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REVIEW (INVITED BY JACQUELINE SHANKS)

Nitrile hydratase as a promising biocatalyst: recent advances and future prospects

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Abstract Amides are an important type of synthetic intermediate used in the chemical, agrochemical, pharmaceutical, and nutraceutical industries. The traditional chemical process of converting nitriles into the corresponding amides is feasible but is restricted because of the harsh conditions required. In recent decades, nitrile hydratase (NHase, EC 4.2.1.84) has attracted considerable attention because of its application in nitrile transformation as a prominent biocatalyst. In this review, we provide a comprehensive survey of recent advances in NHase research in terms of natural distribution, enzyme screening, and molecular modification on the basis of its characteristics and catalytic mechanism. Additionally, industrial applications and recent significant biotechnology advances in NHase bioengineering and immobilization techniques are systematically summarized. Moreover, the current challenges and future perspectives for its further development in industrial applications for green chemistry were also discussed. This study contributes to the current state-of-the-art, providing important technical information for new NHase applications in manufacturing industries.

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Introduction

Compared with traditional chemical catalysts, biocatalysts are of great interest because of their significant advantages in terms of reaction conditions, such as their high efficiency, excellent selectivity (regioselectivity, chemoselectivity and enantioselectivity), and eco-friendly reaction conditions. Thus, "green" biocatalysts have gained much attention, providing another route for the industrial production of bulk chemicals and pharmaceuticals (de Carvalho 2011; Du et al. 2011; Patel 2011; Wang et al. 2012; Lee et al. 2019).

Nitriles, organic compounds widely present in nature, have attracted much attention in the chemical market for the synthesis of important amides (acrylamide (Fleming et al. 2010), nicotinamide (Nikas et al. 2020), cyanoverlamide (Wang et al. 2020b), and drug intermediates (Banerjee et al. 2016). Nitrile hydratase (NHase), which is a key enzyme in the bienzymatic pathway of nitrile degradation, catalyzes the conversion of nitriles to the corresponding amides (Jiao et al. 2020; Cui et al. 2014). NHase has been found in a variety of microbes belonging to various species of diverse genera, including *Actinobacteria*, *Proteobacteria*, *Cyanobacteria* and *Firmicutes*, since it

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was initially identified in the bacterium *Arthrobacter* sp. J1 (now known as *Rhodococcus rhodochrous* J1) in 1980 (Asano et al. 1980). With the increasing demand for NHase, many studies have focused on screening and modifying NHase enzymes for green industrial chemistry (Ma et al. 2024a; Wang et al. 2022; Guo et al. 2024; Zhang et al. 2023). Yamada et al. for the first time achieved large-scale industrial production of acrylamide in three NHase producing strains: *R. rhodochrous* J1, *Rhodococcus* sp. N-774 and *Pseudomonas chlororaphis* B23 (Yamada et al. 1996). To date, the third-generation industrial strain *R. rhodochrous* J1 has dominated in the industrial production of amides, especially acrylamide and nicotinamide. In China, Shen et al. used *Nocardia* sp. to industrialize acrylamide and nicotinamide production (Wang et al. 2007). Additionally, NHase also plays an important role in the textile industry; e.g., it can improve the properties of polyacrylonitrile (PAN) fibres as a synthetic block (Tauber et al. 2000; Guebitz and Cavaco-Paulo 2008). In addition, on the basis of great achievements in synthetic biology, the industrial production of amides could be improved by the use of engineered strains harbour robust NHase genes.

Like many other scientific fields, the history of NHase since its discovery to date is the journey from academia to industry, which has entered a new era to create more possibilities by revealing the secrets of ecological, microbiological, molecular, protein chemistry and bioremediation areas for nearly three decades. Most of the previous NHase reviews invariably covered NHase cloning, structural, and molecular characteristics, mechanisms and applications (Prasad and Bhalla 2010; Supreetha et al. 2019; Cheng et al. 2020b). This review summarizes recent NHase research progress with respect to its natural distribution, enzyme screening, molecular modification, industrial application and recent significant biotechnology. Finally, we briefly discuss the challenges, opportunities, and future prospects for its further development in industrial applications for green chemistry. This review provides useful information and insight for basic research and the industrial application of NHase.

Natural distribution of NHase

In nature, it has been reported that the distribution of NHase-producing microorganisms is very widespread, with bacteria accounting for the majority of producing microorganisms, mainly *Actinomycetes* and *Proteobacteria*. However, the majority of NHases are obtained from various species of *Rhodococcus* (Prasad and Bhalla 2010). Recently, NHase genes have been found in the genomes of some eukaryotes, such as *Monosiga brevicollis* (Tanii 2017). Foerstner et al. explored the NHase gene cluster through sequence-based metagenomic screening method and reported an unusual NHase structure consisting of two usually separated NHase subunits fused in one protein, which might open a new way to study the structure and function of eukaryotic NHases further (Foerstner et al. 2008). However, there is no related research report on gene function identification of NHase in the eukaryote. Therefore, bacteria are still the main source of NHase. Recently, Zhou et al. explored an archaeal NHase from halophilic archaeon A07HB70, which exhibits high tolerance to 3-cyanopyridine and nicotinamide, further broadening our understanding of NHase (Guo et al. 2024).

Screening methods for NHase

Traditional enrichment cultivation

Nitrile compounds, which are metabolic products of biological systems, are ubiquitous in the natural environment and exist in a variety of forms (Legras et al. 1990). It is estimated that there are hundreds of millions of microorganisms in each gram of soil. In order to screen microorganisms harboring NHase, an effective and feasible screening model is required. Conventional screening of NHase has been carried out by enrichment cultivation using selective cultures with nitriles as the sole C/N source. In recent years, owing to the rapid development of gene sequencing technology, the amount of genomic data has increased rapidly, and the researches on screening of metagenomic libraries and genome mining have also become more and more prosperous.

Function- and sequence-based screening of metagenomic libraries

The natural environment contains abundant microbial resources and is an important natural repository for biocatalysts (Kimura and Nobutada 2006). NHase is mainly distributed in microorganisms in natural environment, of which bacteria occupy the majority (Prasad and Bhalla 2010). The strains harbouring NHase isolated and screened thus far have been obtained from environments (such as wastewater, soil, and farmland) through traditional isolation and culture techniques, but less than 1% of the microbial resources in the environment can be cultivated. Metagenomics is the genomic analysis of microbial communities through expression-based or sequence-based methods without culturing; a large amount of genetic information can be obtained without cultivation (Ye et al. 2019). Owing to the use of metagenomic technology, the diversity of the obtained microbial genetic information can be greatly improved, which is conducive to the discovery of many unknown biocatalysts. In particular, the application of high-throughput screening (HTS) greatly enhances screening efficiency and increases the application scope of metagenomic technology. The screening of unknown biocatalysts via metagenomic technology can generally be divided into the following steps: sample collection, DNA extraction, library construction, screening for NHase activity, subcloning and expression, identification and sequencing (Gong et al. 2013).

Genome mining based on the conserved amino acid sequence

The traditional screening methods for NHase mostly involve isolationed from basal medium with nitrile compounds as the only nitrogen source, which is not only time-consuming and labour intensive, but also suffer from low screening efficiency. Gene mining, a network technology, allows the search and screening of homologous sequences with similar functions in the database using the nucleotide or amino acid sequences of known enzyme proteins as probes (Zhao et al. 2023). With this novel method, researchers can design primers according to the known gene sequence, utilize polymerase chain reaction to amplify the target enzyme gene and then perform functional expression of the gene in the host cell (Gong et al. 2013).

In the postgenome era, although gene resources are very abundant, a large number of gene sequences do not have corresponding functional annotations or clear biological functions (Seffernick et al. 2009). The nucleotide sequences of the subunits can be searched in NCBI, which are valuable resources for NHase studying. Genome mining provides technical support for obtaining novel NHase genes.

Molecular modification of NHase based on its characteristics and catalytic mechanism.

NHase characteristics

NHase consists of two allogenic α- and β-subunits (Fig. 1), which are usually present in equimolar

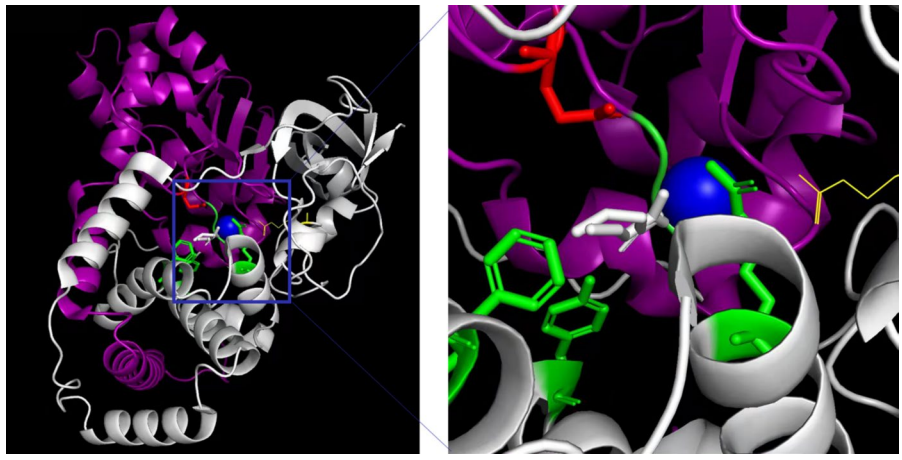


Fig. 1 Crystal structure of nitrile hydratase (NHase) from Pseudonocardia thermophila JCM 3095 (PDB 1ugp). NHase consists of two allogenic α- and β-subunits. The α- subunits and β-subunits are represented in gray and purple, respectively. The square box represents the active-site center as the channel used for entering and exiting of substrate and product molecules

amounts and generally have similar molecular weights, but NHases from different sources are structurally different. Structural analysis of NHases, revealed that the VC(T/S) LCSC(Y/T) in the α- subunit region is highly conserved, and this special site is a metal-binding domain (catalytic domain), that can coordinate with metal ions (Hashimoto et al. 2002; Miyanaga et al. 2004). According to the type of metal ions at the active site, NHases are divided into two categories: (a) Fe-containing NHase and (b) Co-containing NHase (Miller et al. 2024). Fe-NHase generally interacts with the small nitrile compounds, while the Co-NHase is considered to act more strongly on aromatic halogenated molecules (Desai and Zimmer 2004). Furthermore, Co-NHase is more robust and has wider substrate specificity compared with Fe-NHase. These metals are constitutional components of functional NHase, which fulfill a significant role in folding, stability and catalysis of the NHase polypeptide chains (Komeda et al. 1996). It is noteworthy that the nitrile substrate can only be catalysed in the interior of NHase, because the catalytic domain of NHase (the Fe−/Co-ion centre at the active site) is deeply buried in protein scaffold (Prasad and Bhalla 2010).In addition, according to the different protein molecular weight, NHase is divided into two types: low-molecular-weight (L-NHase) and high-molecular-weight NHase (H-NHase). Owing to its excellent thermal stability and organic solvent tolerance, H-NHases have been widely used in the acrylamide and nicotinamide industrial production (Miyanaga et al. 2004; Lan et al. 2017).

The characteristics of the previously reported NHasea are summarized in Table 1. The optimal pH value of the NHase reported to date is 6.5–8.5, and the optimal temperature is 20–35 °C, except for those isolated from thermophilic bacteria, e.g. *Bacillus* RAPc8 (60 °C) (Pereira et al., 1998), *Bacillus pallidus* Dac521 (50 °C) (Cramp and Cowan, 1999), and *Pseudonocardia* (60 °C) (Yamaki et al., 1997). The reaction involving amidase is the main rate-limiting factor for amide production, due to the existing of original amidase, which can further hydrolyse the formed amides into the corresponding carboxylic acids and ammonia. Thus, significant strategies have been developed to overcome this obstacle, for instance, the reaction can be carried out at low temperature (< 25 °C) for reducing amidase activity to negligible levels (Prasad and Bhalla 2010). Besides, cloning and expression of NHases in a heterologous host lacking amidase activity is a widely used method. Furthermore, ceasing the amidase activity through knock-out or interferring the amidase gene in parent strain is another feasible strategy (Ma et al. 2010).

Catalytic mechanism of NHase

In order to illustrate the the complete mechanism of NHase, many studies have focused on experimental and theoretical studies, and some plausible mechanisms have been proposed. To date, four catalytic mechanisms have been proposed for NHase catalysis, as shown in Fig. 2. The inner-sphere mechanism indicates that nitriles initially bind to metal ions, after which the binding group is hydrolysed by water molecules (Fig. 2A). Sugiura and Kuwahara et al. used electron spin resonance spectroscopy (ESR) to analyse the status of Fe ions in *P. chlororaphis* B23 NHase, and reported that the spectra shifted when the acrylic nitrile was added as the optimal substrate. However, when isobutyronitrile (not the catalytic substrate) and the product proacrylamide were added, no change in the spectra occurred, suggesting that the substrate may be directly connected to the metal ion (Fe^{3+}) (Sugiura et al., 1988). The outer-sphere mechanism demonstrated that the hydroxide ion liberated from water molecule coordinated with metal ion and catalysed the nitrile substrate hydrolysis reaction (Prasad and Bhalla 2010) (Fig. 2B). This catalytic mechanism has been confirmed by many studies. Peplowski et al. used a computer-aided molecular docking technique to analyse the conformation of a Co-type NHase from *P. thermophila* JCM 3095 with different substrates and products, and the results supported the outer-sphere mechanism (Peplowski et al., 2007). Kubiak and Nowak et al. used molecular dynamics simulation technology to analyse the action mechanism of NHase derived from *Rhodococcus* sp. N-771, and the results also showed that the water molecules connected with metal ions directly attacked the cyanide carbon atoms in nitrile compounds (Kubiak and Nowak 2008). Yu et al. analysed the Co-type NHase of *P. hermophila* JCM 3095 using a more accurate semiempirical quantum mechanical calculation method, and the results also supported the catalytic mechanism (Yu et al. 2008). Another recently proposed outer-sphere mechanism indicated that the

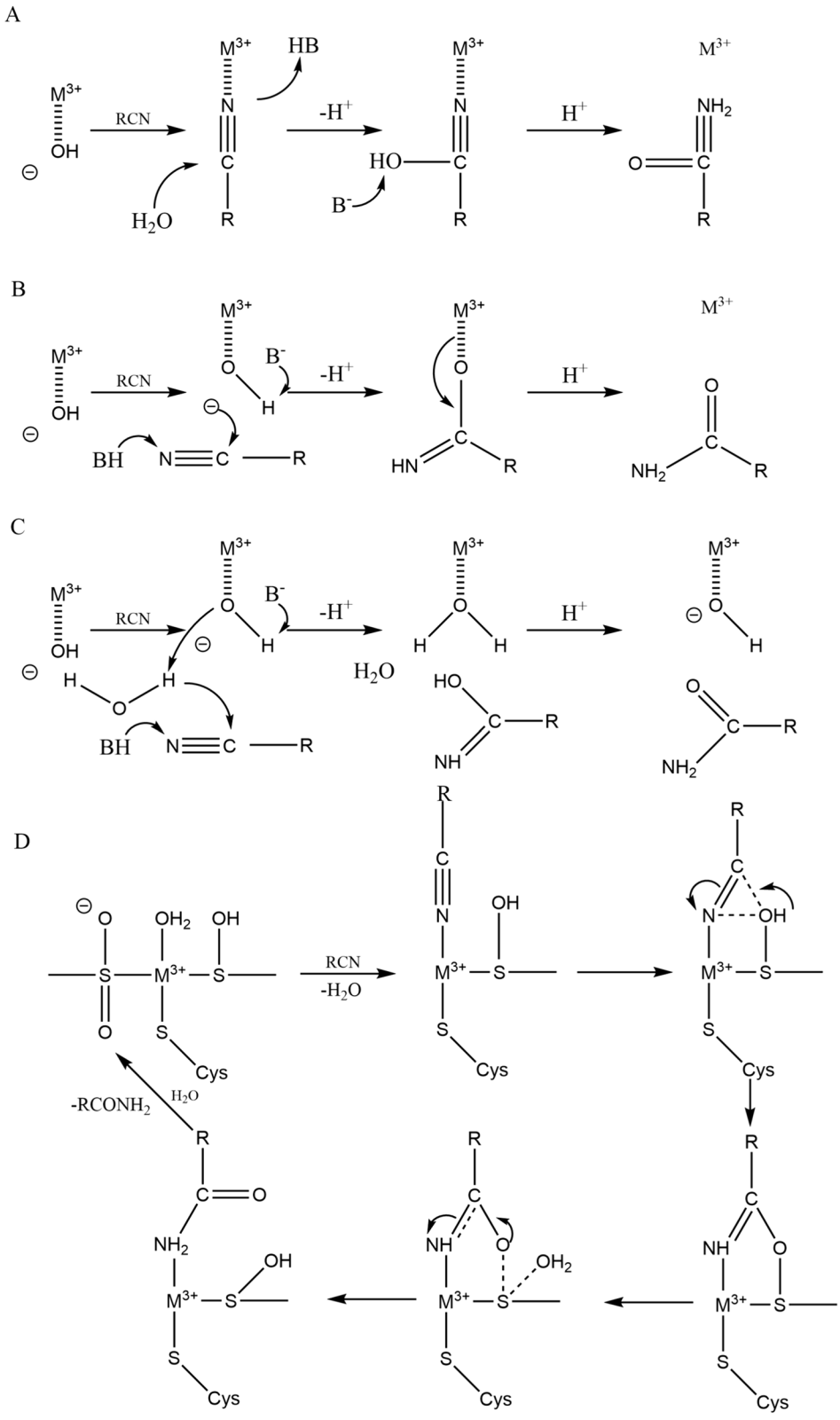
Table 1 The characteristics of the previously reported nitrile hydratase (NHase)

Name of organism		Products	Cofactor	Molecular mass (kDa)		Inducer	Optimum		Reference
				Subunits	Native		pH	Temperature (℃)	
Rhodococcus sp. N774		Acrylamide	Fe	α-28.5 β-29.0	70	Constitutive	7.7	35	(Endo and Watanabe, 1989; Hashimoto et al., 1991)
P. chlororaphis B23		Acrylamide, 5-Cyanovaleramide	Fe	α-22.0 β-24.5	100 (2)	Methacrylamide	7.5	20	(Nagasawa et al. 1987; Hann et al. 1999)
R. rhodochrous J1	H–NHase	Acrylamide, benzamide, 2,6-difluorobenzamide, 2-(1H-indol-2-yl)-acetamide, Nicotinamide, picolinamide, pyrazine-2-carboxamide, isonicotinamide, 2H-thiopyran-6-carboxamide	Co	α-22.7 β-26.3	505	Urea	6.5–6.8	35	(Mauger et al. 1989; Nagasawa et al. 1991)
	L–NHase			α-22.7 β-25.2	101 (2)	Cyclohexanecarboxamide	8.8	40	
R. rhodochrous PA-34		Acrylamide, nicotinamide, Butyramide	Co	α-25.0 β-30.6	86	Acetonitrile	7.5	35	(Prasad et al. 2007; Raj et al. 2007b)

metal-bound hydroxide would activate another free water molecule from the second coordination shell, and that the second water molecule would catalyse the hydrolysis of nitriles (Mitra and Holz 2007; Yu et al. 2008; Yamanaka et al. 2010) (Fig. 2C). More recent research has further demonstrated that post-translational sulfonate (which acts as a nucleophile) initially attacks nitriles and that the source of the product carboxamide oxygen is the protein (Nelp et al. 2016) (Fig. 2D).

Molecular modifications of NHase

Mining novel NHases from nature not only is one way to obtain NHases with high activity and superior stability, but also provides new ideas for the molecular modification of NHases on the basis of the characteristics of novel enzymes. Molecular modification has become one of the most powerful and widespread tools for engineering improved or novel functions in proteins (Ma et al. 2024b). The main molecular modification strategies to improve the robustness of NHase include stabilizing the subunit terminus, stabilizing the mesophilic zone, redesigning the active pocket, and enhancing the hydrophobic network between subunits (Fig. 3). Yokota et al. reported that thermophilins contain more polar amino acid residues and more easily form salt bridges compared with mesophilins (Yokota et al., 2006). With the increase of the total salt bridges and the proportion of the salt bridge network, the heat resistance of the protein is significantly enhanced, indicating that the salt bridge is one of the


A
RCN
HB
-H+
H+
H2O
B-
B
RCN
B-
-H+
H+
BH
C
RCN
B-
-H+
H+
H2O
BH
D
RCN
-H2O
-RCONH2
H2O
Cys

◂**Fig. 2** Four proposed catalytic mechanisms for nitrile hydratase (NHase) catalysis. **A** The inner-sphere mechanism; **B** the outer-sphere mechanism; **C** the newly proposed outer-sphere mechanism (Indirectly Activated Nucleophile); **D** Direct attack of activated sulfenate towards substrate

direct factors affecting the protein stability (Gurry et al. 2010). Directed evolution is an effective modification method, that can modify enzyme-encodinge genes in vitro by simulating natural evolution, error-prone PCR or chemical and physical mutagenesis (Reetz and Carballeira 2007). Zhou et al. developed a high-throughput automatic in vivo screening platform based on a niacin biosensor (NAsensor) for evolving nitrile metabolism-related enzymes (nitrilase, amidase, and NHase) (Han et al. 2022). Homologous recombination is a type of genetic recombination in which nucleotide sequences are exchanged between two similar or identical molecules of DNA.

Using this technology, researchers have integrated homologous protein fragments from different sources into a protein body to design highly variable but still naturally folded chimeric proteins (Carbone and Arnold 2007). NHases with high stability/activity can be screened by constructing a library of hybrid protein mutants.Moreover, the fusion of α- and β- subunits can effectively increase protein stability (Azzam et al. 2012; Xia et al. 2016). Additional molecular modifications of NHase are summarized in Table 2.

NHase applications

Synthesis of fine chemicals

NHase, well-known for its great impact on the revolution wave of acrylamide biosynthesis, has undergone 40 years of academic and industrial utilizationand is one of the most successful cases of biocatalysis in biology. Acrylamide, a synthetic monomer, has attracted much attention in the industrial applications of the leather industry, water treatment, enhanced oil recovery, and many other fields (Taeymans et al. 2004; Jiao et al. 2020). NHase in R. rhodochrous N-774, developed by Japan Nitto chemical industry, was the first biocatalyst for the production of acrylamide. Besides, P. chlororaphis B23 and R. rhodochrous J1, as the new generation of NHase-producing strains, have also been employed as a vehicle for acrylamide industrial production (Yamada et al. 1996). Shen et al. screened a strain Nocardia sp. 86–163 with high production of NHase in 1986, which was successfully applied in the industrial production of acrylamide (Asano 2002; Sahu et al. 2022).

What's more, NHase also serves as an important biocatalyst for nicotinamide industrial production. Nicotinamide is an important vitamin with a wide range of industrial applications in the pharmaceutical, nutraceutical, cosmetic, and feed industries, among other fields (Prasad et al. 2007). Nicotinamide has potential as a safe, well-tolerated, and cost-effective agent to be used in cancer chemoprevention and therapy, such as laryngeal and urinary bladder cancers (Nikas et al. 2020). Among the NHases reported thus far, the NHases from R. rhodochrous J1 and R. rhodochrous PA-34 exhibit high activity towards 3-cyanopyridine (Pratush et al. 2013). Recently, the potential applications of NHase in the synthesis of other valuable amides, such as 2-(1H-indol-2-yl)-acetamide, butyramide, 5-cyanovaleramide, isonicotinamide, picolinamide, indole-3-acetamide, pyrazine-2-carboxamide, lactamide, 2H-thiopyran-6-carboxamide, 2,6-difluorobenzamide, benzamide and adipamide have been disclosed (Table 3).

Environmental bioremediation

NHase is not only widely used in the manufacture of amides, but also fulfills a role in environmental protection. Synthetic nitriles can currently be employed for organic synthesis as starting materials and intermediates. However, most of the nitriles are neurotoxic and belong to mutagenic, teratogenic and carcinogenic compounds in nature, which are continuously released as effluents by industries (Supreetha et al. 2019; Tanii 2017). Thus, removal of nitrile from industrial contaminated soil and water is urgently needed. NHase in combination with amidase or nitrilase is considered promising for potential application in nitrile biotransformation and degradation, which has made significant contributions to the environmental bioremediation in recent decades (Table 1). For instance, Kohyama et al. degraded the acetonitrile wastewater by using the dual-bacteria (R. pyridinovorans S85-2 and Brevundimonas diminuta AM10-C) coupling process, and 90% of the acetonitrile was degraded to acetic acid within

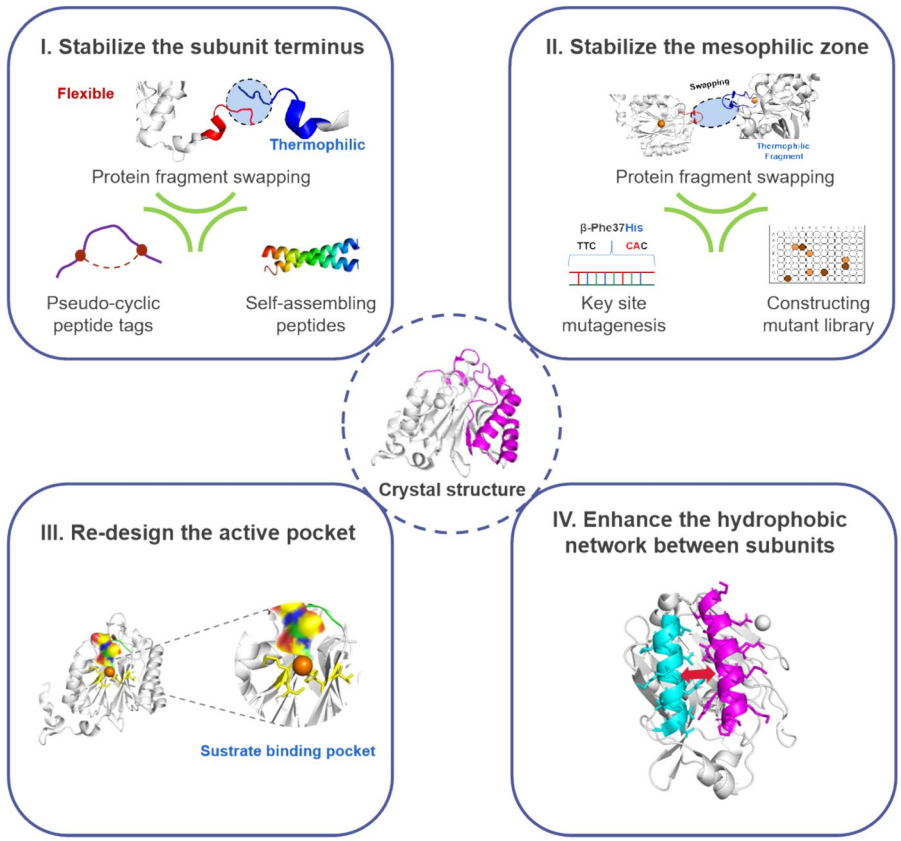


Fig. 3 Strategies used to improve nitrile hydratase (NHase) properties

10 h (Kohyama et al. 2006). Wyatt et al. degraded highly toxic wastewater containing acrylonitrile and other compounds by mixed microorganism producing nitrile converting enzymes (i.e., NHase, nitrilase and amidase). The results revealed that the chemical oxygen demand (COD) decreased by 75%, and 99% of the COD was metabolized by cells as a result of the nitrile compounds (Wyatt and Knowles 1995). Hansen et al. realized the biodegradation of cyanide in a gold tailings environment via nitrilase, NHase and thiocyanate (Welman-Purchase et al. 2024).

Advances in NHase engineering

Whole-cell biocatalysis engineering driven by synthetic biology

NHase has been universally found in a variety of microbes belonging to various species. Compared with other hosts, Rhodococcus strains (e.g., Rhodococcus sp. M8, R. rhodochrous J1 and R. ruber TH) have become increasingly attractive for value-added amides synthesis due to their outstanding characteristics, e.g., high NHase activity and superior stability to high temperature and organic solvents, enabling their wide application in whole-cell biocatalysis (Jiao et al. 2020). Recent advances in synthetic biology have revolutionized the technology to engineer microbial hosts for the production of a wide variety of valuable amides. Thus, it is significant to highlight the achievements in Rhodococcus strains as platform organisms for amides production.

In the past several decades, basic genetic elements, including the development of different promoters, ribosome binding sites (RBSs), reporter genes, and selection markers, have been developed in engineered Rhodococcus sp. to facilitate whole-cell biocatalysis applications (Liang and Yu 2021). What's more, to realize the large-scale amides production at an industrial level, various strategies have been explored in Rhodococcus, ranging from random mutagenesis to precise genome editing, including traditional homologous recombination, bacteriophage

Table 2 Molecular modifications of nitrile hydratase (NHase)

Method	NHase producing organisms	Modification site	Results	Reference
Salt-bridges	R. ruber TH	C-terminal-residue-bridged	Slight enhancement in the expression of β-subunit and enzyme activity, 160% enhancement in thermal stability, 7% enhancement in product tolerance, 75% enhancement in resistance to cell-disruption by ultrasonication	(Chen et al. 2013)
Domain swapping	*P. putida* NRRL-18668	Swapping the corresponding fragments of *Pp*NHase	1.4- to 3.5-fold enhancement in thermostability, 3AB NHases: 1.4 ± 0.05-fold enhancement in enzyme activity	(Cui et al. 2014)
Domain swapping	Bordetella petrii	Swapping the corresponding C-domains	Enhancement in thermal stability	(Sun et al. 2016)
Subunit-fusion	P. putida	Fusing the α- and β-subunits and/or the "activator proteins" of the NHase	Enhancement in thermostability and tolerance to high concentrations of the product amide	(Xia et al. 2016)
Site-saturation mutagenesis	Caldalkalibacillus thermarum TA2. A1	βL48H	Enhancement in enzyme activity	(Cheng et al. 2021)
Site-directed mutagenesis	P. thermophila JCM3095	αI5P/αT18Y/αQ31L/αD92H/βA20P/βP38L/βF118W/βS130Y/βC189N/βC218V	Enhancement in enzyme activity, melting temperature	(Cheng et al. 2020a)

recombinase-assisted recombineering and the CRISPR/Cas9 system (Liang and Yu 2021). In particular, with respect to the CRISPR/Cas9 system, the development and application of CRISPR/Cas9 in Rhodococcusare important. The first CRISPR/Cas9 system in R. ruber for gene deletion, mutation, and insertion was successfully developed by introducing recombinases Che9c60 and Che9c61 in the study of Liang et al., and the editing efficiency reached 75% (Liang et al. 2020).

NHase immobilization

Although biotransformation mediated by free cells or soluble enzymes has been successful, immobilized cells or enzymes have other advantages compared with free enzymes or cells. In particular, immobilization promotes biocatalyst retention and by-product removal, simplifying the process of catalyst separation and product purification (Rangraz et al. 2024). Immobilization can also improve the reusability and stability of biocatalyst, and immobilized cells have also been reported to catalyse a wider range of substrates than free cells do (Dias et al. 2001).

The most mature technology in industry using NHase to convert nitriles into amides is to catalyze acrylonitrile to acrylamide and catalyze nicotinonitrile to nicotinamide by immobilized Rhodococcus (Raj et al. 2010; Wang et al. 2020a). The Swiss company Lonza and Japan's Mitsubishi Corporation have successfully used these two processes to operate on a thousand-ton scale for over a decade.

It is relatively mature to immobilize NHase and cells harboring NHase using traditional matrices (such as calcium alginate, agar, and polyvinyl alcohol) and commercial immobilization materials. In recent years, the application of metal–organic frameworks and biomimetic mineralization in

Table 3 NHase-catalyzed transformation of nitriles to the corresponding amides

Product	Source of NHase	Production (g/L)	Reference
Acrylamide	R. rhodochrous J1	650	(Nagasawa et al. 1991)
	R. rhodochrous PA-34	600	(Prasad et al. 2010)
	Brevibacterium sp. CH2	200	(Lee et al. 1993)
	P. chlororaphis	100	(Nagasawa et al. 1987)
Nicotinamide	R. rhodochrous J1	1456	(Nagasawa et al. 1988)
	R. rhodochrous PA-34	855	(Prasad et al. 2007)
2-(1H-indol-2-yl)-acetamide	R. rhodochrous J1	1045	(Mauger et al. 1989)
Butyramide	R. rhodochrous PA-34	597	(Raj et al. 2007a)
5-Cyanovaleramide	Pseudomonas putida NRRL-18668	99.5% (adiponitrile)	(Cheng et al. 2016)
	Comamonas testosteroni 5-MGAM-4D βF37P mutant	94.1% (adiponitrile)	(Cheng et al. 2016)
	Rhodococcus ruber CGMCC3090	100%	(Cheng et al. 2016)
	R. rhodochrous J1 βY68T/W72Y mutant	70.5% (adiponitrile)	(Cheng et al. 2017)
	P. chlororaphis B23	3150 g/g DCW	(Hann et al. 1999)
Isonicotinamide	R. rhodochrous J1	1099	(Mauger et al. 1989)
Picolinamide	R. rhodochrous J1	977	(Mauger et al. 1989)
Indole-3-acetamide	Ensifer meliloti CGMCC 7333	294.28 U/mg (V-max)	(Zhao et al. 2020)
Pyrazine-2-carboxamide	R. rhodochrous J1	895	(Mauger et al. 1989)
Lactamide	Rhodococcus pyridinivorans NIT-36	14.5 g/g DCW/h	(Singh et al. 2019)
2H-thiopyran-6-carboxamide	R. rhodochrous J1	210	(Mauger et al. 1989)
2,6-Difluorobenzamide	Aurantimonas manganoxydans ATCC BAA-1229	314	(Yang et al. 2019)
	R. rhodochrous J1	360	(Mauger et al. 1989)
Benzamide	R. rhodochrous J1	489	(Mauger et al. 1989)
Adipoamide	*P. putida* NRRL-18668 βL37Y mutant	98.8% (adiponitrile)	(Cheng et al. 2016)
	C. testosteroni 5-MGAM-4D	100% (adiponitrile)	(Cheng et al. 2016)
	R. rhodochrous J1	98.6% (adiponitrile)	(Cheng et al. 2017)

immobilizing NHases is emerging. Common immobilization methods such as covalent binding, embedding, cross-linking, and physical adsorption have also been reported, of which embedding is often used to immobilize the cells containing NHase (Table 4).

Commonly, immobilization of NHase has the advantages mentioned above, but also introduces a series of issues, such as unstable properties of the immobilizing supports under extreme conditions, partial loss of catalytic activity of biocatalyst, the mismatch of immobilization carrier size (Velankar et al. 2010). We hope the future research will make a breakthrough in improving the applicability and stability of the immobilized carrier.

Conclusion and Future Perspectives

The history of NHase since its discovery to date is the journey from academia to industry, and researchers have witnessed the rapid progress of NHase in aspects of industrial application, natural distribution, enzyme screening, molecular modification and significant biotechnology in amide production. NHase, as a green biocatalyst, is launching a revolutionary wave at the forefront of green biomanufacturing, e.g., the agricultural, pharmaceutical, material, and textile industries, along with the fields of chemical engineering and environmentalstudies. What is exciting that NHase has been successfully used for the industrial production of acrylamide,

Table 4 Nitrile biotransformation processes with immobilized cells and enzymes

Immobilization methodology	Support carrier matrix	Substrates for bioconversion	Product	Reference
Cross-linking (immobilized enzymes)	Glutaraldehyde-CLEA	Acrylonitrile	Acrylamide	(van Pelt et al. 2008)
Cross-linking (immobilized enzymes)	Polysulfone hollow-fiber	Acrylonitrile	Acrylamide	(Sun et al. 2004)
Entrapment (immobilized enzymes)	Bio-MOF(Co-Cys)	3-Cyanopyridine	Nicotinamide	(Wang et al. 2020a)
Cross-linking (immobilized enzymes)	PVA/chitosan biocompatible complex	3-Cyanopyridine	Nicotinamide	(Pawar and Yadav 2014)
Cross-linking (immobilized enzymes)	Glutaraldehyde-CLEA	Several nitriles	Corresponding amides	(Kubác et al. 2008)
Adsorption (immobilized cells)	Charcoal	Acrylonitrile	Acrylamide	(Maksimov et al. 2007)
Entrapment (immobilized cells)	LentiKats®	Several nitrile compounds	Corresponding amides and carboxylic acids	(Kubac et al. 2006)
Entrapment (immobilized cells)	Agar	Acrylonitrile	Acrylamide	(Raj et al. 2007b)
Entrapment (immobilized cells)	Alginate and cellulose triacetate	Propionitrile	Propionamide	(Chen et al. 2010)
Entrapment (immobilized cells)	Chitosan-N, N′-Methylene bis-acrylamide	Lactonitrile	Lactamide	(Singh et al. 2020)
Biomimetic mineralization (immobilized enzymes)	Zeolitic imidazolate framework (ZIF-67)	3-Cyanopyridine	Nicotinamide	(Pei et al. 2020)
Entrapment (immobilized cells)	Agar	Butyronitrile	Butyramide	(Singh et al. 2018)

nicotinamide, etc. Although rapid progress has been made in the past decade, the performance of most NHases cannot achieve the goals required for large-scale industrial production because of the notable instability, unsatisfactory catalytic activity, unwanted byproduct formation, etc. In spite of the efforts discussed herein to exploit NHase for various applications, a great deal of work is still necessary to achieve the goals of "Green and Sustainable Chemistry" which are being faced by the scientific community, in both academia and industry. Emerging biological tools and strategies in synthetic biology, protein engineering, and bioinformatics have been developed to not only improve the properties of NHase but also generate novel process technologies, which will be promising for the improvement of the NHase as a robust biocatalyst. Especially, as -omics and other high throughput technologies have been rapidly developed, the promise of applying machine learning (ML) techniques in NHase design has started to become a reality.

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Data availability Data will be made available on request.

Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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