

# APPLICATIONS OF BIOTECHNOLOGY IN FOOD INDUSTRY, AGRICULTURE AND PHARMACEUTICAL - A REVIEW

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**Abstract** - Biotechnology has made many advances in agriculture, pharmaceutical, food production and processing. Biotechnological approaches are available, which may be applied in different food and agriculture sectors. These approaches are gene modification (manipulation) and embryo transfer, altering the genetic material of microbes and enzymes for increasing their efficiency, use of molecular markers, progress of recombinant vaccines and DNA based methods for depiction and diagnosis of diseases, in vitro vegetative propagation and other reproductive technologies in plants and animals. In this way the overall quality, yield and shelf life of food is improved as far as food industry is concerned, the resistance of plants to diseases and their nutritive profile is increased and last but not the least several new drugs are created with low toxicity and high effectiveness at low dosages increased by altering the strains of several microbes.

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**Key words** - Biotechnological approaches, gene modification, disease resistance, strains

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## I. INTRODUCTION

In rising countries, applications of biotechnology to food processing are a subject of debate and deliberations for a long time. Biotechnology has long history of use in food production and processing like fermentation (a form of biotechnology) has been used to produce wine, beer and bread. Selective breeding of essential foods such as rice, corn and wheat have resulted in the creation of thousands of varieties with improved yield in comparison to their wild ancestors. The various biotechnological approaches are gene modification (manipulation) and transfer, use of molecular markers, development of recombinant vaccines and DNA based methods for characterization and diagnosis of diseases, in vitro vegetative propagation of plants, embryo transfer and other reproductive technologies in animals, triploidisation in fish. It may also include the technologies to process the raw food materials produced by crop, fishery and livestock sectors.

There are various unit operations and technologies which are used in food processing to convert quite bulky, perishable and typically inedible raw materials into useful shelf-stable and palatable foods or potable beverages (FAO, 2010). Food processing reduces the food wastes and losses in food supply chain, thereby increasing food availability and marketability. Therefore, it could be said that food processing contributes to food security. Processing of food is also done to improve the quality and safety of the food products (Barrett et al., 1997).

In most of the developing countries, through biotechnological techniques, microbial inoculants are used to enhance the properties of food products such as the aroma, taste, shelf-life, texture and nutritional value of foods. The process whereby microorganisms and their enzymes bring about these desirable changes in food materials is known as fermentation and is widely applied in the production of microbial

cultures, enzymes, flavors, fragrances, food additives and a range of other high value-added products. These high value products are increasingly produced in more technologically advanced developing countries for use in their food and non-food processing applications. Many of these high value products are also imported by developing countries for use in their food-processing applications (FAO, 2010).

## II. APPLICATIONS OF BIOTECHNOLOGY IN FOOD PROCESSING

The use of biotechnology in the food industry has a lot in common with the use of biotechnology in the larger domain of agriculture. A discussion on either subject tends to overlap with common features. However, in this article we intend to specifically focus on biotechnology in food industry, as it has evolved over the years. The use of biotechnology in the food industry is primarily based on the use of enzymes that are to be found in different microorganisms. This is of course nothing new. Although we seldom emphasize this fact, several food products of day to day use that have been around for hundreds of years like alcohol, beer, vinegar, cheese, bread and curd are products of biotechnology, since enzymes and microorganisms have had a role to play in their making (Kumar, 2010). Let's evaluate a few of these food items and see how biotechnology helps in making these products even better.

**Bread:** Take the common bread for instance. Traditionally the method has been that, when bread is made, the dough is used for making the bread comes into contact with yeast cells (these cells feed on the nutrients in the dough) and the process generates alcohol and carbon dioxide with the former responsible for the aroma and latter responsible for

the texture of the loaf. It has been the enzymatic effect of yeast cells that kept bread fresh up to a point. Earlier chemical dough strengtheners and emulsifiers were used until the lipases came on the scene. But now there are lipase enzymes for dough strengthening, conditioning and stability as well. Now these lipase enzymes itself have undergone transformation, which means even better enzymes that permit better high speed mixing of the dough and which do not contribute to the release of fatty acids that are primarily responsible for the stale flavor of bread as opposed to the aroma of the freshly baked bread.

For making bread soft, bakers had been using for a long time  $\alpha$ -amylase enzyme. This has given way to Novamyl a proprietary enzyme which keeps the bread even fresher. Bread can be made more soft using bacterial amylases or other specialty amylases as well. The role of proteolytic enzymes in bread making is significant. Bread is made of wheat flour and gluten happens to be the building block of wheat flour. These enzymes were originally used to make the bread softer and allow better machinability of the dough. But now these proteolytic enzymes of proprietary origin have the ability to offer even better machinability (as for example reduce mixing time for instance) in addition to giving improved color and flavor to the bread.

There are other enzymes that prevent bread from turning stale, which means that bread gets a longer shelf-life and keeps it spongy and fresh for longer periods. The reason why bread gets stale is due to the crystallization of the starch (or what is called starch retrogradation) in the bread. Microbial spoilage of bread is also a possibility. Using current enzyme technology it is now possible to extend the shelf life of bread to more than a week, although consumer acceptance is a different matter altogether.

To sum up, advances in biotechnology plays a key role in the making of bread. Other key enzymes like transglutaminases, oxidases and xylase have a role to play in modern day bread making. In most cases, reference to any of these enzymes means a proprietary blend of enzymes in which the dominant enzyme in the mix contributes the name by which the enzyme product is known. This not only emphasizes the complexity of enzymes that have a role in bread making, but also helps us reckon with the transformational change the bread making industry has undergone over the years thanks to biotechnology (Kumar, 2010).

**Beer:** Let's take the case of beer and see how biotechnology has contributed to beer making process. Traditionally beer has been made from cereal grains (which contain abundant starch and sugar) by breaking them down to form alcohol using yeast by the process of fermentation. What you see at the top of the beer "the froth" is the carbon dioxide gas that the yeast cells produce. Earlier the process of malting

(partial germination of barley for instance in making enzymes that ultimately break these hard to ferment complex sugars) was used to break these unfermentable substances. Malting is considered expensive as it was made hundreds of years ago; water, hops, yeast and barley were needed for making beer. But recent biotechnological advances have altered the structure of the yeast; so for example, you can now get brewer's yeast which can ferment even hitherto unfermentable carbohydrates and now enzymes added to unmalted barley can easily convert complex polysaccharides to simple sugars that yeast can easily ferment and help in faster maturation after fermentation and also help in making lighter beer with less of carbohydrates. Thanks to biotechnology, now there are specialized strains of bacteria for imparting flavor and quality to the beer, enzymes to make the beer making process cheaper and to ensure quality in each and every bottle, help in aging, control alcohol and sugar content. So just as in bread making, in beer making too biotechnology has played a transformational role (Kumar, 2010).

**Fruit and vegetable juices:** Biotechnology has also role in fruit juices making, as for example, the use of proprietary enzymes mostly pectinases helps increase the quantity of antioxidants, color in vegetable and fruit juices made by pressing or by other means. Citrus fruits have some bitter compounds that can be eliminated using certain enzymes too (Kumar, 2010).

**Cheese and other products:** In cheese production, advances in biotechnology have enabled the use of microbial rennet instead of rennet from animal origin. Protease enzymes are used to assist in gaining flavor and in cheese ripening. Coffee whiteners and margarine get their dairy flavor from proprietary fungal lipases. Although the use of enzymes in making better food products offers lower costs and manufacturing advantages, the pace of development of food products using biotechnology will greatly depend on the acceptance of the products already made using this cutting edge technology. This is not to say that bread and beer don't have wide acceptance. But there are the food products from agricultural sector that don't have a wide clientele. That apart, there is an erroneous perception that food product made by using enzymes has enzymes in them. That is not so, as most often enzymes used in the manufacturing process get destroyed in the manufacturing stage itself. If the benefit of biotechnology in the food industry has to gain acceptance then consumer education in that direction is vital. After all civil society's acceptance of biotechnology food products will be the harbinger for more such biotechnological advance to take place (Kumar, 2010).

The first food product resulting from genetic engineering technology to receive regulatory approval, in 1990, was chymosin, an enzyme

produced by genetically engineered bacteria. It replaced calf rennet in cheese-making and is now used in 60% of all cheese manufactured. Its benefits include increased purity, a reliable supply, a 50% cost reduction, and high cheese yield/efficiency (Wieczorek, 2003).

**Improved nutritional value:** Genetic engineering has allowed new options for improving the nutritional value, flavor, and texture of foods. Transgenic crops in development include soybeans with higher protein content, potatoes with more nutritionally available starch and an improved amino acid content, beans with more essential amino acids and rice with the ability produce beta-carotene, a precursor of vitamin A, to help prevent blindness in people who have nutritionally inadequate diets (Wieczorek, 2003).

**Better flavor:** Flavor can be altered by enhancing the activity of plant enzymes that transform aroma precursors into flavoring compounds. Transgenic peppers and melons with improved flavor are currently in field trials (Wieczorek, 2003).

**Fresher produce:** Genetic engineering can result in improved keeping properties to make transport of fresh produce easier, giving consumers access to nutritionally valuable whole foods and preventing decay, damage, and loss of nutrients. Transgenic tomatoes with delayed softening can be vine-ripened for better taste, yet have longer shelf lives through delayed pectin degradation and still be shipped without bruising. Research is under way to make similar modifications to broccoli, celery, carrots, melons, and raspberry. Tomatoes and other produce containing increased levels of certain nutrients such as vitamin C, vitamin E and/or  $\beta$ -carotene, and help protect against the risk of chronic diseases such as some cancers and heart disease. The shelf-life of some processed foods such as peanuts has also been improved by using ingredients that have had their fatty acid profile modified.

Modern biotechnology has offered opportunities to produce more nutritious and better tasting foods, higher crop yields and plants that are naturally protected from diseases and insects. It allows for the transfer of only one or a few desirable genes, thereby permitting scientists to develop crops with specific beneficial traits and reduce undesirable traits. Traditional biotechnology such as cross-pollination in corn produces numerous, non-selective changes. Similarly, introducing genes that increase available iron levels in rice three-fold is a potential remedy for iron deficiency, a condition that affects more than two billion people and causes anemia in about half that number.

**Food Fermentation:** During the production of fermented foods, microorganisms are an integral part

of the processing system. By using traditional and molecular approaches, microbial cultures can be genetically improved. In both developing as well as developed countries, traits considered for commercial food applications are sensory quality (flavor, aroma, visual appearance, texture and consistency) and resistance in the case of dairy fermentations and the ability to produce antimicrobial compounds (e.g. bacteriocins, hydrogen peroxide) for inhibition of the growth of undesirable microorganisms. In many developing countries, the focus is on the degradation or inactivation of natural toxins (e.g. cyanogenic glycosides in Cassava, mycotoxins in cereal fermentation, and anti-nutritional factors (e.g. phytates) (FAO, 2010).

### III. APPLICATIONS OF BIOTECHNOLOGY IN AGRICULTURE

#### Bioengineered Plants

Genetic engineering methods have been extensively used to increase the quantity of different nutrients (vitamins, essential amino acids, minerals and phytochemicals) and enhance their availability in plants (Jube & Borthakur, 2006).

**Essential vitamins:** Vitamins play essential role in human health by controlling metabolism and support the biochemical processes that release energy from foods. They are important in the formation of hormones, blood cells, nervous-system chemicals and genetic material. Vitamins combine with proteins to form metabolically active enzymes important in many chemical reactions. Out of the 13 well-known vitamins, the body can only manufacture vitamin D; all others, such as vitamin A, C and E must be derived from the diet. Insufficient vitamin intake may cause a variety of health problems. Through biotechnology, scientists can increase the content of vitamins in certain crops, allowing the world population to make use of their health benefits.

#### Vitamin A

Nearly two-thirds of the world population depends on rice as their major staple and among them an estimated 300 million suffer from some degree of vitamin A deficiency (WHO, 1997). This is a serious public health problem in a number of countries including highly populated areas of Asia, Africa, and Latin America. The rice endosperm (the starchy interior part of the rice grain) does not contain any  $\beta$ -carotene, which is the precursor for vitamin A and is a component of the visual pigments of rod and cone cells in the retina and its deficiency causes symptoms ranging from night blindness to total blindness. In Southeast Asia, it is estimated that a quarter of a million children go blind every year because of this nutritional deficiency.

Plant foods such as carrots and many other vegetables contain  $\beta$ -carotene. Each  $\beta$ -carotene molecule is

oxidatively cleaved in the intestine to yield two molecules of retinal, which can be then reduced to form retinol or vitamin Ingo Potrykus from the Swiss Federal Institute of Technology, Zurich, Switzerland and Peter Beyer from the University of Freiburg recently developed transgenic rice expressing genes for  $\beta$ -carotene biosynthesis in rice grains (Potrykus, 2001). Rice endosperm naturally contains geranylgeranyl pyrophosphate (GGPP), which is a precursor of the pathway for  $\beta$ -carotene biosynthesis. GGPP can be converted into  $\beta$ -carotene in four steps (Bartley et al., 1994). The bacterial enzyme phytoenedesaturase (EC 1.14.99.30) encoded by the crtI gene can substitute the functions of both phytoenedesaturase and  $\zeta$ -carotene desaturase (EC 1.14.99.30) in plants (Armstrong & Yamazaki, 1986). To reduce the number of genes transformed into rice for the  $\beta$ -carotene pathway, the researchers used the crtI gene from the bacterium *Erwiniauredovora* (Ye, et al., 2000). The psy gene encoding phytoene synthase (EC 2.5.1.32) and the lcy gene encoding lycopene  $\beta$ -cyclase used for transformation originated from the plant daffodil. The plant psy gene (cDNA) and the bacterial crtI gene were placed under the control of the endosperm-specific rice glutelin (Gt1) promoter and the 35S CaMV promoter, respectively, and introduced in the binary plasmid pZPsC. Another plasmid, pZLcyH, was constructed by inserting the lcy gene from daffodil under the control of rice Gt1 promoter and the aphIV gene, for hygromycin resistance, under the control of 35S CaMV promoter. Plasmids pZPsC and pZLcyH were co-transformed into immature rice embryos by *Agrobacterium*-mediated transformation (Ye et al. 2000). All hygromycin-resistant transformants were screened for the presence of the psy, crtI, and lcy genes by Southern hybridization. A few of the transformed plants produced  $\beta$ -carotene in the endosperm, which caused the kernel to appear yellow. The selected line contained 1.6- $\mu$ g  $\beta$ -carotene per gram of endosperm, and was established as "golden rice."

### Vitamin C

Vitamin C or ascorbic acid, found in many plants, is an important component in human nutrition. It has antioxidant properties, improves immune cell and cardiovascular functions, prevents diseases linked to the connective tissue (Davey, et al., 2000), and is required for iron utilization (Hallberg et al., 1989). Most animals and plants are able to synthesize ascorbic acid, but humans do not have the enzyme, L-gulonolactone oxidoreductase (EC 1.1.3.8), necessary for the final step in ascorbic acid biosynthesis. For this reason, ascorbic acid needs to be consumed from dietary sources, especially from plants (Davey et al. 2000). The recent identification of ascorbic acid pathway in plants opened the way to manipulating its biosynthesis and allowed the design of bioengineered plants that produce ascorbic acid at significantly higher levels. The biosynthetic pathway

of ascorbic acid differs from animals to plants. In plants, vitamin C biosynthesis can be accomplished in two ways. First, D-galacturonic acid, which is released upon the hydrolysis of pectin (a major cell wall component), is converted into L-galactonic acid with the help of the enzyme D-galacturonic acid reductase (EC 2.7.1.44). L-galactonic acid is then readily converted into L-galactono-1, 4-lactone, which is the immediate precursor of ascorbic acid (Wheeler et al., 1998; Smirnov et al., 2001). Researchers in Spain (Agius et al., 2003) isolated and characterized galUR, a gene in strawberry that encodes the enzyme D-galacturonic acid reductase. The galUR gene was amplified by PCR as a 956-bp fragment and cloned into a binary vector behind a 35S CaMV promoter. The resulting plasmid was transformed into *E. coli* and delivered to *Agrobacterium* by triparental mating. Finally, the GalUR gene was introduced into *Arabidopsis thaliana* plants via *Agrobacterium*-mediated transformation. The expression of the strawberry GalUR gene in *A. thaliana* allowed the bioengineered plants to increase the biosynthesis of ascorbic acid by 2-3 times compared with the wild-type plants (Agius et al., 2003).

The second way by which plants synthesize vitamin C is through the recycling of used ascorbic acid. During the first step of this recycling, ascorbic acid is oxidized forming a radical called monodehydroascorbate (MDHA). Once MDHA is formed, it can be readily converted back into ascorbic acid by the enzyme monodehydroascorbate reductase (MDHAR) (EC 1.6.5.4), or further oxidized forming dehydroascorbate (DHA). DHA can then undergo irreversible hydrolysis or be recycled to ascorbic acid by the enzyme dehydroascorbate reductase (DHAR) (EC 1.8.5.1), which uses the reductant glutathione (GSH) (Washko et al. 1992; Wheeler et al. 1998; Smirnov and others 2001). Researchers from the University of California, Riverside, hypothesized that by enhancing the expression of DHAR in plants, they could increase ascorbic acid synthesis, because a more efficient ascorbate recycling process would be achieved (Chen et al. 2003). To test their hypothesis, they isolated DHAR cDNA from wheat and expressed the gene in tobacco and maize plants. Tobacco plants were transformed by using *Agrobacterium*. A His tag was added to DHAR, which was then introduced in the binary vector pBI101, behind a 35S CaMV promoter. For maize, a DHAR without a His tag was placed under the control of the maize ubiquitin (Ub) promoter or the Shrunken 2 (Sh2) promoter in the pACH18 vector. Transgenic maize was generated by particle bombardment of the embryogenic callus. DHAR expression was amplified up to 32 times in tobacco, and up to 100 times in maize, resulting in increased ascorbic acid levels of up to four-fold in the bioengineered plants (Chen et al. 2003).

### Vitamin E

Vitamin E is a broad term used to describe a group of eight lipid-soluble antioxidants in the tocotrienol and tocopherol families that are synthesized by photosynthetic organisms, mainly plants (Hess 1993). Both tocotrienol and tocopherol families can be distinguished into four different forms each ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ), based on the number and position of methyl groups in the aromatic ring (Kamal-Eldin and Appelqvist 1996). Tocotrienols and tocopherols protect plants against oxidative stresses and the antioxidant property of these molecules adds functional qualities to food products (Andlauer and Furst 1998). Tocotrienols have more powerful antioxidant properties than tocopherols but are not absorbed as readily. The predominant forms of vitamin E in leaves and seeds are  $\alpha$ -tocopherol and  $\gamma$ -tocotrienol, respectively (Munne-Bosch and Alegre 2002). Vitamin E is an important component of mammalian diet, and excess intake has been shown to produce many beneficial therapeutic properties, including reduction of cholesterol levels, inhibition of breast cancer cell growth in vitro, decrease risk of cardiovascular diseases, and decrease incidence of many human degenerative disorders (Theriault et al. 1999).

The biosynthesis of tocopherols and tocotrienols has been known for many years and their biosynthesis in plants originates from two different precursors. The particular genes that encode for the different enzymes in the pathway have only recently been discovered. Researchers are trying to develop plants with increased vitamin E levels and some positive results have already been achieved.

The first step in the pathway for the biosynthesis of both tocopherols and tocotrienols is the formation of homogentisic acid (HGA) from p-hydroxyphenylpyruvate, catalyzed by the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD) (EC 1.13.11.27) (Grusack and DellaPenna 1999). Tocotrienols are produced from the condensation of HGA and geranylgeranyl diphosphate (GGDP), catalyzed by HGA geranylgeranyltransferase (HGGT) (EC 2.5.1.32), and tocopherols are formed from the condensation of HGA and phytyl diphosphate (PDP), catalyzed by HGA phytyltransferase (HPT) (EC 2.5.1.62) (Sollet et al., 1980; Schultz et al., 1985; Collakova and DellaPenna 2001). Researchers from the Institute of Botany in Germany described the effects of constitutive expression of HPPD cDNA from barley (*Hordeum vulgare*) in tobacco plants. The HPPD gene was cloned into the pBinAR binary vector, in a SmaI cloning site located between the 35S CaMV promoter and the octopine synthase (EC 1.5.1.11) polyadenylation signal. The construct was then introduced into *Agrobacterium* GV3101, which was used to transform tobacco explants. The results showed that transgenic lines had a greater capacity

for overall biosynthesis of homogentisic acid and produced a two-fold increase in the amount of vitamin E in the seeds. Vitamin E content in leaves was not affected (Falk et al., 2003).

In another approach towards vitamin E enhancement, Cahoon et al., (2003) reported the identification and isolation of a novel monocot gene that encodes HGGT, which is so far the only known enzyme specific for the synthesis of tocotrienols. These researchers found that the expression of the barley HGGT enhanced the tocotrienol synthesis by 10- to 15-fold in the leaves of *A. thaliana* and by six-fold in the seeds of corn. The barley HGGT cDNA was placed under the control of the 35S CaMV promoter and the nopaline synthase terminator. The construct was inserted into the binary vector pZS199 to generate plasmid pSH24. The plasmid was then introduced into *Agrobacterium* for transformation into tobacco and *A. thaliana* (Cahoon et al., 2003).

A third way by which vitamin E content in plants can be manipulated involves the last enzyme in the final step of tocotrienols and tocopherols biosynthetic pathway, in which  $\gamma$ -tocotrienol and  $\gamma$ -tocopherol are converted to  $\alpha$ -tocotrienol and  $\alpha$ -tocopherol, respectively. This step is catalyzed by the enzyme  $\gamma$ -tocopherol methyltransferase ( $\gamma$ -TMT) (EC 2.1.1.95) (Shintani and DellaPenna 1998).  $\alpha$ -tocopherol has the highest oxidative property among the members of the vitamin E family (Kamal-Eldin and Appelqvist 1996). Unfortunately, plant oils, which are the main dietary source of vitamin E, contain only a fraction amount of  $\alpha$ -tocopherol but a high level of its precursor,  $\gamma$ -tocopherol. Shintani and DellaPenna over expressed endogenous *A. thaliana*  $\gamma$ -TMT to enhance conversion of  $\gamma$ -tocopherol into  $\alpha$ -tocopherol. They introduced the  $\gamma$ -TMT cDNA construct under the control of a 35S CaMV promoter in a binary vector into *A. thaliana* plants by *Agrobacterium*-mediated transformation.  $\alpha$ -tocopherol content of bioengineered seeds was nine-fold greater than that of the wild-type seeds (Shintani and DellaPenna 1998).

### IV. ESSENTIAL MINERALS

Well-functioning healthy bodies of humans require 17 different essential minerals in their diet. Minerals are inorganic ions found in nature and cannot be made by living organisms. They can be divided into two classes; 1) Macronutrients are the minerals that we need in large quantity, including calcium, phosphorus, sodium, magnesium, chlorine, sulfur, and silicon. 2) Micronutrients, or trace minerals, are the minerals that are required in small amounts, of which iron is the most prevalent, followed by fluorine, zinc, copper, cobalt, iodine, selenium, manganese, molybdenum, and chromium. Although a balanced consumption of plant-based foods should naturally provide these nutrients, mineral deficiency,

especially of iron, is prevalent among the world population.

**Iron:** Even though iron is required in trace amounts, but is the most widespread nutrient deficiency worldwide. It is believed that about 30% of the world population suffers from serious nutritional problems caused by insufficient intake of iron (WHO 1992). Iron is an important constituent of hemoglobin, the oxygen-carrying component of the blood, and is also a part of myoglobin, which helps muscle cells to store oxygen. Low iron levels can cause the development of iron deficiency anemia. In an anemic person the blood contains a low level of oxygen, which results in many health problems including infant retardation (Walter et al., 1986), pregnancy complication (Murphy et al., 1986), low immune function (Murakawa et al., 1987), and tiredness (Bastaet al., 1979). Iron is present in food in both inorganic (ferric and ferrous) and organic (heme and nonheme) forms. Heme iron, which is highly bio-available, is derived primarily from the hemoglobin and myoglobin of flesh foods such as meats, fish, and poultry (Taylor et al., 1986). In humans, reduced iron (ferrous) is taken up more readily than oxidized (ferric) iron. Several approaches have been used in the fight against iron deficiency including nutraceutical supplementation, food fortification, and different methods of food preparation and processing (Maberly et al., 1994). So far, none of these approaches have been successful in eradicating iron deficiency, especially in developing countries. A new tool in the fight against nutrient deficiency is the use of biotechnology to improve essential mineral nutrition in staple crops.

At this time, there are basically two ways in which genetic engineering can be used for this purpose:

1. By increasing the concentration of iron-binding protein ferritin and
2. By reducing the amount of iron-absorption inhibitor phytic acid.

Although iron intake is important for human health, it can be toxic, so the ability to store and release iron in a controlled manner is crucial. The 450 kDa ferritin protein, found in animals, plants and bacteria, can accumulate up to 4500 atoms of iron (Andrews et al., 1992). This protein consists of 24 subunits assembled into a hollow spherical structure within which iron is stored as a hydrous ferric oxide mineral core. The two main functions of ferritin in living organisms are to supply iron for the synthesis of proteins such as ferredoxin and cytochromes and to prevent free radicals damage to cells. Studies have shown that ferritin can be orally administered and is effective for treatment of rat anemia (Beard et al., 1996), suggesting that increasing ferritin content of cereals may solve the problem of dietary iron deficiency in humans. Japanese researchers (Goto et al., 1999) introduced soybean ferritin cDNA into rice plants, under the control of a seed specific promoter, GluB-1,

from the rice seed-storage protein gene encoding glutelin. The two advantages of this promoter are the accumulation of iron specifically in the rice grain endosperm, and its ability to induce ferritin at a high level. The ferritin cDNA was isolated from soybean cotyledons, inserted into the binary vector pGPTV-35S-bar, and transferred into rice using *Agrobacterium*. The iron content of the rice seed in the transgenic plants was three times greater than that of the untransformed wild-type plants.

Phytic acid, or phytate, is the major inhibitor of many essential minerals, including iron, zinc, and magnesium, and is believed to be directly responsible for the problem of iron deficiency (Ravindran et al., 1995). In cereal grains, phytic acid is the primary phosphate storage and it is deposited in the aleurone storage vacuoles (Lott 1984). During seed germination, phytic acid is catalyzed into inorganic phosphorous by the action of the hydrolytic enzyme phytase (EC 3.1.3.8). There is little or no phytase activity in the dry seeds or in the digestive tract of monogastric animals (Gibson and Ullah 1990; Lantschet et al., 1992). In a recent study, it has been shown that phytase activity can be reestablished in mature dry seeds under optimum pH and temperature conditions (Brinch-Pedersen et al., 2002).

A reduction in the amount of phytic acid in staple foods is likely to result in a much greater bioavailability of iron and other essential minerals. Lucca et al. (2002) inserted a fungal (*Aspergillus fumigatus*) phytase cDNA into rice to increase the degradation of phytic acid. Rice suspension cells, derived from immature zygotic embryos, were used for biolistic transformation with the *A. fumigatus* phytase gene. Phytase from *A. fumigatus* was the enzyme of choice because it is heat stable and thus can refold into an active form after heat denaturation (Wyss et al., 1998). The main purpose of this research was to increase phytase activity during seed germination and to retain the enzyme activity in the seed after food processing and in the human digestive tract. Although the researchers achieved high expression levels of phytase in the rice endosperm, by placing it under the control of the strong tissue-specific globulin promoter, the thermo tolerance of the transgenic rice was not as high as expected. It has been speculated that the reason for this unexpected low thermostability of the *A. fumigatus* phytase in transgenic rice is due to the interference of the cellular environment of the endosperm to maintain the enzyme in an active configuration (Holm et al., 2002). Further studies are needed to develop an endogenous phytase enzyme that is thermostable and maintains high activity in plant tissues.

**Essential Amino Acids:** Proteins are the organic molecules formed by building blocks amino acids. The digestive system breaks down proteins into single amino acids so that they can enter into the

bloodstream. Cells then use the amino acids as to form enzymes and structural proteins. There are two types of amino acids; Essential amino acids cannot be synthesized by animals, including humans, therefore, need to be acquired in the diet and nine essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Non essential amino acids; the body can synthesize nonessential amino acids as long as there is a proper intake of essential amino acids and calories. Proteins are present in foods in varying amounts, some foods have all nine essential amino acids in them, and they are referred to as complete proteins (animal products )and some proteins are usually low on or missing certain essential amino acids (Vegetables sources).

In order to provide better nutrition from plant sources, it is essential to increase the content of essential amino acids in seed and tuber proteins. This is particularly important for countries where certain crops, such as rice, potatoes, and corn, are the main dietary source.

**Lysine:** Rice is one of the most important staple crops and is consumed by 65% of the world population on a daily basis (Lee et al., 2003). It is a good source of essential nutrients such as vitamins B<sub>1</sub> (thiamin), B<sub>2</sub> (riboflavin), B<sub>3</sub> (niacin), but it is low in the essential amino acids, lysine and isoleucine (Fickler 1995). Adequate intake of lysine is essential because it serves many important functions in the body including aiding calcium absorption, collagen formation, and the production of antibodies, hormones, and enzymes. A deficiency in lysine may result in tiredness, inability to concentrate, irritability, bloodshot eyes, retarded growth, hair loss, anemia, and reproductive problems (Cooper 1996). Zhenget al. (1995) developed transgenic rice with enhanced lysine content. They accomplished this by expressing the seed storage protein  $\beta$ -phaseolin from the common bean (*Phaseolus vulgaris*) in the grain of transgenic rice. The genomic and cDNA sequences of the  $\beta$ -phaseolin gene from *P. vulgaris* were placed under the control of either a rice seed-specific glutelin Gt1 promoter or the native  $\beta$ -phaseolin promoter. The vectors containing the  $\beta$ -phaseolin gene were transferred into the rice chromosome by protoplast-mediated transformation. Four percent of total endosperm protein in the transgenic rice was phaseolin, which resulted in a significant increase in the lysine content in rice (Zhenget al., 1995).

**Methionine and Tyrosine:** In terms of the global food production, potato (*Solanum tuberosum*) is only behind rice, wheat, and corn on the list of the crop species that are most important for human nutrition worldwide (Chakraborty et al., 2000). There are four main purposes for the production of potatoes: for the fresh food market, for animal feed, for the food processing industry and for nonfood industrial uses

such as manufacture of starch and alcohol (Chakraborty et al. 2000). Potato is a good source of potassium, iron, vitamin C and B, but it is not a rich protein source and is limited in nutritive value for the lack of the amino acids lysine, methionine, and tyrosine (Jaynes et al. 1986). Methionine is also the main supplier of sulfur which prevents disorders of the hair, skin, and nails, helps lower cholesterol levels by increasing the liver's production of the phospholipid lecithin, and is a natural chelating agent for heavy metals (Cooper, 1996). Lack of methionine in a person's diet may result in an imbalanced uptake of other amino acids, as well as retardation in growth and development.

Scientists from the National Center for Plant Genome Research in India isolated and cloned a gene that encodes for a seed-specific protein from *Amaranthus hypochoeroides* called amaranth seed albumin (AmA1) (Chakraborty et al., 2000). The advantages of using the AmA1 seed-protein to improve crops nutritional value are as follows:

1. It is well-balanced in the composition of all essential amino acids.
2. It is a non-allergenic protein
3. It is encoded by a single gene AmA1. This gene was cloned into a binary vector, under the control of a constitutive 35S CaMV promoter (plasmid pSB8) and a tuber specific granule-bound starch synthase (EC 2.4.1.21) promoter (plasmid pSB8G). The AmA1 gene constructs from these two binary plasmids were introduced into potato through *Agrobacterium*-mediated transformation. The amino acid contents in the pSB8-transgenic potato showed a 2.5- to 4-fold increase in lysine, methionine, and tyrosine, while the tissue-specific pSB8G-transgenic potatoes showed a four to eight-fold increase in these amino acids.

**Essential Phytochemicals:** Besides being a major supplier of essential nutrients such as vitamins, amino acids and minerals, plants are also an important source of phytochemicals that are known to be beneficial for health. Some examples of phytochemicals include indoles, isothiocyanates, and sulforaphane, found in vegetables such as broccoli; allylic sulfides, found in onions and garlic; and isoflavonoids found mainly in soybeans. Since the intake of these phytochemicals is not always sufficient, scientists are trying to enhance the nutritional quality of plants through genetic engineering.

**Isoflavonoids:** Flavonoids, which include anthocyanins, condensed tannins, and iso-flavonoids, are a class of phytochemicals that perform a range of important functions for the plants including pigmentation, feed deterrence, wood protection, fungi and insect's defense and induction of genes for root nodulation (Buchanan et al., 2001). Isoflavonoids (or isoflavones) are a type of phytoestrogen, or plant hormone that has a chemical structure similar to

human estrogen. Isoflavonoids are found in soybeans, chickpeas, and many other legumes; however, soybeans are unique because they have the highest concentration of the two most beneficial isoflavonoids, genistein and daidzein (Eldrige and Kwolek 1983, Tsukamoto et al., 1995). In the studies conducted so far, iso-flavonoids show great potential to fight many types of diseases.

The health benefits believed to be provided by isoflavonoids come from the weak estrogenic activity of these molecules in the human body (Jung et al., 2000). They help prevent the buildup of arterial plaque, which reduces the risk of coronary heart disease and stroke (FDA 1999); help reduce breast cancer (Peterson et al., 1991); help prevent prostate cancer by delaying cell growth (Messina and Barnes 1991); fight osteoporosis by stimulating bone formation (Civitelli 1997); and even relieve some menopausal symptoms (Nestelet al., 1999). The main source of iso-flavonoids in human diet comes from the consumption of soybean and its products. It is present in high concentration in unprocessed soybean, whose level can decrease by 50% during seed processing for traditional soy foods (Wang and Murphy 1996). Increasing isoflavonoid concentrations in soybean could solve this problem. Another way to take advantage of iso-flavonoids' health benefits is through the development of other crops that can produce this powerful compound, thereby widening their consumption. Isoflavonoids are synthesized by a branch in the degradation pathway of the amino acid phenylalanine, and its first committed step is catalyzed by the enzyme isoflavone synthase (EC 1.14.13.53). Jung et al. (2000) identified two soybean genes encoding isoflavone synthase, IFS1/IFS2, and expressed these genes in *A. thaliana*, triggering the synthesis of the isoflavonoidgenistein. Although *A. thaliana* does not synthesize iso-flavonoids, it does have the substrate naringenin, which is an intermediate of the anthocyanin biosynthetic pathway. Naringenin can then be converted to the isoflavonoidgenistein by a foreign isoflavone synthase. The soy isoflavone synthase gene IFS1 was cloned in the plasmid pOY204 under the control of the 35S CaMV promoter, and transferred into *A. thaliana* by Agrobacterium-mediated transformation. The introduced ISF1 gene expressed and produced active isoflavone synthase in the transformed plant. The amount of genistein produced was approximately 2ng/ $\mu$ g of fresh plant weight (Jung et al. 2000).

## V. INCREASING YIELD

The Green Revolution succeeded in tripling the food supply but yet it was not enough to feed the growing human population. Increased yields have partly been due to the use of improved crop varieties, but mainly due to the use of better management practices and use

of agrochemicals (fertilizers and pesticides). However, for farmers in the developing world, agrochemicals are often too expensive, and further increases in yield with existing varieties are not possible using conventional breeding. Is there any alternative path that our understanding of genetics can show so that farmers may obtain maximum yield from their fields? Is there a way to minimize the use of fertilizers and chemicals so that their harmful effects on the environment are reduced? Use of genetically modified crops is a possible solution.

Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms (GMO). GM plants have been useful in many ways. Genetic modification has:

- i. Made crops more tolerant to abiotic stresses (cold, drought, salt, heat).
- ii. Reduced reliance on chemical pesticides (pest-resistant crops).
- iii. Helped to reduce post-harvest losses.
- iv. Increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).
- v. Enhanced nutritional value of food, e.g., Vitamin 'A' enriched rice.
- vi. GM has been used to create tailor-made plants to supply alternative resources to industries in the form of starches, fuels and pharmaceuticals.

Other applications of biotechnology in agriculture include the production of pest resistant plants, which could decrease the amount of pesticide used. Bt toxin is produced by a bacterium called *Bacillus thuringiensis* (Bt for short). Bt toxin gene has been cloned from the bacteria and been expressed in plants to provide resistance to insects without the need for insecticides; in effect created a bio-pesticide. Examples are Bt cotton, Bt corn, rice, tomato, potato and soybean etc.

**BT Cotton:** Some strains of *Bacillus thuringiensis* produce proteins that kill certain insects such as lepidopterans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes). *B. thuringiensis* forms protein crystals during a particular phase of their growth. These crystals contain a toxic insecticidal protein. Why does this toxin not kill the *Bacillus*? Actually, the Bt toxin protein exists as inactive protoxins but once an insect ingest it is converted into an active form of toxin due to the alkaline pH of the gut which solubilize the crystals.

The activated toxin binds to the surface of midgut epithelial cells and creates pores that cause cell swelling and lysis and eventually cause death of the insect. Specific Bt toxin genes were isolated from *Bacillus thuringiensis* and incorporated into the several crop plants such as cotton.

The choice of genes depends upon the crop and the targeted pest, as most Bt toxins are insect-group



specific. The toxin is coded by a gene named cry. There are a number of them, for example, the proteins encoded by the genes cryIAC and cryIIAB control the cotton bollworms that of cryIAB controls corn borer (Figure 1).

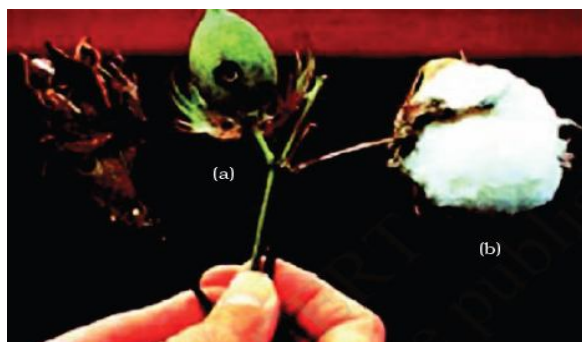


Figure 1. Cotton boll: (a) destroyed by bollworms; (b) a fully mature cotton boll

**Pest Resistant Plants:** Several nematodes parasitise a wide variety of plants and animals including human beings. A nematode *Meloidogyne incognita* infects the roots of tobacco plants and causes a great reduction in yield. A novel strategy was adopted to prevent this infestation which was based on the process of RNA interference (RNAi). RNAi takes place in all eukaryotic organisms as a method of cellular defense. This method involves silencing of a specific mRNA due to a complementary dsRNA molecule that binds to and prevents translation of the mRNA (silencing). The source of this complementary RNA could be from an infection by viruses having RNA genomes or mobile genetic elements (transposons) that replicate via an RNA intermediate.

Using *Agrobacterium* vectors, nematode-specific genes were introduced into the host plant (Figure 2). The introduction of DNA was such that it produced both sense and anti-sense RNA in the host cells. These two RNA's being complementary to each other formed a double stranded (dsRNA) that initiated RNAi and thus, silenced the specific mRNA of the nematode. The consequence was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plant therefore got itself protected from the parasite (Figure 2).

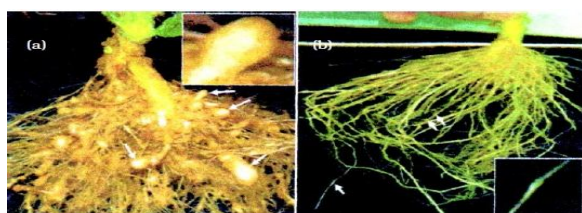


Figure 2. Host plant-generated dsRNA triggers protection against nematode infestation: (a) Roots of a typical control plants; (b) transgenic plant roots 5 days after deliberate infection of nematode but protected through novel mechanism.

### Applications in Pharmaceutical Industries

Biotechnology has continued to advance the state of the art in pharmaceutical research and discovery, especially in the generation and screening of molecular diversity (Moos, Green, & Pavia, 1993). Pharmaceutical biotechnology companies use recombinant DNA technology, which entails genetic manipulation of cells, or a monoclonal antibody for making their biotechnological products. These biotech pharmaceutical products made by the biotech companies are widely used in prevention, diagnosis or treatment of many types of diseases.

The conventional pharmaceutical formulations are relatively simple molecules manufactured mainly through trial and error technique for treating the symptoms of a disease or illness. On the other hand, biopharmaceuticals are complex biological molecules, commonly known as proteins that usually aim at eliminating the underlying mechanisms for treating diseases. However, this is not true in all cases as in the case of type 1 diabetes mellitus where insulin is used to treat only the symptoms of the disease and not the underlying causes. Pharmaceutical biotechnology, essentially, is used to make complex larger molecules with the help of living cells (like those found in the human body such as bacteria cells, yeast cells, animal or plant cells). Unlike the smaller molecules that are given to a patient through tablets, the large molecules are typically injected into the patient's body.

**Pharmaceuticals and Biotechnology- Benefits of Combination:** When the two disciplines, pharmaceuticals and biotechnology, coming together, they result in many advantages for humankind in terms of healthcare. This is possible through Pharmacogenomics (derived from 'pharmacology' and 'genomics') which refers to the study of how the genetic inheritance affects individual human body's response to drugs. Biopharmaceutical drugs aims at designing and producing drugs that are adapted to each person's genetic makeup. Thus pharmaceutical biotechnology companies may develop tailor-made medicines for maximum therapeutic effects. Also, biotechnology drugs can be given to the patients in appropriate dosages as the doctor would know the patient's genetics and how the body processes and metabolizes a medicine. One more benefit of pharmaceutical biotechnology is in the form of better vaccines. Biotech companies design and produce safer vaccines by organisms that are transformed through genetic engineering. These biotech vaccines minimize the risks of infection.

The recombinant DNA technological processes have made immense impact in the area of healthcare by enabling mass production of safe and more effective therapeutic drugs. Further, the recombinant therapeutics does not induce unwanted

immunological responses as is common in case of similar products isolated from non-human sources. At present, about 30 recombinant therapeutics have been approved for human-use the world over. In India, 12 of these are presently being marketed.

**Genetically Engineered Insulin:** Management of adult-onset diabetes is possible by taking insulin at regular time intervals. What would a diabetic patient do if enough human-insulin was not available? If you discuss this, you would soon realize that one would have to isolate and use insulin from other animals.

Think about whether insulin can be orally administered to diabetic people or not. Why?

Insulin used for diabetes was earlier extracted from pancreas of slaughtered cattle and pigs. Insulin from an animal source though caused some patients to develop allergy or other types of reactions to the foreign protein. Insulin consists of two short polypeptide chains: chain A and chain B that are linked together by disulphide bridges. In mammals, including humans, insulin is synthesized as a pro-hormone (like a pro-enzyme, the pro-hormone also needs to be processed before it becomes a fully mature and functional hormone) which contains an extra stretch called the C peptide.

This C peptide is not present in the mature insulin and is removed during maturation into insulin. The main challenge for production of insulin using rDNA techniques was getting insulin assembled into a mature form. In 1983, Eli Lilly an American company prepared two DNA sequences corresponding to A and B, chains of human insulin and introduced them in plasmids of *E. coli* to produce insulin chains. Chains A and B were produced separately, extracted and combined by creating disulfide bonds to form human insulin.

**Gene Therapy:** Gene therapy is a corrective therapy for a person who is born with a hereditary disease. Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child/embryo. Here genes are inserted into a person's cells and tissues to treat a disease. Correction of a genetic defect involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for the non-functional gene.

The first clinical gene therapy was given in 1990 to a 4-year old girl with adenosine deaminase (ADA) deficiency. This enzyme is crucial for the immune system to function. The disorder is caused due to the deletion of the gene for adenosine deaminase. In some children ADA deficiency can be cured by bone marrow transplantation; in others it can be treated by enzyme replacement therapy, in which functional ADA is given to the patient by injection. But the problem with both of these approaches that they are not completely curative. As a first step towards gene

therapy, lymphocytes from the blood of the patient are grown in a culture outside the body. A functional ADA cDNA (using a retroviral vector) is then introduced into these lymphocytes, which are subsequently returned to the patient. However, as these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes. However, if the gene isolate from marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.

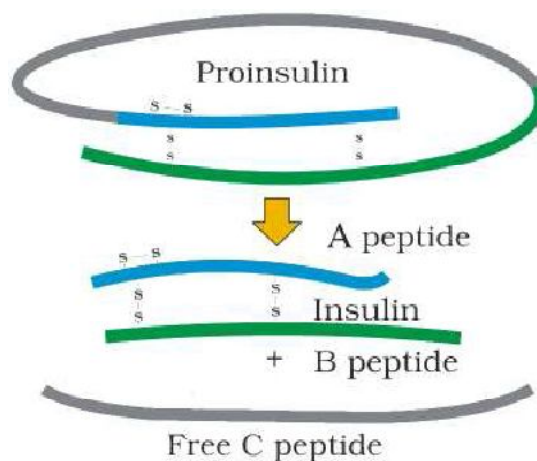


Figure 3. Maturation of pro-insulin into insulin (simplified)

**Molecular Diagnosis:** For an effective treatment of a disease, early diagnosis and understanding its pathophysiology is very important. Using conventional methods of diagnosis (serum and urine analysis, etc.) early detection is not possible. Recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme Linked Immuno-sorbent Assay (ELISA) are some of the techniques that serve the purpose of early diagnosis. Presence of a pathogen (bacteria, viruses, etc.) is normally suspected only when the pathogen has produced a disease symptom. By this time the concentration of pathogen is already very high in the body. However, very low concentration of a bacteria or virus (at a time when the symptoms of the disease are not yet visible) can be detected by amplification of their nucleic acid by PCR. PCR is now routinely used to detect HIV in suspected AIDS patients. It is being used to detect mutations in genes in suspected cancer patients too. It is a powerful technique to identify many other genetic disorders.

A single stranded DNA or RNA, tagged with a radioactive molecule (probe) is allowed to hybridize to its complementary DNA in a clone of cells followed by detection using autoradiography. The clone having the mutated gene will hence not appear on the photographic film, because the probe will not have complementarity with the mutated gene. ELISA is based on the principle of antigen-antibody interaction. Infection by pathogen can be detected by

the presence of antigens (proteins, glycoproteins, etc.) or by detecting the antibodies synthesized against the pathogen.

### Transgenic Animals

Animals that have had their DNA manipulated to possess and express an extra (foreign) gene are known as transgenic animals. Transgenic rats, rabbits, pigs, sheep, cows and fish have been produced, although over 95 per cent of all existing transgenic animals are mice.

### Common reasons for producing these animals and the benefits conferred by them:

- i. Normal physiology and development: Transgenic animals can be specifically designed to allow the study of how genes are regulated, and how they affect the normal functions of the body and its development, e.g., study of complex factors involved in growth such as insulin-like growth factor. By introducing genes from other species that alter the formation of this factor and studying the biological effects that result, information is obtained about the biological role of the factor in the body.
- ii. Study of disease: Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease. These are specially made to serve as models for human diseases so that investigation of new treatments for diseases is made possible. Today transgenic models exist for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's.
- iii. Biological products: Medicines required to treat certain human diseases can contain biological products, but such products are often expensive to make. Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (or genes) which codes for a particular product such as human protein ( $\alpha$ -1-antitrypsin) used to treat emphysema. Similar attempts are being made for treatment of phenylketonuria (PKU) and cystic fibrosis. In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk (2.4 grams per litre). The milk contained the human alpha-lactoalbumin and was nutritionally a more balanced product for human babies than natural cow-milk.
- iv. Vaccine safety: Transgenic mice are being developed for use in testing the safety of polio vaccines before they are used on humans. If successful and found to be reliable, they could replace the use of monkeys to test the safety of batches of the vaccine.
- v. Chemical safety testing: This is known as toxicity/safety testing. Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. They are then exposed to the toxic

substances and the effects studied. Toxicity testing in such animals will allow us to obtain results in less time.

### Pharmaceutical Biotechnology Products

The common pharmaceutical biotechnology products include:

- Antibodies. Antibodies are proteins that are produced by white blood cells and are used by the immune system to identify bacteria, viruses, and other foreign substances and to fight them off. In the recent years, monoclonal antibodies are one of the most exciting developments in biotechnology pharmaceuticals.
- Proteins. Proteins made of amino acids are large, complex molecules that do most of the work in cells and are required for the structure, function, and regulation of the body's tissues and organs. Protein biotechnology is emerging as one of the key technologies of the future for understanding the development of many diseases like cancer or amyloid formation for better therapeutic intervention.
- Recombinant DNA Products. Recombinant DNA is the genetically engineered DNA created by recombining fragments of DNA from different organisms. Some of the Recombinant DNA Products include:
  - Recombinant DNA Vaccines
  - Recombinant DNA Drugs
  - Recombinant DNA Enzymes
  - Recombinant DNA Growth Hormone
  - Recombinant DNA Insulin
  - Recombinant DNA Proteins
  - Recombinant DNA Yeast

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