Review

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Bioengineering Microbes for Improved Nutritional Value and Health Benefits

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Abstract: Microbe bioengineering has the potential to improve the nutritional value and health benefits of food products. Modifying microbial cells to produce specific proteins or compounds that can improve human health is one approach. Penicillin and recombinant insulin are two examples of this. The production of recombinant insulin by genetically modified *E. coli* bacteria has transformed diabetes treatment by providing a consistent and cost-effective source of insulin. Previously, insulin was extracted from the pancreas of animals, which was an expensive process that frequently resulted in impurities that could cause adverse reactions. Bioengineered insulin is now the standard diabetes treatment, providing a safe and effective method of controlling blood glucose levels. Bacteria, for example, can be genetically modified to produce vitamins like B12, which are difficult to obtain from plant sources. Likewise, yeasts can be genetically modified to produce β-carotene, a precursor to vitamin A that is required for vision, immune function, and skin health. Overall, bioengineered microorganisms have the potential to provide significant health benefits by producing essential compounds and proteins. Because of ongoing advances in genetic engineering techniques and understanding microbial metabolism, the possibilities for improving human health through bioengineering are limitless.

Keywords: bioengineered; recombinant; *E. coli*; insulin; penicillin; cancer; vitamin B12; replacement therapy; nutrition; mABs

1. Introduction

Bioengineering microbes is a rapidly expanding field with the potential to transform healthcare and improve the nutritional value of our food. This technology involves manipulating the genetic material of microorganisms such as bacteria or yeast to produce valuable compounds that can be used for therapeutic purposes or to improve the nutritional value of our food.

The production of monoclonal antibodies (mABs) is one of the most important applications of bioengineering microbes. mABs are highly specific proteins with the ability to target specific cells or proteins in the body, making them effective treatments for a variety of diseases [1]. Rituximab, an mAB, is used to treat non-Hodgkin's lymphoma and chronic lymphocytic leukemia by specifically targeting B-cells. Trastuzumab, another mAB, is used to treat HER2-positive breast cancer by targeting HER2 receptors on cancer cells' surfaces [2]. The use of bioengineered microbes to produce mABs has revolutionized cancer treatment by providing highly specific and effective treatments for various types of cancer.

The production of insulin, a hormone that regulates blood sugar levels in the body, is another important application of bioengineering microbes. Insulin was extracted from the animal pancreas before the development of recombinant DNA technology, which was an inefficient and costly process [3]. However, using bioengineered bacteria or yeast to produce human insulin has made diabetes treatment more accessible. Genetically modified bacteria, such as *E. coli*, or yeast, such as *S. cerevisiae*, can produce insulin, which can then be purified and used to treat diabetes [4]. The use of bioengineered microbes to produce insulin has significantly reduced the cost of diabetes treatment while improving the quality of life for millions of diabetics.

Another protein produced through bioengineering is interferon-gamma (IFN-γ). IFN-γ is a cytokine that regulates the immune system and has antiviral and antitumor properties [5].

Researchers have been able to investigate the potential therapeutic benefits of IFN-γ by producing it using bioengineered bacteria or yeast [6]. IFN-γ is currently used to treat chronic granulomatous disease, a rare genetic disorder affecting the immune system [7]. The ability of bioengineered microbes to produce IFN-γ has paved the way for the development of new treatments for a variety of diseases involving immune system dysregulation.

Somatostatin is a hormone that controls the body's release of growth hormones and insulin. Somatostatin can be produced by bioengineering microbes using genetically modified bacteria or yeast, allowing researchers to investigate its potential therapeutic benefits [8]. Acromegaly, a condition in which the body produces too much growth hormone, is treated with somatostatin analogs [9]. The use of bioengineered microbes to produce somatostatin has opened up new avenues for the treatment of various endocrine disorders.

Bioengineering microbes may additionally enhance the nutritional value of food by producing essential nutrients found in animal products, such as vitamin B12. Vitamin B12 is a necessary nutrient for the formation of RBCs and the proper functioning of the nervous system [10]. However, because vitamin B12 is primarily found in animal products, vegans and others who avoid animal products may struggle to get enough of this essential nutrient [11]. Researchers can create a novel form of vitamin B12 accessible to everyone by producing it with bioengineered microbes.

Overall, bioengineering microbes for increased nutritional value and health benefits is an exciting field with many potential applications. Researchers can create new strains of microorganisms by modifying their genetic makeup to produce valuable proteins and other compounds with potential health benefits. These developments have already resulted in the development of new treatments for a variety of diseases and health conditions, and they have the potential to improve the nutritional quality of our food as well. As research in this field continues, we can anticipate even more exciting developments that have the potential to improve people's health and well-being worldwide.

2. Insulin

Human insulin is a protein made up of two polypeptide chains that are joined together by two disulfide links [12]. Under certain conditions, it can exist as a monomer or form dimers and hexamers. Previously, insulin was extracted from the bovine or porcine pancreas, but commercial production of human insulin began in 1982 using an enzymatic method to produce semi-synthetic insulin with the same amino acid sequence as human insulin [13]. This tipping point in the history of diabetes was critical in meeting the global demand for insulin.

It is produced and secreted by the pancreatic islets of Langerhans β cells in the human body and plays a critical role in fat and carbohydrate metabolism [13]. Insulin is crucial for the modulation of glucose metabolism and the management of diabetes [13].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

Direct biosynthesis of human insulin, without using pancreatic tissue from animals is now possible due to rDNA technology. The proinsulin biosynthesis approach has been developed as the preferred method for cloning and expressing human insulin in *E. coli*.

The experiment entails cloning human insulin in *E. coli* via PCR, followed by digestion of the PCR product and vector with BamHI and XhoI and insertion of the product into *E. coli* BL21 (DE3) cells via the calcium chloride heat shock method. Positive clones were grown, and IPTG was used to boost human proinsulin expression [4].

Clinical Uses

Insulin is primarily used in the treatment of diabetes mellitus to regulate blood glucose levels [12].

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Insulin therapy can also be used to treat gestational diabetes during pregnancy. It can also be used to treat hyperkalemia (high potassium levels in the blood) and some rare metabolic disorders, such as glycogen storage disease type 1 [14,15].

Insulin has additionally been investigated for its potential use in other diseases such as Alzheimer's and cancer, though additional studies in these areas are needed [16].

Commercial Uses

Commercial insulin is manufactured in injectable forms such as short-acting, rapid-acting, intermediate-acting, and long-acting insulin. These formulations can be derived from animals or developed using bacteria or yeast cells and rDNA technology. Recombinant human insulin is the most commonly used type of insulin and is available in vials, cartridges, and prefilled pens. Some insulin products also contain other substances that aid in blood sugar control, such as rapid-acting insulin analogs with a swift onset and shorter period of action.

3. Penicillin

Penicillin was the first antibiotic discovered and is still one of the most widely used antibiotics today. Penicillin is a type of antibiotic medication that is mainly used to treat bacterial infections, such as meningitis, pneumonia, gonorrhea, endocarditis, and syphilis [17]. It is a part of the β-lactam class of antibiotic drugs [17]. Penicillin is effective against gram-positive cocci, gram-positive rods, most anaerobes, and gram-negative cocci infections [17].

Penicillin acts by interfering with bacterial cell wall formation by preventing peptidoglycan cross-linking in the cell wall [18]. As the bacteria's cell wall weakens, osmotic pressure forces water into the cell, killing it, while peptidoglycan fragments, which can activate autolysins and hydrolases, further degrade the cell wall [18].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

Penicillin biosynthesis gene cluster amplification is one phenomenon used in high-yielding *P. chrysogenum* strains [19]. Other changes include the over-expression of genes involved in α-aminoadipic acid, side-chain activation, and valine and cysteine biosynthesis [20].

The researchers used protoplast fusion and gene amplification to boost penicillin production in *P. chrysogenum*. Specific genes (*penDE* and *phl*) were amplified with specific primers and introduced into cells with linearized pSW070, pSW071, pSW072, or pSW073 fragments. The cells were then grown in a medium containing only acetamide as a nitrogen source, and gene expression and copy number were determined using qPCR [21].

It was found that by overexpressing the enzyme isopenicillin N acyltransferase (IAT) they were able to increase penicillin production by up to 50% [19].

Clinical Uses

Penicillin is efficacious in the treatment of a wide range of bacterial infections, including strep throat, pneumonia, and skin conditions [17]. One of the most significant clinical implications of penicillin is its role in treating previously fatal bacterial infections such as syphilis and meningitis [17]. Penicillin is also used to prevent infections in people who are at high risk of infection, such as those undergoing surgery or those with weakened immune systems.

Commercial Uses

Penicillin is frequently associated with the treatment of bacterial infections in farm animals and pets like cows, pigs, horses, and so on [22]. It is used in agriculture to prevent and treat bacterial infections in crops via spray or injection into the soil [23]. Penicillin is used in the food industry to control bacterial contamination of food products, such as cheese, yogurt, and meat [24].

4. Monoclonal antibodies

Monoclonal antibodies (mABs) are molecules produced in a laboratory that are intended to mimic the natural antibodies generated by the human immune system. mABS are created through the cloning of B cells [1]. B cells are in charge of producing antibodies, which recognize and bind to a specific antigen [1].

mABs are an excellent example of personalized therapeutics made possible by advances in immunology, molecular biology, and biochemistry [1]. Dr. Edward Jenner used antibody therapy for the first time in 1796 when he inoculated pustular fluid from smallpox lesions to induce immunity in the recipient. Drs Kohler and Milstein did not discover the generation of mAbs for human use until 1975 [1].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

Some of the ways to produce mABs include hybridoma technology, using transgenic animals such as mice, using phage particles, rDNA technology, etc. One such technique is to produce mABs using *E. coli* as a host, more specifically the production of cytoplasmic IgG antibodies [25].

The strain of *E. coli* used for IgG production was SHuffle. The researchers used a bicistronic operon to encode both the heavy and light chains of an antibody, which was then inserted into pET21b, a bacterial expression vector with a strong T7/lac promoter. They optimized synthetic genes for *E. coli* expression and incorporated chaperone genes into the vector to aid protein folding. They tested the expression in both the isogenic and wild-type strains, used various techniques to characterize the expression and folding, and demonstrated the utility of their method by producing functional antibodies against a cancer target. The engineered bacteria were found to be capable of producing and folding the antibody efficiently [1].

Clinical Uses

mABs are highly specific in binding to their particular target, and thus have a wide range of clinical applications. Some mAbs are used to treat cancer by specifically targeting proteins found in cancer cells [26].

mAbs can also be utilized to treat autoimmune disorders like rheumatoid arthritis and multiple sclerosis [2]. mAbs can be radiolabeled and used in diagnostic imaging to detect specific cells or tissues [27].

Commercial Uses

mABs have a high commercial value in a variety of industries and are used for many applications. mAbs are extensively used as therapeutic agents in the biopharmaceutical industry [28]. Trastuzumab for HER2-positive breast cancer and rituximab for non-lymphoma Hodgkin's are two examples [26]. In diagnostics, they are used to detect the presence of specific molecules in a patient's blood or other body fluids, such as pregnancy tests [29].

mAbs can be used to treat infectious diseases by targeting specific proteins found on the surface of viruses or bacteria [30]. Palivizumab for the respiratory syncytial virus [31] and tocilizumab for severe COVID-19 [32] are two examples. They are also used for treating and avoiding animal diseases [33].

5. Leptin

Leptin is a 167 amino acid (16kDa) hormone produced mainly by adipocytes or fat cells and encoded by the LEP gene on chromosome 7 [34]. It regulates appetite and metabolism by acting on receptors in the hypothalamus of the brain [34]. Leptin is produced as a result of changes in fat reserves [34]. Leptin serves several functions in the body. One of its primary functions is to regulate energy balance by signaling to the brain when the body's fat stores and energy reserves are sufficient [34]. Leptin levels rise when fat stores are high, signaling the brain to reduce appetite and increase energy expenditure [34]. When fat stores are low, leptin levels fall, signaling the brain to increase appetite and decrease energy expenditure [34]. Leptin also regulates other physiological processes like fertility, immune function, and bone metabolism [34].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

One common method used to produce recombinant human leptin industrially was through *E. coli*. For cloning and plasmid maintenance, *E. coli* XL1-Blue was utilized. *E. coli* BL21 (DE3) was exploited to express the obese gene from a phage construct. This gene was then subcloned into pUC19 and pET21c, the latter with a NdeI site upstream of the valine codon and a BamHI site downstream of the final cysteine codon, as well as tandem TAA stop codons. The mature obese gene expression was seen after IPTG induction from the strong T7 promoter. DNA manipulation techniques employed included ligation, restriction digestion, and AGE. Optimum culture conditions such as temperature, pH, oxygen concentration, etc. were set to maximize recombinant human leptin production [35].

The mature obese gene was cloned into a pET21c expression vector before being transformed into *E. coli* BL21 cells (DE3). Around 90% of the recombinant human leptin generated after IPTG induction was in the form of inclusion bodies, with leptin levels reaching up to 54% of the entire protein content [35].

Clinical Uses

Leptin deficiency results in severe obesity and metabolic abnormalities in animals and humans due to mutations in the leptin gene or its receptors. In patients with congenital leptin deficiency, recombinant leptin injections can reverse various abnormalities [36]. It has also been shown to regulate the immune system, bone metabolism, and fertility [37,38].

Commercial Uses

Although leptin is not yet currently sold commercially, in rare medical conditions such as congenital leptin deficiency, recombinant human leptin called metreleptin has been approved by FDA for use as a replacement therapy [39].

6. Glucagon

Glucagon is a 29-amino acid single-chain peptide hormone released primarily by pancreatic α cells of the islets of Langerhans [40]. It is obtained from the proglucagon precursor and is crucial in regulating blood glucose levels in the body [40]. Glucagon stimulates the liver's activity, causing it to convert glycogen stored in the liver into glucose, which is then released into the bloodstream via glycogenolysis [40]. It enables gluconeogenesis or glucose production from noncarbohydrate sources like amino acids and fatty acids [40]. By increasing glucose levels in the bloodstream, glucagon helps to raise low blood sugar levels and sustain normal glucose levels in the blood [40].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms.

Several strain improvement methods have been used to increase the production of recombinant glucagon in microorganisms. Some of the methods include culture conditions optimization, genetic engineering, high-density fermentation, media supplementation, etc. One way of production was the expression of recombinant glucagon as a chemically cleavable fusion protein in *E. coli* using the high levels of recombinant human interferon (IFN γ) productivity. This strategy was hence used in *E. coli* to express human recombinant glucagon [41].

The study illustrated that the fusion protein strategy was efficient in producing recombinant human glucagon in *E. coli*. Purification with a sequence-specific enzyme separated the glucagon from the purification tag, yielding a high-purity glucagon product [41].

Clinical Uses

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Glucagon injection stimulates the liver to release glucose into the bloodstream, causing blood sugar levels to rise countering hypoglycemia [42].

Glucagon can relax the esophageal muscles, which can help relieve symptoms and make swallowing easier [43]. By stimulating the heart and increasing blood pressure, glucagon can be used to mitigate the adverse effects of β-blockers [44].

Commercial Uses

GlucaGen is a glucagon injection administered to manage severe hypoglycemia. It comes in a pre-filled syringe or an emergency kit with a syringe and a vial of glucagon powder that must be mixed with sterile water before injection. Baqsimi is a glucagon nasal powder used to treat severe hypoglycemia.

7. Heparin

Heparin is an anticoagulant that is produced naturally in the body by mast cells and basophils in various tissues [45]. It is a highly sulfated anionic glycosaminoglycan with a wide range of relative molecular weight and charge density [45]. These structural characteristics allow heparin to interact preferentially with many proteins, resulting in anticoagulant, antiviral, and anti-inflammatory effects [45]. It is a complex mixture of repeating disaccharide units composed of glucuronic acid (GlcA) or iduronic acid (IdoA) residues linked to N-acetylglucosamine (GlcN) or N-sulfo-N-acetylglucosamine (GlcNS) [45]. It has an important role in the prevention of blood clot formation by inhibiting the activity of various clotting factors such as thrombin, factor Xa, and others.

Molecular Mechanism of Synthesis in Genetically Engineered Microorganisms

Recombinant heparin was produced using an experiment in which microsomal protein was exposed to an *E. coli*-derived polysaccharide to determine sulfation levels [46]. Then, the production of heparin was done using modified *E. coli* K5 as a substrate, as well as the enzymes required [46].

Another method to produce recombinant heparin was to induce the overexpression of heparosan, a heparin precursor. Heparosan was expressed in *E. coli* K5 to manufacture heparin. A defined medium and exponentially fed-batch glucose addition with oxygen enrichment were used to boost heparosan production [47].

Clinical Uses

Heparin is a blood thinner that is used to prevent and cure blood clots in a range of conditions, including, DVT is a disorder in which a blood clot forms in a deep vein, generally in the leg [48].

Heparin may also be given before and after surgery to avoid blood clots. Heparin also possesses anti-inflammatory properties that may be useful in the treatment of certain illnesses, such as sepsis or autoimmune disorders [48].

Heparin derived from genetically modified organisms (GMOs) has not yet received clinical approval. Nevertheless, ongoing research is looking into the potential of GMO-produced heparin for the prevention and treatment of blood clots, as well as its ability to reduce contamination risk and cut costs [48].

Commercial uses

Low molecular weight heparin (LMWH), often known as Lovenox, is a blood thinner. Heparin is utilized to make LMWHs. It is a prescription drug used to treat and prevent chest discomfort and the signs of blood clots [49]. A drug called HepFlush - 10 is used to keep IV catheters open and avoid clotting by preserving normal blood flow. It is a heparin lock flush solution [50].

8. α-Galactosidase

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α-Galactosidase, a glycoside hydrolase enzyme, degrades the terminal α-galactosyl moieties of glycolipids and glycoproteins [51]. The α-galactosidase enzyme is also known as melibiase because it breaks the α-1,6 link between galactose and glucose in melibiose, a reducing disaccharide that releases glucose and galactose [51].

Microorganisms have a strong potential for manufacturing α-galactosidases due to their high levels of expression, extracellular secretion, simplicity of cultivation, and ability to boost production through culture environment optimization [51].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

One of the techniques used was to insert the α-Gal structural genes from *Lb. plantarum* and the legume *Cyamopsis tetragonoloba* (guar) into different *L. lactis* vectors to direct the α-Gal proteins to specific bacterial compartments such as the cytoplasm, cell wall, or medium. The vectors contained strong constitutive or nisin-inducible promoters, signal sequences for membrane translocation, and cell-wall-anchor domains for cell-surface display. The expression of α-Gal genes in *L. lactis* was assessed using chromogenic substrate X-α-Gal and specific antibodies were produced for further analysis [52].

Clinical Uses

Gaucher's disease is a genetic disorder that causes lipid accumulation in the body's cells. To help break down these accumulated lipids, α-galactosidase is used as a replacement therapy [53].

It is used as a replacement therapy for patients with Fabry disease, a genetic disorder that causes glycolipid accumulation in the body's cells. It is administered to aid in the breakdown of accumulated glycolipids [53].

Commercial Uses

As a digestive aid, commercial α-galactosidase is sold under a variety of brand names. Beano, Gas-X Prevention, and Digestive Advantage Gas Defense Formula are some well-known brand names. These products are typically oral supplements.

Furthermore, α-galactosidase is available as a prescription medication to treat Fabry disease and Gaucher's disease under the brand names Fabrazyme and Elelyso, respectively.

9. Erythropoietin

Erythropoietin (Epo) is a 34-kD glycoprotein that promotes the proliferation and development of erythroid cells. It is produced by the peritubular cells present in the kidney [54]. Epo is also produced by the spleen, bone marrow, liver, lungs, and brain in minor quantities [54]. It is usually produced during low levels of oxygen in the body (hypoxia). Epo is essential for maintaining the blood's proper oxygen-carrying capacity [54]. It aids in the production of enough RBCs to meet the body's oxygen requirements. It also helps RBCs survive and avoid death by protecting them from oxidative stress [54].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

Recombinant hEpo (rhEpo) has not been produced using an *E. coli* expression system, despite the possibility of cost-effective therapeutic protein production using this method. This is because rhEpo accumulates in inclusion bodies in the *E. coli's* cytoplasm and is not subjected to post-translational modification. Effects of different protein tags on the solubility of active rhEpo were observed when it was overexpressed in soluble form in *E. coli*. To attain the soluble overexpression of rhEpo in *E. coli*, protein tags like His, GST, TRX, NusA, and MBP were used. The LR recombination cloning method was employed for the creation of vectors expressing rhEpo as hybrid proteins with MBP [55].

Clinical Uses

Epo is a hormone that increases the synthesis of RBCs in the bone marrow. In humans, erythropoiesis can be increased up to 8-fold over baseline levels in many clinical conditions, including hemorrhage, hemolysis, and other types of stress that impair arterial or tissue oxygenation [56]. Epo has been investigated as a possible neuroprotective agent in conditions such as traumatic brain injury, spinal cord injury, and stroke [56]. Another significant benefit of rhEpO is the treatment of anemia in cancer patients undergoing chemotherapy and AIDS patients who develop it as a result of antiviral medications [54]. Epo is used to treat anemia in preterm infants and has been shown to reduce the need for blood transfusions while also improving neurodevelopmental outcomes [57].

Commercial Uses

Epo is sold under a variety of brand names. Epogen, Procrit, and Retacrit are some well-known brand names. There are also biosimilar versions of Epo available under brand names such as Retacrit and Binocrit. These are similar to the original Epo product in terms of safety, efficacy, and quality, but are marketed under a different brand name.

10. Human Growth Hormone (hGH)

Somatotropin, or human growth hormone (hGH), is a 191-amino acid protein that plays an important role in human physiology [58]. hGH, which was discovered in humans in the 1950s, regulates a variety of physiological processes such as skeletal and organ growth, calcium balance, lipolysis, and lean body mass management [58]. During childhood and adolescence, it promotes growth and development, and it regulates body composition, fluids, muscle and bone growth, glucose and lipid metabolism, and heart function throughout life. hGH is a single-chain amino acid hormone that is primarily produced by acidophilic somatotrophs in the anterior pituitary gland. The *GHN* gene, which is part of a gene cluster on chromosome 17, encodes it. The molecular mass of the most common type of hGH is 22kDa. Furthermore, studies have shown that lymphoid cells in nearby lymph nodes can produce hGH[58].

Molecular mechanism of synthesis in Genetically Modified Organisms

Using various strains of *E. coli* is one of the most common methods of producing recombinant hGH. To produce recombinant human growth hormone, researchers used different strains of *E. coli* bacteria along with a plasmid. (rhGH). The plasmid was introduced into *E. coli* cells and allowed to grow in the presence of antibiotics. The researchers used IPTG to induce the cultures to produce rhGH before harvesting the cells and medium. They detected and measured rhGH production using a variety of techniques, including dot blotting, SDS-PAGE, Western blotting, and ELISA. Column chromatography was then used to purify the protein. The resulting rhGH can be used for both therapeutic and scientific purposes [59].

Clinical Uses

hGH therapy benefits adults with growth hormone deficiency (GHD), which can be caused by pituitary tumors, traumatic brain injuries, or other medical conditions. It can also help with bone density and overall health [60]. hGH therapy is used in the treatment of a variety of medical conditions like Turner syndrome, Prader-Willi syndrome, and chronic renal failure [61]. Idiopathic short-stature rhGH is occasionally used to treat children who are shorter than usual but do not have a specific medical disease that is causing their short stature [62].

Commercial Uses

Pharmaceutical companies manufacture and sell rhGH products such as Genotropin, Humatrope, Norditropin, Saizen, and Zomacton, which are injected subcutaneously.

Some rhGH products are prefilled syringes or cartridges, while others are lyophilized powders that must be reconstituted with sterile water before use. The dosing and frequency of rhGH administration also vary according to the individual's medical condition and treatment goals.

11. Spermidine

Spermidine and spermine are positively charged compounds present in eukaryotic cells that are crucial for growth and development. It is a natural polyamine found in various organisms like bacteria, animals, and plants [63]. It has an essential function in protein synthesis, DNA replication, and RNA transcription are examples of biological activities [63]. The history of spermidine can be found in the early 17th century when Antonie van Leeuwenhoek discovered a material in human sperm that he called "animalcules" [63]. Spermidine has now been discovered to be a ubiquitous chemical in living beings and has been intensively explored for its function in cellular processes such as DNA and RNA creation, as well as its potential health advantages. Polyamine levels have long been recognized to decline with age [63]. However, the influence of polyamines, particularly spermidine, on aging has lately been studied.

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

E. coli has been one of the widely used bacteria to produce recombinant spermidine. The research focused on designing and building an efficient method for synthesizing spermidine, which is used to create bioactive compounds in the food and pharmaceutical industries. HSD, CASDH, and CASDC were co-expressed by the researchers to create a whole-cell biocatalytic method for spermidine biosynthesis. For cofactor regeneration, the method employs an NADPH self-sufficient cycle, and a putative CASDC enzyme from *Butyrivibriocrossotus* DSM 2876 was identified and characterized for high decarboxylase activity. Each enzyme's protein expression levels were optimized, resulting in a whole-cell catalyst with high catalytic efficacy [64].

In a 1-L *E. coli* whole-cell catalytic system, the researchers were able to produce a substantial amount of spermidine with a 95.3% molar conversion rate. They achieved this by optimizing temperature, catalyst-to-substrate ratio, and $NADP^+$ amount, resulting in high productivity [64].

Clinical Uses

Spermidine has been studied for its anti-aging properties because it can induce autophagy and improve mitochondrial function, which may aid in preventing age-related diseases like Alzheimer's and Parkinson's [65].

Spermidine has been researched for its potential use in the treatment of certain types of cancer due to its ability to inhibit tumor growth and induce apoptosis in cancer cells [66]. Spermidine has also been shown to have cardioprotective effects and may be useful in the prevention of cardiovascular disease [66].

Commercial Uses

Spermidine is available commercially as a dietary supplement in capsules, tablets, and powders. Its anti-aging and neuroprotective properties, as well as its ability to support cardiovascular and immune system health, are commonly marketed. Some companies also produce and sell spermidine-rich foods as dietary supplements, such as wheat germ and soybeans.

However, it is important to note that regulatory agencies have not thoroughly evaluated the safety and effectiveness of spermidine supplements, and individuals should consult with a healthcare professional before taking any dietary supplements.

12. Human serum albumin (HSA)

The majority of the HSA protein is made up of α-helices, and it has a heart-like configuration. It contains three homologous domains with two long loops and one shorter loop in each. These loops provide functionally distinct subdomains with varying affinity for ligand binding. Subdomains IIA and IIIA, respectively, include Sudlow sites I and II, two significant binding sites [67].

Human Serum Albumin (HSA) is a vital protein in blood plasma that transports hormones, fatty acids, and other substances while assisting in the maintenance of osmotic blood pressure [67]. To enhance the administration of numerous exogenous pharmacological medications, recent research has concentrated on extending the function of albumin as an endogenous ligand transporter [67].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

The albumin gene was cloned into an expression vector and transferred into a host cell, such as bacteria, to produce HSA. The gene was converted to mRNA by the host cell, which was then translated into the human serum albumin by host ribosomes. Folding and post-translational modifications occurred in the protein. After this, the albumin protein was purified from the host cell culture using chromatography, ultrafiltration, and other purification techniques. This technology enabled the efficient and controlled production of large amounts of human serum albumin, which has several applications in various fields [68].

Clinical uses

Albumin is essential for maintaining oncotic pressure in the blood vessels. A drop in albumin levels can cause fluid to extravasate into tissues and cause edema [69]. Albumin is responsible for transporting numerous molecules throughout the blood, such as hormones, fatty acids, medications, and toxins [69].

Decreased amounts of albumin in the blood can be a diagnostic sign for several illnesses, including liver and renal disease, malnutrition, and inflammatory diseases [69].

Many disorders, including hypovolemia, ascites, and hypoalbuminemia, can be managed with albumin infusions. Using rHSA is a safe and effective alternative to plasma-derived albumin in clinical practice [69].

Commercial Uses

Flexbumin is a Human albumin injection used as a priming fluid during cardiopulmonary bypass surgery. Biotinylated Human Serum Albumin Protein, His, Avitag™ is a modified albumin that can be used for a variety of applications, including research into protein-protein interactions, drug delivery, and diagnostic assays. Cellastim-S is an rHSA generated using genetically modified Chinese hamster ovary (CHO) cells. It has a wide range of applications in cell culture.

13. Somatostatin (SST)

The peptide hormone somatostatin (SST), which is created in the brain, inhibits growth hormone (GH) and thyroid-stimulating hormone (TSH), secreted by the anterior pituitary gland. It is also spread out throughout the body, where it has a range of physiological effects. SST is produced as SST-14 and SST-28, two bioactive proteins. The most common type of SST, SST-14, is made up of 14 amino acids connected by a disulfide bond formed by cysteine residues at positions 3 and 14 [8]. The circulatory half-life of SST is only 1.5 to 3 minutes. This suggests that SST-producing cells or SST reserves in nerve endings are typically found near the target cells they affect [8].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

An SST coding sequence was developed with an optimized *E. coli*-preferred codon and was eventually amplified to yield an amplicon comprising the *SST* gene flanked by restriction sites ClaI and SphI. *E. coli* DH5α was used for cloning and plasmid propagation, while *E. coli* BL21 (DE3) and its derivatives were used for protein expression studies [70]. It was discovered that the media containing lactose as a carbon source and the BL21 (DE3) strain of *E. coli* produced the maximum yield of fusion protein without the need for IPTG. SST peptide was produced by further processing the fusion protein and was purified using chromatography [70].

The purity and yield of SST peptide were successfully measured using HPLC and confirmed using MALDI-TOF MS. The results were consistent and reproducible in both small and large-scale tests [70].

Clinical uses

SST reduces gastrointestinal motility. It has been shown to reduce food intake in animals [71]. Some neuroendocrine tumors (NETs) generate high amounts of hormones that manifest a group of symptoms called carcinoid syndrome, which can be treated by SST analogs [71].

Commercial Uses

Patients with acromegaly can utilize Lanreotide to lower their blood levels of growth hormone. Moreover, it acts to treat carcinoid disease and decrease or stop tumor development in individuals with NETs.

Octreotide is a drug used to control and treat acromegaly, thyrotrophinomas, and carcinoid disease. Pasireotide can aid in lowering the excessive production of cortisol by the adrenal gland which is a defining aspect of Cushing's disease.

14. Progesterone

Progesterone is necessary for the maintenance of pregnancy and for the endometrial transition from the proliferative to the secretory stage [72]. It also makes it easier for blastocysts to nest. Diosgenin may be used to make progesterone, which led to the creation of galenic formulations and progesterone-based hormonal therapy [72]. Progesterone is crucial for the circulatory system, CNS, bones, and mammary glands as well as the reproductive system [72].

Throughout the menstrual cycle, progesterone is essential for the endometrium to change from the proliferative to the secretory phase. Throughout the cycle, the expression of progesterone and estrogen receptors in the endometrium fluctuates [72].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

The plasmid used for producing recombinant progesterone contains a gene that codes for the enzyme necessary to convert a precursor molecule into progesterone. The progesterone synthase gene is isolated from the corpus luteum of the ovary. The extracted gene was cloned into a bacterial expression vector, such as pET or pUC, that had a promoter sequence to activate gene expression. The plasmid was inserted into bacteria via heat shock [73].

Using different methods including chromatography, ultrafiltration, and precipitation, the recombinant progesterone was isolated from the culture medium. Using methods like mass spectrometry and HPLC, the recombinant progesterone was characterized and evaluated for quality [73].

Clinical uses

Progesterone is combined with estrogen in hormone replacement treatment for menopausal women who still have their uterus. In women who have low levels of progesterone, progestin replaces natural progesterone and reduces the quantity of estrogen in the uterus during hormone replacement treatment [74].

Progestins are used to treat several disorders, including endometriosis, irregular uterine bleeding, and menopausal symptoms, in addition to contraception [74]. It can be used to treat autoimmune conditions including lupus and multiple sclerosis [75].

Commercial Uses

Act Progesterone Injection is injected intramuscularly and aids in supporting pregnancy in women who have experienced repeated losses or who have undergone certain reproductive procedures like IVF. Bijuva is a capsule containing estradiol and progesterone. It is used to treat mild to severe hot flashes as well as other menopause-related symptoms. Crinone is a vaginal progesterone gel, which is used as part of fertility treatment and hormone replacement therapy.

15. Interleukin - 6 (IL-6)

Interleukin-6 (IL-6) is a pleiotropic cytokine having a variety of biological functions. It helps control immunological reactivity, the acute phase response, inflammation, oncogenesis, and hematopoiesis [76]. It is generated by both lymphoid and nonlymphoid cells. The human *IL-6* gene is located on chromosome 7 and is around 5 kb long [76]. The T and B cell development and activation, as well as the control of immunological responses during inflammation and infection, are all regulated by the IL-6 signaling pathway [76]. It regulates immunological responses, growth and development, and tissue homeostasis among other processes [76].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

Using an expression vector, the researchers attempted to produce human IL-6 in *Pichia pastoris*. Transformants of *E. coli* and *P. pastoris* were chosen, and PCR amplification was used to confirm the presence of the IL-6 gene. The IL-6 cDNA was augmented in a plasmid, and a nucleotide sequence encoding the KEX2 cleavage site was added upstream of the IL-6. The fragment was then introduced into the expression vector, where the alcohol oxidase1 promoter regulated IL-6 gene expression. Sequence analysis confirmed the recombinant construct [77].

Clinical significance

IL-6 is essential for controlling immune response, inflammation, and acute phase response. It is a clinically important predictor of cancer development and a possible tumor marker for cancer detection [76].

IL-6 plays crucial functions in cellular differentiation and expansion, and it can control the production of tumor antigens and immunosuppressive factors [76].

Commercial Uses

IL-6 is available commercially as a research reagent for in vitro studies. Although it is not yet available commercially, various studies are being undertaken to commercialize IL-6.

16. Human Interferon-gamma

Interferon-gamma (IFN-γ) is a cytokine, which also stimulates neutrophils and natural killer cells, and is essential for both innate and adaptive immunity. It serves as the main activator of macrophages. IFN-γ may be secreted by a variety of cell types, although it is typically produced by $CD4^+$ Th1 lymphocytes, NK cells, and $CD8^+$ cytotoxic lymphocytes [78].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

Human IFN-γ is a glycosylated protein. Recombinant hIFNγ, on the other hand, is not glycosylated but is still functional when produced in *E. coli*. For the expression of rhIFN, *E. coli* strain BL21 was employed as the host. The hIFN-γ gene was introduced into the NotI and NdeI sites of the widely available plasmid pET3a, an inducible expression vector (Novagen), to convert this strain. The expression level of rhIFN-γ was measured using polyacrylamide gels [79].

Clinical significance

IFN-γ controls T cell development, activation, expansion, homeostasis, and survival and is crucial for Th1 immune responses [79]. The significance of IFN-γ in the treatment of various malignancies, such as ovarian cancer and bladder carcinoma was investigated. Overall, IFN-γ has the potential as a cancer therapy target [79]. IFN-γ may activate alveolar macrophages, which are crucial for host defense against *M. tuberculosis* and has been studied as a possible therapeutic agent [79].

Commercial Uses

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Actimmune is a drug used to treat severe, malignant osteopetrosis and persistent granulomatous illness. Imukin is a brand name for the medication INF-γ-1b and has been considered a potential therapy for disorders including TB, cystic fibrosis, chronic granulomatous disease, and osteopetrosis.

17. FGF 21

FGF21 is a protein that is mainly synthesized in the liver and has a crucial role in regulating different aspects of homeostasis in various tissues [80]. The FGF family contains 22 proteins that act as signaling molecules with diverse functions [81]. FGFs play complex physiological roles in regulating cell growth, differentiation, tissue repair, inflammatory response, and metabolic modulation [82]. FGF21 belongs to the FGF19 subfamily of hormone-like FGFs [82]. FGF21 plays a significant role in regulating important biological processes, including metabolic modulation and protective effects after injury, in humans and mammals [82].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

The generation of human recombinant FGF 21 (rhFGF21) has potential therapeutic applications for metabolic disorders as an endocrine hormone [83]. The researchers wanted to boost the efficiency of bioactive rhFGF21 production by expressing a codon-optimized rhFGF21 gene in *B. subtilis* under the influence of a strong inducible promoter. A mini-cistron cassette was introduced upstream of rhFGF21 to improve translation start efficiency even further. The adoption of an extracellular proteases-lacking strain of *B. subtilis* Kno6cf aided in the effective accumulation of released rhFGF21. SPdacB exhibited the highest secretion yield of the 11 signal peptides tested from *B. subtilis*. The combinatorially optimized strain produced much more soluble rhFGF21 than the initial production strain [83].

Clinical Uses

Recent research has revealed that FGF21 can inhibit inflammation, reduce oxidative stress, and protect against apoptosis of endothelial cells and cardiomyocytes.

FGF21 activates downstream factors such as AMP-activated protein kinase, Akt, and ERK to regulate heart and brain damage, material metabolism, and metabolic functions such as free fatty acid oxidation and glucose uptake [84].

In-depth research into FGF21's systemic biological roles can aid in the diagnosis and treatment of metabolic illnesses like obesity and diabetes, as well as heart and brain diseases like myocardial IRI and AD, stroke, and TBI.

Commercial Uses

FGF21 is a protein found much higher in those suffering from atherosclerosis, hypertension, or liver disease [85–87]. FGF21 levels are more significant in those with non-alcoholic fatty liver disease (NAFLD), making it a possible diagnostic marker for NAFLD [88]. Adeno-associated viral (AAV) vectors were used to deliver and generate FGF21 for long-term therapy of obesity and insulin resistance [89].

FGF21 is not currently approved for commercial use by the FDA. As prospective therapies for these disorders, some pharmaceutical companies are developing FGF21 analogs or mimetics.

18. Phytochemicals

The word "phytochemicals" encompasses a range of plant-based substances with distinct structures that have the potential to promote good health. Phytonutrients are natural compounds produced by plants, but they are usually not considered nutrients in the conventional sense because they are not made in plants for metabolic processes. Phytonutrients play significant roles in the secondary metabolism activity of plants by serving as natural repellents against pests, protection from ultraviolet radiation, pigments, flavors as well as growth regulators. They are typically found in fewer amounts and often have a pharmacological significance [90].

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Fruits and vegetables have high amounts of phytochemicals, which shield against free radical damage. Phenolic compounds like tannins, flavonoids, and lignins act as antioxidants [91].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

Researchers have developed a bacterial system capable of producing plant alkaloids from simple carbon sources. They created a specific biosynthetic route in *E. coli* cells that can overproduce L-tyrosine to produce alkaloids. The modified strain was able to manufacture 46.0 mg l1 of (S)-reticuline from glycerol using this technique [92].

Experiments have also demonstrated a yeast platform for producing tropine from simple carbohydrates by integrating numerous overexpressed and disrupted genes from plants and bacteria. The de novo tropine production was accomplished by expressing PKS and cytochrome P450. The technology was also used to develop cinnamoyl-tropine, a synthetic tropane ester. The findings lay the groundwork for future engineering efforts aimed at optimizing tropane alkaloid production for industrial uses [93].

Clinical Uses

Phytochemicals exhibit potential in the normal functioning of the immune system, reduction of inflammation, protection of cells from DNA damage, regulation of hormones, and reduction of the growth rate of cancerous cells. It was reported that consuming six types of flavonoids – like flavones, flavonols, anthocyanidins, flavanones, proanthocyanidins, and flavan-3-ols - through the diet can significantly reduce the risk of cardiovascular disorders [94].

A meta-analysis showed that there is a significant relation between the consumption of higher levels of flavonols and a reduced risk of stroke [95]. In a comprehensive analysis, it was found that increased levels of α-carotene, β-carotene, and lycopene were significantly correlated with a reduced risk of breast cancer [96].

Commercial Uses

Urolithin A is a unique natural food metabolite that is effective in human clinical trials. It can stimulate mitophagy, which can improve mitochondrial function [97].

Benzylisoquinoline alkaloids (BIAs) include analgesics such as codeine and morphine, as well as antimicrobial compounds such as palmatine and berberine [92]. Elliptinium is used in the treatment of breast cancer [98]. Vinblastine is utilized in the treatment of numerous cancer types, including lung, breast, and advanced testicular cancer, lymphomas, leukemias, and Kaposi's sarcoma, in tandem with vincristine and other anticancer medications [98].

19. Terpenoids and Terpenes

Terpenoids are the most diverse and abundant natural product class, with the majority coming from plants. Plants utilize terpenoid metabolites for various fundamental purposes in their growth and development, but they allocate the majority for more specific chemical interactions and defense against both non-living and living environmental factors [99]. Certain terpenoids have been identified to serve diverse functional roles in photosynthetic pigments, electron carriers, hormones, mediators of communication, polysaccharide assembly, and defense mechanisms [100]. Terpenes are composed of simple hydrocarbon structures, while terpenoids are considered a modified type of terpenes that contain various functional groups and oxygenated hydrocarbons, with methyl groups that may have been relocated or removed from different positions [101].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

Several genetic techniques can be used for genome engineering in *E. coli* and yeast, with homologous recombination being commonly used. By using this technique to tune down the glyceraldehyde 3-phosphate synthase, the supply of glyceraldehyde 3-phosphate and pyruvate was rebalanced, leading to an increase in lycopene production via the MEP pathway in *E. coli*[102].

MAGE, which can target many genomic loci at once and accurately edit specific loci was employed by researchers to enhance lycopene production in *E. coli* by optimizing the MEP route. A modified form of MAGE has also been successfully employed to generate β-carotene in S. cerevisiae [103].

Scientists integrated genes linked to mitochondrial-targeting signal sequences from the MVA pathway into the genome of *Saccharomyces cerevisiae*. This allowed the yeast to use both cytosolic and mitochondrial acetyl-CoAs through the native cytosolic MVA pathway and a novel external mitochondrial MVA pathway. This genetic modification resulted in the yeast producing more isoprene when compared to using only the cytosolic MVA pathway [104].

Clinical Uses

Fruits and vegetables are rich in terpenoids and have been linked to the prevention and treatment of chronic ailments such as cancer and heart disease [105]. Different terpenoid molecules have anti-inflammatory, antimicrobial, antiparasitic, antiviral, antifungal, antiallergenic, antihyperglycemic, chemotherapeutic, immunomodulatory, and chemotherapeutic properties [100]. Terpenes and terpenoids have been proven to be effective in reducing inflammation in respiratory inflammation, arthritis, neuroinflammation, and atopic dermatitis [101].

Commercial Uses

Taxol® and Artemisinin are well-known drugs derived from terpenes, with Taxol® being used for cancer treatment and Artimesinin used for treating malaria. Among plant derivatives, diterpenoids are the only anticancer drug that has been FDA-approved. Paclitaxel showed potent antimitotic activity against ovarian and breast cancers, and AIDS-related Kaposi's sarcoma [100]. 1,8-cineole has been proven to be beneficial in the treatment of diseases such as sinusitis, bronchitis, and steroid-dependent asthma, and for recurring respiratory infections [100].

The potential use of terpenes and terpenoids as antibacterial agents is highly promising. Limonene is demonstrated to show synergistic modulation effects with antibiotics like gentamycin by inhibiting *S. aureus* and *E. coli* [106,107].

20. Tissue plasminogen activator (tPA)

Mammalian plasma contains an enzyme system that is capable of breaking down fibrin in blood clots. Plasminogen activators are vital constituents of the fibrinolytic system because they produce the active enzyme plasmin through limited proteolysis of the zymogen plasminogen [108]. Tissue plasminogen activator (tPA) is a serine protease with a molecular weight of 64 kDa. It has a length of 527 amino acids with 35 cysteine residues which helps to generate 17 disulfide bonds. There are 5 different structural domains in this protein: a finger-like domain, two kringle domains, an epidermal growth factor-like subdomain, and a catalytic domain [109].

The conversion of plasminogen to plasmin is the primary reaction of plasminogen activators which is crucial for breaking blood clots. The significant increase in t-PA in the area where thrombin is produced enables the effective removal of fibrin accumulations on the inner lining of blood vessels, which is crucial for preventing tissue damage [110].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

There are two prevalent methods to enhance the proper folding of composite proteins in *E. coli*. The first method involves altering the condition of the cytoplasm by transforming its reducing condition into an oxidizing one. The second technique involves majorly the concept of secreting the protein into the periplasm which has a less reducing environment [109].

It was established experimentally that tPA fusion to the stII leader peptide was successful in guiding the mature protein into the bacteria's periplasm. When the tPA gene was linked to another leader peptide called OmpA, a comparable amount of active tPA was produced. The most significant

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amount of functional tPA was detected when the production of DsbC was first induced from a trc promoter, and then tPA expression was induced 30 minutes later.

In another study, a signal peptide was utilized in an *E. coli* strain for disulfide-bonded protein production along with an engineered cytoplasm. A signal peptide was inserted at the 5′ site of the tPA gene creating an expression cassette that eventually transformed into a strain with engineered oxidized cytoplasm. This ensures that the production of protein happens under oxidizing conditions of cytoplasm and the subsequent disulfide bond formation to a certain extent. Later signal peptide transports the synthesized protein to the periplasm creating a more favorable environment for disulfide bond formation, ensuing in the production of a substantial amount of disulfide bonds [109].

Clinical Uses

tPA is instrumental in the clearing of intravascular fibrin deposits as well as other pathological and physiological functions in the brain. It promotes synaptic plasticity, long-term potentiation, learning, as well as neuronal cell migration in the course of development [110]. The administration of tPA has been demonstrated to lower the mortality rate in patients with AMI [111]. It has also been used as a clinical diagnostic marker for aortic valve sclerosis (AVSc) [112].

Commercial Uses

tPA is a beneficial thrombolytic agent due to its very high binding towards fibrin [8]. tPA produced from recombination techniques has been extensively utilized in human studies, animal investigations, and recently in clinical practice [113].

Recombinant tPA has proven to be a life-saving treatment for acute myocardial infarction and other thromboembolic illnesses [114]. Recombinant tPA expression has proven successful in producing adequate levels for clinical usage on a wide scale majorly in patients suffering from AMI, ischemic stroke, major pulmonary embolism, peripheral artery occlusion, and deep vein thrombosis [114].

21. Vitamin B12

Vitamins are essential for optimum health because they participate in several metabolic activities of the body [115]. They are classified as water-soluble or fat-soluble. Vitamin B12, generally known as cobalamin, is available in diverse forms, including cyano-, methyl, deoxyadenosyl-, and hydroxy-cobalamin. Cyano form is majorly found in supplements, but it can also be found in trace levels in food. Vitamin B12 is essential for purine and pyrimidine synthesis, dependent on cofactors such as methylcobalamin and folate. TCI and TCII proteins transport vitamin B12 in the blood, with holo-TC delivering the vitamin to cells [116]. Vitamin B12 is necessary for central nervous system growth, myelination, and function, as well as proper red blood cell creation and DNA synthesis.

Vitamins are crucial elements that contribute significantly to metabolic function and general health. They have broad implications in industries like food, cosmetics, medicine, and feed. Given the expanding global need for vitamins, there is an increased interest in developing innovative production processes. Employing microbial cell factories to create vitamins is a sustainable, ecologically beneficial, and economically viable option [115].

Molecular mechanism of synthesis in Genetically Engineered Microorganisms

Scientists successfully engineered *E. coli* to produce Vitamin B12 by introducing 28 genes from various organisms. These genes were divided into six modules, with each module responsible for producing a different intermediate or enzyme required for Vitamin B12 synthesis. The first two modules produced HBA and CBAD, while the third module helped the bacteria to uptake cobalt. The fourth module synthesized AdoCbi-P, which was then converted to AdoCbl by the enzymes in the fifth module. The sixth module was designed to increase the production of Uro III, a precursor to HBA. Each module was either expressed on its plasmid or integrated into the *E. coli* genome. By doing so, they were able to achieve a high Vitamin B12 yield [117].

Clinical Uses

B12 plays a significant role in cellular metabolism, particularly in DNA synthesis, methylation, and mitochondrial metabolism [116]. It is required for many bodily processes, such as maintaining normal brain and nervous system function, promoting cognitive function, preventing congenital abnormalities, assisting in the genesis of RBCs to prevent anemia, protecting the eyes from macular degeneration, and aiding in energy production.

A shortage of B12 in the body reduces the synthesis of normal red blood cells, leading to anemia, which affects oxygen delivery. Megaloblastic anemia, low blood cell counts, glossitis, weariness, palpitations, pale skin, dementia, weight loss, infertility, and neurological abnormalities including numbness and tingling in hands and feet are all symptoms of vitamin B12 insufficiency [118,119]. Pregnant and breastfeeding women who are deficient in vitamin B12 put their children at risk of neural tube abnormalities, developmental delays, and anemia [119].

Commercial Uses

Vitamin B12 is utilized in nutrition in many forms such as food/feed supplements and grades. Vitamin B12 is mostly utilised as a feed additive for poultry, and cattle [120]. Vitamin B12 is routinely added in morning cereals, energy bars, and plant-based milk replacements to boost the nutritional value of fortified foods and beverages.

It is used in animal feed to improve the growth and development of livestock, which can enhance the quality and quantity of meat and milk produced [120]. Additionally, vitamin B12 is widely used in medical applications such as treating vitamin B12 deficiency, pernicious anemia, and certain neurological disorders [121].

22. Conclusion

In conclusion, using genetic engineering to modify microbes to produce nutritional food, supplements, and pharmaceuticals with improved safety and efficacy has shown promise in improving nutritional value and health benefits. These technologies can potentially revolutionize the food and pharmaceutical industries while improving global public health. However, concerns about safety and regulation must be addressed to ensure that modified microbes do not endanger human health or the environment. Despite these challenges, the potential benefits of bioengineering microbes must be considered, and more research is needed to fully realize this technology's potential.

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