Purification and characterization of a cyanide-degrading nitrilase from *Trichoderma harzianum* VSL291

Jorge RICAÑO-RODRÍGUEZ, Mario RAMÍREZ-LEPE*
Unit for Research and Development in Food, Technological Institute of Veracruz, Veracruz, Mexico

Abstract: An intracellular nitrilase (Nit1) with cyanide-degrading activity was isolated from *Trichoderma harzianum* VSL291, cultivated on benzonitrile as the sole carbon source. Nit1 was purified to homogeneity by ion exchange and gel filtration chromatography with a recovery of 7.15% and a fold of 22.5. The molecular weight was estimated to be 47.7 kDa and the purified enzyme was sequenced with a system of liquid chromatography and mass spectrometry (LC-MS). The enzyme consists of 436 amino acids with a predicted molecular weight of 47.088 kDa. The sequence revealed conserved domains for a nitrilase super family such as putative active and binding sites and a Glu-Lys-Cys catalytic triad. Nit1 exhibited maximum activity (19.6 U mg⁻¹) at 40 °C and a pH of 7.5. Nit1 had a strong inhibition in the presence of Al³⁺, Cu²⁺, Zn²⁺, and Ag⁺ ions and was able to degrade KCN completely at 0.02 mmol/L, 0.05 mmol/L, and 0.1 mmol/L in 15 min, 40 min, and 45 min, respectively. The effect on KCN (0.02 mmol/L) degradation was tested in the presence of Cu²⁺ and Ag⁺ ions (0.025 mmol/L to 1.0 mmol/L) and the enzymatic activity was not affected significantly at 0.025 mmol/L, 0.075 mmol/L, and 0.125 mmol/L concentrations. However, when both ions were combined, the activity of the enzyme decreased significantly.

Key words: Cyanide-degrading nitrilase, bioremediation, enzyme characterization, purification, *Trichoderma*

1. Introduction

Nitrilase enzymes (nitrilases) catalyze the hydrolysis of nitrile (R-CN) compounds to the corresponding carboxylic acid and ammonia (Howden and Preston, 2009). They belong to the nitrilase super family and have been widely acknowledged as valuable alternatives to chemical catalysts because of their ability to transform an immense variety of organic nitriles under mild conditions and often in a stereoselective or regioselective manner (Martínková and Kren, 2010).

Cyanide compounds are widely distributed on earth and are produced naturally by many organisms. They are synthesized by plants as a defense mechanism (Moller, 2010), secreted by fungi and bacteria as an antimicrobial compound (Frapolli et al., 2012), and synthesized by insects as a control over mating behavior (Baxter and Cummings, 2006). However, natural quantities of cyanogenic compounds do not compare to those produced by industrial processes. In addition, these compounds can be used in mining, electroplating, steel manufacturing, polymer synthesis, pharmaceutical production, and other specialized applications including dyes and agricultural products (Dash et al., 2009).

Moreover, many cyanide complexes are highly unstable. Temperature, pH, and light can degrade the components and form free cyanide, which is the most toxic form of cyanide (Rao et al., 2010). There are several chemical methods used to treat effluents containing cyanide before discharging them into the environment. The most common are alkaline chlorination, sulfur oxide/air and hydrogen peroxide processes. However, these methods have significant drawbacks: an inability to treat metal-complexed cyanide, costly reagents, equipment, maintenance, and royalty payments, and the generation of unfavorable by-products such as chlorinated compounds (Maniyam et al., 2011). The biological alternatives for treatment of cyanide containing waste do not require addition of toxic or hazardous chemicals. Several attempts to develop biological processes for cyanide detoxification have been concentrated on cyanide-degrading enzymes obtained from animals, plants, fungi, and prokaryotes (Hong et al., 2008).

As mentioned above, most of the enzymes found in nitrogen-fixing prokaryotes may have disadvantages (e.g. nitrogenases) and require strictly anaerobic conditions due to being subject to the inhibitory effect of oxygen (Goldberg et al., 1987). One example is rhodanese, which
requires thiosulfate to function (Westley, 1981). Nitrilases from fungi, on the other hand, have many properties that make them promising candidates for remediation. They are stable over long periods of time, require no co-factors, are readily expressed at high levels, and can function as purified enzymes or crude extracts (Martínková et al., 2009). In the current study an intracellular nitrilase that hydrolyzes cyanide was purified from *Trichoderma harzianum* VSL291 and the properties of the purified enzyme were characterized.

2. Materials and methods

2.1. Microorganisms and their cultivation conditions

The antagonistic fungi *T. harzianum* strain VSL291 was isolated from soil cultivated with *Agave tequilana*, cv. 'Azul', in the State of Jalisco, Mexico (Sánchez and Rebolledo, 2010). The culture was maintained on potato dextrose agar (BD Bioxon).

2.2. Enzyme purification

A *T. harzianum* VSL291 spore suspension of 1 mL (spore density 1 × 10⁷ spores/mL) from agar slant was inoculated aseptically into 2000-mL culture flasks containing 400 mL of sterilized liquid of potato-dextrose broth (Fluka Sigma St. Louis, MO, USA). Given the fact that *T. harzianum* is a mesophilic microorganism, the flask was incubated at 25 °C and 130 rpm for 7 days, which is an adequate time period for obtaining the required biomass for subsequent experiments.

The culture was filtered using Whatman filter paper no. 41. The mycelium was recovered, washed with sterile distilled water, and inoculated aseptically into 2000-mL culture flasks containing 400 mL of a sterilized minimal medium containing 0.1% (v/v) benzonitrile. The medium composition (expressed as g/L in distilled water) was as follows: NaNO₃, 3; K₂HPO₄×3H₂O, 1.3; KCl, 0.5 MgSO₄×7H₂O, 0.5; FeSO₄×7H₂O, 0.5; ZnSO₄×7H₂O, 0.001; CaCl₂, 0.5; FeCl₃, 0.001; MnSO₄×H₂O, 0.001 and pH was adjusted to 5.0 to minimize the risk of bacterial contamination (Donzelli and Harman, 2001). The flask was incubated at 25 °C and 130 rpm for 7 days, which is an adequate time period for obtaining the required biomass for subsequent experiments.

The culture was filtered using a 0.22-µm (Millipore, Billerica, MA, USA) membrane and the obtained enzymatic extract was filtered using a 500 kDa (Millipore) molecular size weight cutoff membrane. All purification steps were carried out at 4 °C. The protein extract (50 mL) was brought to 80% saturation of ammonium sulfate, and the precipitate was recovered by centrifugation (18,000 × g, at 4 °C, for 40 min), dissolved in a small amount of 50 mmol/L sodium phosphate buffer (pH 7.0), and dialyzed against the same buffer. The resultant dialyzed extract (12 mL) was loaded onto a Sephadex G100 column (1.5 cm by 100 cm; Sigma Chemical Co., St Louis, MO, USA), equilibrated with Tris-HCl (pH 8.0). Fractions (2 mL each) were collected, and the nitrilase-positive fractions (3 fractions) were lyophilized.

During purification, protein concentration was monitored by measuring absorbance at 280 nm, with bovine serum albumin as the standard. The 3 nitrilase-positive fractions (6 mL in total) were loaded onto a DEAE-Sepharose (GE Healthcare, Uppsala, Sweden) column (1.5 cm by 25 cm), pre-equilibrated with 50 mmol/L of sodium phosphate buffer (pH 7.0). The nonadsorbed materials were washed from the column with the same eluting buffer, and the enzymes were fractionated with a linear gradient from 0 mmol/L NaCl to 200 mmol/L NaCl in 50 mmol/L phosphate buffer at a flow rate of 46 mL/h. Twenty fractions (100 µL each) were collected and 6 of them showed nitrilase activity.

2.3. Measurement of enzyme activity and protein concentration

Nitrilase activity was measured with 4-cyanopyridine as a substrate. Enzyme solution (0.1 mL) was added to 0.7 mL of substrate solution, which contained 40 µL of 4-cyanopyridine (50 mmol/L) in a sodium acetate buffer (100 mmol/L, pH 7.5). The mixture was incubated at 37 °C for 20 min and then 100 µL of HCl 1M were added to stop the reaction. A 50-µL volume of reaction mixture was incubated for 5 min with 500 µL of Nessler’s reagent (Sigma-Aldrich, St. Louis, MO, USA). After centrifugation (12,000 × g, for 3 min) samples were taken from supernatant and absorbance was measured at 435 nm in a Bio-Rad SmartSpec 3000 spectrophotometer.

A standard curve of absorbance at 435 nm versus ammonium chloride (10–1000 mM) was prepared and residual ammonium was determined (Benedict et al., 1983). One unit of nitrilase activity was defined as the amount of enzyme that formed 1 µmol of ammonium per 1 min under the above conditions. The specific activity of the enzyme was expressed as the nitrilase activity per milligram of protein. Protein was determined according to Bradford (1976) using bovine serum albumin as standard.

2.4. Molecular weight estimation, LC-MS and bioinformatic analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and native PAGE were used to determine protein purity. The molecular mass of the enzyme was determined under denaturing conditions using a 7.5% acrylamide gel as described by Laemmli (1970). The proteins were Coomassie blue stained. A mixture of high-molecular-weight proteins (HMW electrophoresis standard, Sigma) were used as molecular mass markers. Electrophoresis was performed at 100 V for 30–40 min.
The marker proteins provided protein molecular weight standards in the range of 14.4–97 kDa (GE Healthcare). Proteins were stained with Coomassie blue. The nitrilase-protein was recovered from the polyacrylamide gel, reduced with dithiothreitol (DTT), alkylated with iodoacetamide, and digested with trypsin. The enzyme was sequenced with a liquid chromatography system and mass spectrometry (LC-MS) by the Instituto de Biotecnología, UNAM Cuernavaca, Mor. Mexico. A neighbor-joining (NJ) tree of the amino acid sequence of T. harzianum VSL291 and 19 nitrilase/cyanide hydratases from fungi was performed using Clustal X program. Bioinformatic analyses of homology (i.e. identical amino acids search) were conducted according to BLAST (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi.). The analysis for hypothetical conserved domains was carried out through MASCOT database search. Conserved residues as well as catalytic triad identification, including binding sites, were made according to CDD (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi.).

2.5. Effects of pH and temperature on nitrilase activity and enzyme stability
Enzyme activity was measured in the pH range from 4 to 9 and temperature range from 30 to 50 °C, using 200 µL of 50 mmol/L Britton–Robinson buffer (0.04 M acetic acid/0.04 M boric acid/0.04 M phosphoric acid) and 200 µL of the sample with a time reaction of 1 h. Thermal and pH stability of the enzyme was tested by incubating a sample-buffer aliquot in duplicate at a particular temperature (30 °C to 50 °C) or pH (4 to 9) for 24 h, and assaying its residual activity and plotting the percent residual activity against temperature or pH.

2.6. Effects of effectors on enzyme activity
The effects of (NH₄)₂SO₄, NaN₃ (1 mmol/L), AgNO₃ (1 mmol/L), Al₂(SO₄)₃ (1 mmol/L), CaCl₂ (1 mmol/L), CuSO₄ (1 mmol/L), ZnSO₄ (1 mmol/L), ions and protein inhibitors like DTT (1 mmol/L), cysteine (1%), H₂O₂ (1 mmol/L), and EDTA (10 mmol/L) were determined. Each compound was added to the reaction mixture and incubated for 60 min at 37 °C. The effects of co-solvents (butanol, toluene, acetonitrile, 2-propanol, acetone, ethanol, methanol, and hexane) at 5%, 30%, and 50% v/v on nitrilase activity using 4-cyanopyridine 50 mM as substrate were also determined. All experiments were performed 3 times and relative enzymatic activity was obtained by comparing the enzyme activity with and without the effector.

2.7. Assays for cyanide degradation
Nitrilase was assayed for cyanide degradation. KCN was prepared in 100 mM MOPS, at pH of 7.6. Total volume for each reaction was 1000 µL (60 µL in 50 µL of enzymatic extract, 100 µL of KCN and 750 µL of MOPS buffer, at pH 7.6). Cyanide degradation by nitrilase was tested in 5 different KCN concentrations (0.02 mmol/L, 0.05 mmol/L, 0.1 mmol/L, 0.5 mmol/L, and 1 mmol/L) and the effects of copper, silver, and copper and silver were tested at 0.025 mmol/L, 0.075 mmol/L, 0.125 mmol/L, 0.25 mmol/L, 0.5 mmol/L, 1 mmol/L in 20 µM KCN concentration. For use as a measurement of cyanide concentration at time point zero, all 3 diluted cyanide samples (KCN, KCN + CuSO₄, KCN + AgI, and KCN + CuSO₄ + AgI) were set up at 100%. Samples were incubated at 38 °C for 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 35 min, 40 min, 45 min, 50 min, and 55 min. The percent of degraded cyanide was determined by the picric acid endpoint method (Fisher and Brown, 1952).

3. Results
3.1. Enzyme purification
The purification steps of nitrilase produced by T. harzianum VSL291 are shown in Table 1. Our results showed a yield of 7.15 (per 100), a purification fold of 22.5, and a specific activity of 19.6.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Volume (mL)</th>
<th>Total enzymatic activity (U)</th>
<th>Protein (mg)</th>
<th>Specific activity (U/mg)</th>
<th>Purification fold</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude enzyme</td>
<td>50</td>
<td>35.77</td>
<td>41</td>
<td>0.87</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ precipitation</td>
<td>12</td>
<td>17.52</td>
<td>8</td>
<td>2.17</td>
<td>2.49</td>
<td>48.97</td>
</tr>
<tr>
<td>Sephadex G100</td>
<td>6</td>
<td>8.91</td>
<td>1.5</td>
<td>5.77</td>
<td>6.63</td>
<td>24.90</td>
</tr>
<tr>
<td>DEAE-Sepharose</td>
<td>2</td>
<td>2.56</td>
<td>0.13</td>
<td>19.6</td>
<td>22.52</td>
<td>7.15</td>
</tr>
</tbody>
</table>
activity was present in fractions 6 and 7, which were well separated from other proteins, indicating that the enzyme preparation was relatively pure.

The homogeneity of the enzyme preparation was confirmed by performing a 12.5% SDS-PAGE where a single band was obtained. The molecular weight of the purified enzyme, as determined by plotting the log of molecular weights of the markers versus their relative mobilities, was 47.7 kDa (Figure 1).

3.2. Sequencing and analysis of the purified nitrilase and phylogeny

The protein band was excised from the gel and digested with trypsin, and the generated peptides were analyzed by LC-MS. The results showed that the enzyme consists of 426 amino acids with a predicted molecular weight of 47.088 kDa (assuming an average molecular mass for an amino acid of 108 mass units). This value is similar to the molecular weight calculated by SDS-PAGE (47.7 kDa). The results from the NCBI conserved domains database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) showed that the nitrilase had several conserved regions for the nitrilase super family such as putative active (E42, T131, W163, H165, I166) and binding (M128, K129, P130, M133, D140, A141, S142, Q166, P168, Y169, A177, H188, Y189, S190, S391, P392) sites, as well as a Glu-Lys-Cys catalytic triad (amino acid residues: E127, K134, C162) (Figure 2). Phylogenetic analysis of *T. harzianum* VSL291 nitrilase and other 19 representative fungi nitrilases generated a tree by the neighbor-joining method and formed 2 major groups, where *Pseudomonas aeruginosa* (NP_253032) amidase sequence was used to root the tree (Figure 3).

3.3. Effects of temperature and pH on enzyme activity and stability

The effect of temperature on nitrilase activity was studied in the range of 35–45 °C using 4-cyanopyridine as substrate. The nitrilase was active in the range of 30–40 °C showing optimal activity at that interval, and its activity decreased abruptly at temperatures above 40 °C (Figure 4). Thermal stability studies indicated that the nitrilase retained more than 90% of its activity when exposed to 30–40 °C for 24 h. To evaluate the effect of pH, enzyme activity was measured over a range of 4.0 to 9.0. The optimum pH was found to be around 6.0–8.0 and retained more than 80% of its activity between 6.0 and 8.0 pH values (Figure 5). The highest stability was detected at 24 h at pH 7.5.

3.4. Effects of inhibitors and co-solvents on enzyme activity

The effects of metal ions and protein inhibitors on nitrilase activity were studied by measuring the influence of different ions, protein inhibitors such as EDTA, DTT (Figure 6), and co-solvents (Figure 7). No enzyme activity was observed in the presence of Al³⁺, although a strong inhibition by Ag⁺, Zn²⁺, Ca²⁺, and H₂O₂ ions was detected. On the other hand, nitrilase activity in the presence of cysteine, EDTA, sodium azide, Ca²⁺, Mg²⁺, Fe³⁺, NH₄⁺, and DTT ions decreased due to the decrease of the residual activity of Fe³⁺, NH₄⁺, and DTT (i.e. 98%, 97%, 91%, 90%, 82%, 74%, 60%, and 48%, respectively).
The effects of some of these ions on nitrilase activity are not similar to those reported in other fungal studies. We tested 8 co-solvents at 5%, 30%, and 50% (v/v) concentrations and the highest enzyme activities for all co-solvents were at 5% (v/v) concentrations (Figure 7). When the co-solvent concentrations increased to 30% and 50% (v/v) concentrations and the highest enzyme activities for all co-solvents were at 5% (v/v) concentrations (Figure 7). When the co-solvent concentrations increased to 30% and 50% (v/v) concentrations and the highest enzyme activities for all co-solvents were at 5% (v/v) concentrations (Figure 7). When the co-solvent concentrations increased to 30% and
50% (v/v), enzyme activity decreased significantly. The most suitable co-solvents tested were the following (in descending order): hexane, methanol, ethanol, acetone, 2-propanol, acetonitrile, toluene, and butanol. Interestingly, the enzyme showed 28% activity in hexane and 18% in toluene at high concentrations (50%, v/v).

3.5. Cyanide-degrading assays

The effects of enzyme activity on potassium cyanide degradation are shown in Figures 8A–8D. For all KCN concentrations tested (0.02 mmol/L, 0.05 mmol/L, 0.1 mmol/L, 0.5 mmol/L, and 1 mmol/L) KCN degradation was observed (Figure 8A). At 0.02 mmol/L and 5 min of incubation the KCN concentration in the reaction mixture decreased sharply to less than 10% and at 15 min was not detectable. At 0.05 mmol/L and 1 mmol/L KCN concentrations, the degradation rate decreased and KCN concentration was not detectable after 40 min. Concentrations of 0.5 mmol/L and 1 mmol/L showed ca. 40% and 5% KCN degradation respectively. The effects of Cu²⁺ and Ag⁺ on 0.02 mmol/L KCN degradation by the enzyme are shown in Figure 8B and Figure 8C, respectively.
The results show that as the concentrations of both ions increase from 0.125 mmol/L to 1 mmol/L, the enzyme activity decreases. The combined effect of Cu²⁺ and Ag²⁺ ions significantly affects enzyme activity (i.e., at 0.02 mmol/L KCN concentration the complete time for degradation increases from 20 min to 35 min). Our results show that the nitrilase obtained from *T. harzianum* VSL291 is capable of degrading KCN even in the presence of Cu²⁺ and Ag²⁺ ions.

4. Discussion

In the current study we examined the properties of a cyanide-degrading nitrilase from *T. harzianum* VSL291. The specific activities of some aromatic nitrilases of fungi such as *Fusarium solani* IMI196840, *F. solani* O1, *Aspergillus niger* K10, and *Rhodochrous rhodochrous* J1 using 3-cyanopyridine as a substrate have been estimated at 29.7 U/mg, 3.5 U/mg, 43.7 U/mg, and 59 U/mg, respectively (Kobayashi et al., 1989; Kaplan et al., 2006;
Vejvoda et al., 2008, 2010). Purified nitrilases obtained from Fusarium lateritium, A. niger CBS 513.88, Penicillium marneffei ATCC 18224, Gibberella moniliformis, and Neurospora crassa OR74A have shown molecular weights from 30 to 45 kDa (Cluness et al., 1993; Kaplan et al., 2011; Petricková et al., 2012; Vejvoda et al., 2008, 2010). These results are consistent with those observed for T. harzianum VSL291 nitrilase since Nit1 specific activity was 19.6 U/mg and it had a molecular mass of 47.7 kDa.

In contrast, the molecular weight of a hypothetical cyanide hydratase from F. solani CAC69666 that has at least 2 subunits has been estimated to be more than 300 kDa (Barclay et al., 1998). It has been described that the nitrilase super family hydrolyzes and condenses a variety of nonpeptide carbon-nitrogen bonds, utilizing a characteristic catalytic triad of Glu-Lys-Cys (Basile et al., 2008). This triad has been identified in the T. harzianum VSL291 nitrilase sequence as along with several conserved regions (domains) for the nitrilase super family, such as putative active sites and putative binding sites. Similar sequence architecture is present in some fungal hypothetical nitrilases obtained from the NCBI database (e.g., Trichoderma reesei (EGR46746), Trichoderma virions (EHK18648), Trichoderma atroviride (EHK18468)), cyanide hydratases (F. solani (CAM82815), Aspergillus fumigatus (XP_756085), and Penicillium digitatum (EKV07546)).

As mentioned above, Basile et al. (2008) described the catalytic triad on DNA sequences from degrading-cyanide nitrilases belonging to 4 species of fungi: Neurospora crassa, Gibberella zeae, Gloeocercospora sorghi, and Aspergillus nidulans. Trichoderma harzianum VSL291 nitrilase is grouped with Talaromyces stipitatus nitrilase (X_002478076) and is close to T. atroviride nitrilase (EHK47735) and the hypothetical T. virions nitrilase (EHK17305). The enzymatic parameters described for Nit1 are also consistent with those observed for nitrilases of fungal origin. For example, Aspergillus niger K10 and F. solani 01 nitrilases have higher activities at 40–45 °C and exhibit traces at temperatures close to 50 °C. Likewise, the range of their optimum pH is between 7.0 and 8.5 (Vejvoda et al., 2010; Kaplan et al., 2011).

It has been suggested that Ag⁺ ions act on the cysteine of the catalytic triad of nitrilases, destabilizing their activity (Kiziak et al., 2005). The strong inhibition of the enzymes by these ions is similar to that observed for nitrilase of F. solani (Vejvoda et al., 2008). To date, it is not clear why nitrilases behave differently in the presence of different ions. One of the possible causes could be the different degrees of purification of the studied enzymes, as well as the presence of contaminating proteins on enzymatic extracts (e.g., chaperone proteins). DTT is used commonly as an enzyme stabilizer. However, its effects on Nit1 enzymatic activity (a.c. 50%) and other purified nitrilases (Vejvoda et al., 2008, 2010), suggest that its use in the preparation of nitrilase buffers should be more limited or should be replaced by stabilizers that are less aggressive to the enzyme. In addition, this compound could break disulfide bonds and alter their tertiary structure, which could have a destabilizing effect, as reported in Pseudomonas fluorescens (Kiziak et al., 2005) and A. niger (Kaplan et al., 2011).

The chelating agents of metallic ions such as EDTA showed no important enzymatic inhibition, indicating that Nit1 could lack metal cofactors. Most nitriles are poorly soluble in aqueous media and, therefore, the enzyme's ability to degrade nitriles in organic solvents is very important. Binding of some inhibitors in high concentrations (e.g., 30%–50% v/v), such as butanol, toluene, acetonitrile, and 2-propanol, may prevent the entry of the substrate into the active site of the enzyme and hinder the corresponding reaction. Most of the time enzyme inhibitors react with the enzyme covalently and modify the essential amino acid residues’ structure required for enzyme activity. In contrast, reversible inhibitors bind to the enzyme noncovalently, resulting in different types of inhibition depending on whether the inhibitor binds to the enzyme, the enzyme-substrate complex, or both (Kaplan et al., 2011).

Another important characteristic of nitrilases is that they are able to show activity in the presence of mixtures of organic compounds such as dimethyl sulfoxide, methanol, and heptane (Heinemann et al., 2003). The results of cyanide degrading assays corroborate previous studies that propose the use of nitrilase enzymes obtained from microorganisms as an alternative to detoxification of waste waters (e.g., the plastic industry and the extraction of gold in mines). In bacteria, the genera Rhodococcus, Alcaligenes, Pseudomonas, and Bacillus degrade metallo-cyanide complexes and free cyanide because they use them as nitrogen sources (Barclay et al., 1998; Dubey and Holmes, 1995; Wang et al., 2012; Maniyam et al., 2013).

To date, most of the purified enzymes from fungi and bacteria belong to the xylanase, glucanase, and lipase families (Dobrev and Zhekova, 2012; Pham et al., 2012; Gökbulut and Arslanoğlu, 2013). Several fungal genera that produce cyanide-degrading enzymes have also been identified: Neurospora (Dent et al., 2009), Nocardia (Linton and Knowles, 1986), and Fusarium (Cluness et al., 1993). Demestre et al. (1997) isolated cyanide metabolizing microorganisms from contaminated alkaline wastes and soil, and they found in batch culture that cyanide (2 mM) disappeared in 72 h in an exponential manner in the presence of F. solani. Recombinant forms of fungi cyanide hydratases from N. crassa, A. nidulans, G. zeae, and G. sorghi reached their maximum rate at 60 mM KCN and N. crassa enzyme degraded 90% of the cyanide in less than 5
h (Basile et al., 2008). Ezzi and Lynch (2002) showed that some Trichoderma strains have high capacity to degrade cyanide via both the cyanide hydratase and the rhodanese pathways, and suggest a constitutive nature of both enzymes in this organism. Biodegradation by Trichoderma spp. using cyanide (2000 ppm) as the sole carbon source was achieved in 90 days (Ezzi and Lynch, 2005).

References


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