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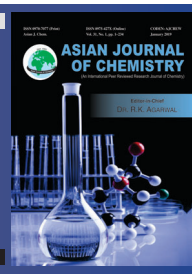

REVIEW

Role of Enzymes in Synthesis of Biologically Important Organic Scaffolds

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Green route of biogenic synthesis of heterocyclic compounds *via* microbes (bacteria, fungi, virus, yeast, algae, *etc*.) has the potential to deliver clean manufacturing technology. The application of biocatalysts for the synthesis of novel compounds has attracted increasing attention over the past few years and consequently, high demands have been placed on the identification of new biocatalysts for organic synthesis. Enzymes play an increasingly important role as biocatalysts in the synthesis of key intermediates for the pharmaceutical and chemical industry, and new enzymatic technologies. The characteristics of biocatalyst can be tailored with protein engineering and metabolic engineering methods to meet the desired process conditions. This review discusses the synthetic application of all the six classes of enzymes which are oxidoreductase, transferase, hydrolase, lyase, isomerase and ligase. Enzymes are highly selective catalysts and their contribution to regio-, chemo- and stereoselectivity of compounds were also discussed.

Keywords: Biogenic synthesis, Biocatalyst, Microorganisms, Epoxidation.

INTRODUCTION

Biocatalysis can be defined as use of whole cells or enzymes in organic synthesis [1-3]. This use of enzymes in synthesis of organic compounds has led to the development of Green Chemistry. Green chemistry or sustainable technology has large potential and can applied in various sectors of chemical industries such as pharmaceutical industry, agrochemical industry and many more [4-11]. Biocatalysis provide various advantages such as reduction in the use of toxic chemicals, saving of energy and minimum production of waste [12]. One of the major challenges faced by synthetic chemists nowadays is the fact that different enantiomers of the same compound are usually produced during synthesis and these may have different interactions in biological systems. Consequently, the production of single enantiomers with specific activity, instead of racemic mixtures becomes an important issue in chemical industries *e.g*. pharmaceutical and agrochemical industries. In addition, chemical synthesis demands expensive equipments due to their high temperature and pressure. Enzymes show activity towards a range of compounds and forms different kinds of structurally related products. Chiral compounds can also be formed because of high efficiency, regio and stereo-selectivity of enzymes [13-15]. Microorganisms are becoming a favoured source of industrial enzymes since the number of enzymes which can be recovered economically from plants and animals are limited. There are two types of microbial enzymes which are extracellular and intracellular enzymes. Extracellular enzymes secrets out from cell and intracellular enzymes remain within cell thus whole cell are used as catalyst. Both bacteria and fungi are great source of various types of enzymes. With the help of recombinant DNA technology large number of recombinant or mutant enzymes was isolated from microbes [16-19]. Microbial enzymes have various advantages over other plants and animals like microbes can grow in extreme environmental conditions and have short generation time. In general, enzymes act as a machinery of nature to synthesize new organic compounds, already discussed by various researchers [20-35]. This review shows some examples of biocatalysts (enzymes) used for the production of various organic compounds and heterocycles. In this review, reactions are categorized according to the class of enzymes used in production which are oxidoreductase, transferase, hydrolase, lyase,

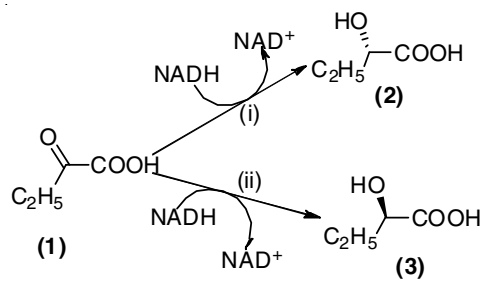
transferase and ligase. In addition, enzymatic engineering allows for the production of enzymes effective in a non-aqueous environment [36]. This kind of environment is used in biocatalysis due to its interesting properties such as increased solubility of the substrate or hydrolytic reaction reversibility.

Classification of enzymes (biocatalysts)

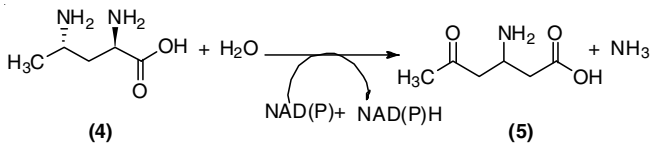
There is a great need of more frequent integration of enzymatic steps in organic synthesis routes for better ecofriendly development [37-40]. Thus, with the help of high through put methods, enzymatic metagenomic libraries and chip technologies, the fourth wave of biocatalysis is approaching day by day [34,41-43]. The summary of classification, types, reaction catalyzed and examples of enzymes are shown in Table-1. About 60 % of biocatalysts used are hydrolases, 20 % oxidoreductases and rest 20 % is for other four classes of enzyme [36].

Oxidoreductases: Oxidoreductase catalyzes the transfer of electron from one molecule to other. Oxidoreductases catalyze reactions similar to the following, $A^- + B \rightarrow A + B^-$ where A is the oxidant and B is the reductant. Oxidoreductase includes oxidases or dehydrogenases. Oxidases are used when molecular oxygen acts as hydrogen or electrons acceptor. Likewise dehydrogenase by transferring hydrogen oxidizes a substrate that is $NAD^+/NADP^+$ or a flavin enzyme. Oxidoreductases enzymes are second most used forms of enzymes in synthesis of organic compounds. This class of enzyme includes various examples like hydroxylases, peroxidases, reductases and oxygenases. Oxidoreductase enzymes also plays very important role in both anaerobic and aerobic metabolism.

Alan *et al*. [44] stated the conversion of 2-oxobutanoic acid (**1**) with the use of L and D form of lactase dehydrogenase to stereospecific isomers of type α-hydroxybutanoic acid (**2**) and (**3**) (**Scheme-I**). L-Lactate dehydrogenase in comparison with D-lactate dehydrogenase has narrower substrate specificity. Both isomers of lactate dehydrogenase use NADH as a catalyst for production of isomers of α-hydroxybutanoic acid. Alan *et al*. [44] also stated that 2,4-diaminopentanoic acid (**4**) can be reduced to 2,4-amino-4-oxopentanoic acid (**5**) with the help of 2,4-diaminopentanoate dehydrogenase (**Scheme-II**). This enzyme acts on CH-NH_2 group of donor and NAD, NADP act as electron acceptor. These enzymes act on various metabolic pathways like arginine and proline metabolism, lysine degradation *etc*.

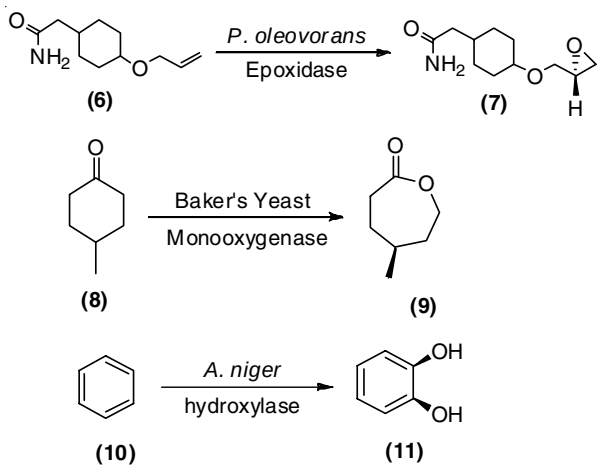


Scheme-I: (i) Action of L-lactate dehydrogenase (ii) Action of D-lactate dehydrogenase



Scheme-II: Reduction of 2,4-diaminopentanoic acid using 2,4-diaminopentanoate dehydrogenase

DeSantis *et al.* [45] also observed that some reactions catalyzed by oxidoreductase which are epoxidation of alkenes through epoxidase isolated from *Pseudomonas oleovorans*, lactonization of cyclohexane by monooxygenase isolated from Baker's yeast and hydroxylation of benzene by hydoxylase isolated from *Aspergillus niger* (**Scheme-III**).

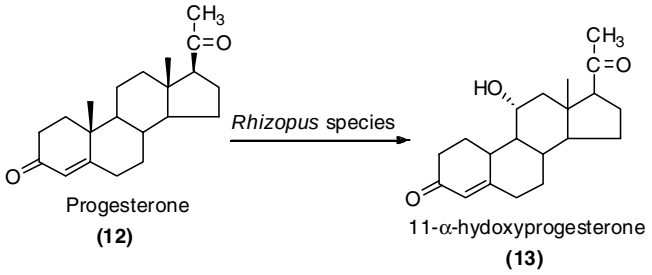


Scheme-III: Epoxidation by epoxidase, hydroxylation by dioxygenase and lactonization by monooxygenase

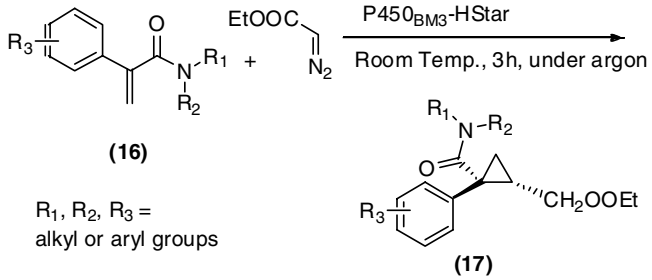
Peterson *et al*. [46,47] found a commercially viable route of synthesizing cortisol that took over the 31-steps chemical synthesis of cortisol from a bile acid and this showed the way for the subsequent commercial success of the steroid hormones. The corticosteriod, 11-hydroxycortisol can be produced from the cheap precursor 11-deoxycortisolusing 11β-monooxygenase. A fungus with genus *Rhizopus* was found which easily add in a single step 11α-hydroxyl group directly on to steroid hormone progesterone (**Scheme-IV**).

TABLE-1
CLASSIFICATION OF ENZYME ACCORDING TO THEIR REACTION

Classes of enzyme	Types of enzyme	Reactions catalyzed by enzymes	Examples
EC 1	Oxidoreductase	Oxidation and Reduction	Dehydrogenase, oxygenase, catalase, oxidase, peroxidase
EC 2	Transferase	Transfer of a group from one molecule to another	Transaminase, transaldolase, glycosyltransferase
EC 3	Hydrolase	Hydrolysis of a chemical bond	Lipase, protease, esterase, hydratase, phosphatase, glycosidase, nitrilase
EC 4	Lyase	Non hydrolytic cleavage of a bond	Dehydratase, decarboxylase, deoxyribosephosphate, aldolase
EC 5	Isomerase	Rearrange the existing atoms of a molecule	Racemase, mutase, epimerase
EC 6	Ligase	Synthesis of a bond with the use of ATP	DNA ligase

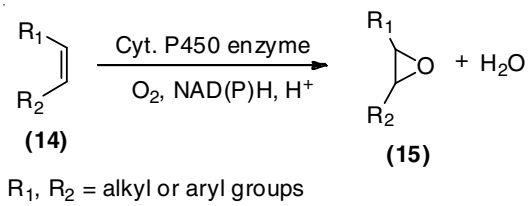


Scheme-IV: Single step hydroxylation using monooxygenase



Scheme-VI: Cyclopropanation of acrylamides using P450 BM3-Hstar

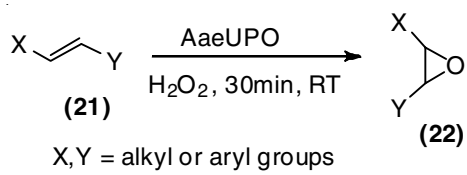
Epoxides are usually formed by epoxidation of alkenes or by halohydrins. For epoxidation three classes of enzymes were used which are (i) enzymes (heme dependent) using molecular H_2O_2 like chloroperoxidase and unspecific peroxidase, (ii) enzymes requiring molecular oxygen like xylene monooxygenase and (iii) enzymes using FAD like styrene monooxygenase or a Baeyer-villiger monooxygenase. Cytochrome P450 enzyme having heme iron centre causes epoxidation. Groves *et al.* [48] stated the mechanism of epoxidation as shown in **Scheme-V**, which was later investigated by Hrycay and Bandiera [49].



Scheme-V: Epoxidation using cytochrome P450 enzyme

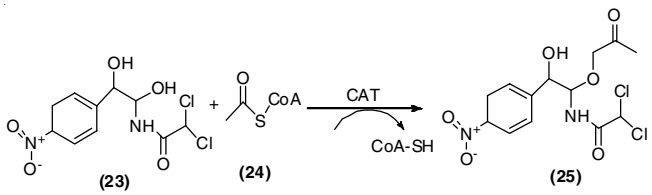
Wang *et al.* [50] reported the cyclopropanation of acrylamides with the use of different of $P450_{BM3}$-Hstar, in which the heme iron's ligand got changed to histidine from cysteine. $P450_{BM3}$-Hstar helps in enantioselective transformation of lots of acrylamide to their corresponding cyclopropanes such as in synthesis of levomilnacipran, an antidepressant [51] (**Scheme-VI**). Dietrich *et al.* [52] reported the advantage of a recombinant $P450_{BM-3}$ G-4 variant in the semi-synthetic development of anti-malarial drug, *e.g.* artemisinin. *E. coli* expressing $P450_{BM-3}$ G-4 variant epoxidizes amorphia-4,11-diene (**18**) into artemisnic-11S,12-epoxide (**19**).This epoxide is further helps in the production of drug artemisinin (**20**) (**Scheme-VII**).

Peter *et al.* [53] reported that an aromatic peroxygenase, isolated from *Agrocybe aegerita* (AaeUPO) which oxidizes alkenes (linear, branched and cyclic) through hydroxylation or epoxidation using hydrogen peroxide. This peroxygenase works by the same mechanism as the P450 enzyme except that it uses hydrogen peroxide for epoxidation rather than NAD(P)H and molecular oxygen (**Scheme-VIII**).



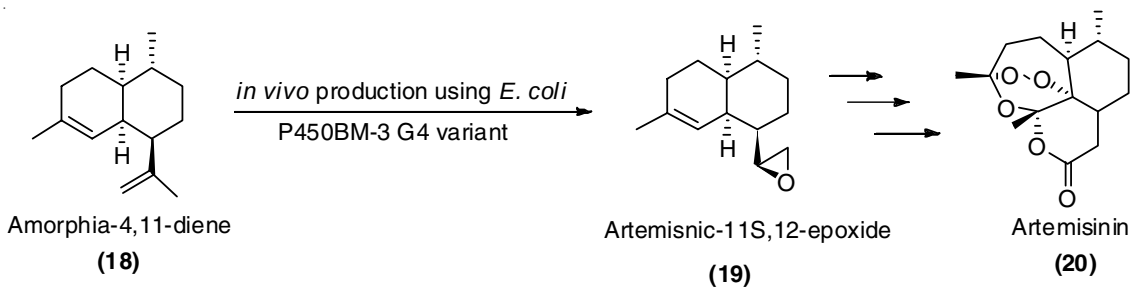
Scheme-VIII: Epoxidation with the help of hydrogen peroxide using peroxygenase

Transferase: Transferases (EC: 2) catalyzes the transfer of groups such as sugar, phosphoryl, aldehyde or ketone and acyl from one molecule to another. Transferases are multifaceted catalysts for synthesis of different types of organic compounds such as transaminases used for the synthesis of amines and amino acids. Rottig and Steinbuchel [54] stated that chloramphenicol acetyltransferases (CAT) catalyzes the acylation of chloramphenicol (**23**) to form 3-acetylchloramphenicol (**25**) by transferring the acetyl group from acetylcoenzyme A (acetyl CoA) (**24**) to the hydroxyl group of chloramphenicol (**Scheme-IX**). CATs were responsible for bacterial resistance to the antibiotic chloramphenicol, which inhibits the activity of ribosomal peptidyl-transferase.



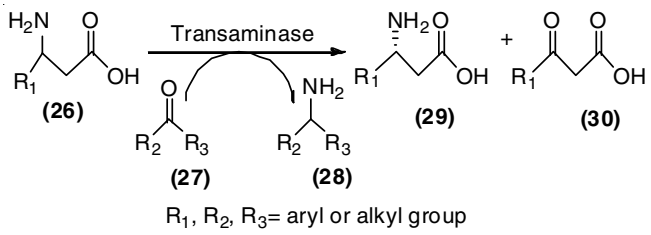
Scheme-IX: Transfer of acetyl group using chloramphenicol acetyltransferases

Horbal *et al.* [55] reported that transaminase catalyzes the transfer of an amino group. This process was used for the preparation and resolution of amino acids and their analogues.



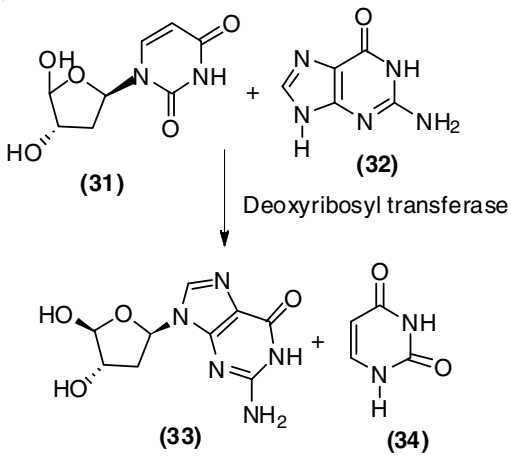
Scheme-VII: Epoxidation using recombinant P450 BM-3 G-4 variant in the development of artemisinin

The transaminases can be applied either in the kinetic resolution of racemic β-amino acids or in asymmetric synthesis of amino acids, starting from the corresponding prochiral β-keto-substrate (**Scheme-X**). Transaminase belong to the large and diverse group of pyridoxal phosphate (PLP)-dependent enzymes and are ubiquitous in living organisms playing an important role in amino acid metabolism.

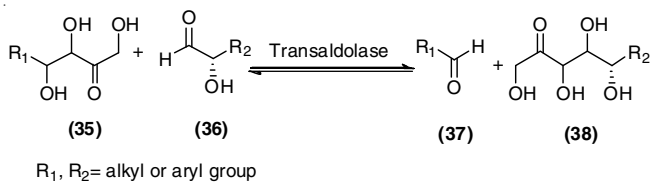


Scheme-X: Transfer of an amino group using transaminase

Some researchers [56,57] reported the preparation of nucleosides analogues (antiviral precursors) can be catalyzed by glycosyl transferase (deoxyribosyltranferase). This reaction involves the transfer of a sugar group from a compound (**31**) to another (**32**) to form a nucleoside (**33**) (**Scheme-XI**). Transaldolase enzyme extracted from *E. coli* catalyzes the transfer of dihydroxyacetone moiety from one donor substrate (**35**) to other acceptor substrate (**36**) (**Scheme-XII**) [55].

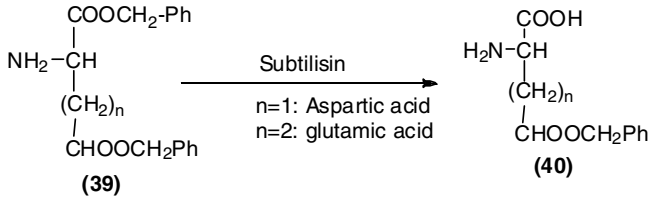


Scheme-XI: Action of deoxyribosyl transferase



Scheme-XII: Transfer of dihydroxyacetone using transaldolase

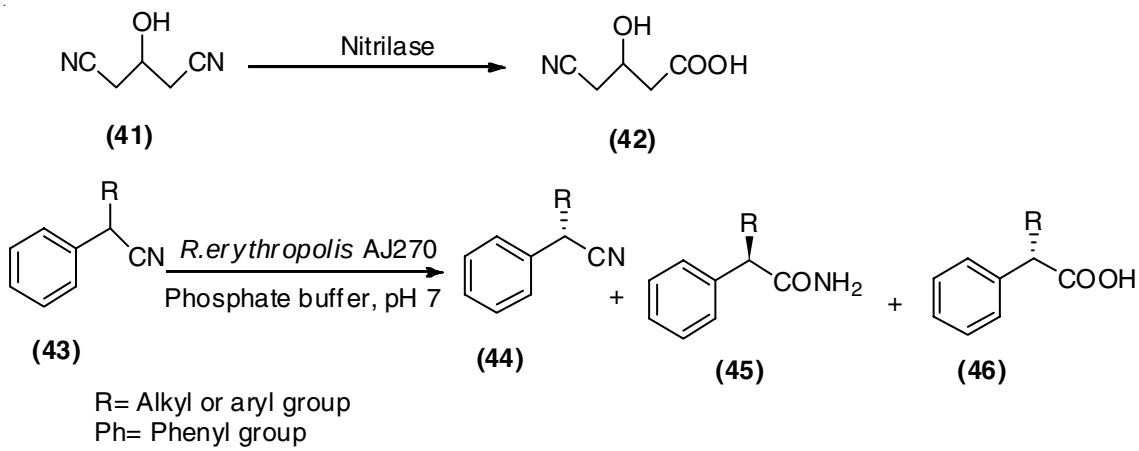
Hydrolases: Hydrolases (EC: 3) catalyze the hydrolytic cleavage of glycosides, anhydrides, esters, amides, peptides, and other C-N moieties. These reactions are referred to as hydrolysis. Tyler *et al*. [13] reported that proteases like papain, α-chymotrypsin and subtilisin were useful biocatalysts for regioselective or stereoselective hydrolytic biotransformations. For example, dibenzylester of glutamic acid and aspartic acid (**39**) at position-1 give their derivatives (**40**) by *Subtilis* in catalyzed hydrolysis (**Scheme-XIII**).



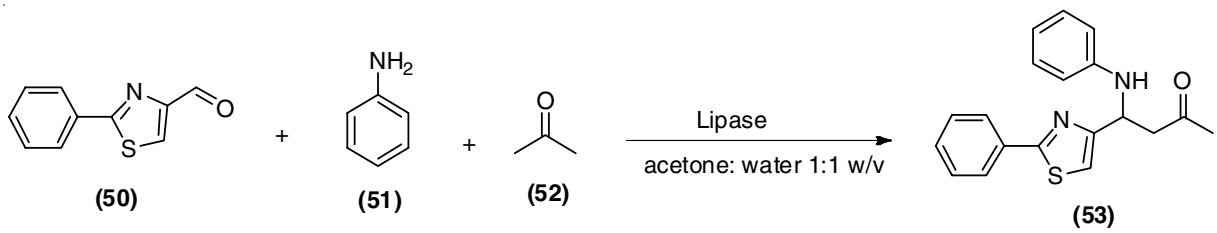
Scheme-XIII: Hydrolytic biotransformation

Nitrilases also play an important function in the preparation, resolution and the conversion of nitrile groups (**41**) to acid groups (**42**). Tyler *et al*. [13] also demonstrated that *Rhodococcus* sp. AJ270 containing a nitrilase was able to catalyzed the stereoselsctive conversion of α-substituted phenylacetonitriles (**43**) under mild conditions into amides (**45**) and carboxylic acids (**46**) (**Scheme-XIV**).

Leonte *et al*. [58] described the biocatalytic synthesis of new Mannich bases containing various heterocyclic rings (thiazole, furane, thiophene, pyridine) by applying the lipase catalyzed trimolecular condensation of the corresponding heterocyclic aldehydes (**50**) with acetone (**52**) and primary aromatic amines (**51**) in mild and eco-friendly reaction conditions (**Scheme-XV**). Penicillin acylase isolated from *E. coli* hydrolyzes the different forms of penicillin such as penicillin G (**58**) into 6-aminopenicillinic acid (6-APA) (**59**) [59,60] (**Scheme-**

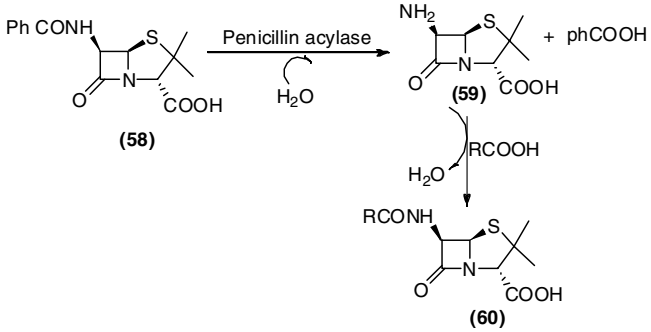


Scheme-XIV: Conversion of nitrile to acid and amid using nitrilase



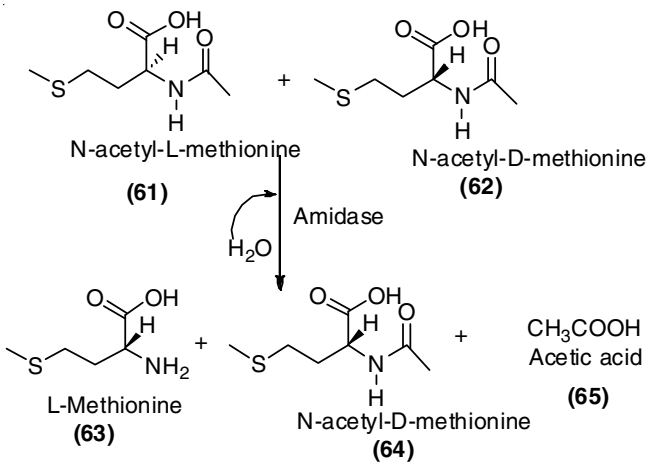
Scheme-XV: Application of lipase in synthesis of Mannich bases

XVI). The enzyme catalyzes the hydrolysis of amide bond in side chain of penicillin to give amine. This 6-aminopenicillinic acid product was then converted into different types of new penicillin derivatives.



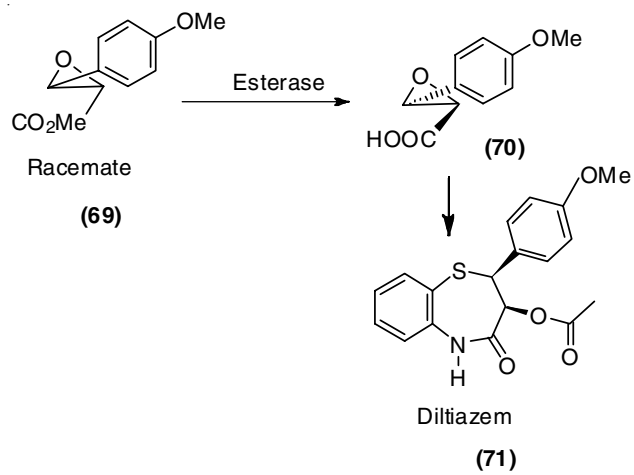
Scheme-XVI: Conversion of penicillin G to 6-aminopenicillinic acid using penicillin acylase

Amidase enzyme extracted from *Aspergillus oryzae* was used for the hydrolysis of acetyl group in *N*-acetylmethionine [61,62]. However, only one of the enantiomer of acetyl methionine was the substrate for amidase. Thus, L-methionine (**63**) was the product of the reaction and D-enantiomer of acetylated methionine (**64**) remains unreacted as like substrate (**Scheme-XVII**) [61].



Scheme-XVII: Hydrolysis of acetyl group using amidase

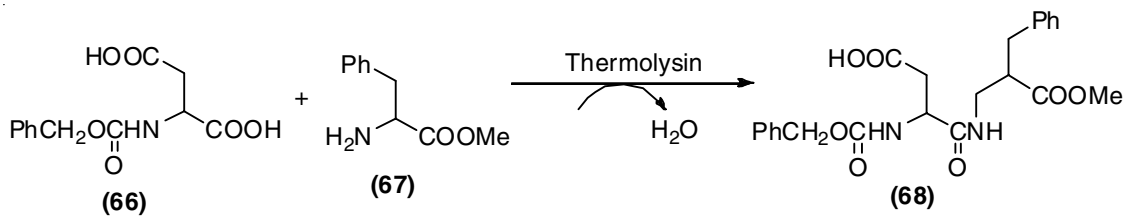
Shadpour *et al*. [62] reported that methyl ester of aspartic acid will hydrolyze under severe conditions. There are two reactive groups in aspartic acid and enzyme needs only of them to take part in condensation reaction. Thermolysin was able to work under extreme conditions such as high temperature, in presence of organic solvents, *etc*. Thermolysin forms the amide bond between two substrates to form product (**68**) (**Scheme-XVIII**). The enzyme esterase selectively hydrolyzes the substrate containing esters to their corresponding acids via specific stereoselectivity. Esterase was capable of differentiating between different isomers of substrate esters. This acid was then further elaborated under mild conditions for the innovative synthesis of final calcium-antagonist drug diltiazem (**71**) (**Scheme-XIX**) [63].



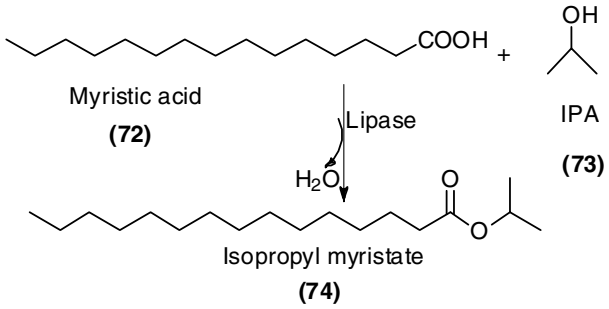
Scheme-XIX: Conversion of esters to acids using esterase

Isopropyl myristate (**74**) was obtained by condensation of myristic acid (**72**) with isopropyl alcohol (IPA) (**73**) (**Scheme-XX**). Isopropyl myristate was an emollient used in skin care products to give a smooth feel to the skin. Lipase used in the condensation, was obtained from a yeast *Candida*. This reaction operates at 60 °C to remove water produced during reaction [64].

Lyases: Lyases (EC: 4) are the enzymes which are responsible to catalyze addition and elimination reactions means it



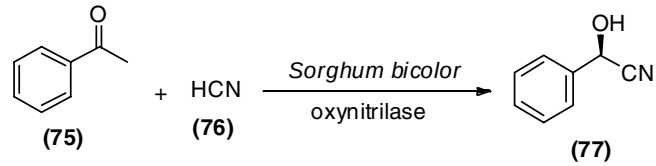
Scheme-XVIII: Formation of amide bond using thermolysin



Scheme-XX: Condensation of two substrates using lipase

catalyzes reactions involving the breaking of a bond between a carbon atom and another atoms such as oxygen, sulfur or another carbon atom. This class of enzyme has great applications in cellular processes such as citric acid cycle and in organic synthesis, such as in the production of cyanohydrins. DeSantis [45] reported the biotransformation of phenylethanone (**75**) to 2-hydroxyl-2-phenylnitrile (**77**) through the catalytic activity of s-oxynitrilase from *Sorghum bicolor* (**Scheme-XXI**). Brovetto *et al*. [65] also reported on the use of benzaldehyde lyase (BAL) to catalyze the transformation of *rac*-benzion (**78**) to *R*-2 hydroxyl-phenylpropanone (**79**) as well as its resolution to *S*-benzion (**80**) (**Scheme-XXII**). Furthermore, Brovetto *et al*. [65] also reported the use of ammonia lyases as efficient biocatalysts for biotransformation by describing the action of phenylalanine lyase and phenylalanine aminomutase in the synthesis of amino acids (**82,84,85**) (**Scheme-XXIII**).

Pelt [66] reported that a lyase known as nitrile hydratase (NHase) was used in the production process of nicotinamide (niacinamide, vitamin B3). The process involves four highly selective, continuous catalytic reaction steps namely (i) cyclization, (ii) dehydrogenation (iii) ammoxidation and (iv) enzymatic



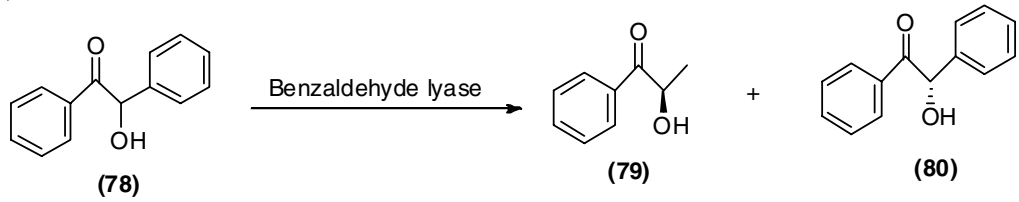
Scheme-XXI: Biotransformation of ketone to nitrile using oxynitrilase

hydration using NHase. The starting material was 2-methyl pentanediamine (**86**), which was a by-product obtained from nylon-66 production. In the last step, hydration of 3-cyano-pyridine (**88**) to nicotinamide (**89**) was carried out by using *R. rhodochrous* J1 whole cells (containing NHase) immobilized in polyacrylamide gel particles (**Scheme-XXIV**).

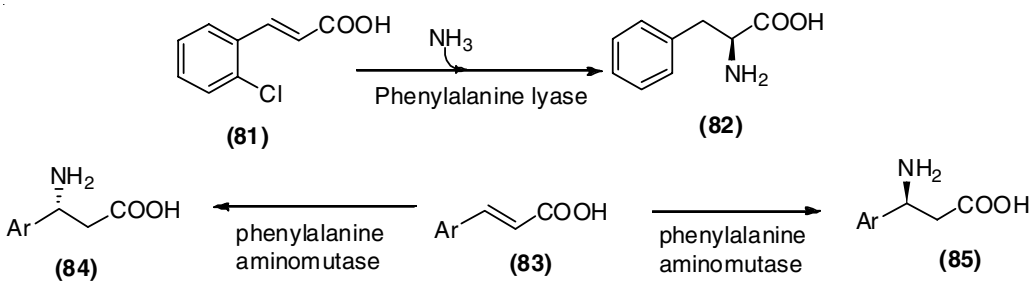
Isomerases: Isomerases are a class of enzyme which catalyzes the structural rearrangement within one molecule. It facilitates intramolecular rearrangements in which bonds are broken and formed. Researches on glucose isomerases are reported and covers the mathematical simulation as well as the establishment of whole-cell processes [65,66]. Epimerase and racemase are the two most commonly used form of enzymes from class isomerase.

Epimerase (EC 5.1.3.8) isolated from *Escherichia coli* facilitates the epimerization of glucosamine. For the synthesis of *N*-acetylneuraminic acid, *N*-acetyl-D-mannosamine serves as an *in situ* generated substrate. Since *N*-acetyl-D-mannosamine (**91**) was quite expensive, therefore, it was synthesized by epimerization at C_2 of *N*-acetyl-D-glucosamine (**90**) (**Scheme-XXV**). By application of *N*-acylglucosamine (GlcNAc) 2-epimerase, it was possible to start with inexpensive *N*-acetyl-D-glucosamine instead of *N*-acetyl-D-mannosamine [67-70].

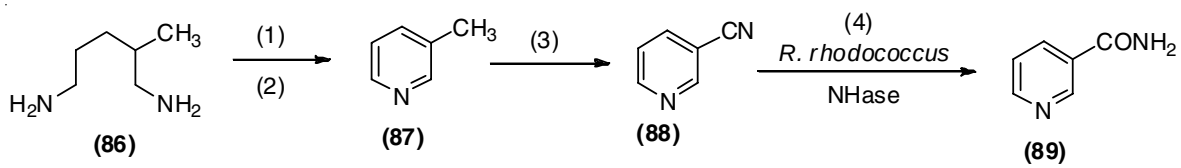
Production of 100 % desired enantiomer in single pot from a given substrate was possible by dynamic kinetic resolution.



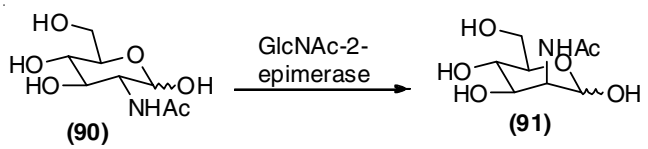
Scheme-XXII: Action of benzaldehyde lyase (BAL)



Scheme-XXIII: Use of phenylalanine lyase and phenylalanine aminomutase in the synthesis of amino acids

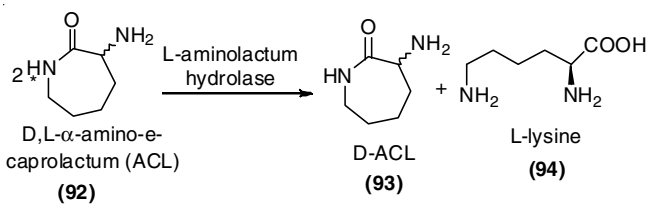


Scheme-XXIV: Production of nylon-66 production using NHase



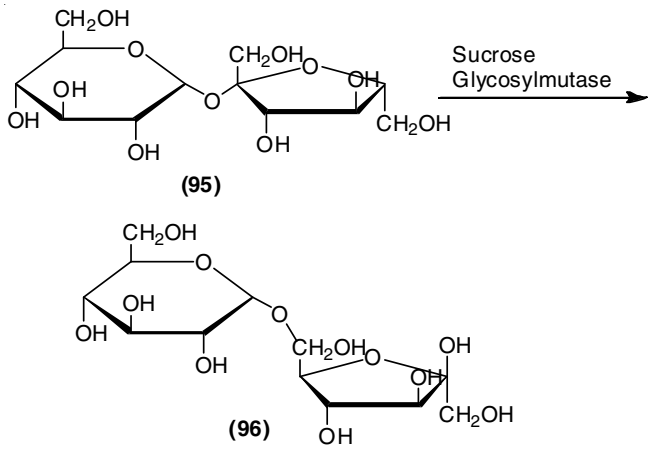
Scheme-XXV: Application of *N*-acylglucosamine (GlcNAc) 2-epimerase

Aminolactamhydrolase (EC 3.5.2.11) and racemase (EC 5.1.1.15) was used for dynamic resolution of α-amino-ε-caprolactam (**92**) (**Scheme-XXVI**). The racemase enzyme for racemization was isolated from *Achromobacter obae* [71,72].



Scheme-XXVI: Action of α-amino-ε-caprolactam

Palatinose, a reducing disaccharide, occurs naturally in low amount in sugarcane extract and honey. Palatinose and its hydrogenated products are used as sweetener with same taste as sucrose, with only half of calorific value and 42 % of sweetness as of sucrose. Thus because of low insulin simulation and lower acid and glucan production, it was used as substitute of sucrose in various food industries. A-Glucosyltransferase (EC 5.4. 99. 11) continuously produces palatinose (**96**) with small amount of trehalulose from sucrose (**95**) (**Scheme-XXVII**) [38].



Scheme-XXVII: Production of palatinose using A-glucosyltransferase

Ligases: Ligases (EC: 6) catalyze the formation of C-O, C-S, C-N, C-C and phosphate ester bonds [25-27]. These enzymes are also known as synthetases. Enzymes from class EC 1 to EC 5 are widely used as catalyst in organic synthesis but application range of EC 6 (ligases) is still not explored yet. This class of enzyme requires ATP as a cofactor, which is easily regenerated in living cell processes, however, *in vitro* reaction is still a challenge [74].

TABLE-2
SUMMARY OF THE ENZYMES USED AS A CATALYST IN VARIOUS REACTIONS

Microbes used	Enzyme	Substrate	Product	Ref.
Bacillus subtilis	Subtilisin	Glutamic acid	Derivatives of glutamic acid	[13]
Rhodococcus species AJ270	Nitrilase	Nitrile	Carboxylic acid	[13]
Aspergillus niger	Lactase dehydrogenase	Oxobutanoic acid	Isomers of α-hydroxybutanioc	[44]
Aspergillus niger	Hydoxylase	Benzene	Catechol	[45]
Aspergillus niger	Epoxide hydoxylase	Benzene	Glycol	[45]
Pseudomonas oleovorans	Oxidoreductase	Alkene	Epoxide	[45]
Clostridium sticklandii	Dehydrogenase	2,4-Diamino pentanoic acid	2,4-Amino-4-oxo-pentanoic acid	[45]
E. coli K2	Oxidoreductase	5,6-Dihydro Uracil	Uracil	[45]
Baker's Yeast	Monooxygenase	Cyclohexane	Lactone	[45]
Rhizopus nigricans	11-α-Hydroxylases	Progesterone	11-α-Hydroxyprogesterone	[46]
Agrocybe aegerita	Cytochrome P450	Alkene	Epoxide	[49]
Bacillus megaterium	P450 BM-3- Hstar	Cylcopropane	Acrylamides	[50]
E. coli	P450 BM-3 G-4	Amorphia-4,1-diene	Artemisnic-11S,12-epoxide	[52]
Agrocybe aegerita	Fungal peroxygenase (AaeUPO)	Alkene	Epoxide	[53]
E. coli	Chloramphenicol acetyltransferases	Chloramphenicol	3-Acetyl chloramphenicol	[54]
E. coli B	Transaminase	Prochiral β- keto amino acids	Racemic β- amino acid	[55]
Lactobacillus helviticus	Glycosyl transferase	2-Deoxy-D-ribosyl-base$_1$ + base$_2$	2-Deoxy-D-ribosyl-base$_2$ + base$_1$	[56]
Candida rugosa	Lipase	Heterocyclic aldehyde and acetone	Mannich base	[58]
E. coli	Penicillin acylase	Penicillin	6-Aminopenicillanic acid	[59,60]
Aspergillus oryzae	Amidase	N-Acetyl L-Methionine	L-Methionine	[61]
Bacillus sterarothermophillus	Thermolysin	Aspartic acid + methyl ester	Aspartame	[62]
Bacillus cereus	Lipase	Myristic acid and isopropyl alcohol	Isopropylmyristate	[64]
Pseudomonas fluorescence	Benzaldehyde lyase	Benzion	*R*-2-Hydroxylphenylpropanone	[65]
Pseudomonas fluorescence	Phenylalanine lyase	Cinnamic acid	Amino acid	[65]
R. rhodochrous	Nitrile hydratase	2-Methyl pentanediamine	Nicotinamide	[66]
Pseudomonas chlororaphis	Nitrile hydrates	Isocyanide	5-Cyanovaleramide (5-CVAM)	[66]
E. coli	Epimerase	N-Acetyl-D-mannosamine	N-Acetylneuraminic acid	[69,70]
Achromobacter obae	Aminolactum hydrolase	D,L-a-amino-e-caprolactam	D-Amino-e-caprolactam + L-lysine	[71,72]
Bacillus coagulans	Glucose isomerase	Glucose	Fructose	[73]

Conclusion

Biocatalysis has emerged as a significant resource for chemical synthesis and it is on the path of exponential growth. In several industries, during past several decades various types of products have been produced by many biocatalytic processes implementation. Among all classes, Class 6 (ligases), have limited applications in organic syntheses. This is because *in situ* regeneration of the cofactor ATP is a challenge. In contrast, enzymes from enzyme classes EC 1-5 are highly efficient catalysts for abroad range of organic synthetic transformations as well as suitable for technical-scale applications Most of the products are produced through the use of natural enzymes, whole cells or microorganisms are summarized in Table-2. The cost and the time for development of new enzymes can be minimized drastically by advancement in protein engineering along with metabolic engineering. These engineered enzymes are used in various pharmaceutical and food industries due to their stereo-selectivity. The advancements in proteomics, genomics and bioinformatics will leads to the biocatalysis development which acts as the integral part of various industrial catalysts.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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