and a sugar ABC transporter and cysteine hydrolase

superfamily member directly downstream. Likewise, the two *Bradyrhizobium* strains in this group have similar gene context, including a downstream TonB-dependent receptor protein and putative hydroxylase. Gene context for all other organisms in this group are highly divergent (Fig. 4A). *Oceanicola granulosus* HTCC2516 and *Agrobacterium vitis* S4 have one copy of the *atzE/F* protein pair in close proximity to the *atzD* genes. The product of the AtzE-catalyzed reaction is allophanate that will spontaneously decarboxylate to form urea, which many organisms can metabolize. This may account for only one AtzE/F homolog being present in these organisms. The other strains, though lacking AtzE/F homologs, have other potential amidases belonging to the cysteine hydrolase or nitrilase superfamilies in close proximity, which could be catalyzing AtzE/F type reactions.

Azorhizobium caulinodans ORS 571 has two cyanuric acid hydrolase/barbiturase family members. The protein encoded by locus AZC_3892 was placed in Group 3, while locus AZC_3203 was not placed in either Clade 1 or 2 (Fig. 3). Upstream of the locus AZC_3892 gene is a metal-dependent amidohydrolase superfamily member similar to proteins that are in the upper pathway in atrazine degradation leading to cyanuric acid production. Downstream of the gene is a urea/short chain amide transport system. A cysteine hydrolase superfamily protein upstream may also be involved in hydrolysis of the AtzD homolog products. Interestingly, the AZC_3203 locus has one *atzE/F* homolog directly downstream of the *atzD* homolog gene.

The identical protein in *Acidithiobacillus ferrooxidans* ATCC 53993 and ATCC 23270 was placed adjacent to Group3 in Fig. 3, but was not formally included in Group 3 for reasons stated above. These strains are γ -Proteobacteria isolated from acid mine drainage. The AtzD homolog has 34-46% identity with the experimentally determined cyanuric acid hydrolases or barbiturases. It has the ability to fix nitrogen, which is common to most of the Group 3 source organisms. The genomes revealed a combination of genes around the AtzD homolog similar to multiple other groups. There are uracil permease and dehydrogenase genes similar to Group 4 (described below), AtzE/F homologs similar to group 5 (described below) and some examples in Groups 2 and 3, and multiple metal-dependent amidohydrolases, which are homologous to the upstream pathway genes in *s*-triazine degradation. This last example demonstrates the variability of the regions found flanking the Clade 1 genes. While some genes, such as those encoding amidase proteins, were observed adjacent to *atzD* homologs with some frequency, no consistent patterns in the genes flanking Clade 1 *atzD* homologs could be identified.

Gene context – Clade 2. Clade 2 consists of proteins found solely in certain Actinobacteria species (Fig. 2). The proteins in this clade share greater than 72% identity with the experimentally determined barbiturase from *Rhodococcus erythropolis* JCM 3132. With such similarity, it might be suspected that these proteins belong in the same group and catalyze the same reaction. However, gene context data suggested the division of this clade into two groups.

Group 4 genes encode proteins that have an upstream enzyme belonging to the amino acid kinase superfamily (Fig. S2). This superfamily includes such enzymes as uridylate, carbamate, asparto-, and acetylglutamate kinase. Uridylate kinase is a bidirectional enzyme that converts uridine 5'-diphosphate (UDP) to uridine 5'-monophosphate (UMP). UMP can then be converted to uridine and then uracil by 5' nucleotidase (EC 3.1.3.5), and uridine nucleosidase (EC 3.2.2.3) or uridine phosphorylase (EC 2.4.2.3) to feed into the pathway for uracil conversion to barbituric acid, and then malonate and urea (Fig. 1). Although the Group 4 proteins have high sequence identity to the known *Rhodococcus erythropolis* JCM 3132 barbiturase (74-86%), no enzyme in this group has been purified and the function of the Group 4 proteins has not been validated. The one organism in this group that lacks the kinase gene is *Nakamurella multipartite* DSM 44233. This organism has transposons flanking the barbiturase homolog, suggesting a mobilization event may have occurred to produce the current gene arrangement.

The second grouping in Clade 2, Group 5, contains the experimentally determined barbiturase from *Rhodococcus erythropolis* JCM 3132 and proteins with greater than 92% identity to this enzyme, and are found within other *Rhodococcus* species and *Saccharomonospora viridis* DSM 43017 (Fig. 3). Gene context data (Fig. S2B) revealed that this group has a conserved amidase homologous to AtzE/AtzF directly after the barbiturase. We propose that, analogous to the cyanuric acid degradation pathway in *P. ADP*, this amidase catalyzes the next step in the barbituric acid degradation pathway, the conversion of ureidomalonic acid to malonate and urea (Fig. 1). Although there is no genome for *Rhodococcus erythropolis* JCM 3132, two other *Rhodococcus erythropolis* species, PR4 and SK121, show conserved uracil/xanthine permease and

uracil/thiamine dehydrogenase (small, middle, and large subunits) in close proximity (Fig. S2B). These enzymes catalyze the transport and conversion of uracil to barbituric acid, respectively. The other *Rhodococcus* strains in this group have these enzymes as well.

Due to a possible gene duplication or horizontal gene transfer event, *Nocardioides* JS614 has two proteins within Clade 2. The protein encoded by gene locus Noca_4270 has the uracil/thiamine dehydrogenase (small, middle, and large subunits), the enzyme producing barbituric acid, encoded directly upstream of the barbiturase homolog. This protein however shares only 72% identity with the experimentally determined barbiturase. Noca_1505 shares 86% identity with barbiturase but has the gene context described in Group 4. It is possible that both group 4 and 5 proteins catalyze the barbiturase reaction, that Noca_4270 encodes a protein having a different function, or that the coding sequence is a pseudogene. Additional experimentation is required to confirm these functions.

Frankia sp. Eu11c is an actinobacterium nitrogen-fixing symbiont found within root nodules of woody trees and shrubs. The cyanuric acid hydrolase/barbiturase homolog in this strain has the lowest sequence identity to the enzymes of known function, 36-40%. The genome sequence does not reveal any genes related to cyanuric acid or barbituric acid metabolism. Experimental investigation is required to determine the function of this protein.

Fig. S2

Whole family sequence alignment. 80% identity are highlighted by the various colors with the most stringent condition being displayed: 80% identity with a single residues are colored with a grey background, negative residue conservation are red (DE), Ser/Thr are cyan, aliphatic are grey with a yellow background (ILV), positive are blue (HKR), tiny are green (AGS), aromatic are blue with a yellow background (FHWY), charged are pink (DEHKR), small are forest green (ACDGNPSTV), polar are light blue (CDEHKNQRSTV), big residues are dark blue with a peach background (EFHIKLMQRWY), and hydrophobic are black with a yellow background (ACFGHILMTVWY).

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Janibacter_sp._HTCC2649      ----MP---E AIEVRKVPPLH SVSDASELAK LIDDGVMEAS RVIAIIGKTE GNGGVNDYR
Intrasporangium_calvum_DSM_430 ----MP---E AIEVRKVPPIH SVADASELAR LIDDGVMEAA RVVAIIGKTE GNGGVNDYR
Noca_1505_1Nocardioides_sp._JS ----MP---D AIEVRKVPPIH SVADASELAK LIDDGVMQAE RVIAIIGKTE GNGGVNDYR
PR4_Rhodococcus_erythropolis_P ----MP---E AIEVRKVPPLH SVSDASELAK LIDDGVLEAD RVIAVIIGKTE GNGGVNDYR
SK121_Rhodococcus_erythropolis ----MP---E AIEVRKVPPLH SVSDASELAK LIDDGVLEAD RVIAVIIGKTE GNGGVNDYR
Barbiturase_Rhodococcus_erythr ----MP---E AIEVRKVPPLH SVSDASELAK LIDDGVLEAD RVIAVIIGKTE GNGGVNDYR
jostii_RHA1_Rhodococcus_jostii ----MP---D AIEVRKVPPLH SVSDAGELAK LIDDGVLEAD RVVAVIIGKTE GNGGVNDYR
B4_Rhodococcus_opacus_B4       ----MP---E AIEVRKVPPLH SVSDAGELAK LIDDGVLEAD RVVAVIIGKTE GNGGVNDYR
Saccharomonospora_viridis_DSM_ ----MP---D AIEVRKVPPIH SVSDASELAK LIDDGVMEAD RIIIAIIGKTE GNGGVNDYR
Nakamurella_multipartita_DSM_4 (11)TP---E PIEVRKIPLH SVSDASELTA LFDAGITDAD RVVAVIIGKTE GNGGVNDYR
Catenulispora_acidiphila_DSM_4 ----MP---E AIEVRKVPPLH HVSDDASGLAE LITAGVVEAD RVVAVVIGKTE GNGGVNDYR
Streptosporangium_roseum_DSM_4 ----MP---E PIEVRKVAIE SVTDASGLTR LIDDGVIEAH RVLAVIGKTE GNGGVNDYR
Thermobispora_bispora_DSM_4383 ----MP---D PIEVRKVPPIE SVTDASGLAK LIDEGIIEAD RVLAVIGKTE GNGGVNDYR
Geodermatophilus_obscurus_DSM_ ----MP---A PIEVRKVPPLH NVSDASELAK LIDDGVMEAD RVVAVIIGKTE GNGGVNDYR
Noca_4270_2Nocardioides_sp._JS ----MT---R PIEVRKVPPIE HVSDDAAGLAD LIDAGVFSAD DVIAVVIGKTE GNGGVNDYR
Aminobacter_aminovorans      -----
Chelatobacter_heintzii       -----
TRZD_Enterobacter_cloacae     -----M QAQVFRVPMS NPADVSGVAK LIDEGVIRAE EVVAVLGGKTE GNGCVNDFR
TrzD_Acidovorax_citrullii     -----M QAQVFRVPMS NPADVSGVAK LIDEGVIRAE EVVAVLGGKTE GNGCVNDFR
Moorella_thermoacetica_ATCC_39 -----MQ KVEVFRIPTA SPDDISGLAT LIDSGKINPA EIVAILGGKTE GNGCVNDFR
Clostridium_asparagiforme_DSM_ -----M KIDVFKIEMS APDDVRELKK LIDAGEVDPG EIIAVLGGKTE GNGCVNDFR
AD25_Arthrobacter_sp._AD25    -----MY HIDVFRIPCH SPGDTSGLED LIETGRVAPA DIVAVMGGKTE GNGCVNDFR
Comamonas_sp._A2             -----
gi|75369013|sp|Q6PMK9|Q6PMK9_9 -----MY HIDVFRIPCH SPGDTSGLED LIETGRVAPA DIVAVMGGKTE GNGCVNDFR
AtzD_ADP_Pseudomonas_sp._ADP -----MY HIDVFRIPCH SPGDTSGLED LIETGRVAPA DIVAVMGGKTE GNGCVNDFR
Pseudomonas_nitroreducens     ----- --GDTSGLED LIETGRVAPA DIVAVMGGKTE GNGCVNDFR
USDA_110_Bradyrhizobium_japoni ----MR--TT SVGVFKIVTK GPGDVSGLMA MFGSGAIDPK SILAVLGGKTE GNGCVNDFR
viciae_3841_Rhizobium_legumino ----MP--SL RAHVFRVPAD GPDDVAGVEA LFASG-LQAN NIVAVLGGKTE GNGCVNDFR
trifolii_WSM1325_Rhizobium_leg ----MP--SL RAHVFRVPAD GPDDVAGVEA LFASG-LQAN NVVAVLGGKTE GNGCVNDFR
Agrobacterium_vitis_S4       ---MTT--CF RASVYRVAAS APDDISGVEA LTGSG-LNPH SIVAVLGGKTE GNGCVNDFR
ORS278_Bradyrhizobium_sp._ORS2 ----MPTLR RAHVHRLPMR SPDDVGALEA AIVSGAIDPA GIVAILGGKTE GNGCVNDFR
BTai1_Bradyrhizobium_sp._BTai1 ----MPTALR RATVHRLPMR SPDDVGALEA ATAQGAIDPA GIIAILGGKTE GNGCVNDFR
4_46_Methylobacterium_sp._4_46 ----MP---R RAELIRLPLMA APDDVSAIAA SLRDGRLDPG DVVAVFAKTE GNGCVNDFR
AZC_3892_2Azorhizobium_caulino ----MP---- IAKVHRIATA SPDDVSGLAA AITATGAIAPA GILAIIFGGKTE GNGCVNDFSR
Paenibacillus_sp._JDR-2      -----MM KCSVFKVPAR SPNDTAAALQR YIDESLINPK DVVAVLGGKTE GNGCVNDFR
Oceanicola_granulosus_HTCC2516 -----M RADVIRFDLP DPGDVSAALAG AIDAGRVDPG RIVAIIGKTEH ENGLVNDYR
Acidithiobacillus_ferrooxidans -----M RTSVYRFFTQ DPADVSGLVR ATAEGVINPQ SIVAIIGKTE GNGMVNDFR
JCM_2831_Methylobacterium_radi -----MP ECLVFRVPAQ HPADVSGVMD LVTSGTVGAP EIVAIIFGGKTE GNGCVNDFR
Micromonas_sp._RCC299        ---VLDKP VAVVAVVDTA HPGDTGPLER WMDAN--PLA DILCVLGGKTE GNGCVNDFR
AZC_3203_1Azorhizobium_caulino -----M KVGVHKLAMS APGDVSELAA LIETGAVNPR EIVAVLGGKTE GNGGANDFR
Bacillus_cellulosilyticus_DSM_ -----M NYELIRCDMD HPGDVSAALAK LLNEEEISAE SIRAIIAQTE GDG----YSR
Frankia_sp._Eu1c             (10)RSARYP KASMHVVPML APNDTAAFRA LFASGAVDPA SVVALIAKSE GSGLHNDHAR
Consensus/80%                ...MMPH... .hpVb+lshp sssDst.l.. blssG.lps. pllAlllGKTE GNGsVNDaTR

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Janibacter_sp._HTCC2649	IIADRAFREV	LIEKG-APA-	----DQVKQV	PIVWSSGGD	IIISPHATT	TVP----	ADK
Intrasporangium_calvum_DSM_430	IIADRAFREV	LVEKG-APA-	----DQVSQI	PIVWSSGGD	VISPHATT	TLP----	ADR
Noca_1505_1Nocardioides_sp._JS	IIADRAFREV	LVEKG-APA-	----EQVKQV	PIVWSSGGD	VISPHATT	TVP----	PED
PR4_Rhodococcus_erythropolis_P	IIADRAFREV	LSAKGNRSP-	----EEVAEV	PIVWSSGGD	VISPHATT	TVP----	ADK
SK121_Rhodococcus_erythropolis	IIADRAFREV	LSAKGNRSP-	----EEVAEV	PIVWSSGGD	VISPHATT	TVP----	ADK
Barbiturase_Rhodococcus_erythr	IIADRAFREV	LSAKGNRSP-	----EEVAEV	PIVWSSGGD	VISPHATT	TVP----	ADK
jostii_RHA1_Rhodococcus_jostii	IIADRAFREV	LSAKGRRSA-	----DEVAEV	PIVWSSGGD	VISPHATT	TVP----	AGK
B4_Rhodococcus_opacus_B4	IIADRAFREV	LSAKGSRST-	----DEVAQV	PIVWSSGGD	VISPHATT	TVP----	AEK
Saccharomonospora_viridis_DSM_	IIADRAFREV	IVEKGSRTM-	----EVRQI	PIVWSSGGD	VISPHATT	TVP----	EDR
Nakamurella_multipartita_DSM_4	IIADRAFREA	IVAGGTRTL-	----DQVRQI	PIVWSSGGD	VLSPHATT	TVP----	ADS
Catenulispora_acidiphila_DSM_4	IIADRAFRET	LMRLGTRSE-	----AEVAEV	PIVWSSGGD	VLSPHATT	TTD----	AEP
Streptosporangium_roseum_DSM_4	IIADRAFREV	LAAKGHPSP-	----ES---V	PLVWSSGGD	VLSPHATT	TTA----	DAE
Thermobispora_bispora_DSM_4383	IIADRAFREDV	LVQKGTRTP-	----EEVAQV	PLVWSSGGD	VLSPHATT	TVD----	PAK
Geodermatophilus_obscurus_DSM_	IIADRAFREV	LLEKGTRSK-	----EEVGEI	PIVWSSGGD	VISPHATT	TLP----	EDA
Noca_4270_2Nocardioides_sp._JS	IIISTHAYRAV	LLEKGRTRSK-	----EEVAQV	PLVWSSGGD	VISPHATT	YAP----	EGR
Aminobacter_aminovorans	-----	-----GVSR-	----QEVGERI	AFVMSGGTEG	VMAPHCTIT	VQKTDNK	QKT
Chelatobacter_heintzii	-----	-----GVSR-	----QEVGERI	AFVMSGGTEG	VMAPHCTIT	VQKTDNK	QKT
TRZD_Enterobacter_cloacae	GYTLAFKVVY	FSEKLGVSR-	----QEVGERI	AFVMSGGTEG	VMAPHCTIT	VQKTDNK	QKT
TrzD_Acidovorax_citrulli	GYTLAFKVVY	FSEKLGVSR-	----QEVGERI	AFVMSGGTEG	VMAPHCTIT	VQKTDNK	QKT
Moorella_thermoacetica_ATCC_39	GFATQSLAMY	LAEKLGISR-	----BEVVKV	AFVMSGGTEG	VMTPHITV	RKDVQ---	EP
Clostridium_asparagiforme_DSM_	ALSTVFSRSL	IMAETGWSE-	----AEVSKV	AFVMSGGTEG	VMSPHATT	CHEDG---	AA
AD25_Arthrobacter_sp._AD25	EYATAMLAAC	LGRHLQLPP-	----HEVEKRV	AFVMSGGTEG	VLSPHHTV	RRP----	AID
Comamonas_sp._A2	-YATAMLAAC	LGRHLQLPP-	----HEVEKRV	AFVMSGGTEG	VLSPHHTV	RRP----	AID
gi 75369013 sp Q6PMK9 Q6PMK9_9	EYATAMLAAC	LGRHLQLPP-	----HEVEKRV	AFVMSGGTEG	VLSPHHTV	RRP----	AID
AtzD_ADP_Pseudomonas_sp._ADP	EYATAMLAAC	LGRHLQLPP-	----HEVEKRV	AFVMSGGTEG	VLSPHHTV	RRP----	AID
Pseudomonas_nitroreducens	EYATAMLAAC	LGRHLQLPP-	----HEVEKRV	AFVMSGGTEG	VLSPHHTV	RRP----	AID
USDA_110_Bradyrhizobium_japoni	EYAVAACTA	LAPQLGLSP-	----BEVEQRI	AFVMSGGTEG	VLSPHITV	RRE----	VER
viciae_3841_Rhizobium_legumino	GYATRSFETL	FSRYG-----	-----VDGV	SIIMSGGTEG	ALSPHWTV	RET----	VET
trifolii_WSM1325_Rhizobium_leg	GYATRSFETL	FSRYG-----	-----VDGV	SIIMSGGTEG	ALSPHWTV	RET----	VET
Agrobacterium_vitis_S4	GFATATFEQL	FARLC-----	-----VTGV	SIVMSGGTEG	ALSPHWTV	RER----	VEA
ORS278_Bradyrhizobium_sp._ORS2	AFAVRSLETL	LGRHLQAD--	----AVRQI	AMVMSGGTEG	ALSPHMIV	AR--T--	GDD
BTAi1_Bradyrhizobium_sp._BTAi1	AFAVRSLOTL	LGRHVDGD--	----AINQI	AMVMSGGTEG	ALSPHMIV	ARDAS--	GET
4_46_Methylobacterium_sp._4_46	PLAVQALRGL	FGPLIGEA--	----ALGRI	AMVMSGGTEG	GLSPHWLV	AR-----	EAA
AZC_3892_2Azorhizobium_caulino	GFAVQSLQML	LRGHMG-A--	----AADEV	CLVMSGGTEG	GMSPHFLV	RA-----	EGN
Paenibacillus_sp._JDR-2	GFAVLALKNL	FASYK-EA--	----TAEPV	SYIMSGGTEG	ILSPHFSV	RSE-S--	LDD
Oceanicola_granulosus_HTCC2516	GYLTQSLKLL	LAARTGEAE-	---AGLQARI	PMVFSGGVEG	VLSPHFIV	VHD----	DED
Acidithiobacillus_ferrooxidans	TLAMMAVSQV	LATHLDCRP-	---BLVEERV	VLSFSGGSEG	VAAPHILV	VSGRA--	AEQ
JCM_2831_Methylobacterium_radi	QLAVMALETA	LATALGCTP-	---AEGARI	ALVMSGGTEG	GLSPHFLI	RRE----	GPA
Micromonas_sp._RCC299	GYATSAIQAA	LGRVRSRRM(12)SSFTADP	AIIMSGGTEG	VLCPHFIV	NSWDE--	ADA
AZC_3203_1Azorhizobium_caulino	GLATLSYQLL	LARHLGLSP-	---BEVGQRI	AFVWSSGGTEG	VLSPHATT	RAP----	DD
Bacillus_cellulosilyticus_DSM_	GYATLAFQLL	LSEKLNISQ--	---QEVFDTI	PMMICKTIGS	LMTPHYTL	EKED---	VEK
Frankia_sp._Eu1c	VFADVSLRTA	LAEARGCP--	---EDLADSV	TVAVSSGGSPS	VISPHVTVT	QEWVADLPAG	
Consensus/80%	hbAs.tbp.h	ls.bh..s..	..Vp...pl	sblbSSGGT-G	lbSPHh*1Fs	...ssNK...	

Janibacter_sp._HTCC2649	TKT P LLTIHT	IRD A KSR G QK	VWTEHT	---H	ESMDLSNGTT	G L GI A VA L G	---	E I EMP	---
Intrasporangium_calvum_DSM_430	TKT P LLTIHT	IRD A KSR G MK	VWTEHT	---H	ESMDLSNGTT	G L GV A VA L G	---	E I EMP	---
Noca_1505_1Nocardioides_sp._JS	TKT P LLTIHT	IRD A KSR G KT	VWTEHT	---H	ESMDLSNGCT	AL G IA V AL G	---	E I EMP	---
PR4_Rhodococcus_erythropolis_P	TKT P LLTIHT	IRD A KSR G KT	VWTEQT	---H	ESMDLSNGT	AL G IA V AL G	---	E I DMP	---
SK121_Rhodococcus_erythropolis	TKT P LLTIHT	IRD A KSR G KT	VWTEQT	---H	ESMDLSNGT	AL G IA V AL G	---	E I DMP	---
Barbiturase_Rhodococcus_erythr	TKT P LLTIHT	IRD A KSR G KT	VWTEQT	---H	ESMDLSNGT	AL G IA V AL G	---	E I DMP	---
jostii_RHA1_Rhodococcus_jostii	TKT P LLTIHT	IRD A KSR G ET	VWTEHT	---H	ESMDLSNGVT	AL G IA V AL G	---	E I DMP	---
B4_Rhodococcus_opacus_B4	TKT P LLTIHT	IRD A KSR G ET	VWTEHT	---H	ESMDLSNGVT	AL G IA V AL G	---	E I DMP	---
Saccharomonospora_viridis_DSM_	TKT P LLTIDT	IRD A KSR G KS	VWTEDT	---L	ESMDLSNGTT	G L GI A VA L G	---	E I DMP	---
Nakamurella_multipartita_DSM_4	TKT P LLTIHT	IRD A K Q R G QT	VWTEHT	---H	ESMDLSNGCT	AL G VA V AL G	---	E I EMP	---
Catenulispora_acidiphila_DSM_4	TKT P LLTIQT	IRE A K A R G ET	VWTEHT	---H	ESMDLSNGCT	AL G IA V AL G	---	E V EMP	---
Streptosporangium_roseum_DSM_4	TKT P LLTLAT	IND A KSR G KD	VVIEDT	---G	PSMDISNSTT	AL G VA V AL G	---	E I EMP	---
Thermobispora_bispora_DSM_4383	TKT P LLTLET	IND A KRR G HT	VVTEDT	---L	KSMDISNSTT	AL G IA V AL G	---	E I EMP	---
Geodermatophilus_obscurus_DSM_	TKT P LLTIET	IRD A K A R G EE	TYDEP	---H	ESMDLSNETT	AL G IA V AL G	---	E I DMP	---
Noca_4270_2Nocardioides_sp._JS	TKT P LLVQDT	IND A ER R GET	VYTHNT	---L	ESMDVSNATT	AL G IA V AL G	---	E I EMP	---
Aminobacter_aminovorans	VKC P LLTAGR	MHD A VER G HT	VATEDT	---Y	ESMGYSRGAS	AL G IA L AL G	---	E V EKANL	---
Chelatobacter_heintzii	VKC P LLTAGR	MHD A VER G HT	VATEDT	---Y	ESMGYSRGAS	AL G IA L AL G	---	E V EKANL	---
TRZD_Enterobacter_cloacae	VKC P LLTAGR	MHD A VER G HT	VATEDT	---Y	ESMGYSRGAS	AL G IA L AL G	---	E V EKANL	---
TrzD_Acidovorax_citrulli	VKC P LLTAGR	MHD A VER G HT	VATEDT	---Y	ESMGYSRGAS	AL G IA L AL G	---	E V EKANL	---
Moorella_thermoacetica_ATCC_39	IKC P LLTAER	IED A KRR G KD	VVVNDT	---Y	KSMAYSRGAS	AL G VA L AL G	---	E I SADKI	---
Clostridium_asparagiforme_DSM_	IKC P LLTSER	ILD A KSR G KD	VVVHDT	---Y	ESMGYNRGAS	AL G VA I AL G	---	E I PAAEV	---
AD25_Arthrobacter_sp._AD25	VKC P LLTPAK	IAS A RSR G CA	PVTTDT	---Y	ESMGYSRGAS	AL G IA L A T E	---	E V PSFML	---
Comamonas_sp._A2	VKC P LLTPAK	IAS A RSR G CA	PVTTDT	---Y	ESMGYSRGAS	AL G IA L A T E	---	E V PSFML	---
gi 75369013 sp Q6PMK9 Q6PMK9_9	VKC P LLTPAK	IAS A RSR G CA	PVTTDT	---Y	ESMGYSRGAS	AL G IA L A T E	---	E V PSFML	---
AtzD_ADP_Pseudomonas_sp._ADP	VKC P LLTPAK	IAS A RSR G CA	PVTTDT	---Y	ES	---	---	---	---
Pseudomonas_nitroreducens	VKC P LLTPAK	IAS A RSR G CA	PVTTDT	---Y	ES	---	---	---	---
USDA_110_Bradyrhizobium_japoni	IKC P LLTSDR	VEA A SAR G NK	TAT TSA	---Y	GSMAYSRGAS	AL G VA V AL G	---	E T G-S D I	---
viciae_3841_Rhizobium_legumino	IKC P LLTSRR	IAE A EA A GRT	VATHDT	---L	KSMGLSRGAS	AL G VA V AL G	---	E I DATSI	---
trifolii_WSM1325_Rhizobium_leg	IKC P LLTSRR	IAE A EA A GRT	VATHDT	---L	KSMGLSRGAS	AL G VA V AL G	---	E I DATSI	---
Agrobacterium_vitis_S4	IKC P LLTSAR	VAE A QAR G KR	TVIAET	---L	KSMGYSRGAS	AL G VA V AL G	---	E L DMDDI	---
ORS278_Bradyrhizobium_sp._ORS2	VKC P LLTMER	IEA A EA R GAR	TAVRDT	---L	KSMGFSRGAS	AL G VA V AL G	---	E L AFDEL	---
BTAil_Bradyrhizobium_sp._BTAil	VKC P LLTMER	IEA A EA R GTT	TAVRDT	---L	KSMFSRGAS	AL G A V AL G	---	E L TLSDL	---
4_46_Methylobacterium_sp._4_46	VKC P LLTSER	IGA A LAR G AA	PATRDT	---L	KSMGLSRAAA	AL G A A LAL G	---	E V PAAAI	---
AZC_3892_2Azorhizobium_caulino	VKC P LLTAMR	VKE A EA R GAT	TATSDT	---L	KSMGLSRGAS	AL G IA L AL G	---	E V AEDAL	---
Paenibacillus_sp._JDR-2	IKC P LLTAEQ	VQE A NLR G AS	TVTEDI	---Y	KSMGYSRGAS	AL G A V AL G	---	E I QRERL	---
Oceanicola_granulosus_HTCC2516	VKG P AFAQA-	--DLA G AAD G	LASDDP	---G	KLWGFCAA	AL G VA R AL G	---	E V PAAEA	---
Acidithiobacillus_ferroxidans	IKG P AIPAP-	--DSTTT C N-	VRDEHL	---R	CDMAWSRGAS	AL G VA L AL G	---	E V TESEL	---
JCM_2831_Methylobacterium_radi	VKC P LLTSAR	IAD A AA R CHG	VATHDT	---Y	ASMGLSRGAS	AL G IA L AL G	---	E V DREQI	---
Micromonas_sp._RCC299	IKC P LLTPER	IAE A AA R GET	CAT TDC	---Y	ESMALSRGAS	SL G AM V A V E	(10)	DARVDDA	---
AZC_3203_1Azorhizobium_caulino	VKG P LLTPAT	IAD A DRR G AR	LVTRDP	---N	GSKPYARGAM	AL G VA L AL G	---	E V AAERI	---
Bacillus_cellulosilyticus_DSM_	VKC P WGVGG-	---	---	---	SLAKATS	AL G SA V A L K	---	E V DREVI	---
Frankia_sp._Eu1lc	VKG P ALSSRA	VAD A LSR G KT	VV T GD Y (4)	M	GSMCWSNDAS	AL G VA V AL G	---	E V KRDLV	---
Consensus/80%	hKsPLLT..p	l.sA..RGps	sh*pcT...b		cSMsbSpGs*	AL G IA L AL G	...	E I ..s.l	---

Janibacter_sp._HTCC2649	TDADVMHNR	FSVASCSS	VELDQAQIV	VVG---NARG	VGGRYRIGHS	VMRDALDADG
Intrasporangium_calvum_DSM_430	SDEDIMHNR	YSVASCSS	VELDQAQVV	VVG---NTRG	IGGRYRIGHS	VMRDALDADG
Noca_1505_1Nocardioides_sp._JS	SDEDVMHDR	YSVASCSS	VELDQAQVV	VVG---NAPG	VGGRYRIGHS	VMKDALDQDG
PR4_Rhodococcus_erythropolis_P	TDEDVMHSRE	FSVASCSS	VELDRAQIV	VVG---NARG	VGGRYRIGHS	VMKDPLDQDG
SK121_Rhodococcus_erythropolis	TDEDVMHSRE	FSVASCSS	VELDRAQIV	VVG---NARG	VGGRYRIGHS	VMKDPLDQDG
Barbiturase_Rhodococcus_erythr	TDEDVMHSRE	FSVASCSS	VELDRAQIV	VVG---NARG	VGGRYRIGHS	VMKDPLDQDG
jostii_RHA1_Rhodococcus_jostii	SDADVMHRR	FSVASCSS	VELDRAQVV	VVG---NARG	IGGRYRIGHS	VMKDPLDQDG
B4_Rhodococcus_opacus_B4	DDADVMHRR	YSVASCSS	VELDRAQIV	VVG---NARG	IGGRYRIGHS	VMTDPLDQDG
Saccharomonospora_viridis_DSM_4	SDEDVTKNR	FSVASCSS	VELDRAQIV	VVG---NARG	VGGRYRIGHS	VMQDALDQDG
Nakamurella_multipartita_DSM_4	GDADVMHDR	FSVASCSS	VELDQAQVV	VVG---NARG	VGGRYRIGHS	VMQDALDADG
Catenulispora_acidiphila_DSM_4	SDEAVLHDRA	YSVASCSS	VELDRAQVV	VVG---NASG	VGGRYRIGHA	VMRDALDAEG
Streptosporangium_roseum_DSM_4	TADQIHRDLS	YSVASCSS	VELDRAQIV	VVG---NVRG	IGGRYRIGHS	VMKDALDADG
Thermobispora_bispora_DSM_4383	REDQIHRDLS	YSVASCSS	VELDRAQIV	VVG---NVRG	IGGRYRIGHS	VMKDALDADG
Geodermatophilus_obscurus_DSM_	EQGQVMRDLS	FSVASCSS	VELDRAQIV	VVG---NARG	HGGAYRIGHS	VMRDALDQDG
Noca_4270_2Nocardioides_sp._JS	TAEQIFHDLS	YSVASCSS	VELDQAQIV	VVG---NARG	VGGFRVGHG	IMKDALDMDG
Aminobacter_aminovorans	SDEVITADYS	YSVASTSA	GIELMNEIIV	VVG---NSRA	WGGDL----	-----
Chelatobacter_heintzii	SDEVITADYS	YSVASTSA	GIELMNEIIV	VVG---NSRA	WGGDL----	-----
TRZD_Enterobacter_cloacae	SDEVITADYS	YSVASTSA	GIELMNEIIV	VVG---NSRA	WGGDLVIGHA	EMKDALDGAA
TrzD_Acidovorax_citrulli	SDEVITADYS	YSVASTSA	GIELMNEIIV	VVG---NSRA	WGGDLVIGHA	EMKDALDGAA
Moorella_thermoacetica_ATCC_39	SNEAICHHDWN	YSVASTSA	VELLNDEIIV	VVG---NSTN	SASDLVIGHS	VMKDALDADA
Clostridium_asparagiforme_DSM_	SQETICHDYS	YSKVASTSA	VELLNCEIIV	VVG---NSVN	STSDYRIGHS	VMEDALDFKA
AD25_Arthrobacter_sp._AD25	VDESVLNDWS	SSSLASASA	GIELEHNVVI	VVG---MSEQ	ATSELVTAHG	VMSDALDAAS
Comamonas_sp._A2	VDESVLNDWS	S-----	-----	-----	-----	-----
gi 75369013 sp Q6PMK9 Q6PMK9_9	VDESVLNDWS	SSSLASASA	GIELEHNVVI	VVG---MSEQ	ATSELVTAHG	VMSDALDAAS
AtzD_ADP_Pseudomonas_sp._ADP	VDESVLNDWS	SSSLASASA	GIELEHNVVI	VVG---MSEQ	ATSELVTAHG	VMSDALDAAS
Pseudomonas_nitroreducens	-----	-----	-----	-----	-----	-----
USDA_110_Bradyrhizobium_japoni	SDGDVLRYYD	FSKVASTSA	GIELMHNVI	VVG---NSAA	SASEFETGHA	VMNDALDAAA
viciae_3841_Rhizobium_legumino	NDADICTRFD	FSRCASTSS	VELTDHEIIV	VVG---MSAK	WSGPLSDHA	VMRDALDAHS
trifolii_WSM1325_Rhizobium_leg	GDADICTRFD	FSRCASTSS	GELTDHEIIV	VVG---MSAK	WSGPLSDHA	VMLDALDAHS
Agrobacterium_vitis_S4	KDTDICTRLD	YSARASTSA	VELTDHEIIV	VVG---MSDH	WSGPFAMTHT	VMFDAMDAPS
ORS278_Bradyrhizobium_sp._ORS2	SDADICADYS	RYSERAAATSG	VELLDHEIM	VAG---MSAR	WTGPLADHG	VMRDALDIEP
BTAil_Bradyrhizobium_sp._BTAil	SDAMICADYA	RYSERAAATSG	VELLDHEIM	VAG---MSAD	WTGPLADHA	VMADALDIEP
4_46_Methylobacterium_sp._4_46	GETVAETDPG	RHARRCGASA	VELLDHEVV	VVG---MSPD	WTGPLVDHA	VMADALDLRP
AZC_3892_2Azorhizobium_caulino	SDAVICADYG	WSARASCSS	GIELLGHEIV	VVG---MSEG	WSGPLATAHG	VMADALDVTP
Paenibacillus_sp._JDR-2	QDTDICHSWE	YSKVASTSA	GSELAYCEVI	VVG---NSAY	AEGPYFDHD	VMKDALDAGA
Oceanicola_granulosus_HTCC2516	NEAAVLRDFS	VRNVASCSS	GELVSCNEVI	VVG---MAPG	WSGPLATAHA	PMADALDVAA
Acidithiobacillus_ferroxidans	SDDVINQDWS	YSRASVSA	KPLQRSEMV	LFA---NSCW	WEGDLVTAHG	VMQDILDVPA
JCM_2831_Methylobacterium_radi	TDPAIGTRRD	FSGRASASA	GIELMRNEIIV	VVG---NAPG	WTGPLATAHR	VMDDGIDLPA
Micromonas_sp._RCC299	VYAKTCEDYS	VFSDHVSSA	VELMHCELL	MML(6)SKT	PRSKFRMARG	RMDDALDLAG
AZC_3203_1Azorhizobium_caulino	TPDMIACDME	VFSAVASTSA	GELTKCEVL	LFG---NAPG	ATSAFRIGHG	VLKDALDVAG
Bacillus_cellulosilyticus_DSM_	DEDSLINEDHS	FSVKTSVSA	QEQVAARVI	VVG---NSVK	STSNLYTSG	VMKDALDLKG
Frankia_sp._Eu1lc	ADDRIRSDWD	FSAAVATSS	GGEKRGGEVL	LLA---NSAQ	SASELRIGHG	ITRDMADTEG
Consensus/80%	s-.slhpcbs	LaSshASsSt	GIEL.psp1l	VhG....t..	hst.b.ItHt	VMpDt1D..t

Janibacter_sp._HTCC2649	IWEATKDAGL	ELP--DRPHT	SDIQGRLVN	FLKCEVSQD	QVRCRRNAML	DDSDVHWH-R
Intrasporangium_calvum_DSM_430	IWEATKDAGL	DLP--ERPHW	TDIDGRLVN	FLKCEVSQD	MVHGRRNAML	DDSDVHWH-R
Noca_1505_1Nocardioides_sp._JS	IWEATKDAGL	DLP--ERPRT	SDLDGRLVN	FLKCEASQD	LVRGRRNAML	DDSDVHWH-R
PR4_Rhodococcus_erythropolis_P	IWAATRDAGL	ELP--ERPHS	SDLDGQLVN	FLKCEASQD	TVRCRRNAML	DDSDVHWH-R
SK121_Rhodococcus_erythropolis	IWAATRDAGL	ELP--ERPHS	SDLDGQLVN	FLKCEASQD	TVRCRRNAML	DDSDVHWH-R
Barbiturase_Rhodococcus_erythr	IWAATRDAGL	ELP--ERPHS	NDLDGQLVN	FLKCEASQD	TVRCRRNAML	DDSDVHWH-R
jostii_RHA1_Rhodococcus_jostii	IWAATRDAGL	ELP--ERPHT	SDLDGQLVN	FLKCEASQD	TVRCRRNAML	DDSDVHWH-R
B4_Rhodococcus_opacus_B4	IWSAIRDAGL	ELP--ERPHT	SDLDDRLVN	FLKCEASQD	TVRCRRNAML	DDSDVHWH-R
Saccharomonospora_viridis_DSM_	IWQATRDAGL	KLP--ENPHP	KDIDGRLVN	FLKCEASPD	TVRCRRNAML	DDSDVHWH-R
Nakamurella_multipartita_DSM_4	IWAATRDAGL	DLP--DRPHP	RDLDGRLVN	FLKCEASQD	QVRCRRNAML	DDSDVHWH-R
Catenulispora_acidiphila_DSM_4	IWDATRAACL	ELP--ERPRS	SDLDGRLVN	FLKCEASTD	MVRCRRNAML	DDSDVHWH-R
Streptosporangium_roseum_DSM_4	IWEATRSGI	DLP--DRPHP	SDLGGRLVN	FMKCEADPS	SVRCRRNIML	DDSDVHWH-R
Thermobispora_bispora_DSM_4383	IWEATRNASL	DLP--ERPHP	SDLKGRLVN	FLKCEADPT	RVRCRRNIML	DDSDVHWH-R
Geodermatophilus_obscurus_DSM_	IWNATRDAGL	DLP--ERPHP	SDLGDRLVN	FLKCEADPT	YVRCRRNAML	DDSDVFWH-R
Noca_4270_2Nocardioides_sp._JS	VWAATRDAGL	DDMPVDCIHP	RHIKGRLVNL	FLKCEADPT	RVRCRRNIML	DDSDVAWH-R
Aminobacter_aminovorans	-----	-----	-----	-----	-----	-----
Chelatobacter_heintzii	-----	-----	-----	-----	-----	-----
TRZD_Enterobacter_cloacae	VRQALRDVGC	CEN---DLPT	VDELGRVVN	FAKAEASPD	EVRNRRHTML	DDSDINST-R
TrzD_Acidovorax_citrulli	VRQALRDVGC	CEN---DLPT	VDELGRVVN	FAKAEASPD	EVRNRRHTML	DDSDINST-R
Moorella_thermoacetica_ATCC_39	VRAALKDAGL	KFD---CCPP	AEELAKIVN	LAKAEAASS	TVRCRRNTML	DDSDINHT-R
Clostridium_asparagiforme_DSM_	VIEATQSACL	EVD---KYPT	DEQAKIIVN	FAKAEADST	YVRCRRNTML	DDSDINHT-R
AD25_Arthrobacter_sp._AD25	VRRTIESLGL	IRSD---DE	MDR---IVNV	FAKAEASPD	VVRCMRHTML	SDSDINST-R
Comamonas_sp._A2	-----	-----	-----	-----	-----	-----
gi 75369013 sp Q6PMK9 Q6PMK9_9	VRRTIESLGL	IRSD---DE	MDR---IVNV	FAKAEASPD	VVRCMRHTML	SDSDINST-R
AtzD_ADP_Pseudomonas_sp._ADP	VRRTIESLGL	IRSD---DE	MDR---IVNV	FAKAEASPD	VVRCMRHTML	SDSDINST-R
Pseudomonas_nitroreducens	-----	-----	-----	-----	-----	-----
USDA_110_Bradyrhizobium_japoni	VTSALKCVG	LGVA---PQ	AEAGRELVNI	FAKAEASPD	SVRCFRHTML	EDTDISST-R
viciae_3841_Rhizobium_legumino	VRKARERLP	-----	-ENS-RLAAV	LAKAEPDPS	EIDGRRHTML	DDSDIAGT-R
trifolii_WSM1325_Rhizobium_leg	VRKARERLP	-----	-ENS-RLAAV	LAKAEPDPS	RIDGRRHTML	DDSDIAGT-R
Agrobacterium_vitis_S4	VRAAWSMDP	-----	-LG--KLSAV	LAKAEPDPR	TVRCRRHTML	EDSDISGT-R
ORS278_Bradyrhizobium_sp._ORS2	ARAALSRLGL	DGP--GQLPA	ASRG-RIAAV	LAKAEAAQS	KVRCRLHTML	DDSDVSSST-R
BTAil_Bradyrhizobium_sp._BTAil	ARAALARLGI	DSA--GQVPA	AARG-RIAAV	LAKAEAAQS	RLRCQRHTML	DDSDIAST-R
4_46_Methylobacterium_sp._4_46	VAACLGRLGL	LGPD-GFVDE	AGRA-RLAAL	LAKAEASAD	AIRGRRHTML	DDSDIAPT-R
AZC_3892_2Azorhizobium_caulino	VKAALSALG	-----	AEAG-EATIV	LAKAEPSRS	RIRGKRHTML	DDSDISPT-R
Paenibacillus_sp._JDR-2	LKRILEKYP	-----	--DA-EMVQV	LAKAEADPT	YIRGQRHTML	DDSDINHT-R
Oceanicola_granulosus_HTCC2516	VHHALAGVGL	AAAP--QLAA	GDAA-RLRAV	FVKGEAARS	LIRGRGHTML	NDGDDQQ-R
Acidithiobacillus_ferroxidans	IHAVLNQLCL	QPVN-GQLAD	VDTK-KVLAV	FAKSDADPR	HIRHRRHTML	TDADISDM-R
JCM_2831_Methylobacterium_radi	ILGVLSDLGF	PEAG--PLSR	QESE-RVIAL	LVKAEPSHD	LIRGRRHTML	DDSDINAT-R
Micromonas_sp._RCC299	VTRCVDAAR	-----	GSRG-TNTIV	TAIVKADPAA	SVRCRRHTML	SDSDIHAT-R
AZC_3203_1Azorhizobium_caulino	VKEALRSAGL	SFHG---TPS	EEQAHEIVAV	FAKAEAPVN	RIRGRRHTML	SDADIHYE-R
Bacillus_cellulosilyticus_DSM_	MQSAFKDAGI	NHS---DTLS	EEQAQKIVQV	FVNAGADAIN	EVRGRRHTML	TDALAMHS-I
Frankia_sp._Eu1lc	IKTARTAGV	DFD---CCLS	PAQQAQVVQV	FGKFVLPDGS	VLRGQHITAML	DDHEAHV--
Consensus/80%	lb.slpsHgl	..ssV.p...	-.sclssV	bhKsEss.sG	.lRGpRpsML	sSDSlp.pGR

Janibacter_sp._HTCC2649	QIKSCVGGVT	ASVTGDPAVF	VSVSA--AHQ	GFDCGGPVAA	IIDLGE(12)
Intrasporangium_calvum_DSM_430	QIKAAVGGVT	AAVTGDPAVF	VSVSA--AHQ	GFDCGGPVAA	IIDLGE(9) -
Noca_1505_1Nocardioides_sp._JS	QIKSCVGGVT	AAVTGDPAVF	VSVSA--AHQ	GFDCGGPVAA	IVDLG-----
PR4_Rhodococcus_erythropolis_P	QIKSCVGGVT	AAVTGDPAVF	VSVSA--AHQ	GFDCGGPVAA	IVDLGQ----
SK121_Rhodococcus_erythropolis	QIKSCVGGVT	AAVTGDPAVF	VSVSA--AHQ	GFDCGGPVAA	IVDLGQ----
Barbiturase_Rhodococcus_erythr	QIKSCVGGVT	AAVTGDPAVF	VSVSA--AHQ	GFDCGGPVAA	IVDLGQ----
jostii_RHA1_Rhodococcus_jostii	QIKSCVGGVA	SSVTGDPAVF	VSVSA--AHQ	GFDCGGPVAA	IVDLGQ----
B4_Rhodococcus_opacus_B4	QIKSCVGGVA	SSVTGDPAVF	VSVSA--AHQ	GFDCGGPVAA	IVDLGQ----
Saccharomonospora_viridis_DSM_	QIKACVGGVT	AAVTGDPAVF	VSVSA--VHQ	GFAGGGPVAA	IVDLGD-(8) -
Nakamurella_multipartita_DSM_4	QIKACVGGVT	AAVTGDPAAF	VSVSA--AHQ	GFDCGGPVAA	IVDLG-----
Catenulispora_acidiphila_DSM_4	QIKACVGGVA	ASVTGDPAVF	VSVSA--AHQ	GFDCGGPVAA	IVEVGPGA--
Streptosporangium_roseum_DSM_4	QIKATVGGVA	ASVTGDPAVF	VSVAA--VHQ	GFSGGGPVAA	IADLG-----
Thermobispora_bispora_DSM_4383	QIKAAVGGVA	ASVTGDPAVF	VSVAA--VHQ	GFAGGGPVIA	IVDHG-----
Geodermatophilus_obscurus_DSM_	QIKATVGGVA	ASVTGDPAVF	VSVAA--VHQ	GFSGGGPVAA	IYKA-----
Noca_4270_2Nocardioides_sp._JS	QIKACVGGVV	AAVSGDPMNF	VSVAA--VHQ	GFSGGGPVIA	IYDLEA----
Aminobacter_aminovorans	-----	-----	-----	-----	-----
Chelatobacter_heintzii	-----	-----	-----	-----	-----
TRZD_Enterobacter_cloacae	HARAVVNAVI	ASIVGDPMVY	VSGGG--EHQ	GFAGGGPVAV	IARTA-----
TrzD_Acidovorax_citrulli	HARAVVNAVI	ASIVGDPMVY	VSGGG--EHQ	GFAGGGPVAV	IARTA-----
Moorella_thermoacetica_ATCC_39	SARAVVNAVI	ASVVGDPMVY	VSGGG--EHQ	GFDCGGPIAV	IARV-----
Clostridium_asparagiforme_DSM_	HARAVVNGVI	AAIVNDPMVY	VSGGG--EHQ	GFDCGGPVAV	IYKR-----
AD25_Arthrobacter_sp._AD25	HARAVTGAAI	ASVVGHTVY	VSGGG--EHQ	GFAGGGPFVAV	IARA-----
Comamonas_sp._A2	-----	-----	-----	-----	-----
gi 75369013 sp Q6PMK9 Q6PMK9_9	HARAVTGAAI	ASVVGHTMVY	VSGGG--EHQ	GFAGGGPFVAV	IARA-----
AtzD_ADP_Pseudomonas_sp._ADP	HARAVTGAAI	ASVVGHTMVY	VSGGG--EHQ	GFAGGGPFVAV	IARA-----
Pseudomonas_nitroreducens	-----	-----	-----	-----	-----
USDA_110_Bradyrhizobium_japoni	HARAAVGGLI	AGLAFGTGAVY	VSGGG--EHQ	GFAGGGPVAV	IARLSD----
viciae_3841_Rhizobium_legumino	HARAFVGGVL	AGIFGITDLY	VSGGG--EHQ	GFPGGGPVAI	IYKEQ-----
trifolii_WSM1325_Rhizobium_leg	HARAFVGGVL	AGIFGITDLY	VSGGG--EHQ	GFPGGGPVAI	IYKEQ-----
Agrobacterium_vitis_S4	HARAFVGGVL	AGVFGETDLY	VSGGG--EHQ	GFPGGGPVAI	IYREDVI--
ORS278_Bradyrhizobium_sp._ORS2	HARAFVGGAL	AGLFGFTDLF	VSGGG--EHQ	GFDCGGPVAI	IYERR-----
BTAi1_Bradyrhizobium_sp._BTAi1	HARAFVGGAL	AGLFGFTDLF	VSGGG--EHQ	GFDCGGPIAI	IYVERT----
4_46_Methylobacterium_sp._4_46	HARAFVAGAL	AGLVGATDLF	VSGGG--EFQ	GFDCGGPVAV	IARRSGAASP A
AZC_3892_2Azorhizobium_caulino	HARAFVAGAL	AGVVGHTETIY	VSGGG--EHQ	GFDCGGPVAV	IYAARTMG---
Paenibacillus_sp._JDR-2	HARAVVGGVL	ASVCGDPMIY	VSGGG--EHQ	GFAGGGPVAV	IYFRN-----
Oceanicola_granulosus_HTCC2516	HIRAALGAVV	AATLGETQLF	VSGGG--EHQ	GFAGGGLVAV	IYAERT-----
Acidithiobacillus_ferroxidans	YSRCVVSALL	AGITGETGVY	VSTRA--EHQ	GFQGGGPVAV	IYGYAS-----
JCM_2831_Methylobacterium_radi	HARALVGGVA	AAAIERTDLF	VSGGG--EHQ	GFDCGGPVAV	IYARVRD----
Micromonas_sp._RCC299	HARAAGGVV	AAAVGDCRVY	VSGGG--EHQ	GFDCGGPVAV	IYRVGGAR--
AZC_3203_1Azorhizobium_caulino	HARAAGGAVI	ASVTGDPAIF	VSGGT--EHQ	CAPGGAAPIA	IYVRA-----
Bacillus_cellulosilyticus_DSM_	VAKAVANAVV	GSYVGETRIIL	CSAGS--EHQ	GFQGSNLVAA	IYRVGGIR--
Frankia_sp._Eu1lc	-AKAVGGALV	VSITGQPMSE	IYGGERNSHM	GFPGGNPVAA	IYVRLPA---
Consensus/80%	ph+thVtllh	AtlsGcs.la	VSstARN.HQ	GPsGGGPVAs	Isc...s.GP A

Fig. S3

Mass spectra of cyanuric acid hydrolase reaction products using (A) unlabeled and (B) labeled cyanuric acid substrate. Peak distribution from electrospray ionization in negative mode over the approximate reaction time course for m/z of 244.0193 ± 0.058 Da and 247.0196 ± 0.038 Da for unlabeled and labeled, respectively. These values are the averaged ion mass corresponding to the carboxybiuret anion complexed with potassium and acetate ions.

