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A New Nitrilase-Producing Strain Named *Rhodobacter sphaeroides* LHS-305: Biocatalytic Characterization and Substrate Specificity

Chunsheng Yang · Xuedong Wang · Dongzhi Wei

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Abstract The characteristics of the new nitrilase-producing strain *Rhodobacter sphaeroides* LHS-305 were investigated. By investigating several parameters influencing nitrilase production, the specific cell activity was ultimately increased from 24.5 to 75.0 μmol g^{-1} min^{-1}, and hereinto, the choice of inducer proved the most important factor. The aromatic nitriles (such as 3-cyanopyridine and benzonitrile) were found to be the most favorable substrates of the nitrilase by analyzing the substrate spectrum. It was speculated that the unsaturated carbon atom attached to the cyano group was crucial for this type of nitrilase. The value of apparent K_m, substrate inhibition constant, and product inhibition constant of the nitrilase against 3-cyanopyridine were 4.5×10^{-2}, 29.2, and 8.6×10^{-3} mol L^{-1}, respectively. When applied in nicotinic acid preparation, the nitrilase is able to hydrolyze 200 mmol L^{-1} 3-cyanopyridine with 93% conversion rate in 13 h by 6.1 g L^{-1} cells (dry cell weight).

Keywords *Rhodobacter sphaeroides* · Nitrilase · 3-Cyanopyidine · Nicotinic acid · Biocatalyst

Introduction

In the past two decades, nitrilases have been acknowledged as useful biocatalysts for the hydrolysis of nitriles in a mild and environmentally friendly manner, which also assure a significantly cleaner process and specificity with high yield. For these reasons, nitrilases have been widely applied in the pharmaceutical, chemical engineering, and agricultural industries [1, 2].

Researchers and scientists started to exploit new nitrilases from many years ago, because of their huge demand in industry. After the first one was discovered in a plant in 1960s [3, 4], an increasing number of nitrilases from different sources have been discovered, especially those from microorganisms, which possesses most in nature [5, 6], such as

C. Yang · X. Wang (✉) · D. Wei
State Key Laboratory of Bioreactor Engineering, New World Institute of Biotechnology,
East China University of Science and Technology, Shanghai 200237, People's Republic of China
e-mail: xdwang@ecust.edu.cn

Acinetobacter, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Nocardia*, *Pseudomonas*, *Rhodococcus* [7–9], the fungus *Fusarium* [10, 11], etc. Many of the nitrilases from microorganisms have been applied successfully in industry. For example, the nitrilase from *Alcaligenes faecalis* ATCC8750 [12] is an effective catalyst for the stereoselective hydrolysis of mandelonitrile to (R)-(−)-mandelic acid and the nitrilase from *Acidovorax facilis* 72W [13] can convert glycolonitrile to glycolic acid with high efficiency.

Although too many nitrilases are reported and have been applied for manufacturing production, seeking for new sources of nitrilases has never stopped, because there is also a considerable number of undiscovered nitrilases in nature and some of them may have higher activities or may be from new source. For example, Mei Shen isolated a novel nitrilase-producing strain *Arthrobacter nitroguajacolicus* ZJUTB06-99 [14] from soil samples in Zhejiang province in 2009, which is able to convert acrylonitrile to acrylic acid with high efficiency.

In this paper, the characteristics of a novel isolated nitrilase-producing strain *Rhodobacter sphaeroides* LHS-305 was investigated, including its morphology, molecular weight, substrate specificity, and the apparent enzyme kinetics. The significant application prospects for producing useful acids were studied.

Materials and Methods

Chemicals

3-Cyanopyridine (AladdinBiotech, China), benzonitrile, phenylacetonitrile, *p*-methoxy phenylacetonitrile, β-amino propionitrile, and acrylonitrile (Sinopharm Chemical Reagent Co., LtdS, China) were used in this study. All the other chemicals were of analytical grade.

Medium and Cultivation

Seed medium (L): glycerol 3.3 g, yeast extract 5 g, fish peptone 5 g, NaCl 1 g, K_2HPO_4 2 g, $MgSO_4$ 0.2 g, $FeSO_4{\cdot}7H_2O$ 0.03 g, acetonitrile 5 mmol.

Enzyme production medium (L): Na_2HPO_4 2 g, KH_2PO_4 1 g, glycerol 3.25 g, $MgSO_4$ 0.2 g, $FeSO_4{\cdot}7H_2O$ 0.03 g, yeast extract 0.5 g, fish peptone 0.5 g, pH 7.0, autoclaved at 115 °C for 20 min.

Acetonitrile was added into the medium as inducer with the concentration of 10 mmol L^{-1} after seed culture was transferred into the enzyme production medium.

General Procedure of Biotransformation Using Rest Cells

The strain was incubated in seed medium on a rotary shaker at 160 rpm, 30 °C for 24 h. Subsequently, the inoculum (10%, *v*/*v*) was transferred into a fresh enzyme production medium for 24 h cultivation. After being collected by centrifugation at 4 °C, 10,000 rpm for 7 min, the cells were washed with sodium phosphate buffer (50 mmol L^{-1}, pH 7.0) and stored at 4 °C.

The standard reaction mixture was composed of 50 mmol L^{-1} sodium phosphate (pH 9.0), 200 mmol L^{-1} 3-cyanopyridine, and an appropriate amount of the cells or enzyme. Each reaction was carried out at 30 °C for 20 min at 200 rpm in an Erlenmeyer flask and stopped by adding 50% ethanol (*v*/*v*) to the reaction mixture. The cells were centrifuged and collected, and washed with distilled water, then dried in an oven at 100 °C to determine the dry cell weight.

Optimization of Culture Conditions for Nitrilase Production

To determine the optimal conditions for nitrilase production, different factors were tested during the cultivation, including temperature (20–37 °C), pH (5.0–9.0), inducers, etc. First, 10 mmol L^{-1} inducers were added into the enzyme production medium at the beginning of the cultivation at 30 °C and pH 7.0. Subsequently, all factors influencing enzyme production were analyzed by changing one factor at a time.

Characteristics of Nitrilase in Cells as a Biocatalyst

The specific cell activity was determined in the pH range of 4.0–10.6 at 30 °C, using the buffers Na_2HPO_4/KH_2PO_4 (pH 4.92–9.18) and $Na_2CO_3/NaHCO_3$ (pH 8.77–10.57).

The thermostability of intracellular nitrilase was assessed at temperature of 30 °C, 37 °C, and 50 °C. The rest cells were stored in the temperatures above, whose 3-cyanopyridine hydrolyzing activities were determined every 10 h under the optimum conditions. The half-life of the nitrilase was estimated according to the presence of the specific cell activity at each temperature, which was plotted against time.

The following nitrile compounds were tested for substrate specificity under optimum reaction conditions: 3-cyanopyridine, benzonitrile, phenylacetonitrile, *p*-methoxy-phenylacetonitrile, mandelonitrile, Acrylonitrile, β-amino propionitrile, and butyronitrile.

Reaction of 3-Cyanopyridine to Nicotinic Acid in a Batch Reactor by *R. sphaeroides*

The *R. sphaeroides* LHS-305 was used to catalyze 3-cyanopyridine in a batch reactor under optimum operational conditions to achieve the highest conversion rate with 6.1 g L^{-1} cells (dry cell weight) under six different concentrations of substrates (50–500 mmol L^{-1}).

Separation of *R. sphaeroides* LHS-305 Nitrilase

All steps were performed at 4 °C. Potassium phosphate pH 9.0 was used as buffer and centrifugation was carried out for 90 min at 20,000×*g* in all purification steps. Cells from the culture broth were rinsed twice with buffer and suspended in 50 mmol L^{-1} buffer, disrupted with an ultrasonic oscillator and then centrifuged. The supernatant was fractionated by ammonium sulfate precipitation (20–50%). The farther separations were carried out by hydrophobic chromatography (Phenyl Sepharose, 20 mmol L^{-1} Tris–HCl buffer pH 7.2, 0.8–0 mol L^{-1} ammonium sulfate gradient elution) and gel filtration chromatography (Sephacryl S-200 HR, 50 mmol L^{-1} potassium buffer pH 7.2). The fractioned samples were detected by activity analysis and SDS–PAGE.

Analytical Methods

The nicotinic acid, the product of the reaction mixture, was quantitatively analyzed through an HPLC system (Agilent) equipped with C18 reverse phase column (4.6×250 mm), and loaded with the mobile phase, 0.01 M H_3PO_4 buffer (pH 2.0)/acetonitrile (95:5, *v*/*v*) flowing at a rate of 0.8 ml min^{-1} at room temperature.

One unit of specific cell activity is expressed as 1 μmol g^{-1} min^{-1}, defined as 1 μmol of nicotinic acid released by 1 g of dry cell weight in 1 min under optimum reaction conditions and 1 U of the enzyme activity per volume (u L^{-1}) for the cell culture is defined as 1 μmol of nicotinic acid released by 1 L cell culture or enzyme solution in 1 min.

Results and Discussion

Morphological Characteristics and Background of the Strain

In a previous work, the strain LHS-305 was isolated from soil samples after enrichment with glycerol as the carbon source and 3-cyanopyridine as nitrogen source [15]. The strain was identified as *R. sphaeroides* through analysis of the 16S rDNA sequence and physiological and biochemical experiments.

It was easy to cultivate the strain in rich medium (such as seed medium). Colonies cultivated on agar plates were light yellow, convex, smooth, dry, and with a single edge. The cells were in the form of rods, with a length of about 0.8–1.0 μm, with capsules and gram negative.

R. sphaeroides is a purple non-sulfur phototrophic bacterium, belonging to the α-3 subgroup of the *Proteobacteria* [16]. The strain was originally collected from Delft, Holland, and California from a variety of enrichment cultures. Members of *R. sphaeroides* exhibit substantial metabolic versatility [17] and genomic complexity. Under aerobiosis, *R. sphaeroides* grows as a chemoheterotroph, possessing a terminal respiratory chain, and morphologically resembles a typical gram-negative bacterium [18]. *R. sphaeroides* has been shown to detoxify a number of metal oxides and oxyanions. At present, it is the subject of intensive investigations worldwide as regards the structure, function, and regulation of its photosynthetic membranes, its mechanisms for CO_2 fixation, nitrogen fixation, cytochrome diversity, and electron transport systems. A considerable number of applications are reported for this bacterium, such as coenzyme Q_{10} production [19, 20], biohydrogen production [21, 22], and biodegradation of chlorobenzene [23] from industrial wastewater.

Identification of *R. sphaeroides* LHS-305 Nitrilase

The previous work showed that the free cells of *R. sphaeroides* LHS-305 exhibited nitrile-hydrolyzing activity towards 3-cyanopyridine without any nicotinamide detected, and was not active towards nicotinamide.

With the purpose of identification for the enzyme, preliminary separation was performed. The separated enzyme gave only one band on sodium dodecyl sulfate/polyacrylamide gel electrophoresis analysis, corresponding to a molecular mass of 38 kDa (Fig. 1). The enzyme was able to hydrolyze 3-cyanopyridine to nicotinic acid without any nicotinamide formed and did not show any activity towards nicotinamide. Consequently, it can be concluded that the enzyme showed nitrilase activity and could be identified as nitrilase.

Optimization of Nitrilase Production Conditions

The initial specific cell activity and enzyme activity per volume of the cell culture induced by 3-cyanopyridine was 24.5 μmol g^{-1} min^{-1} and 58.8 u L^{-1}, respectively at pH 7.0, 30 °C. Several parameters were optimized for nitrilase production such as temperature, ions, pH (data not shown), inducers, etc., among which inducer types play the most important role. Acetonitrile was proven to be the best inducer, which caused the enhancement of the specific cell activity to 65.3 μmol g^{-1} min^{-1}, more than twice of that induced by the initial inducer 3-cyanopyridine (Table 1). Table 1 also showed that phenylacetamide caused the highest enzyme activity per volume for the cell culture with the value of 155.0 u L^{-1}, but we finally chose acetonitrile as the inducer for the cultivation, because of its cheaper price and good effectivity. Other factors, including pH, temperature (Fig. 2), ions, etc., were also investigated

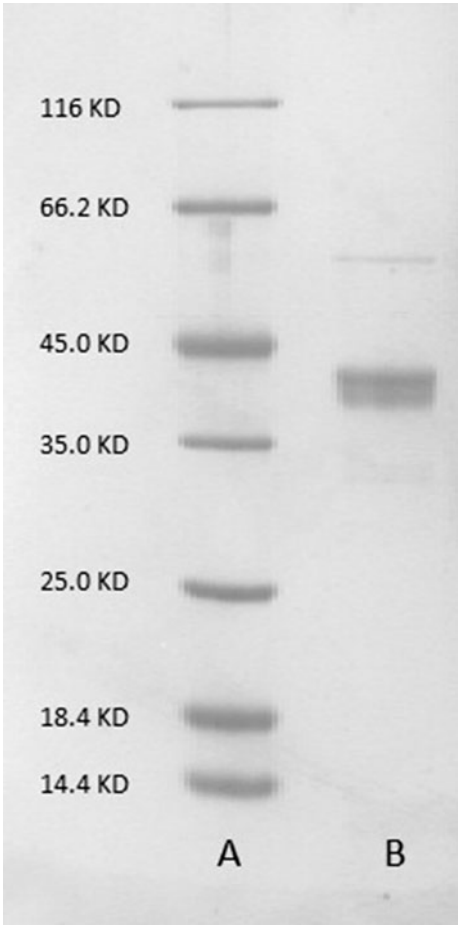

Fig. 1 SDS/PAGE of the purified *Rhodobacter sphaeroides* LHS-305 nitrilase. **A** Marker protein. The molecular weight of each band was shown on the left side of the picture. **B** The purified enzyme

and the specific activity of cells was improved from 65.3 to 75.0 μmol g^{-1} min^{-1}. The result showed that all other factors were not as significant as the inducer. Finally, the optimum pH and temperature were established as 7.0 °C and 28 °C, respectively, and the best ion for nitrilase production was Fe^{2+}. Several ions, including Fe^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, and Co^{2+}, were tested for the optimization and the culture added Fe^{2+} led to highest enzyme activity, so Fe^{2+} was considered as the best ion for nitrilase production. After the optimization of all factors for nitrilase production, the specific activity of cell was enhanced from 24.5 to 75.0 μmol g^{-1} min^{-1}, with the activity of culture from 58.8 to 155.0 u L^{-1}.

Characteristics of the Nitrilase in Rest *R. sphaeroides* LHS-305 Cells as a Biocatalyst

Effects of Different pH on the Specific Cell Activity

Figure 3 showed that *R. sphaeroides* preferred alkalescent conditions rather than neutral in biocatalytic reaction. When Na_2HPO_4/KH_2PO_4 buffer (pH 9.0) used, the cells exhibited the highest activity at 75.0 μmol g^{-1} min^{-1}. When the reaction was carried out in the buffer $Na_2CO_3/NaHCO_3$ at higher pH [10, 11], a sharp decrease of the specific cell activity was observed.

Table 1 The effects of different inducers on nitrilase production

Inducers	Cell activity (μmol g^{-1} min^{-1})	Dry cell weight (g L^{-1})	Enzyme activity per volume (u L^{-1})
None	20.2	3.3	66.7
3-Cyanopyridine	24.5	2.4	58.8
Nicotinic acid	22.0	1.2	26.4
Acetonitrile	65.3	2.2	143.7
Acrylonitrile	38.9	2.1	81.7
Phenylacetamide	64.6	2.4	155.0
Urea	15.4	1.7	26.2
Nicotinamide	24.0	2.5	60
Benzonitrile	40.4	2.2	88.9
Phenylacetic acid	22.4	0.3	6.7
Phenylacetonitrile	6.0	0.4	2.4
Acrylic acid	17.9	1.5	26.8
Benzoic acid	17.8	0.3	5.3

The activity was determined at 30°C with 10 mmol L^{-1} substrate added for the initial 20 min

Hence, the optimum pH 9.0 buffer Na_2HPO_4/KH_2PO_4 was selected as the best one. Usually, the hydrolysis of nitriles by nitrilase was generally performed in neutral environments, with pH ranging from 6.0 to 8.0. An example is that from *Arthrobacter nitroguajacolicus* ZJUTB06-99, which catalyzes acrylonitrile to acrylic acid at the optimum pH of 6.5 [14].

Thermostability of the R. sphaeroides as a Biocatalyst

The specific cell activity was determined at intervals when strains were stored at temperatures 30 °C, 37 °C, and 50 °C as shown in Fig. 4. The half-life ($t_{1/2}$) of biocatalyst at each temperature was calculated according to the specific cell activity curve against time and found to be 25 h (30 °C), 21 h (37 °C), and 8 h (50 °C). Therefore, the *R. sphaeroides* as a nitrilase-producing strain is unable to endure high temperatures since the specific cell

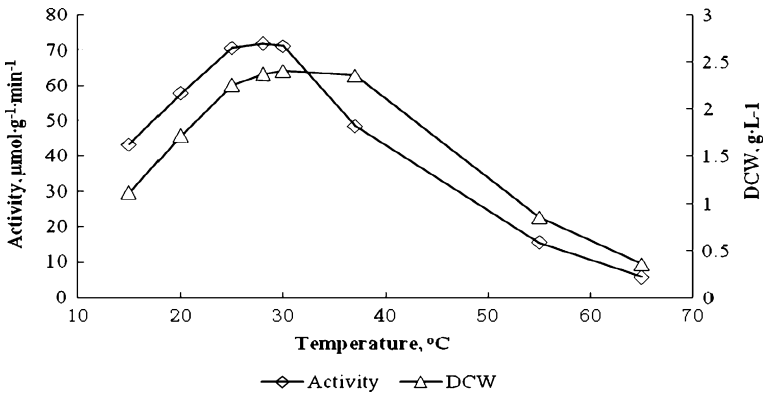

Fig. 2 Effect of temperature on nitrilase production. The cells were cultivated under the condition below: acetonitrile 50 mmol L^{-1}, glycerol 5 g L^{-1}, $FeSO_4$ 0.03 g L^{-1}, pH 7.0, rotation rate 160 rpm. The activity was determined at 30°C with 200 mmol L^{-1} substrate and 20 min reaction time

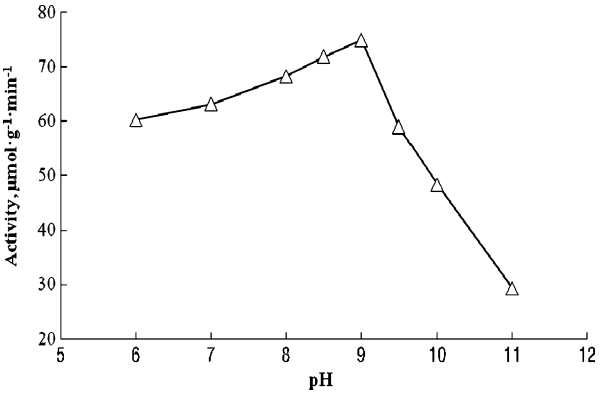

Fig. 3 Effect of different pH on cell activity. The activity was determined at 30°C with 200 mmol L^{-1} substrate and 20-min reaction time

activity decreased sharply at 50 °C. However, there are also some nitrilases which possess both good thermal stability and 3-cyanopyridine hydrolyzing activity in nature, such as that produced by *Bacillus pallidus* Dac521, which demonstrated an optimum temperature of 60 °C to convert 3-cyanopyridine [24].

For most enzymatic reaction, the higher the temperature, the higher the activity that could be achieved, but enzymes may be inactivated rapidly as the temperature goes up. The strain studied in this paper is in that case. Consequently, 30 °C was selected for the catalytic reaction in further studies.

Substrate Spectrum

Currently, three types of nitrilases have been reported according to the characteristics of the hydrolyzed nitriles, namely aliphatic nitrilases, aromatic nitrilases, and arylacetonitrilases [25]. Most nitrilases belong to one or two kinds of them, although particular cases exist, such as that in *Bacillus subtilis* ZJB-063 [26] which exhibited the hydrolyzing activities against almost all kinds of nitriles. In Table 2, eight substrates were studied and it was found that the *R. sphaeroides* nitrilase could hydrolyze the aromatic nitriles like 3-cyanopyridine and benzonitrile effectively, but the activity against benzonitrile was not as high as that towards 3-cyanopyridine. This is probably due to the toxicity of the latent solvent (DMSO, 4%), which is used to increase the solubility of benzonitrile. No products were found when phenylacetonitrile and *p*-methoxy phenylacetonitrile served as substrates for the reaction, so was β-amino propionitrile. However,

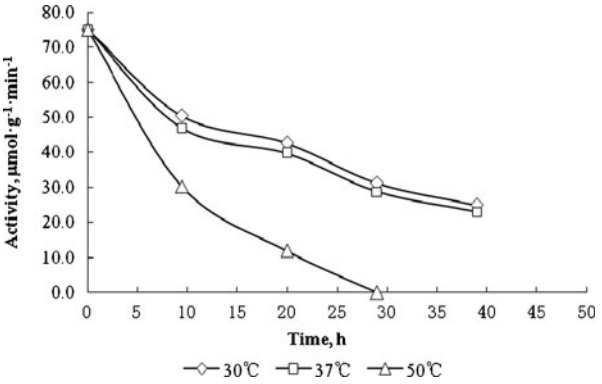

Fig. 4 The effects of different temperatures on stability of the cell activity. *Diamonds*, *squares*, and *triangles* stand for the temperatures of 30 °C, 37 °C, and 50 °C, respectively. The activity was determined at 30 °C, pH 9.0 with 10 mmol L^{-1} substrate added for the initial 20 min

Table 2 Cell activity towards different substrates

Nitriles	Activity (μmol g^{-1} min^{-1})
3-Cyanopyridine	75.0
Benzonitrile	7.5
Phenylacetonitrile	ND
p-Methoxy-phenylacetonitrile	ND
Mandelonitrile	ND
Acrylonitrile	22.0
β-Amino propionitrile	ND
Butyronitrile	ND

The activities were all detected at the optimum conditions served with 200 mmol L^{-1} substrates

acrylonitrile (aliphatic nitrile) was hydrolyzed at the activity of 22.0 μmol g^{-1} min^{-1}, which indicates that the nitrilase of *R. sphaeroides* was active against some of the aliphatic nitriles as substrates. It was speculated that the structure of acrylonitrile with unsaturated carbon atom could be the reason. These substrates had conjugated double bonds offered by the cyano group and its neighbor group: aromatic nucleus or alkyl group with a double bond. Consequently, the *R. sphaeroides* nitrilase might prefer certain nitriles with the cyano group attached to unsaturated carbon atom.

Apparent Kinetics of the Biocatalyst for the Hydrolysis of 3-Cyanopyridine

In Fig. 5, the initial reaction velocity of the rest cell was examined under 3-cyanopyridine's concentrations of 100–600 mmol L^{-1}. The results suggested that substrate inhibition appeared when the substrate concentration exceeded 400 mmol L^{-1}, and the initial reaction velocity decreased dramatically when the 3-cyanopyridine's concentration increased more. However, the relationship between the velocity and the 3-cyanopyridine's concentration showed clear linearity when 3-cyanopyridine is below 400 mmol L^{-1}, as illustrated by the Lineweaver–Burk plot [27–29] in Fig. 6. Meanwhile, apparent K_m and r_{max} were given as 4.5×10^{-2} mol L^{-1} and 7.7×10^{-5} mol L^{-1} min^{-1}, respectively. Subsequently, MATLAB (7.0) was applied to calculate the apparent substrate inhibition constant K_I through Eq. 1 using the least-squares estimation which is estimated to be 29.2 mol L^{-1}. The results indicate that *R. sphaeroides* nitrilase did not exhibit a high affinity against 3-cyanopyridine due to its high K_m value compared with other nitrilases, such as that in *Pseudomonas putida* with a K_m of 3.61×10^{-3} to 13.39×10^{-3} mol L^{-1} towards three different substrates. Consequently,

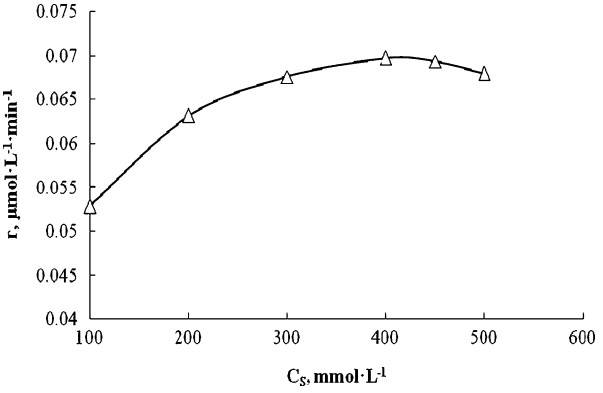

Fig. 5 The effect of substrate concentration on initial reaction velocity. Reaction was carried out by 0.63 g L^{-1} (dry cell weight) cells at pH 9.0, 30 °C

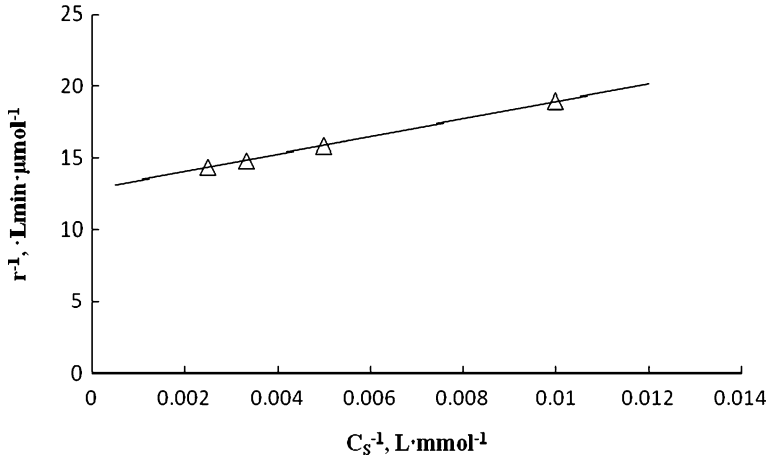

Fig. 6 Method of Lineweaver–Burk to calculate the apparent kinetic constants K_m and r_{max} with 0.63 g L^{-1} (dry cell weight) cells added to each system at pH 9.0, 30°C. r the initial reaction velocity, C_s the substrate concentration

although the *R. sphaeroides* possessed high 3-cyanopyridine hydrolyzing activity, a zero-order reaction could not be obtained easily because of the low substrate affinity. The apparent r_{max} was attained when the substrate concentration was significantly higher than the K_m value. However, for the *R. sphaeroides* nitrilase, the high concentration was unfavorable when it exceeded 400 mmol L^{-1} due to the substrate inhibition (Fig. 5).

The typical product inhibition is shown in Fig. 7. The experimental data fitted the curve well, which is described by Eq. 2. The product inhibition constant K_{PI} was then estimated at 8.6×10^{-3} mol L^{-1} using the method above.

$$r = \frac{r_{max} \bullet C_s}{K_m + C_s\left(1 + \frac{C_s}{K_I}\right)} \tag{1}$$

$$r = \frac{r_{max} \bullet C_s}{K_m\left(1 + \frac{C_P}{K_{PI}}\right) + C_s} \tag{2}$$

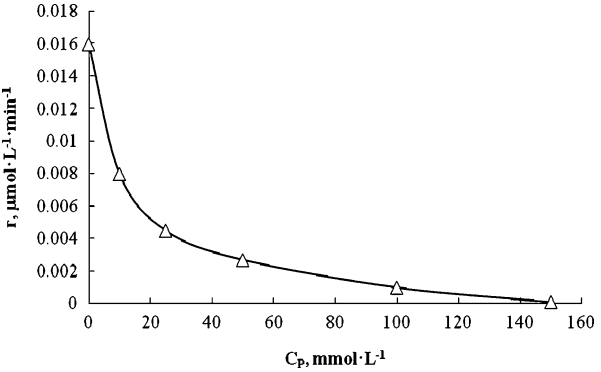

Fig. 7 The effect of product concentration on the initial reaction velocity. C_p is product concentration (mmol L^{-1}). Reaction was carried out by 0.63 g L^{-1} (dry cell weight) cells with 10 mmol L^{-1} 3-cyanopyridine added at pH 9.0, 30 °C

Table 3 Comparison of the specific cell activity of different strains towards 3-cyanopyrine

Strain	Optimum reaction pH	Optimum reaction temperature (°C)	Specific cell activity (μmol g^{-1} min^{-1})	Maximal substrate tolerance (mmol L^{-1})
Nocardia globerula NHB-2	7.5	30	310	40
Bacillus pallidus Dac521	8	60	76	300
Rhodococcus sp. NDB 1165	7.2	30	117	50
Rhodobacter sphaeroides LHS-305	9	30	75	400

The highest initial reaction velocity appeared when 400 mmol L^{-1} substrate was added into the buffer (Fig. 5), which means that the nitrilase showed the highest activity at that substrate concentration. Table 3 shows several nitrilase-producing strains which possess the ability to hydrolyze 3-cyanopyridine, such as *Nocardia globerula* NHB-2, *B. pallidus* Dac52, and *Rhodococcus* sp. NDB 1165 [30–32]. It is found that the nitrilase's specific cell activity from *R. sphaeroides* LHS-305 is 75 μmol g^{-1} min^{-1}, a little lower than others, but it shows the best substrate tolerance. Among them, only *B. pallidus* Dac521 nitrilase could convert 3-cyanopyridine at the high concentration of 300 mmol L^{-1}, and all others showed weak 3-cyanopyridine tolerance (Table 3). This property of the enzyme suggests that it is with great potentiality for industry production of nicotinic acid, because high substrate concentration is of great benefit of enhancing production efficiency and reducing the cycle time.

Application of R. sphaeroides LHS-305 to Convert 3-Cyanopyridine in a Batch Reactor

In Table 4, 3-cyanopyridine could be hydrolyzed almost completely with concentrations below 100 mmol L^{-1} using 6.1 g L^{-1} cells (dry cell weight), while incomplete conversion was obtained when the substrate concentration exceeded 200 mmol L^{-1}. As described above, both substrate and product inhibition existed. High substrate concentrations (above 400 mmol L^{-1}) could decrease the reaction rate at the beginning, and the effect of product inhibition increased as the reaction progressed. Considering that the effect of inhibition could be lowered by high cell concentration, a high density of cells (6.1 g L^{-1}, dry cell weight) was used. A 200 mmol L^{-1} substrate concentration was finally selected for future application in the batch reactor due to its high conversion rate of 93%.

Table 4 Application of *Rhodobacter sphaeroides* nitrilase to covert 3-cyanopyridine in batch reactor towards different substrate concentrations

Substrate concentration (mmol L^{-1})	Conversion rate (%)	Reaction time (h)
50	99	6
100	99	9
200	93	13
300	83	16
400	75	19
500	58	24

Conclusions

In summary, an isolated nitrilase-producing *R. sphaeroides* was identified in hydrolyzing aromatic nitriles such as 3-cyanopyridine. The specific cell activity against this substrate was established at 75.0 μmol g^{-1} min^{-1} after optimization of the enzyme production and reaction conditions. Although the activity was not higher than the strains reported performing nitrilase activity towards 3-cyanopyridine, the strain showed the best 3-cyanopydine tolerance, which was very important for industrial production of nicotinic acid. This shows that the *R. sphaeroides* LHS-305 as a nitrilase-producing strain could serve as a good biocatalyst. By comparing the conversion rate in batch reactions, concentration of 200 mmol L^{-1} was considered as the most suitable for the substrate. Both the nitrilase's substrate and product inhibition exists towards 3-cyanopyridine, and the product inhibition effects more on the reaction, which should be considered when the nitrilase was applied in bioreactors. In future, further researches could be taken in the significant application prospects for producing this useful acid.

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