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PROJECT FOR MICROBIAL BIOTECHNOLOGY (MBIO566)

MICROBIAL BIOTECHNOLOGY

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Introduction :

Biotechnology is the part of natural science, which manages the control through genetic engineering of living of living organisms or their components to produce helpful products for different applications in natural sciences. Therefore, microbial biotechnology and its applications in feasible advancement of agribusiness and ecological wellbeing are getting better attention. Microorganisms/miniature living beings are generally microscopic little animals are put in various gatherings such bacteria, parasites, protozoa, micro-algae and viruses. These living beings live in soil, water, food, animal digestion tracts and other various conditions. Different microbial habitats reflect an enormous variety of biochemical and metabolic traits that have arisen by genetic variation and natural selection in microbial populations. They was utilized some of microbial variety in the production of fermented food sources like bread, yogurt, and cheddar. Some soil microorganisms release nitrogen that plants need for growth and emit gases that maintain the critical composition of the Earth's atmosphere. Microbial biotechnology is an important area that promotes for advances in food safety, food security, value-added products, human nutrition and functional foods, plant and animal protection, and overall fundamental research in the agricultural sciences. A genome is the totality of genetic material in the DNA of a specific organism. Genomes vary significantly in size and succession across various organic entities. Acquiring the total genome succession of a microorganism gives critical data about its biology, however it is just the initial move toward understanding a microbe's biological capacities and adjusting them, if necessary, for agricultural purposes. Microbial biotechnology, enabled by genome studies, will lead to breakthroughs, for example, improved vaccines and better disease-diagnostic tools, improved microbial agents for biological control of plant and animal pests , changes of plant and animal pathogens for reduced virulence, development of new industrial catalysts and fermentation organisms, and improvement of new microbial agents for bioremediation of soil and water contaminated by agricultural runoff (16). Biotechnology is the utilization of living systems and organisms to develop or make products, or "any technological application that utilizes biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use" (UN Convention on Biological Diversity, Art. The American Chemical Society characterizes biotechnology as the application of biological organisms, systems, or processes by various industries to learning about the science of life and the improvement of the value of materials and organisms, for example, pharmaceuticals, crops, and livestock. According to European Federation of Biotechnology, biotechnology is the integration of natural science and organisms, cells, parts there of, and molecular analogues for products and services. Advancement of biotechnology applications was started in 6000 BC, however improvement of genetic tools and cellular and tissue engineering gave the start in 1970s then after numerous the speed of biotechnological work gain momentum (2). Microorganisms are the gathering of tiny organism that are available everywhere in soil air and in water. Human being is exploiting microbes since ancient era. The science of producing curd, bread and alcohol was existed before modern civilization. Early man used to de-formed agricultural and kitchen waste by burying them in the soil and letting them for many months that now a day known as composting (17).

Microbial biotechnology to sustainable development:

Sustainable development involves diverse and complex approaches Human stewardship of the planet, in particular its biosphere, is wanting: the trajectories of deterioration of critical features of the biosphere (loss of biodiversity, climate change, desertification, unbalanced N and P cycles, water quality and quantity) and the quality of the human condition (hunger, poverty, regional conflicts, refugees, human trafficking, rising health costs, diminishing urban security), so planet Earth and human life are becoming increasingly unsustainable (‘Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs). To counteract this divergence, and to reorient evolution of the biosphere towards a more sustainable trajectory, internationally accepted Sustainable Development Goals have been formulated by the United Nations that take into account the fact that all key biosphere and relevant human behavioural processes are interconnected, interdependent and hence must be steered via a systems approach. As a consequence, sustainable development goals are exceptionally diverse, ranging from poverty elimination to moderation of climate change, via safe cities, sustainable use of aquatic and terrestrial systems, to adoption of renewables. As all goals encompass environmental, economic and social aspects, efforts to achieve sustainability are of necessity highly complex (8). Microbial diversity and microbial technology are critical to achieve a majority of Sustainable Development Goals (SDGs), mainly due to the central role of microbes in the provision and regulation of ecosystem services. For example, microbial communities play a vital role in primary productivity, via nutrient cycling, and disease regulation, thereby impacting human, animal and plant health, and hence farm productivity and food security. Microbes and microbial technology are now increasingly employed in disease prevention and therapy, and to sustainably increase farm profitability, productivity and nutrient quality which directly contribute to SDGs 1, 2, 3. Similarly, microbes have critical roles in regulating climate via their contributions to both production and consumption of greenhouse gas (GHG) emissions and they create environments (e.g. soil structure) to support the growth of other organisms on land and water. A number of microbial technologies are used or in developmental pipeline for removal of GHGs and for purification of waste water for consumption. Microbes are a source of an exceptional range of chemicals and chemical catalysts, bioactive substances, biomaterials and of important forms of bioenergy, including hydrocarbons and electricity. They are key agents of pollutant removal and recycling. Microbes have been a source of industrial productivity throughout human history and currently are main drivers of the bioeconomy and industry, which are worth several trillion dollars, thereby contributing directly to SDGs 8 and 9 (8). Using microbiota for therapeutic purposes contributes directly to Sustainable Development Goal. Microbiome therapies and , live biotherapeutics products LBPs have opened opportunities and perspectives that are clearly aligned with the directions of sustainable development. Their impact on SDG 3 – 'Good Health and Well-being' – is significant as articulated in the previous sections. In addition, these new tools are providing a different approach for professionals such as researchers, medical doctors, clinicians and pharmaceutical experts alike. Interestingly, this angle has the potential to change the mindset of workers in the field as well as anyone involved in high-level professional development and education (11). The teaching of microbiome therapies and their underlying concepts means that the human body will be considered more carefully not as a single organism but as a host of a whole community with equilibrium and balance.

Role of microbes in sustainable agriculture:

Biotechnology is a very fast growing division in biological sciences in present days and it also expanded in sustainable agriculture production. Beneficial microbes in agricultural systems are used as biofertilizers, biopesticides, bio-herbicides, bioinsecticides, fungal based bioinsecticides, bacterial based bioisecticides and several viral based bioinsecticides for the enhance production and protection of cereals and other plants. With the help of new biotechnological tools, exploitation of microbial genomes as well as invention of many new, creative ways are utilized for the production of new important beneficial microbes. The research challenge is to meet sustainable environmental and economical issues without compromising yields. exploiting the agro-ecosystem services of soil microbial communities appears as a promising effective approach(4).

Biofertilizers:

Biofertilizers are basically live formulates which include living microorganisms which, when pertained to seed, plant surfaces, root, or soil, inhabit around the rhizosphere and boost the bioavailability of nutrients and escalating the microflora through their biological activities, and thereby promoting plant's growth. Biofertilizers are formulations that readily progress the fertility of land using biological agents. Biofertilizers are collected and prepared from biological wastes and are not hazardous to soil. Biofertilizers are not only valuable for the enriching soil quality but also facilitate to fight with the pathogens. Microorganisms that are usually employed as biofertilizers constituent are nitrogen fixers (N-fixer), potassium solubilizer (K-solubilizer), and phosphorus solubilizer (P-solubilizer), or with the mixture of molds or fungi. Most of the bacteria included in biofertilizer have close association with plant roots. Rhizobium has symbiotic interaction with legume roots, and rhizobacteria reside on the root surface or in rhizosphere soil. The chief resources of biofertilizers are bacteria, fungi, and cyanobacteria (blue-green algae). The association of these organisms have with plants is referred to as symbiosis. In this case, both collaborators derive benefits from each other .It is also very important to state that there are few instances when they have least or no effects.. The fungal inoculate progress crop yield because of improved availability or uptake or absorption of nutrients, stimulation of plant growth by hormone accomplishment or antibiosis, and by decomposition of organic deposits .***Penicillium*** species have been also used as a fungal biofertilizers which improve plant growth. These biofertilizers are PSMs that progress phosphorus absorption in plants and encourage plant growth (4) .

Biopesticides and plant growth regulators:

Biopesticides are certain types of pesticides obtained from such natural materials as animals, plants, bacteria, and certain minerals. In commercial terms, biopesticides comprise microorganisms that manage pests (microbial pesticides), naturally occurring substances that control pests (biochemical pesticides), and pesticidal materials produced by plants containing added genetic material (plant incorporated protectants). Biopesticides are engaged in agricultural use for the purposes of insect control, disease control, weed control, nematode control, and plant physiology and efficiency. Biopesticides are usually inherently less toxic than conventional pesticides. They provide growers with valuable tools by delivering solutions that are highly effective in managing pests, without creating negative collisions on the environment. They generally affect only the target pest and closely related organisms, in contrast to the broad range conventional pesticides that may affect organisms as different as birds, insects, and mammals. Overall, the biopesticides have very partial toxicity to birds, fish, bees, and other wildlife thus helping in maintaining beneficial insect populations(4). Chemical pesticides, such as halogenated, carbamate and organophosphorus compounds have been used widely for agriculture system. Their use as pest control results in several problems, such as toxic effect on wild life, human and domestic animals; chemical changes on undesired insects/pests on their predators, parasites and contamination of ground water . Biopesticides include biofungicides (Trichoderma), bioherbicides (Phytopthora) and bioinsecticides (***Bacillus thuringiensis, B. sphaericus***) are preferred over conventional pesticides due to biodegradable nature, highly effectiveness, target specificity and less environmental risks . Biological pesticides based on pathogenic microorganisms specific to a target pest and pose less threat to the ecosystem. The potential benefits to agriculture and public health programmes through the use of biopesticides are considerable. Over 100 bacteria have been identified as insect pathogens, among them ***Bacillus thuringiensis*** (Bt), a gram-positive endospore forming bacteria, has got the maximum importance as an insecticidal bacterium to control caterpillar pests, fly and mosquito larvae, and beetles . Bt produces insecticidal endotoxin protein during spore formation that binds to and destroy the cellular lining of the digestive tract, causing the insect to stop feeding and die. The protein kills mainly caterpillars of the Lepidoptera (butterflies and moths), mosquito larvae, and simuliid blackflies. Bt sprays are used on fruit and vegetable crops, on broad-acre crops such as maize, soya bean and cotton . **Biofungicides** have been used in both the phylloplane and rhizosphere to control plant diseases caused by fungi, bacteria or nematodes including some insect pests and weeds. The most common commercial fungal bio-pesticides used in agricluture and forestry are ***Trichoderma spp***. and ***Beauveria bassiana. Trichoderma harzianum*** is an antagonist of ***Rhizoctionia, Pythium, Fusarium*** and other soil-borne pathogens***. Beauveria bassiana*** and ***Metarhizium anisopliae*** are parasitic fungi found on many insect species***. Beauveria bassiana*** has been proved effective in controlling crop pests such aphids, thrips and whitefly pesticide resistant strains. ***Metarhizium anisopliae*** is used against spittlebugs on sugarcane and grassland and furthermore for the control of locust and grasshopper pests in Africa and Australia. ***Coniothyrium minitans*** is a mycoparasite applied against ***Sclerotinia sclerotiorum*** (15-17) .

Molecular biology of relevance in in industrial microbiology and biotechnology:

The availability of complete genomes from many organisms is a major achievement of biology. Aside from the human genome, the complete genomes of many microorganisms have been completed ,One technology important in studying functional microbial genomics is the use of DNA Microarrays. **Microarrays** are microscopic arrays of large sets of DNA sequences that have been attached to a solid substrate using automated equipment. These arrays are also referred to as microchips, biochips, DNA chips, and gene chips. DNA microarrays are small, solid supports onto which the sequences from thousands of different genes are immobilized at fixed locations. The supports themselves are usually glass microscope slides, silicon chips or nylon membranes may also be used. The DNA is printed, spotted or actually directly synthesized onto the support mechanically at fixed locations or addresses. The spots themselves can be DNA, cDNA or oligonucleotides. The process is based on hybridization probing. Single-stranded sequences on the microarray are labeled with a fluorescent tag or flourescein, and are in fixed locations on the support**. In microarray assays** an unknown sample is hybridized to an ordered array of immobilized DNA molecules of known sequence to produce a specific hybridization pattern that can be analyzed and compared to a given standard. The labeled DNA strand in solution is generally called the target, while the DNA immobilized on the microarray is the probe, a terminology opposite that used in Southern blot. Microarrays have the following advantages over other nucleic acid based approaches:

a. High through-put: thousands of array elements can be deposited on a very small surface area enabling gene expression to be monitored at the genomic level. Also many components of a microbial community can be monitored simultaneously in a single experiment.

b. High sensitivity: small amounts of the target and probe are restricted to a small area ensuring high concentrations and very rapid reactions.

c. Differential display: different target samples can be labeled with different fluorescent tags and then hybridized to the same microarray, allowing the simultaneous analysis of two or more biological samples.

d. Low background interference: non-specific binding to the solid surface is very low resulting in easy removal of organic and fluorescent compounds that attach to microarrays during fabrication.

Microarray technology is still young but yet it has found use in a some areas which have importance in microbiology in general as well as in industrial microbiology and biotechnology, including disease diagnosis, drug discovery and toxicological research. Microarrays are particularly useful in studying gene function. A microarray works by exploiting the ability of a given mRNA molecule to bind specifically to, or hybridize to, the DNA template from which it originated. By using an array containing many DNA samples, it is possible to determine, in a single experiment, the expression levels of hundreds or thousands of genes within a cell by measuring the amount of mRNA bound to each site on the array. With the aid of a computer, the amount of mRNA bound to the spots on the microarray is precisely measured, generating a profile of gene expression in the cell. It is thus possible to determine the bioactive potential of a particular microbial metabolite as a beneficial material in the form of a drug or its deleterious effect. When a diseased condition is identified through microarray studies, experiments can be designed which may be able to identify compounds, from microbial metabolites or other sources, which may improve or reverse the diseased condition (10).

Therapeutic recombinant proteins:

Many years have passed since recombinant human insulin, the first medicine made via recombinant DNA technology, was approved by the FDA (6). Today we are witnessing the continuous rise in the number of approved protein therapeutics (19) and there is little doubt that biopharmaceuticals have the potential to become the medicines of the future.Recombinant DNA technology not only allows therapeutic proteins to be produced on a large scale but using the same methodology protein molecules may be purposefully engineered. Genetic modifications introduced to a protein have many advantages over chemical modifications. Genetically engineered entities are biocompatible and biodegradable. The changes are introduced in 100% of the molecules with the exclusion of rare errors in gene transcription or translation. The preparations do not contain residual amounts of harsh chemicals used in the conjugation process. Low cost and simplicity of cultivating bacteria make the E. coli expression system a preferable choice for production of therapeutic proteins both on a lab scale and in industry. In addition straightforward recombinant DNA technology offers engineering tools to produce protein molecules with modified features. The lack of posttranslational modification mechanisms in bacterial cells such as glycosylation, proteolytic protein maturation or limited capacity for formation of disulfide bridges may, to a certain extent, be overcome with protein engineering. Protein engineering is also often employed to improve protein stability or to modulate its biological action. More sophisticated modifications may be achieved by genetic fusions of two proteins(7).

Glycosylation:

One of the major types of posttranslational modifications absent in E. coli is glycosylation. The biological roles of glycans added to a protein in the glycosylation process span the spectrum, from those that appear to be relatively subtle, to those that are crucial for the development, growth, functioning, or survival of the organism that synthesizes them . Obviously these proteins for which the glycosylation plays an important role in their biological activity must be expressed in mammalian cells. However there are glycoproteins which do not require glycans to exert their function. In such cases the E. coli expression system may be successfully employed. It was shown for instance that the recombinant human interleukin-2 (IL-2) produced in E. coli has the same biological activity as the glycosylated IL-2 version isolated from cultured mammalian cells 3. Indeed aldesleukin (recombinant IL-2) which was marketed by Chiron Corporation (part of Novartis since April 2006) to treat patients with metastatic melanoma under the brand name Proleukin is produced in E. coli. Recombinant non-glycosylated IL-2 retains its full biological activity but the presence of the glycans substantially improves this protein's solubility. Limited solubility of the non-glycosylated IL-2 is a serious problem not only because the dose of the therapeutic protein has to be increased to compensate for the lost activity but also because subsequent aggregation may cause immunogenicity (14).

The application of microorganisms:

Microorganisms are of immense importance to environment and essential to all life forms, and are primary source of nutrients and act as chief recycler in environment . Microorganisms are present in extremely large sphere of environment and thrive from abyssal zone to stratosphere and in a wide range of temperatures ranging from arctic ice to boiling volcanoes . These microscopic organisms are used in the preparation of variety of foods and also used as a source of food and feed supplements. For example, amino acids are obtained from Corynebacterium, ***Brevibacterium*** and ***Escherichia coli*** vitamins from ***Propionibacterium*** and ***Pseudomonas*** organic acids from ***Aspergillus, Lactobacillus, Rhizopus***, and enzymes from ***Aspergillus, Bacillus*** . Microbes have been recognized considerably for their potential in the development of bioprocess technologies for unhindered production of food products and supplements to meet increasing demand by continuously growing world population. In addition, microorganism-based methodologies do not constitute a major source of pollution, and therefore, are preferred alternative for overcoming serious environmental problems, which arise from the conventional chemical methods(15).

Vitamins:

Vitamins are fundamental micronutrients needed in follow sum to keep up ordinary physiological capacity of the body. Vitamins fundamental supplements are not blended by vertebrates, and in this way, dietary enhancement is important from outer sources to keep up the reasonable digestion taking all things together living creatures . A few Vitamins are needed as coenzymes to encourage the biochemical responses catalyzed by the proteins. Nutrients are created during standard digestion of microorganisms and broadly utilized as food added substances, wellbeing enhancements, and restorative specialist and so forth Nutrients are delivered financially either through direct maturation or consolidated compound and microbiological measures utilizing fitting microorganisms . The chief microorganisms used in the fermentative production of riboflavin are two closely related ascomycetes, ***Eremothecium ashbyii*** and ***Ashbya gossypii***. A. gossypii is an efficient and preferred source of riboflavin production as it can produce 40,000 times more vitamin than required for its own growth. Vitamin K is required for normal blood clotting and also to activate receptor to facilitate transcription mechanisms in bone tissues, and to treat osteoporosis . Vitamin A is required as precursor to rhodopsin and other visual pigments, and also associated with specific gene transcription activation that facilitates growth and development . Vitamins are produced during regular metabolism of microorganisms and widely used as food additives, health supplements, and therapeutic agent etc (3 – 15).

Amino acids:

The amino acids are building squares of protein atoms and consequently utilized in dietary and feed enhancements of human and creatures, individually . These natural particles have different job, like creature feed added substances (lysine, methionine, threonine), flavor enhancers (aspartic corrosive, monosodium glutamate, serine), cell reinforcements (cysteine, L-tryptophan and L-histidine), as sugars (aspartame produced using aspartic corrosive and phenylalanine), and fixings in corrective and therapeutic items . Moreover, amino acids are recommended as dietary enhancements for lifting weights, bruxism, gloom, tranquilizer, premenstrual dysphoric problem, consideration shortage hyperactivity issue, and smoking discontinuance. Unnecessary amino acids can be integrated by human body yet fundamental amino acids can't be orchestrated in human body yet are needed for protein combination and accordingly, dietary enhancement is vital from outside sources. Henceforth, creation of fundamental amino acids at modern scale utilizing microbial sources is promising and alluring. The overall creation innovation for amino acids is overwhelmed by microbial aging and enzymatic cycles inferable from cost-adequacy, biological agreeableness and straightforwardness to deliver enantiomerically unadulterated amino acids . The commercial production through fermentations and enzymatic transformations mainly use Corynebacterium glutamicum and Escherichia coli to produce L-glutamic acid (monosodium glutamate), L-aspartic acid, Lphenylalanine, L-lysine, L-methionine, L-threonine, and Ltryptophan. L-lysine is a preferred additive to animal feed and approximately, 1.3 million tons of lysine is produced annually through microbial fermentation using ***C. glutamicum***(15-12-18).

Enzymes:

Catalytic activities of microbes have been utilized since ancient times for production of bread, wine, and beer. Enzymes derived from microorganisms have drawn significant attention for extensive applications in food, chemical and healthcare industries due to ease of production, stability, and other technical advantages and therefore, microorganisms are utilized in industries as the preferred source of enzymes than other sources of enzymes production, such as animals and plants The use of biocatalytic activities in several industries including food, feed, leather, textiles are increasing rapidly due to time saving process, cost effectiveness, biodegradable nature and environmental friendly characteristics . Besides, microbial enzymes are also involved in the potential degradation of toxic chemical compounds, such as phenolic compounds, nitriles, amines etc. of industrial and domestic wastes . The biotechnological developments have led to the manipulation of the microorganisms through recombinant DNA technology, protein engineering and their production in appropriate quantities to meet the demand . According to a market analysis, the global market of industrial enzymes was estimated $4.2 billion in 2014 and is predicted to grow at compound annual growth rate (CAGR) of 7% from 2015 to 2020 . In healthcare industries, enzymes are used in therapeutic management of health disorders caused due to enzyme deficiencies in humans . For example, phenylalanine ammonia lyase is used in degradation of phenylalanine in persons with inherited phenylketonuria disorder . Besides, sacrosidase (b-fructofuranoside fructohydrolase) enzyme is given to facilitate digestion of sucrose in patients with genetic congenital sucrase-isomaltase deficiency as they are incapable of digesting sucrose . Utilization of microbial enzymes for different purposes in food, pharmaceutical, textile, paper, leather, and other industries are extensive and incessantly increasing over conventional methods owing to higher effectiveness.The prospects of enzymes of microbial origin for industrial applications have grown significantly in the 21st century and their valuable contribution in food industry may be used to meet incessantly growing demand of food supply for rapidly growing population. Furthermore, these are used in development of alternative fuel supply to overcome the issues associated with depletion of natural resources and in the development of green environment. Microbial Enzymes in Food Industry Microorganisms have been used in food fermentation since ancient times and fermentation processes are still applied in the preparation of many of the food items . Microbial enzymes play a major role in food industries because they are more stable than plant and animal enzymes. They can be produced through fermentation techniques in a cost-effective manner with less time and space requirement, and because of their high consistency, process modification and optimization can be done very easily . Many of these enzymes find numerous applications in various industrial sectors, e.g. amylolytic enzymes find applications in food, detergent, paper and textile industries . They are used for the production of glucose syrups, crystalline glucose, high fructose corn syrups, maltose syrups, etc. In detergent industry, they are used as additives to remove starch-based stains. In paper industry, they are used for the reduction of starch viscosity for appropriate coating of paper . In textile industry, amylases are used for warp sizing of textile fibres . Similarly, enzymes like proteases, lipases or xylanases have wide applications in food sectors. The following sections give detailed and updated information about various food enzymes of microbial origin (15).

α-AMYLASES:

α-Amylases are starch-degrading enzymes capable of hydrolyzing α-1,4 glycosidic bonds of polysaccharides, which results in the production of short-chain dextrins. These enzymes are widely distributed in all living organisms. Majority of α-amylases are metalloenzymes and require calcium ions for their activity, stability as well as integrity .Wide applications of α-amylases in food industry include baking, brewing, starch liquefaction as well as a digestive aid .They are widely used in baking industry as flavour enhancement and antistaling agent to improve bread quality. During baking, α-amylases are added to the dough for conversion of starch to smaller dextrins, which are subsequently fermented by yeast. It improves the taste, crust colour and toasting qualities of bread α-Amylases are also used in the manufacture of high-molecular-mass branched dextrins. They are used as a glazing agent for the production of rice cakes and powdery foods In starch industry, they also find application for starch liquefaction, which converts starch into glucose and fructose syrups. Enzymatic conversion of starch involves three steps: gelatinization, liquefaction and saccharification. Gelatinization involves formation of a viscous suspension by dissolution of starch granules. This is followed by a liquefaction process, which reduces viscosity and involves partial hydrolysis. Glucose and maltose are further produced by saccharification. This requires highly thermostable enzymes and most of the starch saccharification is carried out with α-amylases from Bacillus amyloliquefaciencs, Bacillus stearothermophilus or Bacillus licheniformis . For the production of ethanol, starch is converted to fermentable sugars by the action of α-amylases and further fermentation of the sugars to alcohol is carried out by Saccharomyces cerevisiae. Other applications of α-amylases include clarification of fruit juices, which is carried out in the presence of cellulases and pectinases to improve yield as well as to make the process cost-effective (13).

Antibiotics :

The antibiotic period began in 1929 with the discovery of penicillin from a fungal sp. Penicillin notatum and its commercial production flourished in the 1940s . Later, a number of antibiotics have been discovered specifically from fungi and actinomycetes in quest of more effective pharmacological properties and to combat new pathogens. Continuing research in this area led to the discovery of a series of antibiotics, such as cephalosporin, tetracycline, macrolids, ansamacrolids, aminoglycosides, chloramphenicol, glycopeptides, peptide inhibitors, anthracyclins an antifungal antibiotics . Nowadays, antibiotics are used for a wide range of applications, such as in chemotherapy, veterinary, plant pathology, food preservation and in research laboratories. These compounds inhibit several pathways, such as nucleic acid synthesis, protein synthesis, cell wall formation, and electron transport pathway . Some of the microbial species have been reported for their efficient ability to produce a variety of antibiotics. The ***actinomycetes*** are responsible for the production of largest proportion (75%) of antibiotics and the Streptomycetes are the largest producer of antibiotics . Besides, ***Streptomycetes*** are also involved in the potential production of a range of pharmaceutical agents including antiparasitic, anticancer, immunosuppressive and enzyme inhibitors. Antibiotics can be classified in different ways, such as based on their chemical structure, biosynthesis pathway, source, mode of action, route of administration, and their effective range. Tetracyclines, the most extensively used broad-spectrum antibiotics, are used considerably in the treatment of diseases caused by a wide range of microorganism including gram-positive and gram-negative bacteria, protozoan parasites, chlamydiae, rickettsiae and mycoplasma (15).

Vaccine:

A vaccine is a biological preparation that provides active acquired immunity to a particular pathogen. The agent stimulates the immune system to recognize itself as a foreign threat and thus destroys and remembers it, so that the immune system can easily destroy any of these pathogens when they later invade into the body. The following vaccine characteristics may be altered or enhanced by biotechnologies. Biotechnology is mainly used in three ways as follows: separation of a pure antigen using a specific monoclonal antibody, synthesis of an antigen with the assistance of a cloned gene; and synthesis of peptides to be used as vaccines(20).

Reverse vaccinology:

The basic idea of reverse vaccinology is that an entire pathogenic genome can be sequenced and screened by employing bioinformatics methods to explore genes. Functional genomics approaches, such as DNA microarrays, proteomics, and comparative genome analysis, are used for the identification of virulence factors and novel vaccine candidates. This new computational approach allows prediction of all antigens, independent of their abundance and immunogenicity during infection. The first attempt at reverse vaccinology began with Meningococcus B (MenB) vaccine. Moreover, it has been used on several other bacterial vaccines such as antibiotic-resistant ***Staphylococcus aureus and Streptococcus pneumoniae*** (1).

Recombinant subunit vaccination:

The gene cloning is a powerful tool to synthesize protein materials to subunit vaccine by recombinant DNA techniques. Recombinant subunit vaccines are made from a fragment of protein (antigen) expressed in the laboratory using the viral DNA, for example, hepatitis B (HB) vaccine. The hepatitis B virus (HBV) gene that codes for the antigen is inserted into baker's yeast genome and then expresses the antigen protein. The antigen protein is harvested and purified to be used for the vaccine. This technique is also being used to explore a vaccine against hepatitis C (9).

Recombinant protein vaccination:

Upon infection, a pathogen produces proteins to elicit an immune response from the infected body. The gene encoding such a protein is isolated from the causative organism and used to develop a recombinant DNA which is expressed in a heterologous expression system (e.g., bacterium, yeast, or insect). Recombinant protein vaccines, such as cholera vaccine, diphtheria toxoid, and tetanus toxoid, are composed of protein/toxin antigens that have either been produced in another host organism or purified from large amount of pathogens. The vaccinated persons produce antibodies to the protein/toxin antigen to protect themselves from diseases(20).

Deoxyribonucleic acid (DNA) vaccination:

DNA vaccination is a technique for protecting against diseases through the direct injection of genetically engineered DNA. The gene responsible for the immunogenic protein is cloned with a corresponding expression vector. This DNA will trigger an immune response and the individual is successfully vaccinated. DNA vaccines may have the ability to induce a wider range of immune response types over conventional vaccines(20).

Food Products:

Foods typically contain a variety of bacteria of which some may be beneficial, such as those preserving foods through products of fermentation, and others may be harmful by causing human illness or food spoilage. Lactic acid bacteria are among the most important groups of microorganisms used in food fermentations and are largely included in the genera ***Carnobacterium, Enterococcus, Lactobacillus, Lactococcus , Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus, and Weissella.*** The essential feature of lactic acid bacteria metabolism is efficient carbohydrate fermentation coupled to substrate-level phosphorylation. These bacteria can degrade a variety of carbohydrates, with lactic acid being the predominant end product. Many lactic acid bacteria also produce bacteriocins that have antimicrobial activity that is antagonistic to other bacteria, especially toward bacteria closely related to the bacteriocin-producing strain. Bacteriocins are peptides that are produced ribosomally by bacteria and released extracellularly. Starter cultures, which are largely comprised of lactic acid bacteria, are food-grade microorganisms that are used to produce fermented foods of desirable appearance, body, texture, and flavor. Types of fermented foods for which commercial starter cultures are currently used include dairy products (cheese, sour cream, yogurt), meat products (sausages), and vegetable products (pickles, sauerkraut, olives). For starter cultures to be effective during food fermentations, they must dominate over naturally occurring microflora and produce the desired end products of fermentation. Many of the activities essential for food fermentations, including lactose metabolism, proteinase activity, oligopeptide transport, bacteriophage-resistance mechanisms, bacteriocin production and immunity, bacteriocin resistance, exopolysaccharide production, and citrate utilization, are encoded on plasmids harbored by lactic acid bacteria. Advances in molecular technology have enabled the construction of superior strains of starter cultures for food fermentations. Improved features of these strains include bacteriophage resistance, genetic stability, and reduced variation and unpredictability in performance. Another application for beneficial microbes used in foods is adding probiotic microorganisms to provide a health benefit to consumers. Many beneficial health effects for probiotics have been reported and include protection against enteric pathogens, improved digestion by means of enzymes to metabolize otherwise indigestible food nutrients (e.g., lactase to hydrolyze lactose in lactose intolerant consumers), stimulation of the intestinal immune system, and improvement of intestinal peristaltic activity. Lactic acid bacteria are the most common types of probiotic microbes being used. Probiotics have been largely delivered in fermented foods such as yogurt and fermented milk products; however, growing consumer interest in probiotics is leading to using other types of foods such as fruit and vegetable juices, cereal-based products, and even ice cream, as delivery vehicles. Fermented foods are an important part of the food processing industry and of many consumers' diets and are largely produced by lactic acid bacteria that have been selected for their ability to produce desired products or changes in the food. Many advances have been made during the past decade in developing improved bacterial strains for starter culture application, which largely have been made possible through advances in molecular technology. The use of lactic acid bacteria to enhance the quality and safety of foods is a rapidly evolving field. With the discovery of new bacteriocins and the development of more efficient approaches to deliver them to foods, the importance of lactic acid bacteria in preserving and providing enhanced safety of food will continue to increase for the foreseeable future(5).

Conclusion:

In conclusion was reviewed about microbial of biotechnology, and its applications e.g : vitamin, enzymes , food products , antibiotic ,amino acid and its role in Microbial biotechnology to sustainable development and in sustainable agriculture as Biopesticides and plant growth regulators and biofertilizers. Also, review about Molecular biology of relevance in in industrial microbiology and biotechnology, Therapeutic recombinant proteins .all of these gives an impression of the importance of biotechnology by microorganisms in the microbiology.

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