Characterization of a nitrilase from

*Arthrobacter aurescens* CYC705 for synthesis of iminodiacetic acid

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A nitrilase gene cyc705 from *Arthrobacter aurescens* CYC705 for synthesis of iminodiacetic acid (IDA) was cloned. This gene contained a 930 bp ORF, which encoded a polypeptide of 310 amino acids. A recombinant *Escherichia coli* BL21(DE3)/pET28a-cyc705 was constructed to achieve the heterologous expression of cyc705. This recombinant nitrilase was purified to homogeneity with a molecular weight of 36.7 kDa on SDS-PAGE and mass spectrometry, and characterized to be an oligomer of 14 subunits by gel permeation chromatography. Using iminodiacetonitrile (IDAN) as the substrate, the $V_{\text{max}}$, $K_m$, $k_{\text{cat}}$ and $k_{\text{cat}}/K_m$ were 9.05 U mg$^{-1}$, 43.17 mM$^{-1}$, 94.1 min$^{-1}$ and 2.18 $\times$ 10$^3$ min$^{-1}$ M$^{-1}$, respectively. The optimum temperature and pH were 25°C and 5.8. The suitable substrates for the purified nitrilase were short-chain aliphatic dinitriles. High concentration of IDAN could be hydrolyzed to IDA in a shorter time.

Key words: *Arthrobacter aurescens*; characterization; iminodiacetic acid; nitrilase

Introduction

Nitrilase (EC 3.5.5.1), as an important industrial enzyme, belongs to the nitrilase superfamily (Brenner, 2002). Nitrilases are widely derived from various organisms, including bacteria, fungi and plants. Initial investigations suggested nitrilases to be specific for aromatic nitriles. But now, depending on the substrate spectrum, nitrilases are reported from different sources (Banerjee et al., 2002), which can be active on aromatic or heterocyclic nitriles (Vejvoda et al., 2010) and aliphatic nitriles (Bayer et al., 2011) as well as arylacetonitriles (Sosedov et al., 2010). Nitrilases can convert nitriles directly into the corresponding carboxylic acids and ammonia. Compared with chemical methods of producing carboxylic acids, nitrilases are widely used due to the mild reaction conditions, regioselectivity (Wang et al., 2014) and enantioselectivity (Vejvoda et al., 2010). Therefore, they are used to catalyze the production of various valuable carboxylic acids.

IDA is an important fine chemical intermediate. It has wide applications and is mainly used in producing glyphosate herbicides, chelating agents and surfactants (Ni et al., 2009). Currently, the production of IDA mainly uses chemical methods, including the hydrocyanic acid method (Rmon, 1991), diethanolamine method (Bornscheuer and Kazlauskas, 2006), and chloroacetic acid method (Farbwerke Hoechst, 1969). These methods produce a large amount of waste water and byproducts, which are unfriendly to the environment (Duan et al., 2007). Enzymatic production of IDA has some advantages, such as the mild reaction conditions, environmental friendliness and low cost. So far, only a few papers have reported nitrilase-catalyzed production of IDA. A nitrilase-produced strain named *Alcaligenes faeacalis ZJB-09133* was screened by using IDAN as the carbon source, and the whole cells were used to catalyze IDAN to IDA, which showed the conversion only reached 65.3% at 8 h after optimization of the reaction conditions (Liu et al., 2011). The *Acidovorax facilis* nitrilase was cloned and expressed in *Escherichia coli* BL21 (DE3), and the recombinant cells were entrapped in polyvinyl alcohol and sodium alginate copolymer and used to catalyze IDAN to...
IDA, which showed a maximum yield of 68% at 8 h (Liu et al., 2012). A nitrile-hydrolyzing Alcaligenes faecalis ZJUTBX11 was screened through a high-throughput screening model, and the whole cells were used to catalyze IDAN to IDA after optimization of the culture conditions, which gave a conversion of more than 95% and a yield of only 79% after 500 min (Zhang et al., 2012). The nitrilase activity of Alcaligenes faecalis WBX11 was improved by low-energy ion beam implantation mutagenesis, and the Alcaligenes faecalis WBX11-MD271mutant was used to catalyze IDAN to IDA, which showed 150 mM of IDAN could be completely converted in 8 h, but only about 72% of 250 mM of IDAN could be converted after 750 min (Zhang et al., 2013). Based on homology modeling and ‘hot spot’ mutations, one mutant M3 from Acidovorax facilis nitrilase was identified, and used to catalyze the transformation of IDAN to IDA, which resulted in a conversion of 96% and a yield of 73.6% at 8 h (Liu et al., 2012). As can be seen from the above reports, the enzymatic production of IDA also faces some problems, such as low nitrilase activity in the wild strain, low substrate specificity to IDAN and the intermediate of 2-((cyanomethyl)amino)acetic acid (CCA) or by-product of amide compound accumulation. Therefore, it is still very important and meaningful to obtain a more effective nitrilase and investigate its properties for the production of IDA.

Over 500 soil samples from different environments were collected in our laboratory. Using IDAN as the sole carbon source, a nitrilase-produced strain was ultimately screened from a primeval forest soil sample, which could efficiently catalyze IDAN to IDA without CCA or amide compound accumulation. This strain was identified as Arthrobacter aurescens (named CYC705) through biochemical identification and 16S rDNA gene sequencing. To the best of our knowledge, nitrilase has not yet been reported from Arthrobacter aurescens. Arthrobacter aurescens CYC705 was used for calibration.

For the nitrilase activity assay, enzymes were prepared by sonification on ice for 5 min, and the lysate was centrifuged at 4,000 ×g for 20 min to remove the cell debris. The resulting supernatant was passed through a 0.22 μm filter, and then was applied onto a Ni-NTA Superflow column (1 ml, Qiagen) previously equilibrated with a binding buffer (50 mM sodium phosphate buffer, 300 mM NaCl, 10 mM imidazole, pH 8.0). Unbound proteins were washed out from the column with a washing buffer (50 mM sodium phosphate buffer, 300 mM NaCl, 20 mM imidazole, pH 8.0). Then, the nitrilase was eluted from the column with an elution buffer (50 mM sodium phosphate buffer, 300 mM NaCl, 250 mM imidazole, pH 8.0). Enzyme fractions were analyzed by 12% SDS-PAGE and visualized by staining with Coomassie blue R250. Protein concentration was determined by using the Bradford method, with bovine serum albumin as the standard.

Determination of oligomer molecular weight. The purified nitrilase was analyzed with an UltraFLEX III MALDI-TOF/TOF mass spectrometer (Bruker-Daltonics) to determine the monomer molecular weight. The molecular mass of the purified native nitrilase was determined by gel permeation chromatography on a Superdex 200 gel filtration column (Pharmacia, Stockholm, Sweden) using a Dionex HPLC system. A gel filtration HMW calibration kit (GE Healthcare, Chalfont St. Giles, UK) with the range of 158–669 kDa was used for calibration.

**Nitrilase assay.** Nitrilase activity was routinely assayed with IDAN as the substrate. The standard assay mixture consisted of 25 μg of the purified nitrilase and 1.0% (w/v) of the IDAN in 500 μl of sodium phosphate buffer (100 mM, pH 7.2). The reaction was carried out at 30°C for 15 min.

The DNA and protein sequence similarities were analyzed by using the BLASTN and BLASTP programs (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), respectively. Multiple sequence alignments were performed with Clustal X. A phylogenetic tree was constructed by using the neighboring-joining algorithm in MEGA ver 5.0.

**Expression and purification of Arthrobacter aurescens CYC705 nitrilase.** The resulting cyc705 gene fragments were ligated into the expression vector pET28a (+) and transformed into the expression host Escherichia coli BL21 (DE3). The recombinant Escherichia coli BL21(DE3)/pET28a-cyc705 was routinely cultured in Luria-Bertani (LB) medium containing 50 μg/ml kanamycin at 37°C. The expression of cyc705 was induced by the addition of 0.1 mM isopropyl-β-D-thiogalactoside (IPTG) when the OD600 of the culture broth reached 0.6. The induction was carried out at 20°C and 180 rpm for another 24 h. Then, the cells were harvested by centrifugation at 4°C, 8,000 ×g for 10 min.

The cell pellets were resuspended in 50 mM sodium phosphate buffer (pH 7.4). The cell suspension was disrupted by sonification on ice for 5 min, and the lysate was centrifuged at 8,000 ×g for 20 min to remove the cell debris. The resulting supernatant was passed through a 0.22 μm filter, and then was applied onto a Ni-NTA Superflow column (1 ml, Qiagen) previously equilibrated with a binding buffer (50 mM sodium phosphate buffer, 300 mM NaCl, 10 mM imidazole, pH 8.0). Unbound proteins were washed out from the column with a washing buffer (50 mM sodium phosphate buffer, 300 mM NaCl, 20 mM imidazole, pH 8.0). Then, the nitrilase was eluted from the column with an elution buffer (50 mM sodium phosphate buffer, 300 mM NaCl, 250 mM imidazole, pH 8.0). Enzyme fractions were analyzed by 12% SDS-PAGE and visualized by staining with Coomassie blue R250. Protein concentration was determined by using the Bradford method, with bovine serum albumin as the standard.

**Materials and Methods.** IDAN, IDA, nitrile substrates, and other chemicals of the highest purity were purchased from TCI (Tokyo, Japan), Sigma-Aldrich (St. Louis, MO) or Merck (Darmstadt, Germany). The strain of Arthrobacter aurescens CYC705 was stored in our laboratory. The plasmid pET-28a (+) (Novagen, Darmstadt, Germany) carrying an N-terminal 6× His-Tag sequence and Escherichia coli strain BL21 (DE3) (Invitrogen, Karlsruhe, Germany) were used for cloning and expression of the nitrilase.

**Cloning and analysis of Arthrobacter aurescens CYC705 nitrilase gene.** The nitrilase gene was amplified from the genomic DNA of Arthrobacter aurescens CYC705 by PCR using the following two oligonucleotide primers: Forward: 5'-GGCGGATCCATGACCAAAGTAGCAGTAGTCC-3' (underlined bases indicate the BamHI digestion site); Reverse: 5'-GGCGAGCTTGTTTATACGGTCGCAGCGCCGTTC-3' (underlined bases indicate the HindIII digestion site). The sequence of the oligonucleotide primers was designed on the base of the DNA sequence of a putative nitrilase from Arthrobacter aurescens TC1 (GenBank accession no. YP946154). PCR conditions were denaturation at 94°C for 10 min, followed by the addition of Taq DNA polymerase (TaKaRa, Otsu, Japan); each of the following 30 cycles included 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min; and the final step was 72°C for 10 min. All cloning experiments were performed according to the Sambrook molecular cloning manual.

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with shaking (150 rpm). Aliquots (100 µl) were withdrawn and supplemented with 5 µl of 6 M HCl to stop the reaction, and then centrifugated at 8,000 × g for 10 min. Subsequently, the supernatant was subjected to HPLC analysis to quantify the amount of IDA formed. One unit of nitrilase activity is defined as the amount of enzyme that hydrolyzes IDAN to release 1 µmol of IDA per minute under the standard assay conditions. All assays were performed in triplicate if not specified.

**HPLC analysis of IDA.** The HPLC method with pre-column derivatization was used to determine the IDA. 2,4-dinitro-fluorobenzene (DNFB) was used as the derivatization agent (Li et al., 2011). Samples were microfiltered with a 0.45 µm membrane (Millipore, Billerica, MA). The 0.1 ml flow out was diluted by 2 ml 0.25 M NaHCO₃ and then mixed with 0.4 ml of 1% (w/w) DNFB dissolved in acetonitrile. The mixture was incubated at 60°C for 30 min, and 7.5 ml H₂O was added before further HPLC analysis. Dinitrophenyl-IDA (DNP-IDA) was directly quantified by HPLC (Aglient 1100; Agilent, Santa Clara, CA) equipped with an ultraviolet (UV) detector and a XDB-C18 column (250 mm × 4.6 mm, Agilent). The mobile phase consisted of 55% (v/v) methanol and 45% (v/v) 50 mM CH₃COONa-CH₃COOH (NaAc-HAc) buffer (pH 5.0). The flow rate of mobile phase was controlled at 0.8 ml/min, and the detection was performed at 365 nm.

**Effects of temperatures and pH values on the purified nitrilase.** For the determination of temperature effect, a mixture of purified nitrilase (25 µg) with IDAN (100 mM) in 500 µl of sodium phosphate buffer (100 mM, pH 7.2) was incubated at temperatures ranging from 15 to 70°C. Aliquots (100 µl) were taken after 1 and 4 h intervals, and acidified with 5 µl of 6 M HCl. The formed IDA was quantified by HPLC analysis after conversion to DNP-IDA.

As for thermal stability determination, a solution of 25 µg of purified nitrilase in 500 µl of sodium phosphate buffer (100 mM, pH 7.2) was kept at temperatures ranging from 20 to 70°C for 0.5 and 1 h separately. After heat-treatment, IDAN was added to the enzyme solution to a final concentration of 100 mM. The resulting mixture (500 µl) was incubated at 30°C for 4 h. Aliquots (100 µl) were acidified with 5 µl of 6 M HCl. The formed IDA was quantified by HPLC analysis after conversion to DNP-IDA.

The pH effect was investigated using the following buffers: sodium citrate buffer (pH 5.21, 5.83 and 6.35), potassium phosphate buffer (pH 6.35, 6.90, 7.23 and 7.89) and sodium bicarbonate buffer (pH 7.87, 8.50 and 9.22). A mixture of purified nitrilase (25 µg) with IDAN (100 mM) in 500 µl of the respective buffer (100 mM) was incubated at 30°C. Aliquots (100 µl) were withdrawn after 1 and 4 h intervals, and acidified with 5 µl of 6 M HCl. The formed IDA was quantified by HPLC analysis after conversion to DNP-IDA.

The pH stability of the purified nitrilase was determined by incubating the enzyme in different pHs ranging from 5.15 to 9.06 for 0.5 and 1 h separately. Then IDAN was added to the enzyme solution to a final concentration of 100 mM. The resulting mixture (500 µl) was incubated at 30°C for 4 h. Aliquots (100 µl) were acidified with 5 µl of 6 M HCl. The formed IDA was quantified by HPLC analysis after conversion to DNP-IDA.

**Determination of the kinetic parameters.** The kinetic parameters of the purified nitrilase were calculated from the initial-velocity data determined under different IDAN concentrations. The reactions were initiated by addition of purified nitrilase (25 µg) to 500 µl of sodium phosphate buffer (100 mM, pH 7.2) with different concentrations of IDAN (50–300 mM) in a 1.5 ml capped vial at 30°C and 150 rpm for 15 min. Aliquots (100 µl) were withdrawn and acidified with 5 µl of 6 M HCl, and then centrifuged at 8,000 × g for 10 min. The formed IDA was quantified by HPLC analysis after conversion to DNP-IDA. Initial velocity was determined by calculating the initial rate of IDAN hydrolysis. The kinetic constants Kₘ and Vₘₐₓ values were determined from Lineweaver-Burk plots using standard linear regression techniques. The kₗₑᵃᵗ was calculated from the ratio of Vₘₐₓ to enzyme concentration.

**Substrate scope.** The specific activities of the purified nitrilase towards different nitriles with structural diversity were measured by the quantification of the amount of ammonia released during the hydrolysis. The purified nitrilase (50 µg) was incubated with 50 mM of different nitriles in 500 µl of sodium phosphate buffer (100 mM, pH 7.2) at 30°C and 150 rpm. The conversion was determined by measuring the amount of ammonia produced in the reaction using the Bertheoay assay (Weatherburn, 1967).

**Biotransformation of IDAN to IDA with recombinant Escherichia coli BL21(DE3)/pET28a-cyc705 cells.** The biotransformation reactions using recombinant Escherichia coli BL21(DE3)/pET28a-cyc705 cells (0.01 g dry cells) as a biocatalyst were performed in different concentrations of IDAN solution (100 mM, 200 mM, 300 mM, 400 mM and 500 mM) in 5 ml of sodium phosphate buffer (100 mM, pH 7.2) at 30°C and 150 rpm. Aliquots (100 µl) were withdrawn from separate reaction mixtures, and acidified with 5 µl of 6 M HCl, and then centrifuged at 8,000 × g for 10 min. The supernatant was subjected to HPLC analysis to quantify the concentration of IDA formed.

**Results and Discussion**

**Cloning and sequence analysis**

The nitrilase gene cyc705 was amplified from the genomic DNA of Arthrobacter aurescens CYC705. The open reading frame of cyc705 consisted of 930 bp, which encoded a polypeptide of 310 amino acids. The gene cyc705 had been amplified from the genomic DNA of Arthrobacter aurescens CYC705. The open reading frame of cyc705 consisted of 930 bp, which encoded a polypeptide of 310 amino acids. The gene cyc705 had been submitted to GenBank database and assigned the accession number of KC961317.

Sequence analysis indicated that the nitrilase gene cyc705 from Arthrobacter aurescens CYC705 had identity of 99%, 61% and 59% with the Arthrobacter aurescens TC1 putative nitrilase (YP946154.1), Alcaligenes faecalis nitrilase (WP003805062.1) and Pseudomonas sp. M1 nitrilase (WP009622954.1), respectively. Phylogenetic analysis (Fig. 1) also showed that Arthrobacter aurescens CYC705 nitrilase formed a cluster together with nitrilases from Arthrobacter aurescens TC1, Corynebacterium lubricantis, Alcaligenes faecalis sp., Burkholderia gladioli and Pseudomonas sp.

**Expression and purification**

The amplified gene fragment cyc705 was digested with
BamHI and HindIII, and ligated into the plasmid pET28a (+). The recombinant plasmid pET28a-cyc705 was heterologously expressed in *Escherichia coli* BL21 (DE3). SDS-PAGE analysis of the recombinant *Escherichia coli* BL21(DE3)/pET28a-cyc705 expression revealed a distinct band at 37 kDa which was the same as the predicted molecular weight of CYC705 nitrilase (Fig. 2). In addition, SDS-PAGE analysis also showed that the CYC705 nitrilase constituted as much as 30% of the soluble protein in the supernatant.

Based on the N-terminal 6 × His affinity tag, the recombinant nitrilase was simply purified to homology by using a Ni-NTA column (Fig. 2). A total of 10.2 mg nitrilase was purified from 4.0 g of wet cells, which was purified 12.01-fold with a yield of 23.3% (Table 1).

**Oligomer molecular weight**

The purified CYC705 nitrilase gave a single band on SDS-PAGE with a molecular mass of about 37 kDa (Fig. 2, Lane 3). The monomer (subunit) molecular weight was determined to be 36.7 kDa by the mass spectrometry analysis. The molecular weight of the holoenzyme was estimated by gel permeation chromatography to be 520 kDa. This suggested that CYC705 nitrilase self-aggregated to the active form with the native structure being 14 subunits of

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Fig. 1. Phylogenetic analysis of *Arthrobacter aurescens* CYC705 nitrilase and closely related nitrilases.

Unrooted phylogenetic tree showing relatedness and levels of homology between the nitrilase amino acid sequences. The tree was constructed using the neighbor-joining algorithm in MEGA ver 5.0. Confidence for the tree topology was estimated using the bootstrap values based on 1,000 replicates. The estimated genetic distance between sequences was proportional to the lengths of the horizontal lines connecting one sequence to another.

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Fig. 2. SDS-PAGE analysis of the recombinant CYC705 nitrilase. Lane 1: protein marker; Lane 2: supernatant of the whole cell lysate of the recombinant *Escherichia coli* BL21 (DE3)/pET28a-cyc705; Lane 3: purified CYC705 nitrilase.
identical size. Most known nitrilases consist of a single polypeptide with a molecular mass in the range of 32–45 kDa, which self-associate to form active enzymes. The preferred native forms of nitrilases seem to be large aggregates of 6–26 units (Banerjee et al., 2002; O’Reilly et al., 2003). The present results are consistent with the previous observations.

**Kinetic parameters**

The kinetic parameters of the purified nitrilase for IDAN were determined as described in MATERIALS AND METHODS. The Lineweaver-Burk plot is shown in Fig. 3. The $V_{\text{max}}$ and $K_m$ for IDAN were 9.05 U mg$^{-1}$ and 43.17 mM, respectively. The catalytic constant $k_{\text{cat}}$ and overall catalytic efficiency $k_{\text{cat}}/K_m$ were 94.1 min$^{-1}$ and 2.18 $\times$ 10$^3$ min$^{-1}$M$^{-1}$. $K_m$, $k_{\text{cat}}$, and $k_{\text{cat}}/K_m$ revealed that the CYC705 nitrilase was very active for the hydrolysis of IDAN to the corresponding IDA.

**Effects of temperatures and pH values**

Nitrilases are catalysts with biological activity, which are always affected by circumstances. The effects of temperature on the purified nitrilase activity were investigated at varying temperatures with IDAN as a substrate. As shown in Fig. 4(A), nitrilase activity increased at temperatures ranging from 15 to 25°C and reached its maximum at 25°C, after which the nitrilase activity decreased. When the temperature was above 45°C, the nitrilase activity decreased sharply. As can be seen, the CYC705 nitrilase is inclined to hydrolyze the IDAN at room temperature, which is different from the optimum temperatures of other nitrilases reported at 30–50°C (Kato et al., 2000; Kim et al., 2009; Zhang et al., 2013).

Since the optimal temperature of CYC705 nitrilase was about 25°C, a question was raised as to how thermostable this enzyme would be. Therefore, the thermostability of CYC705 nitrilase was investigated by using IDAN as the substrate. The result is presented in Fig. 4(B). It can be seen that the yield of IDA decreased sharply after the enzyme was heat-treated at 40°C for 30 min and 1 h. The enzyme completely lost its activity after heat-treatment at 60°C for 30 min. However, this enzyme was relatively stable at the temperature range from 20 to 30°C. Most of the nitrilases reported in the literature were labile at higher temperatures. For example, the nitrilase from *Pseudomonas putida* was quite labile at higher temperatures as revealed by its half-life value of 9 min at 50°C (Banerjee et al., 2006). The nitrilase from *Aspergillus niger* K10 was fairly stable at 30°C, but the

### Table 1. Purification of the recombinant nitrilase from *Arthrobacter aurescens* CYC705.

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein (µg)</th>
<th>Total activity (U)</th>
<th>Specific activity$^a$ (U µg$^{-1}$)</th>
<th>Yield (%)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-free extract</td>
<td>$5.26 \times 10^5$</td>
<td>$2.9061 \times 10^4$</td>
<td>0.05525</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Ni-NTA column</td>
<td>$1.02 \times 10^4$</td>
<td>$6.7677 \times 10^3$</td>
<td>0.6635</td>
<td>23.30</td>
<td>12.01</td>
</tr>
</tbody>
</table>

$^a$Enzyme activity was assayed with 100 mM iminodiacetonitrile.
The enzymes strongly depend on the pH of the reaction media. Generally, they have an optimal pH, and higher or lower pH values can lead to a partial inactivation of the enzyme. Extreme pH might change the tertiary structure of the enzyme and result in the loss of catalytic activity. The effect of pH on the purified CYC705 nitrilase was studied. As shown in Fig. 5(A), this enzyme was highly active in the acidic pH region from 5.5 to 7.0 with an optimum around pH 5.8. A sharp activity decrease was observed at slightly alkaline pH values, only trace activity being observed at pH 8.5. This pH optimum was not like the pH optima of other nitrilases reported in the range of 7.0 to 9.0 (Mueller et al., 2006; Vejvoda et al., 2010; Kim et al., 2009). However, it was similar to that of the nitrilase from *Rhodococcus rhodochrous* K22, the pH optimum of which was reported at 5.5 (Kobayashi et al., 1990).

The purified enzyme showed high and moderate stabilities at pH 5.1 and 7.0 after incubation for 1 h (Fig. 5B). The stability decreased as the pH increased. Lower stability of the nitrilase in the alkaline pH region signified the importance of maintaining the pH of the reaction mixture near neutral throughout long biocatalytic reactions. As for biotransformation of IDAN to IDA, nitrilases with an acidic pH optimum and stability are favorable because the production of IDA will lead to a pH incline in the reaction system.

**Substrate specificity**

The specific activities of the purified CYC705 nitrilase towards a variety of nitriles were measured by the quantification of the amount of ammonia released during the hydrolysis. For the convenience of the comparison, the activity to IDAN was defined as 100. The activities to other nitriles are presented in Table 2. The CYC705 nitrilase had a narrow substrate spectrum, and showed high specific activities towards some short-chain aliphatic dinitriles (Entries 14–16). A similar behavior was found in nitrilase Nt1 from a metagenomic library (Bayer et al., 2011). The activity of CYC705 nitrilase towards aromatic dinitriles (Entry 19), aliphatic substituted mononitriles and medium-chain aliphatic mononitriles (Entries 9–13) was far less than that of short-chain aliphatic dinitriles. This nitrilase also showed low activities towards aromatic nitriles and heterocyclic nitriles (Entries 1–8). This phenomenon also proved this enzyme prefers short-chain aliphatic dinitriles. Only a few dinitrile-converting enzymes have been identified from *Rhodococcus rhodochrous* K22 (Kobayashi et al., 1990), *Comamonas testosterone* (Levy et al., 1995), *Synechocystis* sp. PCC6803 (Heinemann et al., 2003), *Acidovorax facilis* 72W (Chauhan et al., 2003), *Bradyrhizobium japonicum* (Zhu et al., 2008) and *Pseudomonas fluorescens* Pf-5 (Kim et al., 2009).

**Biotransformation of IDAN to IDA**

To determine the effectiveness of recombinant *Escherichia coli* BL21 (DE3)/pET28a-cyc705 cells in the production of IDA from IDAN, the time course of the yield was investi-
Characterization of a nitrilase

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References


Conclusion

The Arthrobacter aurescens CYC705 nitrilase gene was cloned and overexpressed in Escherichia coli. The enzyme was characterized with the native structure being 14 subunits of identical size. This enzyme could be classified as an aliphatic nitrilase, and was a high-efficiency biocatalyst for the hydrolysis of short-chain aliphatic dinitriles. Taking IDAN as the substrate, the $V_{\text{max}}$, $K_{\text{m}}$, $k_{\text{cat}}$, and $K_{\text{cat}}/K_{\text{m}}$ were 9.05 U mg$^{-1}$, 43.17 mM$^{-1}$, 94.1 min$^{-1}$ and 2.18 $\times$ 10$^{4}$ min$^{-1}$ M$^{-1}$, respectively. In this investigation, IDAN could be efficiently biotransformed to IDA in a shorter time than those of the previous studies.


