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NITRILASES – AN ATTRACTIVE NITRILE DEGRADING BIOCATALYST

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ABSTRACT

Nitrile compounds are found everywhere in nature. They are synthesized naturally as well as xenobiotically. Nitrilases are nitrile degrading enzymes and belong to nitrilase superfamily. They convert nitriles into corresponding acids in a single step pathway. Members of this superfamily are present in both prokaryotes and eukaryotes and have characteristics αββα fold. They pose conserved Cysteine, Glutamate and lysine in catalytic triad and exist as inactive dimer and further oligomerize to become active. The enzyme is being used as an attractive biocatalyst for the production of fine chemicals and pharmaceuticals. Due to their high regioselectivity and enatioselectivity, the utility of nitrilases have progressed from the application of nitrilase in biotransformation to its importance in bioremediation. Nitrilases are also used in the treatment of toxic effluents, herbicides and in cyanide remediation. This review, gives large information about nitrilases and steps taken to improve the biotransformation potential of nitrilases.

Key words: Biocatalyst, Enantio-selectivity, Nitriles, Nitrilases, Regio-selectivity

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INTRODUCTION

Chemical compounds containing Cyano (-CN) functional groups are called nitriles. These nitriles are formed naturally as well as xenobiotically. They show wide range of application in synthesis of plastics, cosmetics, pharmaceuticals and other organic chemicals but are also highly poisonous in nature[1, 2]. Nitrile compounds can be degraded chemically or by microbial systems. Chemical procedure to convert nitriles into acids and ammonia require harsh environment which lead to production of many unwanted by-products along with inorganic waste. Therefore, biological transformation has become a new rage. Biotransformation is conversion of one substance acting as substrate to another (product) by microorganism. Biological degradation of the nitriles show greater specificity as well as lack of production of secondary by-products along with easy purification of product and sometimes the energy requirements of reactions are also reduced than conventional chemical reactions. Microbes use different enzymatic pathways to catabolise nitrile. Either it can be two steps conversion of nitrile to carboxylic acid using enzymes, nitrile hydratase and amidase with amide as intermediate or it can be a single step conversion of nitriles into corresponding acids by nitrilase as shown in Figure 1 [3]

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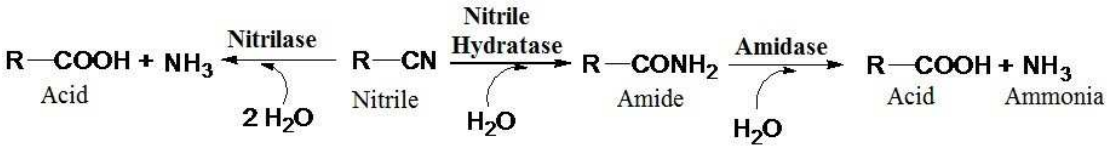

Figure 1
Nitrile hydrolyzing enzymes.

Most of the nitrilases are characterized in terms of regio-(positional) and enantio-selectivity. Enantio-selectivity involves the biotransformation of the enantiomeric substrate (R and S enantiomers) with their difference in *Gibbs* free energy around 1-3 kj/mole [4]. The regioselective properties are exploited for the production of chiral compounds. Therefore, due to such unique qualities of nitrilase and convenience of single pathway degradation compared to combine action of nitrile hydratase and amidase, it has attracted lot of attention.

Nitrilases belong to nitrilase superfamily and expressed in both prokaryotes and eukaryotes. Members of this superfamily have been divided into 13 branches based on the amino acid sequence analysis, in which nitrilases belong to 1st branch along with the cyanide hydratase and cyanide dihydratase. Nitrile hydratase which too hydrolyzes nitriles, do not belong to any branch of this superfamily [5]. The remaining 12 branches show amidase activity with varying specificity. Aliphatic amidase, amino-terminal amidase, biotinidase and β-ureidopropionase fall in 2nd, 3rd, 4th and 5th branches, respectively. Carbamylase belong to branch 6. Branch 7 and 8 consist of prokaryotic and eukaryotic NAD^+ synthetase. These enzymes associate with the amidase domain in order to utilize liberated ammonia from the glutamine as a source of nitrogen for NAD^+ synthesis. Enzymes of branch 9 are apolipoprotein N-acyltransferase and catalyze reverse amidase (condensation) reaction *in vivo* [6]. Branch 10th, 11th, 12th and 13th constitute of Nit and NitFhit, NB11, NB12 and nonfused outliners, respectively [4].

SOURCES OF NITRILASE ENZYMES

Nitrilases are present in both prokaryotes and eukaryotes. Among plant kingdom, it is mainly present in Gramineae, Cruciferae, and Musaceae. Nitrilase was first described 40 years ago in barley leaves catalyzing the conversion of indole acetonitrile (IAN) to indole acetic acid (IAA) [7]. The first prokaryotic source for nitrilase was *Pseudomonas.* It was isolated by selection on the naturally occurring nitrile, ricinine as a sole carbon source [8]. Later on large number of microbial isolates showed the presence of nitrilase which is well documented in Table 1. A recombinant *Pyrococcus abyssi* nitrilase overexpressed in *E.coli* represent the first archeal nitrilase [9]. Different factors such as effect of different carbon sources, nitrogen sources, inducers as well as optimal conditions may influence the properties of nitrilases. In *Rhodococcus rhodochorus* NCIMB 11216 propionitrile induced synthesis of nitrilase hydrolyzed 3-cyanobenzoate and both the nitrile groups in dicyanobenzoate whereas, benzonitrile induced nitrilase synthesis hydrolyzed only one of the nitrile groups in 1, 3 dicyanobenzoate and did not affect 3-cyanobenzoate [10]. When *Arthrobacter* sp. strain J-1 was grown in acetonitrile as sole carbon and nitrogen, it led to production of nitrile hydratase and amidase whereas in case of benzonitrile as sole carbon and nitrogen source, aromatic nitrilase was expressed [11]

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Table 1
Microbial nitrilases and their various properties.

Organism	Nature of expression	Substrate specificity	Optimum temperature (ºc)	Optimum pH	Size Of Subunit (Kda)	Complexity (No. Of Subunits)	References
Fusarium solani	Inducinble (Benzonitrile)	Aromatic	25	7.8-9.1	76	8	56
Nocardia (*Rhodococcus*) N.C.I.M.B. 11215	Inducible (p-Hydroxybenzonitrile)	Aromatic	30	7.0-9.5	45	12	57
Arthrobacter sp. strain J1	Inducible (Benzonitrile)	Aromatic	40/30	8.5/7.5	30/23	1/-	11
Rhodococcus rhodochorus J1	Inducible (Isovaleronitrile)	Aromatic	45	7.6	41.5	2	58
Rhodococcus rhodochorus ATCC 39484	Inducible (Isovaleronitrile)	Aromatic	40	7.5	40	14	59
Rhodococcus rhodochorus PA-34	Inducible (Propionitrile)	Aromatic	35	7.5	45	1	60
Rhodococcus rhodochorus NCIMB 11216	Inducible (Propionitrile)	Aromatic	30	8.0	45.8	12	10
Bacillus pallidus DAC 521	Inducible (Benzonitrile)	Aromatic	65	7.6	41	14	43
Aspergillus niger K10	Inducible (2-Cyanopyridine)	Aromatic	45	8	38.5	-	61
Exophiala oligosperma R1	Inducible (Phenylacetonitrile)	Aromatic	30	4	-	-	62
Fusarium solani O1	Inducible (2-Cyanopyridine)	Aromatic	40-45	8	~40	14	63

Fusarium solani IMI196840	Inducible (2-Cyanopyridine)	Aromatic	45	8	40	-	64
Rhodobacter sphaeroides LHS-305	Inducible (Acetonitrile)	Aromatic	30	9.0	38	1	42
Alcaligenes faecalis JM3	Inducible (Isovaleronitrile)	Arylacetonitrile	45	7.5	44	6	16
Alcaligenes faecalis ATCC 8750	Inducible (n-Butyronitrile)	Arylacetonitrile	45	7.5	32	14	65
Pseudomonas fluorescens EBC191	Recombinant enzyme, Inducible (L-Rhamnose)	Arylacetonitrile	50	6.5	37.7	-	17
Pseudomonas putida	Inducible (Acetonitrile)	Arylacetonitrile	40	7	43	10	20
A. faecalis ZJUTB10	Inducible (n-Butyronitrile)	Arylacetonitrile	40	7-8	41.5	-	66
Rhodochoccous rhodochorus K22	Inducible (Crotoonitrile)	Aliphatic nitrile	50	5.5	41	15-16	14
Acidovorax facilis 72W	Constitutive	Aliphatic	65	8-9	40	14	67, 68
*Streptomyces sp.*MTCC 7546	Inducible (Benzonitrile)	Mono and di aliphatic nitrile	50	7.4	-	-	1
Klebsiella pneumonia ssp. *Ozaenae*	Recombinant enzyme	Bromoxynil specific nitrile	35	9.2	37	2	69
Pyrococcus abyssi	Inducible (Isopropyl-β-D-thiogalactopyranoside)	Aliphatic	60-80	6-8	29.8	2	9
Synechocystis sp. strain PCC6803	Recombinant enzyme, Inducible (L-Rhamnose)	Aliphatic and Aromatic	50	7	40	10	70

SUBSTRATE SPECIFICITY OF NITRILASES

Nitrilases hydrolyze different types of nitriles. Therefore, due to their broad substrate specificity they are classified into three main categories: Aromatic, aliphatic and arylacetonitrilase as shown in Figure 2. The best described aromatic nitrilases are present in *Rhodococcus rhodochorus* J1 where amino acid at position 142 is responsible for their substrate specificity. Conjugated Π electron system of the aromatic ring of substrate or amino acid could hydrolyze aromatic or aliphatic nitriles whereas point mutation at 142 with non aromatic, non charged amino acid show activity only against aromatic nitriles [12]. It is also reported that the positively charged amino acid at position 129 in *R. rhodochrous* ATCC 33278 is active against m- substituted benzonitriles whereas neutral or negatively charged amino acids at 129 has no activity against any aromatic nitriles [13].Very few literatures are available on nitrilases acting preferentially on aliphatic nitriles. *R. rhodococcus* K22 was the first aliphatic nitrilase producing isolate [14]. Some microbes showed activity against both aromatic as well as aliphatic nitriles [15]. The first arylacetonitrilase was isolated from the isovaleronitrile induced cells of *Alcaligenes faecalis* [16]. Arylacetonitrilase activity depends on the presence and position of aromatic ring as well as type of alkyl side chain. In *Pseudomonas fluorescens* EBC191, a direct correlation was observed between increased amide formation and increased negative inductive effect due to substitution at the 2-position in ring [17]. In *Pseudomonas fluorescens* EBC191 when alanine residue directly adjacent to the catalytic triad of nitrilase was replaced with larger substituent Ala165Phe, an increased proportion of 'R' enantiomer was observed where as variant with smaller substituent Ala165Gly showed preference for synthesis of 'S' enantiomer [18]. In the same enzyme the amount of amide formation increased when Cys-163 which is in close proximity to catalytic triad was changed to asparagine or glutamine [19]. Arylacetonitrilase activity from *Pseudomonas putida* and *Alcaligenes faecalis* showed that the substitution at the para position of phenyl ring enhances the activity whereas substitution at ortho position decreases the activity due to steric hindrance [20]. Nitrilase from *Klebsiella pneumonia* subsp. *ozaenae* is highly specific for hydrolysing herbicide bromoxynil (3, 5-dibromo-4-hydroxybenzonitrile) to 3, 5-dibromo-4-hydroxybenzoic acid and use liberated ammonia as the source of nitrogen for its growth. The bxn gene encodes for bromoxynil specific nitrilase. The gene was incorporated into the leaves of tobacco plants. Transgenic plants showed resistance against herbicide Buctril^{R} (commercial bromoxynil) [21].

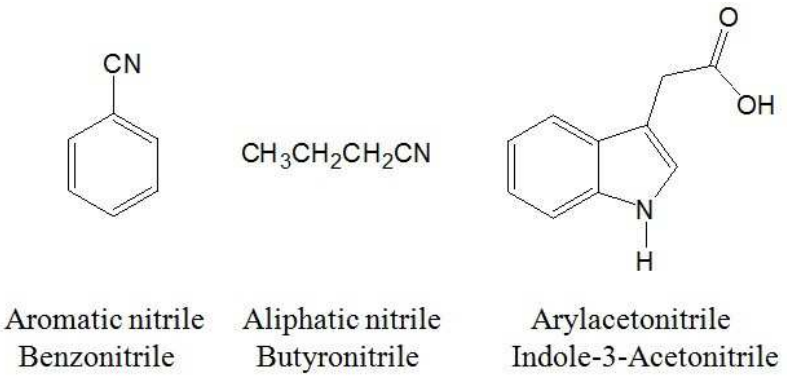

Figure 2
Types of different nitriles.

MOLECULAR ANALYSIS OF NITRILASE

In microbes the gene responsible for expression of nitrilase is nitA. In isovaleronitrile dependent induction of nitrilase in *Rhodococcus rhodochorus* J1, this gene is transcriptionally regulated by the protein expressed by the nitR at 1.4 kb downstream of nitA. Protein encoded by nitR is positive regulator of nitA [22]. In *Pseudomonas fluorescens* EBC 191, nitA is physically linked with the genes encoding enzyme arylacetonitrilase responsible for mandelonitrile biotransformation [17]. In case of plants like *Arabidopsis thaliana,* there are four genes nit1, nit2, nit3 and nit4 that regulate the expression of nitrilases [23]. Nit1, nit2 and nit3 are 85% identical to each other and are present on the chromosome III. Therefore nit1, 2 and 3 are considered to be nit1 homologs. Nit1 homologous enzymes are active on different types of aliphatic and aromatic nitriles and show no activity against β-cyanoalanine. Nit4 is only 65% identical to nit1/2/3 and is present on the chromosome V. Nitrilase 4 homolog is considered to be primordial nitrilase found in all plant species. Unlike nit1 homologs, nit4 homologs are active against β-cyanoalanine and take part in cyanide detoxification. Expression of each nit gene is localized and conditional [24, 25]. PinA, a plant induced nitrilase is found in plant growth promoting bacteria *P. fluorescens* SBW27 using *in vivo* expression technology. This gene shows homology to formation [27].

nit4 of plant nitrilase and may be helpful during colonising plant roots [26].

MECHANISM OF ACTION

Nitrilases from *R. rhodochorus, Pseudomonas putida, Alcaligenes faecalis* and *Fusarium solani* have shown that thiol compounds such as dithiothreitol and 2-mercaptoethanol are required for maximum activity of enzymes whereas, thiol specific reagents such as $AgNO_3$, $HgCl_2$, p-hydroxymercuribenzoate and phenyl mercuribenzoate retard the enzymatic activity suggesting presence of sulphur containing amino acid in the catalytic site. Metal chelating reagent such as EDTA, cyanide, azide, disodium 4, 5 dihydroxy-m-benzenedisulfonate doesn't affect the activity of nitrilase indicating absence of metal ion in the enzyme. In nitrilases cysteine, glutamate and lysine at positions 165, 48 and131, respectively form the catalytic triad. In calatytic triad Cys-165 attack the cyano group as nucleophile, role of general base is assumed by Glu-48 whereas, Lys-131 is involved in stabilization of a tetrahyadral intermediate by forming hydrogen bond with the nitrogen of the nitrile substrate [12]. Figure 3 shows mechanism of Nitrilase catalysis. It is proposed that the scissile bond in thioimidate tetrahydral intermediate is not well defined which can lead to amide

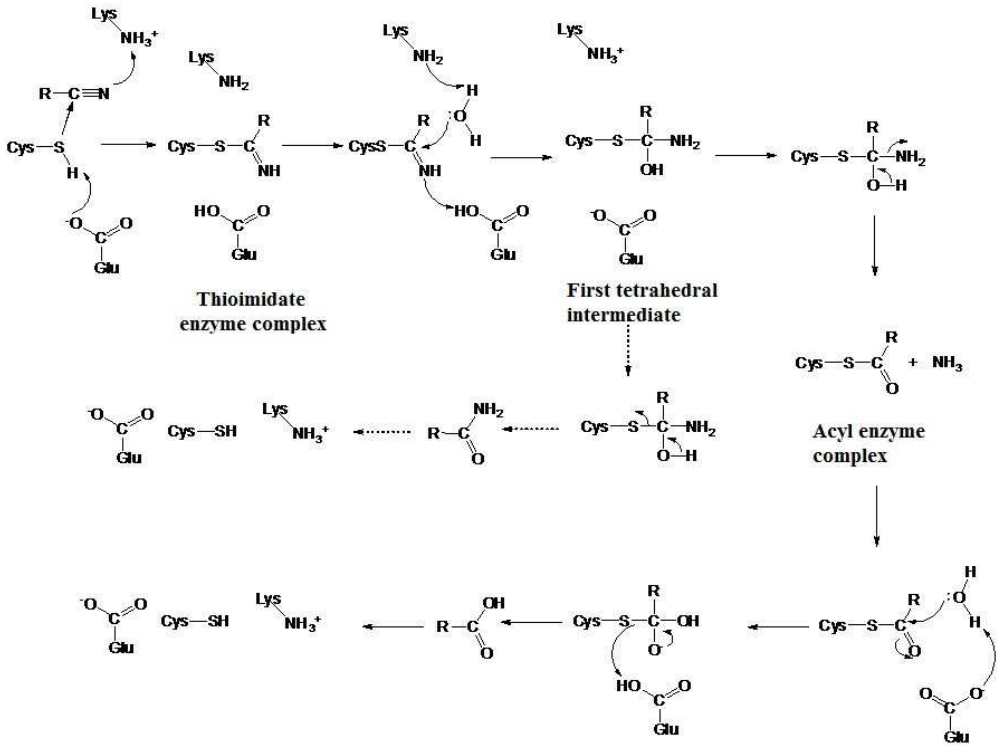

Figure 3
The mechanism of nitrilase action. The dashed arrow shows the possible sissile cleavage which lead to amide formation in some nitrilases.

STRUCTURE OF NITRILASE ENZYME

The microbial nitrilases generally exist as inactive dimers which after activation form large homo-oligomer spirals with a varying number of subunits. Monomer form of nitrilase exist in four layered αββα fold which associate to form eigth layered αββα-αββα dimeric form which further oligomerizes to form active enzyme [28]. The recombinant enzyme from *R. rhodochorus* J1 expressed in *E. coli* undergoes post translation cleavage at residue 327 removing around 39 C terminal amino acids from wild type enzyme, which results in formation of active oligomeric structure [29]. The interacting regions in the subunit of nitrilase superfamily has been designated as A, B, C, D, E and F. A, C, D and F have two fold axes symmetry and take part in spiral formation whereas E surface is asymmetric. 'B' surface is present only in NitFhit protein, N-carbamyl-D-aminoacid aminohydrolase and the prokaryotic XC1258 Nit protein. The α5 and α6 helices of 'A' surface associated together to form dimer. Interaction at 'A' surface can be further strengthened by the association of additional α helices at its C-terminal end. 'A' surface also brings the catalytic cysteine residue within the active site pocket. The 'C' surface is located almost at right angle to 'A' surface and is involved in elongation of spiral and association of dimers to form spiral quaternary structure. Conformational flexibility in this region leads to formation of different types of oligomeric structure. Mutation at C surface can render enzyme inactive. D surface interaction is mainly to strengthen the association between dimers and occurs after spiral completes one turn. Mutation at D region does not affect the enzyme activity. F surface interacts to increase helical twist [27]. Asymmetric interaction at E region leads to termination of the spiral form by filling the gaps which can be available for the further subunit to be added [30].

Assembling of the dimers into large spirals increased by increase in salt concentration, organic solvent, and enzyme concentration and at high temperature whereas $HgCl_2$ inhibits thiol group and so inhibits enzyme assembling as observed in nitrilase of *R. rhodochorus* J1 [31].

INDUSTRIAL APPLICATION OF NITRILASE

Nitrilases act at milder physiological conditions though some thermostable enzymes with better properties have also been reported**.** Nitrilases show regio-, enantio-selectivity as well as also have broad substrate specificity. Enantioselective biotransformation of nitriles is important in pharmaceutical industry, to form bioactive molecules such as Ibuprofen. *Acinetobacter* sp. AK226 hydrolyzes racemic 2-(4'-isobutylphenyl) propionitrile (Ibu-CN) to S-(+)-2-(4'-isobutylphenyl) propionic acid also known as S-(+)-ibuprofen [32]. Nitrilases also catalyze desymmetrization of 3-hydroxyglutaryl nitrile to (R)-4-cyano-3-hydroxybutyric acid. This ethyl ester synthesized by nitrilase is an intermediate to the cholesterol lowering drug, Lipitor. But unlikely, low enantiomeric excess (ee) was observed when the substrate concentration increased to a certain concentration that affects the large scale process for economic production. A recombinant nitrilase enzyme with ideal properties was made using gene site saturation mutation (GSSM) where a single alanine residue at position 190 was changed with histidine which led to 10% increase in enantiomeric excess at commercially relevant 3M substrate concentration [33]. Another recombinant enzyme was made after point mutation at T210A and T210C in *Acidovorax facilis* 72W resulted in increased specific activity for biotransformation of 3-hydroxyvaleronitrile to 3-hydroxyvaleric acid by 7.3 and 6.2 fold respectively. The hydroxyacids are commonly used in the synthesis of polyesters whereas the combined mutation at F168V and L201N in nitrilase of above said organism enhanced specific activity by 15.3 fold for glycolic acid production, commonly used in medical and industrial products [34, 35]. Regioselective properties of nitrilases provide the synthesis of chiral substances for chemical synthesis with varied applications. The enzyme from *Acidovorax facilis* 72W regioselectively catalyzes (E, Z) -2 - methyl -2-butenenitrile to (E) -2- methyl-2-butenoic acid, commonly known as tiglic acid [36]. R-(-)mandelic acid is an optical resolving reagent and source of semisynthetic cephalosporins pharmaceuticals. Nitrilase from *Alcaligenes faecalis* ATCC 8750, *Pseudomonas putida, Microbacterium paraoxydans* and *Microbacterium liquefacians* have shown production of R-(-)mandelic acid [4]. An autodisplay of *Alcaligenes faecalis* ATCC 8750 on *E. coli* was used to construct whole cell biocatalyst for the synthesis (R)-mandelic acid with an ee >99% [37]. Nitrilase from *R. rhodochorus* J1 could convert cyanopyrazine into pyrazinoic acid which showed antimicrobial action against *Mycobacterium tuberculosis* and is also used as precursor of pyrinzamide which is an active pharmaceutical drug [38]. It can also produce acrylic and methacrylic acid from acrylonitrile and methacrylonitrile, respectively [39]. Production of nicotinic acid from 3-cyanopyridine has been reported from various sources [40 - 44]. Nitrilases are also involved in biotransformation of Indole-3-acetonitrile (IAN) to Indole acetic acid (IAA). Conversion of IAN to IAA is last step in the IAN mediated pathway for IAA synthesis [45]. *Streptomyces griseoviridisi* K61 and *Streptomyces lydicus* WYEC108 are used for commercial production of IAA under the trade name mycostop using tryptophan as main substrate [46].

BIOREMEDIATION

Many man-made nitriles have entered the global environment via various ways such as herbicides, agricultural wastes as well as from exhaust of various automobiles [2]. Nitriles are very toxic, carcinogenic, mutagenic and teratogenic. Their exposure can lead to disorder of central nervous system, hepatic, cardiovascular, renal and gastrointestinal systems in mammals [47]. Therefore, it has become necessary to monitor the discharge of different nitriles into the environment. Nitrilase with its ability to convert nitriles into non toxic products have shown its potential role in

bioremediation. Li *et al.* (2007) used a consortium for degradation of organonitriles to corresponding acids [48].

Some herbicides are the analogues of the dihalogenated benzonitrile such as dichlobenil, bromoxynil and ioxynil. Nitrile hydratase coverts these halogenated benzonitrile into corresponding benzamide which is more soluble in water and less biodegradable than parent compound, therefore pose threat to environment [49]. In groundwater of Denmark, BAM (2, 6 dichlorobenzamide) which is a catalyzed product of dichlobenil by nitrile hydratase is most frequently encountered contaminant [50]. As discussed earlier, bxn gene of nitrilase from *Klebsiella pneumonia* sp. *oxaenae* was used to develop genetically modified bromoxynil and ioxynil resistant plants [21]. Nitrilase from isobutyronitrile induced cells of *R. rhodochorus* PA-34, *Rhodococcus* sp. NDB 1165, *Nocardia globerula* NHB-2 could also convert dihalogenated benzonitrile into acid [50].

COMMERCIAL PRODUCTION OF NITRILASE

Biocatalysts have huge impact in the chemical world. Chemical manufacturers are increasingly using enzymes to synthesize chiral compounds and other pharmaceutically active biomolecules. In fact, five most selling drugs in the world are single enantiomers. The traditional chemical method to produce carboxylic acid from nitriles meet with many challenges such as harsh environment, undesired by-poducts as well as low selectivity, whereas nitrilase can easily synthesize these important acids without altering other functional groups. Nitrilases are also employed in the industrial production of acrylamide, nicotinic acid and certain amino acids. Therefore, large production of nitrilase is needed to meet the demand of commercially important products. A thorough literature review was performed and it was noticed that most of the nitrilases are produced at shake flask level and very little emphasis was given on the production at bioreactor level. The enzyme from *P. putida* 5110 was produced in a 6 L lab scale bioreactor with a specific activity of 2.17 U/mg [51]. Similarly, Jain *et al*. (2012) investigated the production of nitrilase from recombinant *E.coli* expressing nitrilase gene of *Alcaligenes faecalis* in a 7 L lab scale bioreactor [52]. The effects of various process parameters such as pH, temperature, concentration of inducer, aeration, agitation etc. are crucial during the scale up of enzyme production. Some of the known companies involved in production of nitrilases are listed in Table 2.

Table 2
Commercial nitrilase manufactures

Nitrilase	Organisms	Price	Name of manufacturer
Nit01	*Bradyrhizobium japonicum* USDA 110 Nitrilase	50 mg/€300	Nzomics Biocatalysis,UK
Nit02	*Rhodopseudomonas palustris* CGA009 Nitrilase	50 mg/€300	Nzomics Biocatalysis,UK
Nit03	*Bacillus cereus* ATCC 14579 Nitrilase	50 mg/€300	Nzomics Biocatalysis,UK
Nit04	*Silicibactor promeroyi* DSS-3 Nitrilase	50 mg/€300	Nzomics Biocatalysis,UK
PRO-E0263	*Silicibactor promeroyi* DSS-3	10 mg/£70.00	Prozomix Limited, UK
PRO-E0260	*Bradyrhizobium japonicum* USDA 110	10 mg/£70.00	Prozomix Limited, UK
PRO-NITRP	_	50 mg/€535.50	Prozomix Limited, UK

LIMITATIONS OF NITRILASES

The first nitrilase mediated bioprocess was performed in 1988 using *R. rhodochorus* J1 for the conversion of 3-cyanopyridine to nicotinic acid. But, irrespective of nitrilase's potential ability of regio- and enatioselectivity, the commercialization had been rather slow. The possible bottleneck causes for nitrilase large scale production are slow conversion rate, low product yield and low substrate tolerance [53]. The enantioselective hydrolysis of mandelonitrile to mandelic acid by recombinant nitrilase was severely affected when substrate concentration reached more than 300 mM [54]. It was observed that most of the nitrilases produced were from mesophillic sources and hence, witnessed low thermal & solvent stability and narrow substrate specificity. It has been predicted that nitrilase from thermophillic sources have higher thermal stability, broad substrate specificity as well as improved enantio- and regioselective properties. Immobilized cells of thermostable nitrilase from *Bacillus pallidus* strain Dac 521 showed 100% conversion rate of cyanopyridine for 100 hours at 50˚ C. Since cells were immobilized, the problem of washing out was removed [55]. The other possible limiting factors are lack of proper crystal structure of nitrilase and loss of activity after storage.

CONCLUSION

Nitrilases are one of the hydrolytic enzymes which act on nitriles and convert them into corresponding acids for different applications. They pose unique qualities such as regio- and enantioselectivity so, their role in pharmaceutical and chemical industries are immense. These enzymes have been used in production of many high value products which are very difficult to manufacture using traditional chemical methods. Nitriles are obnoxious in the nature therefore; this enzyme also plays a vital role in environment protection. Considering the economic importance and wide applicability of nitrilases, the studies on biotransformation of nitriles are scanty. The potential application of this enzyme can be enhanced further by increasing its specific activity for substrates, decreasing the loss of activity during storage and with the knowledge of its proper crystal structure by *in silico* study. It has also been viewed that substrate specificity of the nitrilases varies widely inspite of their conserved structure. Therefore, lot more study is needed to increase the applicability of nitrilase which can lead to formation of enzymes with better properties.

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