A Novel Deaminase Involved in Chloronitrobenzene and Nitrobenzene Degradation with Comamonas sp. Strain CNB-1

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Comamonas sp. strain CNB-1 degrades nitrobenzene and chloronitrobenzene via the intermediates 2-amino-5-chloromuconate and 2-aminomuconate, respectively. Deamination of these two compounds results in the release of ammonia, which is used as a source of nitrogen for bacterial growth. In this study, a novel deaminase was purified from Comamonas strain CNB-1, and the gene (cnbZ) encoding this enzyme was cloned. The N-terminal sequence and peptide fingerprints of this deaminase were determined, and BLAST searches revealed no match with significant similarity to any functionally characterized proteins. The purified deaminase is a monomer (30 kDa), and its Vmax values for 2-aminomuconate and 2-amino-5-chloromuconate were 147 μmol · min−1 · mg−1 and 196 μmol · min−1 · mg−1, respectively. Its catalytic products from 2-amino-muconate and 2-amino-5-chloromuconate were 2-hydroxymuconate and 2-hydroxy-5-chloromuconate, respectively, which are different from those previously reported for the deaminases of Pseudomonas species. In the catalytic mechanism proposed, the α-carbon and nitrogen atoms (of both 2-amino-muconate and 2-amino-5-chloromuconate) were simultaneously attacked by a hydroxyl group and a proton, respectively. Homologs of cnbZ were identified in the genomes of Bradyrhizobium japonicum, Rhodopsseudomonas palustris, and Roseifexus sp. strain RS-1; these genes were previously annotated as encoding hypothetical proteins of unknown function. It is concluded that CnbZ represents a novel enzyme that deaminates xenobiotic compounds and/or α-amino acids.

2-Aminomuconate deaminase (2-aminomuconate aminohydrolase; EC 3.5.99.5) converts 2-aminomuconate to 4-oxaloconatone (2-hydroxymuconate) and ammonia. It is involved in biodegradation of natural compounds, such as tryptophan (4), as well as xenobiotic compounds, such as nitrobenzene (6, 7), aminophenol (17), and 2-nitrobenzoate (11). 2-Aminomuconate deaminases have been identified in several Pseudomonas strains (17) and Burkholderia cepacia (4). A new type of deaminase has recently been discovered in Bordetella sp. strain 10d that is highly specific for 2-amino-5-carboxymuconic 6-semialdehyde; 2-aminomuconate is not deaminated (12). Neither the nucleotide nor amino acid sequence of this deaminase has been reported.

Certain bacteria or microbial consortia can utilize 4-chloronitrobenzene (4-CNBr) as the sole source of carbon and nitrogen (9, 14, 22). 2-Amino-5-chloromuconate is an intermediate in the 4-CNBr degradation pathway of Comamonas sp. strain CNB-1 (21). cnbH occurs in the degradative cnb cluster and encodes 2-amino-5-chloromuconate deaminase (21). However, the specific activity of CnbH expressed in Escherichia coli is 7 nmol min−1 · (mg of cellular protein)−1 · (2-aminomuconate as the substrate) (21), an activity that would appear to be too inefficient for bacterial growth. Indeed, the deaminase activity of cellular lysates of Comamonas sp. strain CNB-1 approximates 2.5 μmol min−1 · (mg of cellular protein)−1 · (2-aminomuconate as the substrate) (unpublished data). These contrasting observations indicate that Comamonas sp. strain CNB-1 might contain an alternative deaminase and prompted the present study.

MATERIALS AND METHODS

Bacterial strains, cultivation, and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. Comamonas sp. strain CNB-1 (20, 21) was isolated from activated sludge and was usually cultivated with Luria-Bertani (LB) medium or mineral salts basic broth (MSB) (10) containing 2 mM 4-CNBr as the sole source of carbon and nitrogen. All Escherichia coli strains were cultivated in LB medium. When necessary, ampicillin and kanamycin were added at final concentrations of 100 and 30 μg ml−1, respectively.

Purification of deaminase from Comamonas sp. strain CNB-1. Cellular lysates were prepared from 1.2 g of cells (wet weight) of Comamonas sp. strain CNB-1 grown in LB broth. The cell pellet was suspended in 10 ml buffer A (pH 8.0) composed of 20 mM Tris-HCl, 10% (vol/vol) ethanol, 1 mM dithiothreitol, and 0.5 mM L-ascorbic acid. The suspended cells were disrupted by sonication. Cellular debris was removed by centrifugation at 17,000 × g for 10 min at 4°C. The supernatant fluid was subjected to (NH4)2SO4 fractionation. Proteins that precipitated between 20% and 50% (NH4)2SO4 saturation were dissolved in 2 ml buffer A and subsequently applied to a Superdex 200 column, which was operated with an AKTA fast protein liquid chromatography (FPLC) system (Amerham Biosciences). The proteins were separated with buffer A at a flow rate of 0.5 ml min−1. Eluant (1 ml per fraction) was analyzed for protein concentration and enzyme activity. Fractions (from 28 ml to 30 ml) with 2-amino-5-chloromuconic acid deaminase activity were pooled and loaded onto a Mono Q HR5/5 ion-exchange column (Amerham Biosciences). The column was washed with buffer A and proteins were eluted at a flow rate of 0.4 ml min−1 with a linear gradient (50 to 500 mM) of NaCl. Eluants of 300 μl per tube were collected. The 2-amino-5-chloromuconate deaminase was recovered in tubes 1 to 4.

Determination of native molecular mass and N-terminal sequence. The native molecular mass of the deaminase was estimated by gel filtration chromatography with a Superdex 200 column (Amerham Biosciences) at a flow rate of 0.5 ml...
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min⁻¹ with Tris-HCl (50 mM; pH 8.0)-NaCl (0.1 M) buffer. Carbonic anhydrase (29 kDa), albumin (66 kDa), alcohol dehydrogenase (150 kDa), β-amylase (200 kDa), apoferritin (443 kDa), and thyroglobulin (669 kDa) (all from Sigma) were used as molecular mass standards.

To check the purity and determine the N-terminal sequence of 2-amino-5-chloromuconate deaminase, the FPLC-purified enzyme was subjected to sodium difluoride membrane by electroblotting and visualized by Coomassie brilliant amide). The resulting single protein band was transferred to a polyvinylidene chloromuconate deaminase, the FPLC-purified enzyme was subjected to sodium chloride (12%) acrylamide). The resulting single protein band was transferred to a polyvinylidene difluoride membrane by electroblotting and visualized by Coomassie brilliant blue staining. The protein band was cut out and sequenced by the Edman degradation method using an Applied Biosystems Procise 491 protein sequence.

**Operation of Q-TOF-MS and peptide identification.** For identification of the peptide fingerprint and obtaining more information on the sequence of the purified deaminase, the deaminase was subjected to digestion by trypsin. The tryptic peptides were separated on a Waters capillary liquid chromatography system. The system was coupled on-line with a quadrupole time-of-flight (Q-TOF) Ultima Global mass spectrometer (Waters), and the samples were system. The system was coupled on-line with a quadrupole time-of-flight (MS/MS) data search was processed using Mass Lynx 4.0 and an online MS/MS ion-searching program.

**Plasmid DNA extraction and sequencing.** The plasmid pCNB1 was isolated from strain CNB-1 by using the modified alkaline lysis method (16). The plasmid DNA was sequenced using a shotgun method. A contig was obtained containing the plasmid pCNB1. The PCR product was purified, treated with NdeI and HindIII, and ligated into pET2a (+) which had been digested with the same restriction enzymes. The resulting plasmid, designated pETcnBZ, was used to transform E. coli BL21(DE3). Synthesis of recombinant protein in E. coli BL21(DE3) cells was initiated by addition of 1 mM isopropyl 1-thio-β-d-galactopyranoside (IPTG) when the culture reached an optical density of 0.6 to 0.8. Cultivation was continued for an additional 4 h after the addition of IPTG. Cells were harvested by centrifugation and broken by sonication as described above. Recombinant cnbZ-derived protein was purified with a His-Blind protein purification kit (Novagen, Madison, WI) according to the manufacturer’s instructions.

**Cloning, overexpression, and purification of the deaminase (CnbZ) in E. coli.** PCR primers (Table 1) were designed according to the DNA sequence of cnbZ to amplify the entire 2-amino-5-chloromuconate deaminase gene (753 bp) from the plasmid pCNB1. The PCR product was purified, treated with NdeI and BamHl, and ligated into pET2a (+) which had been digested with the same restriction enzymes. The resulting plasmid, designated pETcnBZ, was used to transform E. coli BL21(DE3). Synthesis of recombinant protein in E. coli BL21(DE3) cells was initiated by addition of 1 mM isopropyl 1-thio-β-d-galactopyranoside (IPTG) when the culture reached an optical density of 0.6 to 0.8. Cultivation was continued for an additional 4 h after the addition of IPTG. Cells were harvested by centrifugation and broken by sonication as described above. Recombinant cnbZ-derived protein was purified with a His-Blind protein purification kit (Novagen, Madison, WI) according to the manufacturer’s instructions.

**Deaminase activity and extinction coefficient of 2-amino-5-chloromuconic acid.** 2-Aminomuconate deaminase activity was determined as described by He and Spain (7). 2-Amino-5-chloromuconate deaminase activity was determined similarly, but ΔA415 was used as the maximum absorption of 2-amino-5-chloromuconate (CnbZ) was monitored. The extinction coefficient of 2-amino-5-chloromuconate was determined by a combination of the extinction coefficient of 2-hydroxy-5-chloromuconate (ε = 12,650 M⁻¹·cm⁻¹) (15) and of a complete enzymatic conversion of 2-amino-5-chloromuconate to 2-hydroxy-5-chloromuconate. The extinction coefficient of 2-amino-5-chloromuconate was 22,000 M⁻¹·cm⁻¹ based on the Beer-Lambert law.

**RESULTS**

Purification of 2-amino-5-chloromuconate deaminase. A typical purification (Table 2) yielded a 25.7-fold purification with a recovery of 3.3% of the 2-amino-5-chloromuconate deaminase activity. An analysis of the purified protein by SDS-PAGE revealed a single band with a molecular mass of 28 kDa. Gel filtration chromatography revealed a native molecular mass of 30 kDa, indicating that this enzyme is a monomer.

**Alignment of proteins.** Alignment of CnbZ and other related putative proteins from genomes of different bacterial species was carried out with CLUSTALW (18). Nucleotide sequence accession number. The DNA sequence reported here is available at GenBank under accession number DQ875599.
mined to be PDAVVSFP. BLASTN and BLASTP searches at the GenBank and PDB databases revealed no match with significant similarity. This purified deaminase was further analyzed with Q-TOF-MS, and six peptides (SAGLARHVFELER, VVFVLEE MESR, GLAAEPGFNG, LVAFGDTSPEGLR, AVVFSSLS SR, VVFVLEEFESR) were obtained. Efforts to discover other known proteins with similar peptide fingerprint patterns by using the MASCOT tool revealed no match with a significant score.

Cloning of the 2-amino-5-chloromuconate deaminase gene. Before the plasmid pCNB1 was sequenced, various efforts to obtain the deaminase gene from genomic DNA were carried out with pairs of degenerate primers based on the N-terminal sequence and the six peptides but did not succeed. Meanwhile, it was found that a mutant of strain CNB-1, namely, strain CNB-2, which had lost plasmid pCNB1 (21), had also lost deaminase activity. This indicated that the deaminase gene was possibly located on plasmid pCNB1. As the pCNB1 sequence project was completed, examination of the sequence of pCNB1 revealed a contig flanked by two transposases (Fig. 1). In addition to the transposases, this contig contained a hypothetical protein that has an N-terminal sequence identical to that of the purified deaminase, as well as the previously identified six peptides, according to Q-TOF-MS. This putative gene (named cnbZ, herein) was PCR amplified and cloned onto vector pET28a(+), resulting in pETcnbZ. E. coli BL21(DE3) cells harboring pETcnbZ showed high deaminase activity upon induction with 1 mM IPTG. This result clearly indicated that cnbZ encodes the previously purified deaminase.

Further analysis of the DNA fragment containing cnbZ indicated that putative promoter regions (−35 and −10 sites), as well as a putative ribosome binding site, are located upstream of cnbZ (Fig. 1). It is deduced that cnbZ is independently transcribed from its neighboring genes. The genes upstream of cnbZ are putative genes for arsenate resistance, which are oriented divergently to cnbZ. Downstream of cnbZ is an ORF, orfZ, whose function is unknown (Fig. 1 and Table 3). All ORFs and their homologs with the highest similarities from GenBank are listed in Table 3. Very interestingly, Orf1 showed 100% identity to a hypothetical protein that was recently identified from an ongoing genome project for Acidovorax sp. strain JS42 (Table 3).

Identification of CnbZ homologs from various microbial genomes. With the entire amino acid sequence of CnbZ, extensive searches in GenBank and PDB databases for homologs to CnbZ were carried out. Three genome-encoded hypothetical proteins were found. They were BAC48130 (NCBI accession number) from Bradyrhizobium japonicum USDA 110, ABD04858 from Rhodopseudomonas palustris HaA2, and ETA26300 from Roseiflexus sp. strain RS-1. Their identities to CnbZ were 52.3%, 52.5%, and 38.8%, respectively. Alignment of CnbZ and its homologs indicated some conservative amino acid residues and a putative conservative glycosyl hydrolase domain (family 6) in CnbZ (YTVPTTSAAKRPSFQLAGGG). It was concluded that CnbZ represents a novel deaminase.

### TABLE 2. Purification of 2-amino-5-chloromuconate deaminase from Comamonas sp. strain CNB-1

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein (mg)</th>
<th>Total activity (µmol · min⁻¹)</th>
<th>Sp act (µmol · min⁻¹ · mg⁻¹)</th>
<th>Purification (fold)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell extract</td>
<td>179</td>
<td>453</td>
<td>2.5</td>
<td>1.0</td>
<td>100</td>
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<tr>
<td>(NH₄)₂SO₄</td>
<td>79</td>
<td>358</td>
<td>4.5</td>
<td>1.8</td>
<td>79</td>
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<tr>
<td>Superdex 200</td>
<td>0.71</td>
<td>26</td>
<td>36.6</td>
<td>14.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Mono Q HR5/5</td>
<td>0.23</td>
<td>15</td>
<td>65.3</td>
<td>25.7</td>
<td>3.3</td>
</tr>
</tbody>
</table>

FIG. 1. Organization of the genetic fragment containing cnbZ and comparison to other genetic fragments that contain cnbZ homologs. The sequence upstream of cnbZ is shown; the predicted promoter consensus sequence is underlined, the transcription start codon is boxed, and the translational start codon is shown in italics. cnbZ and the putative arsenate resistance genes (arsRABC) are flanked by two putative transposase genes. Sequence identities of ArsC, ArsB, ArsA, and ArsR from Comamonas sp. strain CNB-1 to their counterparts from B. japonicum strain USDA 110 are 59%, 15%, 48%, and 37%, respectively. The sequence identities of Orf2 to Blr7786 and OrfJ are 44% and 41%, respectively; however, the C termini of Blr7786 and OrfJ were not found in Orf2. RBS, ribosome binding site.
CnbZ has novel catalytic properties different from previously known deaminases of *Pseudomonas* species. The 2-amino-5-chloromuconate deaminase used 2-amino-5-chloromuconate and 2-aminomuconate as substrates. Methyl-3-aminocrotonate and vinylglycine as well as saturated $\beta$-amino acids such as glutamic acid, glutamine, aspartic acid, and asparagines were not used as substrates (at concentrations of 50 and 100 $\mu$M). The $V_{\text{max}}$s of CnbZ for 2-amino-5-chloromuconate and 2-aminomuconate were 196 and 147 $\mu$mol $\cdot$ min$^{-1}$ $\cdot$ mg$^{-1}$, respectively, and the $K_m$s were 3.9 and 8.8 $\mu$M, respectively. These data indicated that CnbZ was more adapted for 2-amino-5-chloromuconate than 2-aminomuconate. Cysteine residue-modifying agents such as $p$-chloromercuribenzoate (0.2 mM) and the SH-binding metals ($2$ mM) $\text{Zn}^{2+}$, $\text{Ni}^{2+}$, and $\text{Cu}^{2+}$ completely inhibited deaminase activity, whereas $\text{Ca}^{2+}$, $\text{Mg}^{2+}$, and the histidine residue-modifying agent diethyl pyrocarbonate (at 2 and 10 mM) inhibited the activity by 23 to 50%. EDTA (at 5 and 50 mM) and dithiothreitol (10 mM) did not exert observable effects on deaminase activity. Based on these results, it was deduced that an SH group(s) was probably involved in catalysis.

Typical spectral changes during CnbZ-catalyzed deamination of 2-aminomuconate and 2-amino-5-chloromuconate are shown in Fig. 2. Based on the observations that the maximal absorption of 2-hydroxy-5-chloromuconate occurs at 306 nm and that the maximal absorption of 2-oxopent-5-chloro-3-enoate occurs at 245 nm (15), the product of 2-amino-5-chloromuconate deamination was identified as 2-hydroxy-5-chloromuconate (Fig. 2A). Spontaneous isomerization of 2-hydroxy-5-chloromuconate into 2-oxal-5-chloromuconate did not happen, unless tautomerase CnbG (21) was added to the assay buffer (data not shown). It was found that 2-hydroxymuconate spontaneously isomerized into 4-oxalcrotonate, and the isozomerization rates were almost equally high (3.2 nmol $\cdot$ min$^{-1}$ for spontaneous isomerization of 2-hydroxymuconate) as for 2-amino-5-chloromuconate (3.7 nmol $\cdot$ min$^{-1}$), as determined in this study. Therefore, it was concluded that the true product during 2-amino-5-chloromuconate deamination catalyzed by CnbZ was most probably 2-hydroxymuconate, and this product spontaneously converted into its keto form, 2-oxalmuconate (Fig. 2B).

RT-PCR results indicated that *cnbZ* but not *cnbH* was transcribed during growth on 4-CNB. In a previous study, a differ-

### Table 3. Annotation and identification of homologs of putative genes of the *cnbZ* genetic fragment from *Comamonas* sp. strain CNB-1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position in sequence (bp)</th>
<th>Gene product</th>
<th>No. of residues</th>
<th>Homolog</th>
<th>Source</th>
<th>% Identity</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>tpnA1</em></td>
<td>1–1611</td>
<td>Transposase</td>
<td>536</td>
<td>TpnA</td>
<td><em>Pseudomonas</em> sp. strain ADP</td>
<td>95</td>
<td>NP_862476</td>
</tr>
<tr>
<td><em>orf1</em></td>
<td>1674–2663</td>
<td>Hypothetical protein</td>
<td>329</td>
<td>AjsDRAFT_1040</td>
<td><em>Acidovorax</em> sp. strain JS42</td>
<td>100</td>
<td>ZP_01383765</td>
</tr>
<tr>
<td><em>arsC</em></td>
<td>2686–3042</td>
<td>Putative arsenate reductase pump protein</td>
<td>118</td>
<td>CtesDRAFT_0032</td>
<td><em>C. testosteroni</em>KF-1</td>
<td>80</td>
<td>ZP_01521815</td>
</tr>
<tr>
<td><em>arsB</em></td>
<td>3052–4341</td>
<td>Putative arsenate membrane protein</td>
<td>429</td>
<td>DaciDRAFT_5631</td>
<td><em>D. acidovorans</em> SPH-1</td>
<td>81</td>
<td>ZP_01578294</td>
</tr>
<tr>
<td><em>arsA</em></td>
<td>4536–5096</td>
<td>Low-molecular-weight phosphotyrosine protein phosphatase</td>
<td>186</td>
<td>AjsDRAFT_0386</td>
<td><em>Acidovorax</em> sp. strain JS42</td>
<td>71</td>
<td>ZP_01384477</td>
</tr>
<tr>
<td><em>arsR</em></td>
<td>5122–5448</td>
<td>Putative regulatory protein</td>
<td>108</td>
<td>AjsDRAFT_3852</td>
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<td>71</td>
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<tr>
<td><em>cnbZ</em></td>
<td>5577–6332</td>
<td>Deaminase</td>
<td>251</td>
<td>Blr2865</td>
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<tr>
<td><em>orf2</em></td>
<td>6368–6805</td>
<td>Hypothetical protein</td>
<td>145</td>
<td>Blr7786</td>
<td><em>B. japonicum</em> USDA 110</td>
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<tr>
<td><em>tpnA2</em></td>
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<td>Transposase</td>
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<td><em>Pseudomonas</em> sp. strain ADP</td>
<td>100</td>
<td>NP_862476</td>
</tr>
</tbody>
</table>

![FIG. 2. Typical spectral changes during deamination of 2-amino-5-chloromuconate (A) and 2-amino-5-chloromuconate (B) by purified deaminase CnbZ and proposed catalytic mechanisms. Absorption was recorded every 30 s. The amount of purified CnbZ in the reaction was 2.7 $\mu$g of protein. Below the graphs is a proposed putative mechanism of CnbZ-catalyzed deamination, which includes simultaneous attacks on the $\alpha$-carbon and nitrogen atoms of 2-amino-5-chloromuconate, 2-aminomuconate, respectively, by a hydroxyl group and a proton.](http://jb.asm.org/Downloaded from)
ent deaminase (CnbH) was identified at the degradative cnb cluster by genetic cloning and expression in *E. coli* (21). However, CnbH was not detectable during the purification of deaminase proteins. To determine whether *cnbH*, *cnbZ*, or both were functional during growth on 4-CNB, transcription of the two genes was examined with RT-PCR. The results clearly showed that only *cnbZ*, not *cnbH*, was transcribed during growth on 4-CNB (Fig. 3). Based on this result and the fact that CnbH was not detected during purification from 4-CNB-grown cells, it is proposed that *cnbZ* is the functional gene for 4-CNB degradation.

**DISCUSSION**

In this study, a novel deaminase CnbZ involved in 4-CNB and nitrobenzene degradation was purified from *Comamonas* sp. strain CNB-1, and its gene was cloned and functionally expressed in *E. coli*. Sequence analysis revealed several unique features for this CnbZ deaminase: (i) CnbZ does not possess any known binding sites for cofactors required by other deamination-catalyzing enzymes, such as the pyridoxal phosphate domain of aminotransferase (5), the NAD⁺ binding site of glutamate dehydrogenase (19), and the Zn⁺ coordinate site of adenosine deaminase (2); (ii) CnbZ does not show any significant similarities to previously known deaminases; (iii) a putative conservative domain of glycosyl hydrolases was identified in CnbZ through alignments with sequences in the Conserved Domain Database (http://www.ncbi.nlm.nih.gov/structure/cdd), but the necessity of this domain for deaminase activity needs to be confirmed. Moreover, three hypothetical proteins from the genomes of *B. japonicum* USDA 110, *R. palustris* HaA2, and *Roseiflexus* sp. strain RS-1 have modest similarities (38.8 to 52.5%) to CnbZ. Phylogenetic analysis revealed that CnbZ and these hypothetical proteins are closely related, and their evolutionary relationships to previously known deaminases are distant (Fig. 4). In order to find clues to any physiological function(s), the genes neighboring those encoding these hypothetical proteins were subjected to bioinformatics analysis with various biosoftware tools. Results indicated that the genes corresponding to the CnbZ homologs, i.e., BAC48130 from *B. japonicum* USDA 110 and ABD04858 from *R. palustris* HaA2, were located at genetic clusters putatively involved in metabolism of xenobiotic compounds and amino acids. Therefore, it was deduced that BAC48130 from *B. japonicum* USDA 110 and ABD04858 from *R. palustris* HaA2 were putative deaminases involved in assimilation of xenobiotic compounds and amino acids in these bacteria.

The genetic organization of the DNA fragment containing *cnbZ* is interesting: the whole fragment is flanked by two transposase genes, but sequence alignment indicated that these transposase genes are truncated. Thus, it was deduced that this DNA fragment was not likely to be transposable. To obtain any clues about the origin and evolution of *cnbZ*, homologs of *cnbZ* neighbors were searched for in the public databases. Homologs of the *cnbZ* neighbors, a putative arsenate resistance gene cluster (*arsRABC*) and *orf2*, were found in the genomes of *B. japonicum* USDA 110 (Fig. 1) and *Acidovorax* sp. strain JS42 (Table 3), but these genes are located far apart in strain USDA 110, and their relative positions in strain JS42 are currently unknown. The most striking match from the data bank was a hypothetical protein in strain JS42 with 100% identity to Orf1 in strain CNB-1. In addition, a homolog (OrfJ) to Orf2 was also identified from a degradative genetic fragment (GenBank accession no. AB029044) of *Comamonas testosteroni* TA441. These findings may suggest that the *cnbZ*
mutagenesis of CnbZ is in progress. Further work on crystallization and a putative hypothesis to illustrate the CnbZ-catalyzed deamination is proposed. This is different from previously characterized homologs of the eukaryotic 3-hydroxyanthranilate 3,4-dioxygenase and 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase in the 2-nitrobenzoate degradation pathway of Pseudomonas fluorescens strain KU-7. The formation of different products by CnbZ may reflect a different catalytic mechanism, and a putative hypothesis to illustrate the CnbZ-catalyzed deamination is proposed. Further work on crystallization and mutagenesis of CnbZ is in progress.

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