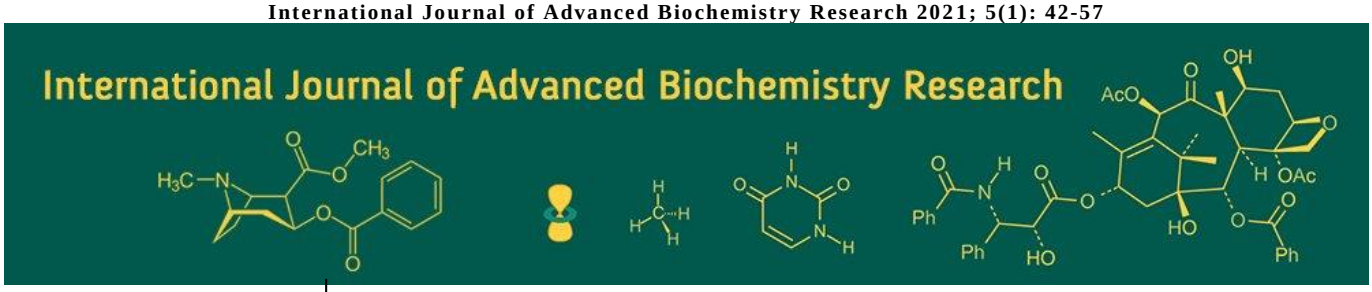

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Isaac John Umaru
Department of Biochemistry, Federal University, Wukari, Taraba, Nigeria

Baba Rufaidat Adam
Department of Biochemistry, Federal University, Wukari, Taraba, Nigeria

Bilyaminu Habibu
Department of Biochemistry, Federal University, Wukari, Taraba, Nigeria

Kerenhappuch Isaac Umaru
Department of Biochemistry, Federal University, Wukari, Taraba, Nigeria

Barrah Collins Chizaram
Department of Biochemistry, Federal University, Wukari, Taraba, Nigeria

Corresponding Author:
Isaac John Umaru
Department of Biochemistry, Federal University, Wukari, Taraba, Nigeria

Biochemical impact of microorganism and enzymatic activities in food and pharmaceutical industries

Isaac John Umaru, Baba Rufaidat Adam, Bilyaminu Habibu, Kerenhappuch Isaac Umaru and Barrah Collins Chizaram

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Abstract
Bio catalytic activity of microorganisms and enzymes have been explored for centuries to yield bread, wine, vinegar and with some common products without knowledge about the biochemical basis of their properties. Due to their established catalytic activity, stability, simplicity of biosynthesis and higher optimization when compared with plants and animal enzymes, microbes and enzymes are now widely used in the food and medical industries. Enzymes usage across diverse industries (such as food, agriculture, chemicals, and medicines) is rapidly on the increase owing to their characteristic rapid processing time, low energy requirements, affordability, non-toxic and environmental friendliness. Therefore, toxic chemicals from the domestic environment and various industries (e.g. phenols, nitriles and amines) can be degraded or converted by microbes. This review is geared towards discussing the present-day technical and scientific involvement of ubiquitous organisms (microorganisms) and the production of enzymes with their present status in global enzyme market among various industries.

Keywords: Biochemical, microorganism, enzymatic, food, pharmaceutical, industries

1. Introduction
Microbes have been put to use since the dawn of human civilization, with the Babylonians and Sumerians using yeast in commercial quantities as early as 6000 BC to make alcoholic beverages from barley. The broad utilization of microbial enzymes in many different industries, including medicine, energy, food, chemicals and agriculture, has earned them respect on a global scale. Because of their decreased processing times, low energy requirements, cost effectiveness, non-toxic nature and environmental friendliness, enzyme-mediated procedures are quickly gaining popularity (Li *et al*., 2012 and Choi *et al*., 2015) [1, 3]. Additionally, the development of recombinant DNA technology and protein engineering has made it possible to alter and produce these microbes in vast numbers to meet rising demand in businesses and/or industries (Liu *et al*., 2013) [2].

In exclusion of catalytic RNA molecules (ribozymes), biological molecules (enzymes) are proteinaceous in origin and function as catalysts for almost all of the chemical reactions necessary for life's processes (Cech and Bass, 1986) [4]. Enzymes are essential macromolecules that sustain life since they help in speeding up certain chemical reactions by lowering the activation energy without undergoing a permanent change. These enzymes are known to be highly selective (Fersht, 1985; Piccolino, 2000 and Aldridge, 2013) [5, 6, 7]. Because they are biodegradable and harmless, they are employed as an alternative to harmful chemical pollutants and often demand moderate conditions of temperature and pressure for catalyzing reactions (Mojsov, 2011; Illanes, *et al*., 2012 and Choi *et al*., 2015) [8, 9, 3]. Enzyme usage in healthcare and other industries has setbacks in addition to its merits over conventional approaches. The ideal temperature and pH for many mammalian enzymes are 37 ˚C and 7.4, respectively, and changes in these variables have a significant impact on how active these enzymes are. These macromolecules can only be used in non-physiological settings up to a higher temperature of 40 ˚C and a significant pH difference from the physiological pH of 7.4. They can also be subject to product or substrate inhibition, and some of their final products can lead to allergic reactions. The cost implication of enzyme separation and purification is high as well as the difficulty in recovering them for later usage may hamper on their overall usage (Johannes *et al*., 2006) [10].

Industrial enzymes are frequently derived from microorganisms due to their easy availability and rapid development rate. Recombinant DNA technology makes it simple to genetically modify microbial cells for higher enzyme production and scientific advancement (Illanes *et al*., 2012) [9]. Production of microbial enzymes is a crucial process in the industrial sectors because of the high and outstanding performances of enzymes from diverse microorganisms, which work successfully under a wide range of varied physical and chemical circumstances. Additionally, medical diseases brought on by genetically based human enzyme deficits are treated by microbial enzymes (Vellard, 2003 and Anbu *et al*., 2015) [11]. For instance, sacrosidase (b-fruc to furano side fruc to hydrolase, EC 3.2.1.26) enzyme is administered orally to patients who have inherited congenital sucrase-isomaltase impairment and are unable to digest sucrose (Treem *et al*., 1999) [12]. Additionally, the hereditary phenylketonuria condition uses phenylalanine ammonia lyase (EC 4.3.1.24) to breakdown phenylalanine (Sarkissian *et al*., 1999) [13].

Enzymes are large macromolecules with molecular masses ranging from kilo to mega Dalton and comprise of polymers of amino acids linked by amide bonds. These macromolecules' selectivity for their substrate is determined by the location of their catalytic site, which is frequently hidden deep within hydrophobic recesses (Sarkissian, *et al*., 1999, Jordan 1929, Gurang *et al*., 2013) [13-15]. The basis for an enzyme's classification and nomenclature is its unique ability to drive the catalytic reactions between one types of chemical component over another. After 1940, biochemistry saw significant advancements that led to the isolation and characterization of several new enzymes, necessitating the regulation of enzyme nomenclature (Kamini *et al*., 1999) [16].

In order to oversee the nomenclature and systematic classification of enzymes, the International Union of Biochemistry and Molecular Biology (IUBMB) and International Union for Pure and Applied Chemistry (IUPAC) founded the Enzyme Commission (EC) (Liese *et al*., 2006, David, 2016) [17, 18]. The enzyme commission has divided enzymes into six major classes based on the sort of reaction they catalyze. Enzymes, especially those of microbial origins, can be cultured primarily by gene modification through genetic engineering, depending on their requirements for various applications in the manufacturing industries. Microbial enzymes' application in the pharmaceutical, food, textile, paper, leather and other sectors are enormous and they are expanding quickly in comparison to conventional techniques because they cause less environmental damage, more effective and produce goods of higher quality (Jordon, 1929; Kamini *et al*., 1999 and Gurung *et al*., 2013) [14-16].

2. Impact of microorgansm in food industry

In addition to agriculture, the food business has many wonderful uses for microorganisms. The amount and quality of the food produced are significantly influenced by bacteria. Pasteurized milk is inoculated with a particular culture of bacteria to cause fermentation. Yogurt and cheese are two examples of the various fermented dairy products that may be made (Dave & Shah, 1997) [19]. They are really employed to modify a substance's nature into one that may be utilized safely as food. For instance, the manufacture of wine and bread from sugar and the production of cheese and yoghurt from milk. A few notable industrial food. Below is a description of a few significant industrial food products:

2.1 Benefits of use of microbes in food industry

In employing microorganisms as sources of food, the following are the advantages (Caplice and Fitzgerald, 1999) [35]:

- Microbes develop incredibly quickly and don't require as much area as they do in traditional methods.
- The cells of microbes have a high protein content. They include between 40 and 50 percent protein in bacteria and between 20 and 40 percent in algae.
- They assist in recycling waste materials, which helps to clean up waste items.
- They have a high capacity for yield.
- They are less affected by environmental influences for example, the climate has less impact on them.
- The bacterial proteins possesses the requisite amino acids.
- A broad variety of low-cost agricultural waste products, industrial by-products, such as methanol, ethanol, other petroleum products, sugar, molasses, paper mill waste, etc., can be used to support their growth.
- Yeasts, in particular, have a large amount of vitamins.

2.1.1 Yoghurt production by bacteria

Yogurt is a milk-based food that is made by inoculating milk with bacterial culture. Any type of milk may be used to make it, although cow's milk is most frequently utilized. Yogurt may be made using a range of milk types. This includes whole, dry, skimmed, semi-skimmed milk or evaporated (Dave and Shah, 1997) [19].

- Bacteria such as Streptococcus thermophiles, Streptococcus salivarius and Lactobacillus bulgaricus ferment lactose, the milk sugar and create lactic acid as a by-product. These microorganisms are also referred to as LABs, or Lactic Acid Bacteria (Oyeleke, 2009) [20]. The lactose present in milk is used up by bacteria to yield lactic acid as a waste product throughout the feeding process.
- Lactic acid produced as a result bacteria consuming casein in milk causes it to change into a solid substance called curd. Yogurt's flavor and gelly texture are produced by the fermentation process of lactose sugar into lactic acid (Sandoval-Castilla O, *et al*., 2004) [21]. The production of lactic acid in yogurt increases its acidity, which inhibits the spread of other potentially hazardous bacteria. Either pasteurized or unpasteurized milk can be used to make yogurt. Full fermentation can be achieved by combining two or more bacterial cultures. The fruits can either be arranged at the bottom or followed by the flavored and sweetened yogurt (Chollet M, *et al*., 2013) [22].

2.1.2 Cheese production by bacteria and fungi

Milk is infused with a microbial culture containing certain bacteria to create cheese. Cheese is a solid meal that may be made from the milk of many different animals, although cow milk is often chosen. You may also utilize milk from goats, sheep, reindeer and water buffalo. Lactic acid is produced during milk fermentation as a result of the inoculation of bacterial cultures and this acid gives the milk its sour flavor. Casein, or milk protein, coagulates as a result of it. The liquid element of the coagulated milk is known as whey, while the solid component is known as curd. (Holmes, D.G., *et al*., 1997) [23].

The curds are then divided and given the required shape. The liquid component, known as whey, is then utilized as a source of food for yeasts, which are afterward, processed to create bovine feed and are a good source of vitamins and proteins. By introducing an inoculum of either bacteria, fungus, or both, the cheese becomes ripe. The pH is decreased, the texture is changed, and a taste is developed when these bacterial or fungal inoculums are introduced (Chapman HR, *et al*., 1974) [24]. The rennin enzyme, which is present in rennet pills, can be used to regulate coagulation. Actually, calves' stomachs contain the rennin enzyme. However, today's production relies on genetically modified microorganisms (Desobry-Banon S. *et al*., 1994) [25]. Lemon juice and vinegar can also be combined to create coagulation. There are several varieties of cheese (Maubois JL and Mocquot G. 1975) [25, 26]. Cheese comes in the following varieties according on the bacteria that are added:

a) Bacteria are used in the preparation of cheddar cheese to enhance flavor and texture.
b) Mould fungi are used in the production of blue cheese and Roquefort cheese.
c) Camembert cheese production involves the use of both bacteria and fungi.
d) The production of Swiss cheese is achieved using Propionibacterium shermanii.

Most cheeses range in color from off-white to yellow in their natural state. The cheese can also be flavored with herbs and spices (Roudot-Algaron, *et al*., 1994) [27] Different milk fat concentrations, processing methods, aging duration variations, and animal breeds are other elements that greatly influence the need style and flavors of cheese.

2.1.3 Bread production by yeast

Yeast is a saprotrophic, one-celled fungus. By secreting enzymes, yeast cells may digest foods including minerals and sugar. In the production of bread, yeast is used (Edema *et al*., 2005) [28]. The addition of yeast culture to add to flour, water and other substances leads to the production of carbon dioxide. The dough made of flour then traps this carbon dioxide. CO_2 is used to rise the dough in order to make bread. Most often, starch-containing wheat flour is utilized. Starch serves as the yeast's energy source. Additionally, the gluten protein in the wheat causes the sticky, elastic threads to form as the yeast inoculum reacts with the starch. These threads cause the bread to rise by capturing CO_2 (Zhang *et al*., 2007) [29].

Yeast has been used as a leavening agent in the bakery industry for a long time. *Saccharomyces cerevisiae* is the yeast species that is most frequently utilized because of its capacity to rapidly multiply and ferment sugar in dough (Asyikeen, *et al*., 2013) [30]. The dough rises as a result of the CO_2 produced during fermentation. Baker's yeast is produced in large quantities under regulated pH, humidity, and temperature conditions (Baldo and Baker. 1998) [31].

2.1.4 Chocolate production by yeast and bacteria

Microbes are used in the production of chocolate. Actually, cacao tree seeds are used to make chocolate. These seeds may be found in the cocoa tree's white, meaty pods (Motamayor *et al*., 2002) [32]. The pods are first fermented with naturally occurring microorganisms, mostly bacteria and yeasts, namely Acetobacter and Lactobacilli, in order to extract the seeds from these pods. Because of the increase in temperature during the fermentation process, the microorganisms' by products, namely the ethanol generated by yeast, kill the beans and contribute to the flavor of the chocolate (Zhao and Fleet, 2014) [33]. The fermentation process is what gives chocolate that fragrance, flavor and deep colors. Acetic acid fermentation and alcoholic fermentation are the two steps that make up this process. In the first step of this procedure, yeast activity in the cocoa pulp transforms the sugar into alcohol. The alcohol is then oxidized by microorganisms, yielding acetic acid (Schwan and Wheals, 2004) [34]. The strong correlation that exists between particular illnesses and microbial activity can be used to support the involvement of microorganisms. Various advancements in microbiology have resulted into numerous discoveries and inventions, as well as good outcomes in the discovery of drugs and in medical practices. However, several microorganisms play a substantial function in the immunological and digestive systems in addition to causing a variety of microbial infections and infectious illnesses like HIV. Pharmacists and microbiologists are collaborating to develop medication treatments that must focus on the bacteria that cause opportunistic infections, rather than the human body's host cells.

A protein found in the plasma membrane of *Halobacterium salinarum* (called bacteriorhodopsin), is one of the significant medicinal products produced by using microorganisms like bacteria (Caplice and Fitzgerald, 1999) [35].

2.2 Enzymes in food processing

The extraction of enzymes from living cells started in the 20th century, which led to an extensive commercial manufacturing and more widespread usage in the food industry (Leader *et al*., 2008, Mathhijis *et al*., 2019, WHO, 2015) [36, 37, 38].

Currently, the most important source of commercial enzymes is microorganisms. Despite not having the same enzymes as plants or animals, it is typically possible to find a microbe that can manufacture a similar enzyme that will catalyze the necessary process. Through natural selection and conventional breeding methods, enzyme producers have improved microbes for producing enzymes (Lartigue, *et al*., 2019, Bonten *et al*., 2015, Gaynes, 2015) [39, 40, 41]. In recent years, application of biotechnology in the food industry has expanded to encompass gene cloning of animals and plants as well as further advancements in genetically modified food products (Sulakyelidze *et al*., 2020, Reese &Maguire 1996, Mahdinia *et al*., 1996, Delves *et al*., 1996, Agarwal and Sahu, 2014) [42-47].

Due to the fact that they may function as catalysts and change basic materials into better food products, enzymes have long been crucial to food technology. According to Ward and Moo-Young (1988) [48], the primary benefits of enzymes are their substrate specificity, catalytic efficiency, and a rate boost of 1010 or more compared to chemical processes (Burbaum *et al*., 1989) [49] when performing under mild temperature, ion concentration and pH conditions.

Enzymes have many uses in the processing and manufacture of many sorts of food items because they may change and enhance the nutritional, functional and sensory aspects of components and finished goods. The enzymes chosen by food technologists are ones that can enhance a certain step in the food manufacturing process. These enhancements include replacing milk in calf feed with fish protein hydrolysates, saving money and energy during the production processes and changing the functional characteristics of proteins (Diaz-

Castaneda & Brisson, 1989) [50]. (Christensen, 1989, Adler-Nissen *et al*., 1983) [51, 52]. Today, a growing number of enzymes used in food technology are made from specifically chosen or genetically altered microbes that are cultivated in fermenters on an industrial scale. In the food business, 64 enzymes are utilized for technical purposes, 57 enzymes are used in feeds and 24 enzymes are employed in three different industrial fields. The majority of commercial enzymes-nearly 75% are hydrolytic.

Carbohydrases, proteases and lipases account for more than seventy percent (70%) of all enzyme purchases and dominate the enzyme market.

2.3 Enzymes in dairy industry

India is recognized for having the largest milk output in the world, and as a result, the food and dairy businesses in our nation have been forced to look for methods of turning the liquid milk into goods that are of the best quality. These products are created by enzymatic and biochemical mechanisms. One of the first exogenous usage of enzymes in the food manufacturing industry was the use of rennet in the production of cheese. Recently, proteinases have been shown to have other uses in dairy technology, such as expediting cheese ripening, changing functional qualities, and creating dietetic products (IDF, 1990). In order to produce cheeses of the highest quality, animal rennet (bovine chymosin) is traditionally utilized in the dairy business (having a characteristic good flavour and texture). Rennin can affect milk both enzymatically and non-enzymatically through a two-step reaction. This ultimately causes milk to coagulate (Bhoopathy, 1994) [53].

Lactose, a sugar mostly present in whey and milk, and its related hydrolase, lactase or β-galactosidase, have been the subject of intense research during the past 10 years (Mehaia, 1987) [54]. Plants, animal organs, microbes, yeast (an intracellular enzyme) and molds can all be sources of lactose. Since aminopeptidases have demonstrated the ability to release individual amino acid residues from oligopeptides, which are created by extracellular proteinase activity, they are important in the production of flavor in fermented milk products (Law and Haandrikman, 1997, Whitehust, 2002, Bloom, *et al*., 2005, Fernandes, 2010b, Riberiro *et al*., 2010) [55-58].

Proteases and lipases have also demonstrated important functions in the dairy food sector. Other small enzymes with sporadic uses in the processing of dairy products include lactoperoxidase, glucose oxidase, catalase, superoxide dismutase, sulfhydryl oxidase and lysozymes. In some foods, catalase and glucose oxidase are typically combined as a preservative (Riberiro *et al*., 2010) [59].

2.3 Enzymes in brewing

Alcoholic drinks like beer and wine are created by the yeast fermentation of sugar molecules. After water and tea, beer is the third most popular drink in the world and the most consumed alcoholic beverage overall (Nelson, 2005) [60]. Beer is usually made from barley, but wine is made from grapes. The ripe grapes already have the sugar required for the necessary fermentation, whereas barley contains starch. Before the yeast can produce alcohol from this starch, the starch must be converted into fermentable sugar molecules. Enzymes surely play essential roles in the brewing process, particularly the starch from the leaven that encourages some noticeable changes throughout the saccharification phase. Some enzymes, such as amylases, are already present in barley, whereas-amylases and proteases are made during germination. All the enzymes required for turning "grains" into a fermentable liquid (wort) are present in the final malt. The enzymes employed in the brewing industries are necessary for the saccharification of starch (fungal and β-amylases), hydrolysis of protein (neutral protease), breakdown of barley β-1,3-and β-1,4-linked glucan (β-glucanase), all of which accelerate the rate of (later) fermentation, notably in the manufacture of high-gravity beer where more protein is added (Aastrup *et al*., 2004) [61]. Cellulases are also infrequently employed, especially when wheat is added as a supplement and to aid in the breakdown of the β-glucans in the barley. The B. amyloliquefaciens β-amylase is extremely heat stable, hence in applications where it is employed, the wort must be heated for 30 minutes to turn its activity off before fermentation. Papain is utilized in the final post-fermentation phases of brewing beer to avoid the formation of "chill-haze," which would then, develop after the beer is cooled. This haze contains proteins and tannins.

2.4 Enzymes in potable wine and alcohol production

Grape juice is fermented to produce wine. Enzymes enormous usability in the production of wine can never be overemphasized. Many of these enzymes, which are necessary in the production of wine, come from the grape itself, the natural microflora on the grape, and the microorganisms available throughout the wine-making process.

The enzymes used often in commercial wine production and their contributions include the following:

Pectinases, xylanases, glucanases and proteases: they aid in the improvement of wine processing and clarifying;

Glycosidase: Aids in the precursor compound-mediated release of varietal fragrances.

Urease: Decreasing the production of ethyl carbamate is one of the functions of urease.

Glucose oxidase: Aids in lowering alcohol levels (Mojsov, 2013) [62].

The pectinase family gives rise to the majority of industrial processes used today in winemaking preparations. They are polygalacturonase, pectin methyl-esterase, and pectin lyase (PL) (PG). Industrial enzymes used for food offer significant manufacturing process improvements, which boost the total economic value of the finished goods. Quantitative advantages of industrial enzymes include higher free run and press juice yields. According to Ducasse *et al*. (2010) [64], Main *et al*. (2007) [65], and Romero-Cascales *et al*. (2008) [67], Main and Morris (2007) [65], Bucelli (2006) [63], Parley *et al*. (2001) [66] and Watson *et al*. (1999b) [68], Canal-Llaubères. (1989) [69], among the qualitative advantages include improvements in the general aging process of wines, i.e. taste enhancement. According to several studies (Revila and Gonzàlez-SanJosé, 2002, Capaunova and Drdak, 2002; Rogerson *et al*., 2000; Plank and Zent, 1993; Villettaz and Dubourdien, 1991 and Canal-Llaubères, 1989) [71-74], the benefits of processing led to a reduction in the overall time required for the maceration, settling, and filtration.

The grape pulp and skin cells may be broken down by pectic enzymes, which are also essential for breaking down the chains and links between the chains in saccharides (Whitaker, 1984) [75]. Pectinase was the most popular enzyme used commercially utilized in the wine-making industry (Rombouts and Pilnik, 1980) [76]. Today, it has been

determined that pectic enzymes alone produce around 25% of the world's food enzymes. The majority of pectic enzymes used in commercial preparations come from fungi (Alkorta *et al*., 1994) [77]. The most significant phenolic groups in the manufacture of red wine are tannins and anthocyanins. Tannins greatly enhance the mouth feel of wines, but they also work with anthocyanins to create coloured polymers. This relationship is necessary to supply the stable pigments needed to maintain the color of red wine over the long term. Red pigments known as grape anthocyanins are found in the initial exterior layers of the hypodermal tissue, primarily in the vacuoles of the cell and in unique structural elements known as anthocyanoplasts (Barcelo *et al*., 1994, Pecket and Small, 1980) [78, 79].

2.5 Utilization of enzymes in bakery technology

The progressive development of bakery has remained a significant milestone in human history. After the nineteenth (19^{th}) century, as agriculture became more industrialized, bread quality improved and price declined, bringing white and rye bread into the price range of almost everyone.

The use of industrial enzymes in the baking process, of which bakery enzymes are an important subset, had a considerable impact on the expansion of the baking market. The enzyme market for baked goods is likely to grow from 420 million dollars in 2010 to 900 million dollars in 2020, but it is predicted to maintain its representation in this segment, which ranged from 34.4 percent in 2010 to 35.7 percent in 2020, according to the global bakery and enzyme demand between 2000 and 2020. (The Freedonia Group, Inc, 2015) [80].

Endogenous enzymes found in flour, added to the dough and chiefly involved in the metabolism of the most prevalent microorganisms remains the three sources of enzymes used in the bakery industry (Van Rensburg, 2015) [81].

It is common practice to add enzyme improvers (also known as technical enzymes) to flour and dough for the standardization of flour and as a baking aid. Typically, addition of enzymes to dough is needed to alter its gas retention, rheology and crumb softness when making pastries, bread and biscuits, as well as to alter product softness when making cakes and reduce the generation of acrylamide in bakery goods (Cauvain & Young, 2006) [82]. The enzymes can be used alone or in intricate mixes, and they can cooperate to create baked foods. They frequently exist at extremely low levels (Di Cagno *et al*., 2003) [83].

Within the mixing stage in bread-making process, enzymes are routinely added to flour as a technical booster. The most often used β-amylases in the manufacture of bread come from a variety of sources (SanzPenella *et al*., 2008) [84]. Amylases may degrade starch and produce minute amounts of dextrins that yeast can use. Enzymes like xylanases, hemicellulases, oxidases and lipases can strengthen the gluten network directly or indirectly, which will likewise, enhance the quality of the final bread. With the correct dose, dough can become more malleable and workable by increasing its machinability. The presence of functional lipases modifies the natural lipids present in wheat, enhancing their capability to stabilize the dough. According to some reports, adding lipases will delay the rate at which baked goods stale. Cauvain and Young (2006) [85]. Lipoxygenases are also employed to improve the dough handling and mixing tolerance properties. The action of the lipoxygenase enzyme can give bread a disagreeable taste. Linkso *et al*. (2007) [86]. Glucose oxidase is also used as an alternative oxidizing agent for baking bread in place of potassium bromate. The addition of increasing glucose oxidase concentrations had a considerable impact on the quality of the bread and dough, and the extent of the effect was considerably impacted by both the enzyme concentration and the quality of the original wheat flour. Additionally, glucose oxidase restored the capacity of damaged gluten to generate bread. Asparaginase is said to have a strong ability to reduce the formation of acrylamide during baking (Meghavarnam *et al*., 2011) [87].

2.6 Enzymes in fruit and vegetable

2.6 Enzymes in fruit and vegetables processing and juice extraction

When it comes to the production of fruits and vegetables, India is the second-largest producer of fruits and vegetables, coming after China. 162 million tons of vegetables and 81.2 million tons of fruits were produced overall in 2012-2013. Second preliminary projections predict that in 2013-2014, such quantities will be 84.4 million tons and 170.2 million tons, respectively. The market for fruit and vegetable juices is expected to expand by 3.7 percent yearly in the following years. Between 2007 and 2013, the market grew on average by 3.5 percent annually. The largest markets for fruit and vegetable juice are in France, France, China, Germany, the United Kingdom, and the United States, while the highest annual growth is anticipated in Morocco (30.5%), India (18.0%), Rwanda (17.0%), Egypt (13.8%) and Moldova (12.0 percent) (Smith and Knutsen, 2005) [88].

Enzymes serve as common aids for fruit processing industries, particularly for making clear fruit juice and concentrate. Enzymes can, among other things, boost the yield of solid recovery during pulp washing, improve the recovery of essential oils from peel, clarify lemon juice, facilitate the creation of highly concentrated citrus bases, and raise the value of waste products (Hamza, *et al*., 2015) [89]. One of the most crucial new enzymes for the commercial sector, particularly for the fruit juice business, is pectinases. Juices with higher yields, more stability and better clarity are certain to be produced as a result (Girard and Fukumoto (1999) [90]. Amylases, glucoamylases, cellulases, hemicellulose, laccase, naringinase and limonene are other examples of enzymes utilized in the juice industry. During the processing season, when apples have starch, amylases and pectinases are added. Due to this, in order to process vegetable juice and reduce viscosity to the appropriate level for juice extraction, additional cellulases in addition to pectinases are required (utilizing the decanter). Using Peclyve LI (Lyven) or Rapidase Vegetable Juice (DSM), which both include pectinases and cellulases, is advised for the extraction of vegetable juice.

2.7 Enzymes in meat processing

The most important qualitative quality that sets meat apart in the eyes of consumers is thought to be softness. (Zor, *et al*., 2009) [91]. Meat gets its suppleness via a combination of loosening and breakdown of collagen-rich connective tissue inside muscle fibers, which is primarily brought on by enzyme activity. Numerous pre- and post-slaughter elements as well as how they interact affect the meat's softness (Destefanis *et al*., 2008) [92]. The meat business and the catering industry are the two main industries that use enzymes to break down proteins. Transglutaminases (TGase), an enzyme that crosslinks proteins, have been employed for

a very long time now to improve texture. Emerging enzyme technologies in the food business include structural engineering with oxidative enzymes and taste design using lipases, glutaminases, proteases and peptidases. According to Whitehurst and Van Oort (2010) [93], one of the promising areas in the processing of meat is the use of enzymes like bromelain and papain, which are derived from plant sources like papaya and pine apple plant, respectively. Actomycin, collagen and elastin are frequently responsible for the hardness of meat. Meat portions that are considered to be of inferior quality because of their roughness are just as nutrient-dense as good quality meat, which may be tenderized using enzymes. Even the flavor of the meat is influenced by the peptides and amino acids contained in it. For instance, proteases are employed as marinating and tenderizing enzymes. From a variety of numerous by-products of meat, as bones, sheep visceral mass poultry by-products or bovine by-products, proteases can be utilized to make protein hydrolyzates (Bhaskar *et al*., 2007) [94].

2.8 Enzymes in starch processing

Starch is a typical renewable resource. It serves as a storage component in the leaves, tubers, seeds, and roots of several plants. Typically, the starch is altered chemically or enzymatically to create a variety of derivatives. The industrial breakdown of starch is often initiated by β-amylases (β-1, 4-glucanohydrolases), a rather common enzyme in microorganisms. Pullulanases and β-amylases are both starch-degrading enzymes that belong to the same family. Glycosyl hydrolases 13 (Hanrissat and Bairoch, 1996) [95]. Pullulanases specifically target β-1, 6-linkages to release linear oligosaccharides with glucose residues bound by β-1, 4-bonds. While type II pullulanases hydrolyze both 1, 4 and 1, 6 connections and principally generate maltose and maltotriose, type I pullulanases exclusively hydrolyze 1, 6 linkages and produce branched dextrins (Doman-Pytka and Bardowski, 2004) [96]. Although they are inert against pullulan, these enzymes preferentially hydrolyze amylopectin or glycogen's β-1, 6 glycosidic linkages (Yokobayashi *et al*., 1970 and Amemura *et al*., 1988) [97, 98]. Saccharification, liquefaction and gelatinization are the three primary phases of the enzymatic conversion of starch. The two types of exo-acting hydrolases that are typically used for starch saccharification are glucoamylases and-amylases. Because-1, 6-linkages cannot be broken down by B-amylases, maltose and-limit dextrin are the final products. As a result, the elimination of amylopectin is incomplete. Glucoamylases primarily cleave-1, 4-linkages, while they can also do so with-1, 6-glycosidic linkages, albeit at a considerably slower pace. As a result, glucoamylases are able to entirely convert starch into glucose (Synowiecki, 2007) [99]. It has a higher sweetness and is often used in a range of food and beverage goods. Syrup is made by isomerizing glucose derived from starch. Most of the time, xylose isomerase, which is produced from immobilized glucose, acts as a catalyst in a continuous process to create fructose syrup. Sugars are fractionated by cation exchange to produce syrup with the necessary fructose content (55%) (Synowiecki, 2007, Crabb and Mitchinson, 1997) [99, 100]. Maltose can be converted into isomaltooligosaccharides by the use of certain glucosidases (IMO). From the non-reducing end, these exo-acting enzymes hydrolyze oligosaccharides including maltose, amylose and amylopectin to yield glucose (Whitehurst and Oort, 2010) [101].

3. Impact of microorganism in pharmaceutical industries

The following is a description of the useful goods that are produced by using microorganisms in pharmaceutical industries:

3.4 Impact of enzymes in pharmaceutical industries

Production of pharmaceutical enzymes

According to Saxena *et al*. (2004) [102], Saxena *et al*. (2006) [103] and Patel *et al*. (2017) [104], fermentation of suitable microbial strains produces the majority of enzymes by industries, including those used in pharmacological processes. This is because bacteria and fungi are easy to handle, grow quickly, and scale up easily in large vessels (fermenters).

The most widely used microorganisms for enzyme production by the biotechnology industries worldwide are bacteria like Escherichia coli, lactic acid bacteria, filamentous fungi like Aspergillus oryzae, Bacillus subtilis, Aspergillus niger, Pichiapastoris, Trichoderma atroviride and yeast like Sac-charomyces cerevisiae, and others (Yang 2017 and Patel *et al*., 2017) [105, 104]. Due to advancements in genetic engineering, the strains produce huge amounts of enzymes. The selection of suitable microbial strains is a crucial element in the development of successful commercial applications for a variety of industrial enzymes. The ideal scenario is for the generating microbial strain to release the enzymes in the fermentation medium since this simplifies and makes downstream processing more economically feasible; however, this may not always be the case with most commercial strains. Using fermentation technology, pharmaceutical enzymes are produced mostly using bacteria and fungi, which are classified as generally recognized as safe compounds (Yang 2017) [105]. In this business, submerged fermentation (SmF) and solid-state fermentation (SSF) are the two principal methods (Meghwanshi and ashishtha, 2018, Thomas & Pandey, 2013) [106, 107]. Both approaches have benefits and shortcomings of their own. Despite a rising interest in SSF for some specific areas, most businesses have embraced the SmF process for making enzymes (Patel *et al*., 2017 and Thomas, 2013) [107, 108]. A set volume of medium is utilized during the whole fermentation process, and no more medium additions are performed after that (except the addition of alkali, antifoam, acid). During continuous culture, the fermentation process is initiated in batch mode. After a certain period of time, fresh medium or nutrient concentrate is provided at a rate that nearly matches the growth rate of the used microorganisms. The medium accommodating the product and biomass is continuously emptied from the fermenter's overflow line (Kargi, 2001) [109]. Fresh media are continually injected into the process while used media and product are removed in a perfusion culture, similar to a continuous culture technique. The approach is different from the previous one in that it preserves a lot of live cells either by adhering the cells to a substrate in the fermenter or utilizing alternating tangential-flow and regular tangential-flow filtering (such as capillary fibres, membranes, micro carriers in fixed bed, etc.). 2016 (Challenger). When utilizing a fed batch culture, the concentrated nutrient components are delivered to the batch culture in minute amounts on a regular schedule or as necessary for the procedure (Balbas, 2001) [110].

3.5 Applications of enzymes in pharmaceutical industries
The Synthesis of antimicrobials using enzymes

Synthesis of 6-amino penicillanic acid by penicillin acylases

When penicillin acylases split the acyl chain of penicillins, six-amino penicillanic acid (6-APA) and the corresponding organic acid are created (Shewale and Sivaraman, 1989) [111]. A screening of these taxa revealed that bacteria, actinomycetes, yeasts, and fungi produce penicillin acylase (Sudhakaran and Borkar, 1985a; Sudhakaran and Borkar, 1985b and Illanes and Valencia, 2017). Based on their ability to recognize certain substrates, three kinds of penicillin acylases have been identified: Penicillin G acylases and penicillin.

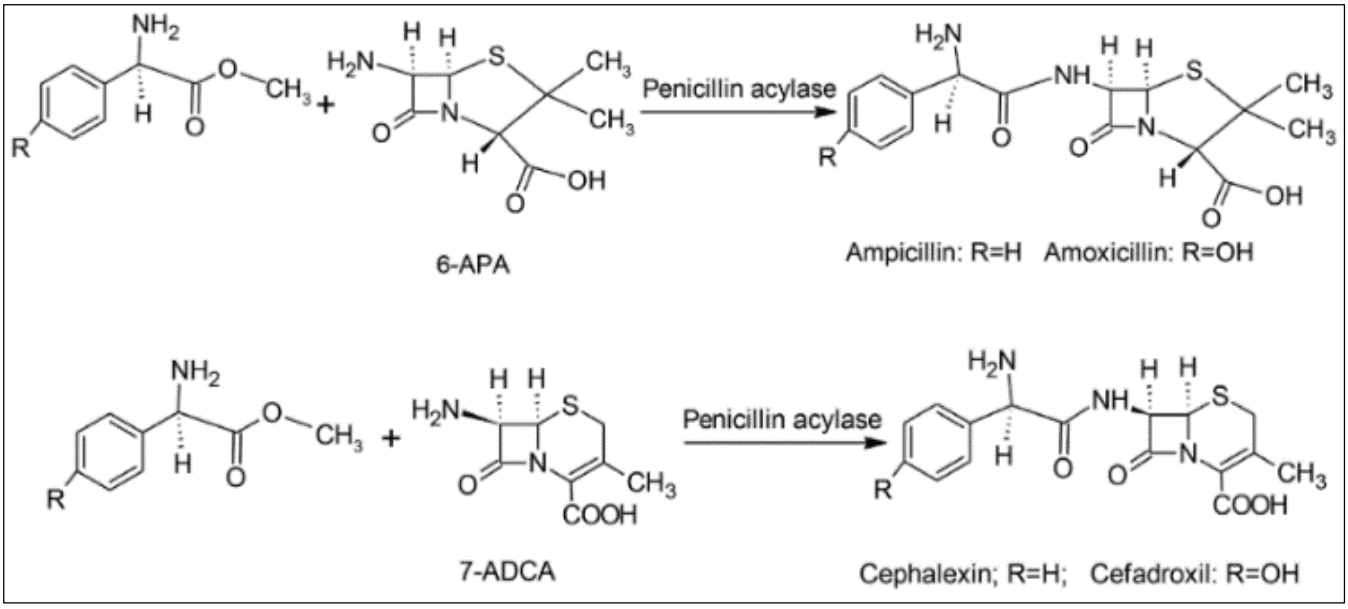

Fig 1: Synthesis of 6-amino penicillanic acid by penicillin acylases

Some significant semi-synthetic-lactam antibiotics were produced with the assistance of penicillin acylase (Arroyo *et al*., 2003) [112].

Examples include V acylases and ampicillin. The penicillin acylase-catalyzed procedures for 6-APA production have taken the place of the traditional chemical processes. Beginning in the 1970s, the conventional method of 6-APA synthesis depended on a fermentation-based penicillin G diacylation procedure that called for the use of potentially hazardous chemicals and solvents. The subsequent 15 to 20 years saw this manufacturing process in use before it was generally replaced by a procedure based on penicillin G acylase because it generated 6-APA in enormous numbers. According to certain investigations, a new process based on penicillin V acylase and penicillin V might be used to produce 6-APA (Shewale and Sudhakaran, 1997 and Avinash *et al*., 2015) [113, 114]. The advantage of this approach resided in the fact that the penicillin V showed better stability at a lower pH when it was removed from the fermented broth, which boosted the generation of 6-APA. Furthermore, compared to penicillin G acylases, penicillin V acylase-based methods permitted the production of 6-APA even at higher concentrations of the substrate. Furthermore, their wider optimal pH range reduced the requirement for pH regulation during hydrolysis (Demain, 2000, Illanes & Valencia, 2017) [115, 116]. Using immobilized versions of the enzymes, 6-APA may be produced inexpensively. It saves a lot of money since the enzyme may be reused, is simple to extract from the product, and has enhanced stability. Penicillin acylases from E. coli, Bacillus megaterium and Alcaligenes faecalis have been immobilized for efficient 1. usage (De Vroom, 1997; Bianchi *et al*., 1998; Wedekind *et al*., 1998; Vroom, 2000; Parmar *et al*., 2000, Roberts, *et al*., 2017, and Zhang *et al*., 2017) [115-121].

3.6 Synthesis of semi-synthetic penicillins by penicillin acylases

Semi-synthetic penicillins are more stable, easier to absorb, have less side effects and are more resistant to adaptive microbial antibiotic resistance than penicillin G and V. (Grulich *et al*., 2017) [122]. The large-scale production of semi-synthetic antibiotics generated from penicillin is based on the condensation of the β-lactam nucleus with the appropriate D-amino acid, catalyzed by penicillin acylases (Martens and Demain 2017) [123]. The kinetic enantioselective acylation of the racemic azetidinone intermediate for the creation of the carbacephalosporin antibiotic Loracarbef, a Cefaclor and Xemilofiban analogue, is another use for penicillin G acylase (Zmijewski 1991 and Cainelli *et al*., 1997) [124, 125]. Additionally, it has been demonstrated that penicillin G acylase may enantioselectively acylate the l-enantiomers of the methyl esters of phenylglycine and 4-hydroxyphenylglycine in organic solvents (Basso *et al*., 2000) [126] A quick approach for isolating the d-enantiomer was created by the process, which is helpful in the creation of -lactam antibiotics.

3.7 Synthesis of β-lactam antibiotic key intermediate (7-Aminocephalosporanic Acid)

The heterocyclic primary ingredient of cephalosporins, one of the most significant and effective therapies for bacterial infections and illnesses, has been used to create a wide range of drugs today (Von Nussbaum 2006a and Nussbaum 2006b) [127, 128]. Several semi-synthetic-lactam antibiotics based on cephalosporins have been made commercially accessible, including Cefotaxime, Ceftriaxone, Cefuroxime and Cefdinir. Treatment of severe MRSA infections is now possible because to recent advancements in ceftobiprole and Ceftaroline fosamil (methicillin resistant Staphylococcus

aureus). Cephalosporin C remains the most practicable material used for initiation in the synthesis of 7-aminocephalosporanic acid (7-ACA), since it can be produced by microbial fermentation in the required volumes and at a competitive price. By cleaving an amide link, cephalosporin C also serves as a direct precursor of 7-ACA (Groger *et al*., 2017) [129]. During the combined enzymatic and spontaneous reaction cascade that results in the enzymatic synthesis of 7-ACA, the d-amino acid oxidase catalyzes the oxidation of the amino acid side chain into the appropriate α-keto acid. Following that, spontaneous decarboxylation occurs on the α-keto acid. Finally, the intermediate is hydrolyzed by glutaryl-7-ACA hydrolase to yield the phenyl glycine 4-hydroxy esters and 7-ACA methyl esters (Resch *et al*., 2010) [132].

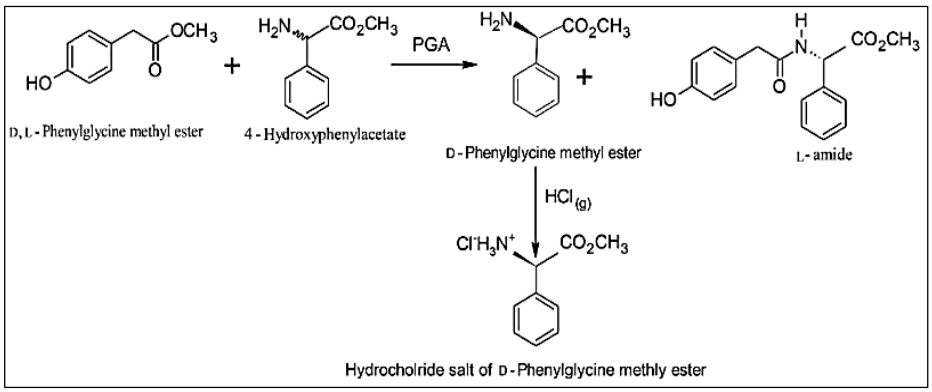

Fig 2: Synthesis of B-Lactam Antibiotic Key Intermediate, 7-Aminocephalosporanic Acid

3.8 Enzymes involved in the synthesis of amino acids

Amino acids are crucial for the health and nutrition of both people and animals since they are the building blocks of life. As a result of their chirality, they are also very important biochemically and helpful in various chemical synthesis. Among the many amino acids, the nine essential ones, which neither humans nor animals are able to synthesize, are: L-isoleucine, L-leucine, L-valine, L-lysine, L-methionine, L-histidine, L-threonine, L-phenylalanine and L-tryptophan. Enzymes and whole cell biocatalysts have been used to produce proteinogenic and non-proteinogenic D-and L-amino acids, as well as enantiomerically pure amino acid derivatives, which are essential building blocks for the active ingredients used in the production of pharmaceuticals, cosmetics, and agrochemicals (Ya-Ping *et al*., 2018) [130]. The cost-effective synthesis and separation of amino acid products, which began in the 1980s, are mostly to blame for the amino acid market's explosive growth (Fan *et al*., 2017) [131]. Due to their affordability and environmental friendliness, the two most common techniques of producing amino acids are fermentation and enzymatic catalysis (Cheong *et al*., 2016) [132].

3.9 Enzymatic production of proteinogenic amino acids

In Japan, enzymes have been used for the last 40 years to produce L-amino acids (Fan *et al*., 2017, Leuchtenberger, 2005) [133, 134]. Enzymatic resolution using Aspergillus oryzae acylase has been utilized to produce L-methionine, which is employed in special diets (Woeltinger *et al*., 2005) [135]. In order to reduce enzyme loss, several hundred tons of L-methionine and L-valine are currently generated annually utilizing enzyme membrane reactor technology.

L-aspartic acid is another amino acid that is best produced by enzyme catalysis. Aspartate is an enzyme that facilitates the addition of an amino group from ammonia to fumaric acid.

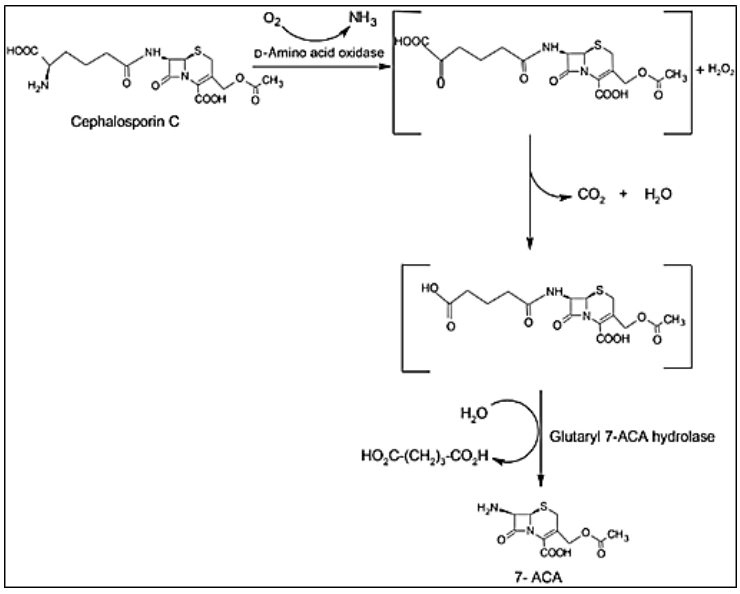

Fig 3: Enzymatic transformation of cephalosporin C into 7-ACA catalyzed by D-amino acid oxidase and glutaryl-7-ACA acylase. (Groger *et al*., 2017) [136]

L-aspartate, which is utilized on an industrial scale to make the artificial sweetener L-aspartame, is directly produced by acid. With the help of the enzyme aspartate-decarboxylase, L-aspartate is converted to L-alanine (Calton, 1992) [137]. Electrochemical reduction is the major method used to create L-cysteine from L-cystine. With the help of three enzymes-ATC racemase, S-carbamoyl-L-cysteine hydrolase and L-ATC hydrolase-enzymatic hydrolysis and racemization of the substrate DL-2-amino-2-thiazoline-4-carboxylic acid (ATC) has made its commercial manufacturing possible (Pae, 1992) [138]. Recently, fermentation of an *E. coli* strain with a defective L-cysteine regulon, which is the yci W gene, resulted in significant L-cysteine synthesis (Kawano *et al*., 2015) [139].

3.10 Enzymatic production of non-proteinogenic amino acids

Enzymatic synthesis of D-amino acids and non-proteinogenic L-amino acids is a growingly well-liked and ecologically friendly approach. D-amino acids can be produced as a by-product of the enzymatic resolution of racemic mixtures of D L-amino acids used to manufacture L-amino acids (Leuchtenberger *et al*., 2005) [140].

However, it is also feasible to directly create D-amino acids from racemic acetyl amino acids, for instance, by utilizing a d-specific acylase (Woeltinger *et al*., 2005) [141]. The production of the D-phenylglycine and p-hydroxy-D-phenylglycine building blocks for the semi-synthetic antibiotics ampicillin and amoxycillin, which are synthesized utilizing the enzyme system hydantoinase/carbamoylase, is yet another technique of economic significance. Recent developments in current molecular biological techniques (directed evolution) have made it feasible to convert hydantoinase from having a D-specificity to an L-specificity (May *et al*., 2002) [142].

Furthermore, racemases may now coexpress with D- or L-selective hydantoinase and carbamoylases in highly efficient recombinant whole-cell systems that can synthesize a variety of D-and L-amino acids (May *et al*., 2000) [143]. A more effective enzymatic method for producing D-amino acids has recently been reported using N-succinyl amino acid racemase and D-succinylase, two enzymes that carried out dynamic kinetic resolution of N-succinyl amino acids and enantioselectively hydrolyzed the N-succinyl-D-amino acids to their corresponding d-amino acids (Sumida *et al*., 2017) [144].

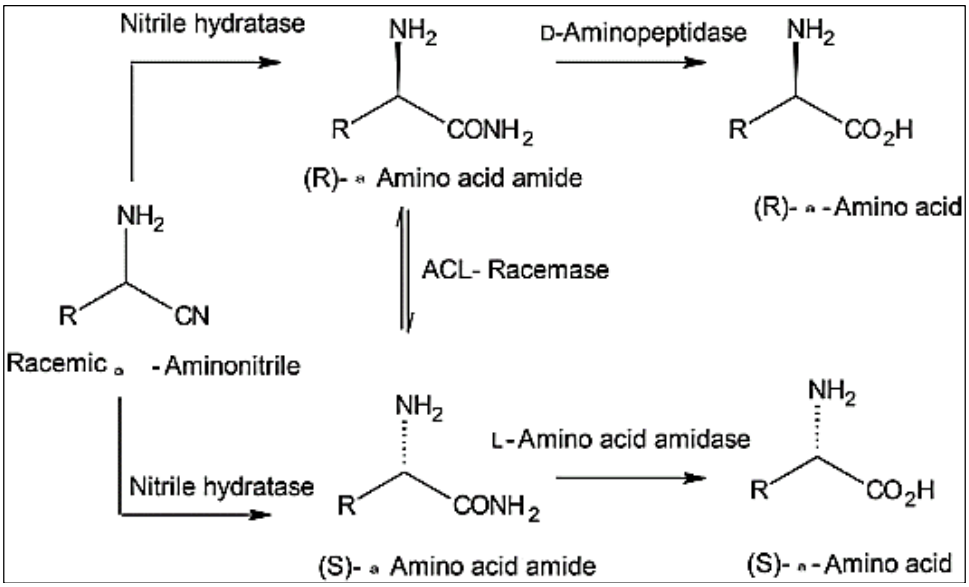

Fig 4: Dynamic kinetic resolution of α-aminonitriles to produce chiral α-amino acids. (Yasukawa *et al*., 2011) [145]

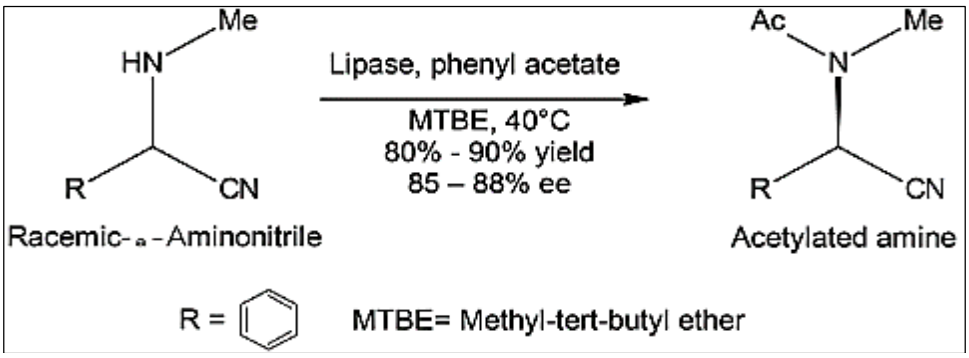

Fig 5: Kinetic resolution of α-aminonitriles catalyzed by lipase. (Vongvila *et al*., 2011) [146].

3.11 Enzymatic synthesis of pregabalin intermediate, (s)-3-cyano-5-methylhexanoic acid

Pregabalin is a potent anticonvulsant that is used to treat fibromyalgia, neuropathic pain, and seizure disorders. Only the (S)-enantiomer of pregabalin has pharmacological action (Silverman, 2008) [147]. Consequently, the pharmaceutical sector has placed a lot of emphasis on its asymmetric synthesis the important intermediary (S)-3-cyano-5-methylhexanoic acid of (S)-pregabalin has been synthesized using many chemocatalytic and biocatalytic processes. With

reasonable yields and good enantiomeric excess, i.e., 98 percent and 97 percent respectively, the asymmetric synthesis of (S)-3-cyano-5-methylhexanoate has been characterized utilizing the reagents bisphosphine rhodium and nitrilase. However, in terms of the environment and cost, neither strategy was successful. Racemic kinetic resolution of the ester was catalyzed by an esterase from *Arthrobacter* sp. ZJB-09277 (whole cell catalysis). To produce (S)-3-cyano-5-methylhexanoic acid in 44.6 mm with 95.1 percent yields of (S)-3-cyano-5-methylhexanoic acid, 3-cyano-5-methylhexanoic acid esters were used (Zheng *et al*., 2014) [148]. The sitagliptin intermediate (R)-3-amino-4-(2, 4, 5-triflurophenyl) butanoic acid is created by an enzyme. Significant anti-diabetic drugs including sitagliptin, retagliptin and evogliptin are produced using the amino acid (R)-3-amino-4-(2, 4, 5-triflurophenyl) butanoic acid (3-ATfBA) (Ramisetti *et al*., 2016) [149]. Two-stage enzymatic synthesis of 3-ATfBA has been described, with the first step requiring Candida rugosa lipase's conversion of the β-ketoester substrate to β-keto acid. The ω-Transaminase (ω-TA) of Ilumatobacter coccineus then aminated this β-keto acid to its equivalent β-amino acid. The substrate, a 100 Mm β-keto ester, was converted to 3-ATfBA at a conversion rate of around 92.3 percent. During the reaction's scale-up, excellent conversion (81.9%) and enantioselectivity were observed (99 percent) (Yoon *et al*., 2019) [150].

Boc-(R)-3-amino-4-(2, 4, 5-trifluorophenyl) butyric acid, a crucial sitagliptin intermediate, has been discovered to be produced by through enzymatic reactions (Hau *et al*., 2016) [151]. In 24 hours, the procedure led to an 82 percent conversion at a scale of 100 mm. The amino ester product was further processed to yield boc-(R)-3-amino-4-(2, 4, 5 trifluorophenyl) butyric acid, a crucial step in the production of sitagliptin.

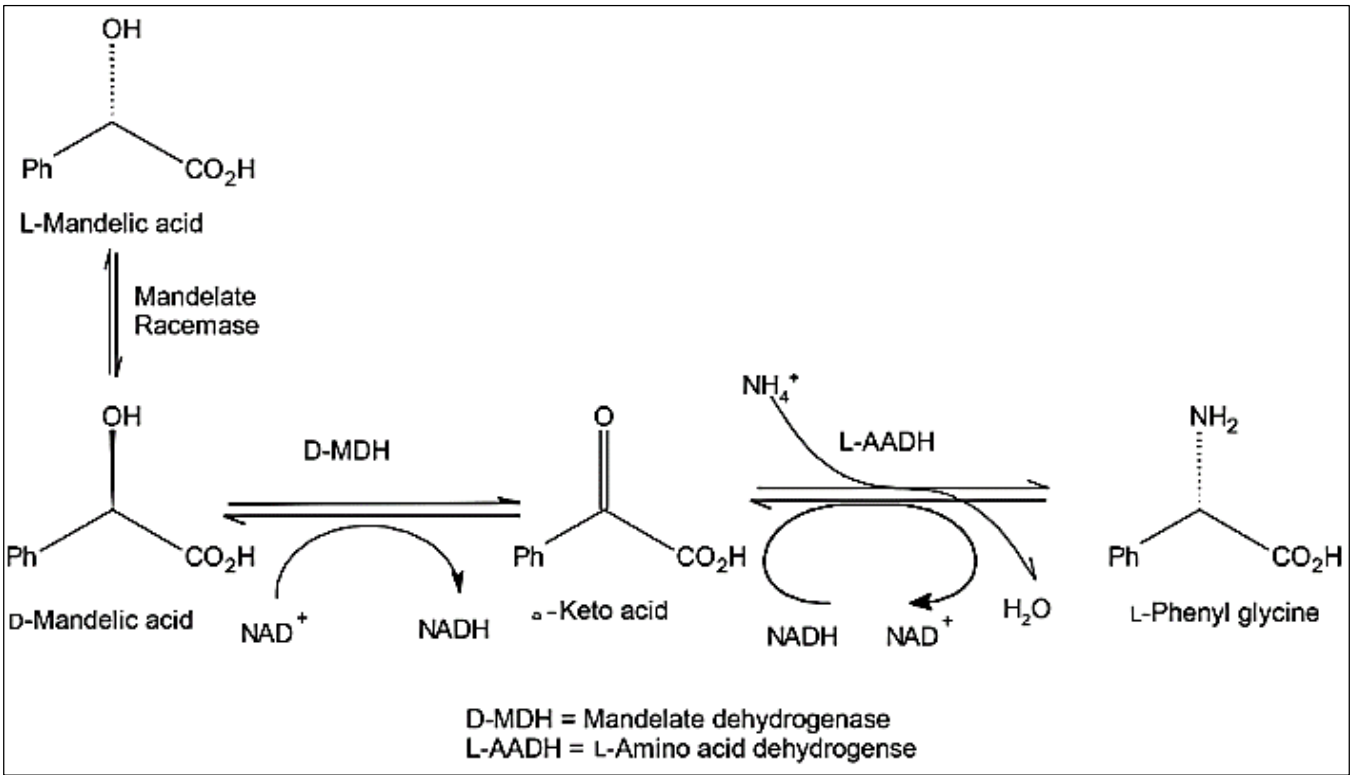

Fig 6: Dynamic kinetic resolution of mandelic acid (Resch *et al*., 2010) [152].

3.12 Enzymatic synthesis of l-tert-leucine

L-tert-leucine serves as the fundamental chiral building block for many pharmaceutically useful compounds. These compounds include atazanavir, boceprevir and telaprevir, which are potent protease inhibitors for the Human Immunodeficiency Virus (HIV) protease, hepatitis C virus genotype 1 protease and hepatitis C NS3-4A serine protease, respectively (Li *et al*., 2014 and Patel, 2018) [153, 154]. Exiguobacterium sibiricum leucine dehydrogenase (LeuDH) and Bacillus megaterium glucose dehydrogenase (GDH) are effectively coexpressed in E. coli BL21 for the production of L-tert-leucine. Here, LeuDH was used to convert the substrate trimethylpyruvic acid into L-tert-leucine, while GDH was used to convert the substrate NAD+ into the coenzyme NADH, which was required to complete the synthesis step. Utilizing co-expressed whole cells, they were able to create a decagram of L-tert-leucine at a scale of 1 L from 0.6 M (78.1 g L^{-1}) of substrate, reaching 99 percent conversion after 5.5 H, 80.1 percent yield, and >99 percent efficiency. In another investigation, the synthesis of L-tert-leucine from trimethylpyruvic acid and NADH regeneration were catalyzed by recombinant Thermoactinomyces intermediates LeuDH and Pichia pastoris format dehydrogenase (FDH). With this system, a substrate input of 100 g/L resulted in a reaction yield of 95% and an efficiency of 99.5%. (Vollmer & Rosenfield, 1983) [155].

3.13 Enzymatic synthesis of statin intermediates

Statins like rosuvastatin and atorvastatin are used primarily to lower cholesterol and prevent cardiovascular diseases (Patel, 2018) [154]]. These drugs particularly block the enzyme known as hydroxymethylglutaryl coenzyme A reductase, which transforms HMG-CoA into mevalonate as a rate-limiting step in the synthesis of cholesterol (Wu *et al*., 2015; Xingyuan *et al*., 2013) [156, 157]. Two intermediates that are converted to atorvastatin and rosuvastatin are discussed here. The first and second are, respectively, t-butyl 6-chloro-(3R, 5S)-hydroxyhexanoate and (3S, 5R)-dihydroxy-6-(benzyloxy)

hexanoic acid, ethyl ester. The alcohol dehydrogenase (KleADH) from Klebsiella Oxytoca was combined with recombinant E. coli bacteria to create t-butyl 6-chloro-(3R, 5S)-dihydroxyhexanoate. Without using any expensive cofactors, KleADH was able to complete the conversion of t-butyl 6-chloro-(5S)-hydroxy-3-oxanoate to t-butyl 6-chloro-(3R, 5S) dihydroxyhexanoate in 24 hours with over 99 percent diastereomeric excess (de). The 3, 5-dioxo-6-(benzyloxy) hexanoic acid, ethyl, or tert-butyl esters of the respective diketoesters may also be reduced enantioselectively. Recombinant E. coli cells containing the ketoreductase from *Acinetobacter calcoaceticus* catalyzed this process (Yokobayashi *et al*., 1970) [158].

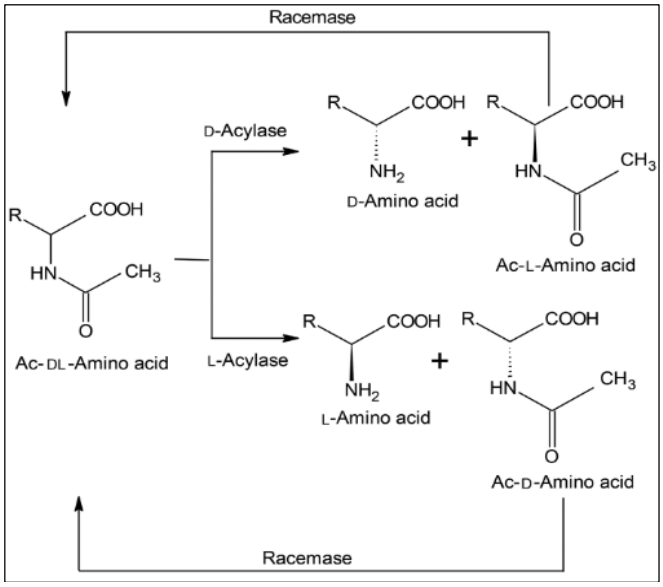

Fig 7: Enzymatic Synthesis of Statin Intermediates

3.14 Enzymatic synthesis of antiplatelet agent

L-Amino acid/D-amino acid production using acylases Xemilofiban Penicillin G amidohydrolase (penicillin G acylase) from *E. coli* was used to resolve a racemic mixture of ethyl 3-amino-5-(trimethylsilyl)-4-pentynoate to produce the S-isomer, which may be employed as a chiral synthon for the production of the antiplatelet drug Xemilofiban (Topgi *et al*., 1999) [159]. Desilylatedphenyl acetamide racemic ethyl 3-amino-5-(trimethylsilyl)-4 pentynoate synthesis is the initial stage, a nonenzymatic process. Phenylacetamide is enzymatically degraded in the second phase to give (R)-amide with a quantitative yield and (S)-amine with a 90% yield.

4.1 Conclusion

One of the largest industries in the world is that of microbes and enzymes. On the worldwide market view, there is constant need for the discovery of enzymes with new and better synthetic activity. The abundant of potential microbes and enzymes that are produced by the enormous species of microorganisms has a positive impact on a variety of industries, including food processing, fine chemicals and pharmaceutical, feed, paper and pulp, polymer and textile, detergent, therapeutic sectors and biofuel.

4.2 Recommendation

Since naturally occurring enzymes lack the microbial and enzymatic features mentioned above, which are necessary for the bio catalytic phenomena, they must be further tailored or redesigned in order to supplement the catalytic qualities. The catalytic characteristics and enzymatic capacities of enzymes obtained from microbes and other harsh environmental sources need to be improved. I advise more thorough application-focused research study in order to efficiently manipulate their full biotechnological potential. In order to examine these enzymes' effects in their natural habitat for maximum output in the agricultural, pharmaceutical and medical sectors, it may be possible to prospect for the variety and distribution of microbial enzymes in their natural sources.

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