

## Role of Biotechnology in Improving Human Health

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### Abstract

Biotechnology has offered modern medical devices for diagnostic and preventive purposes, which include diagnostic test kits, vaccines and radio-labeled biological therapeutics used for imaging and analysis. Human health is a major growing concern worldwide because of infectious diseases. Biotechnology has played a dynamic role in improving the challenges regarding to human health as it has flexibility to reduce global health differences by the provision of promising technologies. Health, life quality and expectancy of life have been increased worldwide through the services provided by biotechnology. Malnutrition mainly arises due to the lack of essential nutrients and vitamins in food and ultimately results in death. Biotechnology has play a major role in eliminating these problems by producing nutrients enriched food such as Golden Rice, Maize, potato and soybean etc. Biotechnology has also played an important role in controlling the environmental pollution through biodegradation of potential pollutants. This review sketches improvement of human health by the use of biotechnological advances in molecular diagnostics, medicine, vaccines, nutritionally enriched genetically modified crops and waste management.

**Keywords:** Biotechnology; Human health; Infectious diseases; Molecular diagnosis; Vaccines; Drugs

### Introduction

Biotechnology contributes much towards the growing public and global health needs. It has revolutionized mankind since its existence. It provides effective diagnostics, prevention and treatment measures including production of novel drugs and recombinant vaccines [1]. It gives effective drug delivery approaches, new methods for therapeutics, nutritionally enriched genetically modified crops and efficient methods for environmental cleanup. Health, life quality and expectancy of life have been increased worldwide through the services provided by biotechnology [2,3]. Parasitic and infectious diseases like Acquired Immunodeficiency Syndrome (AIDS) [4-6] and tuberculosis (TB) have been diagnosed rapidly at relatively low cost. Molecular diagnostic tools including polymerase chain reaction (PCR) [7], recombinant antigens and monoclonal antibodies have been used for this purpose. Biotechnology has offered modern diagnostic test kits, rickettsial, bacterial and viral vaccines along with radiolabelled biological therapeutics for imaging and analysis. Vaccines have eliminated small pox, polio and other deadly diseases for the last hundred years. Biotechnology has made advancements in vaccination by making recombinant vaccines that have the potential to eradicate non-communicable diseases like cancer [8,9]. Naked DNA vaccines, viral vector vaccines [10] and plant-derived vaccines [11] are found to be most effective against a number of bacterial and viral disorders [12].

Therapeutic proteins have a large influence on non-communicable diseases responsible for over 60% of deaths in developing countries [13]. Transgenic bacteria, yeasts, plants [2,14] and mammals [15] have been used as a factory of recombinant therapeutic proteins. New desired genes are introduced into these systems to produce therapeutic proteins of interest in large quantities and are then purified [16]. The most important recombinant therapeutic proteins include erythropoietin for anemia treatment. Interferon alpha against leukemia and viral diseases [17,18] and insulin against type 1-Diabetes Mellitus [19] have been produced. Other therapeutic agents include growth hormones [20], cytokines, recombinant blood products, monoclonal antibodies [21], gene therapy products, molecular pharming agents and engineered tissue products. Xenografts [22], bone grafts, collagen and heart valves have been successfully engineered.

Biotechnology offers relatively cheaper drug and vaccine delivery tools. They eliminate blood-borne infections caused by re-use of needles. Drugs and vaccines are delivered efficiently in a controlled manner, thus avoiding the use of needles. Drugs can be propelled into the body speedily by gas jets, can be diffused into the body and can also be inhaled through nasal sprays. Drugs are efficiently delivered into the body using nano-particles [23]. Recent advances in biotechnology have reduced drug dosage required for a particular treatment. Genetically modified crops are a beneficial product of biotechnology which fulfills the requirement of essential nutrients and vitamins in the body. They are helpful in the physical and cognitive development of children. Novel and desired genes are introduced into these crops which become nutritionally enriched to cope with growing needs of health care among the people worldwide. Examples include iron rich rice, which fulfills the requirement of iron, thereby preventing anemia. Golden rice is rich in vitamin A, the deficiency of which causes blindness among a lot of children in developing countries [24]. Potato has also been genetically engineered to have all the vitamins, thereby satisfying the nutritional needs of every individual. Genetically modified foods can become a part of staple food to increase the life quality. Other enriched genetically modified foods include flax seeds, corn, maize [25], tomatoes, papaya, squash, soybeans, sugar beets, canola and cotton seeds. Biological fungicides, herbicides and insecticides have also been produced to control pests. Biotechnology has tremendous applications in environmental cleanup for the sake of human health. Contaminated soil, water and air are being cleaned by using microorganisms or plants in a process of bioremediation [8,26]. Microorganisms and plants have the ability to detoxify organic waste pollution and heavy metal pollution caused by mercury, cadmium, and lead. Radioactive waste,

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Received August 24, 2016; Accepted November 06, 2016; Published November 08, 2016

Citation: Afzal H, Zahid K, Ali Q, Sarwar K, Shakoor S, et al. (2016) Role of Biotechnology in Improving Human Health. J Mol Biomark Diagn 8: 309. doi: 10.4172/2155-9929.1000309

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acid mine drainage and oil spills are also treated effectively by using biotechnology. Plant biomass can be converted to ethanol, which can then be used as biofuel, offering much less air pollution. Biological enzymes can make the processing of paper and pulp industry easy, efficient and rapid [27].

### Biotechnology regarding health improvement

Followings are the biotechnological techniques and their derived health products which are helpful in the treatment of different diseases.

#### Molecular diagnostics

Nearly 40% deaths are due to infectious and parasitic diseases (malaria, tuberculosis, and AIDS) each year. Spread of these diseases can be overcome by the development of quick and accurate diagnostic tools. These developments result in increase in survival rate as well as help to prevent the waste of resources on non-suitable treatment. Many conventional diagnostic tools are inaccurate, time consuming, laborious and expensive. In contrast, modern biotechnology comprised of molecular diagnostic tools sketches recent advances in biology for the detection of diseases [28,29]. Molecular diagnostics was being ranked by the scientific panel in the University of Toronto as the most ideal set of technologies for improving the health status. Biotechnology is based on following diagnostic techniques; PCR, Monoclonal antibodies and microarrays. These are simple, quick, cost effective and have high sensitivity and specificity [30,31]. PCR requires small volume of sample to amplify and identify the DNA sequence of pathogen (that cause the disease). It is identified in a very quick and accurate manner as compared to conventional diagnostics. The infectious or dangerous organisms (HIV, Mycobacterium and plasmodium) that are difficult to grow in culture are identified by the PCR [32]. Multiplex PCR are used to detect the pathogens which cause the broad range of diseases at once and hence it saves both time and resources [33].

Nanotechnology is advancement in biotechnological techniques for detection without amplification at molecular level. Mostly blood sample is placed between two electrodes in the presence of probe (complementary of detected DNA) coated on gold particles. These probes anneals to pathogen's DNA sequence. If present, circuit is closed by Gold particles to produce detectable signals. This technique is more sensitive as compared to conventional diagnostic methods [34]. Development of simple and quick dipstick coated by antibody have been increased the applications of molecular diagnostics. It can be used anywhere without any laboratory facilities. "The program for appropriate technology in health (PATH)" have been developed the dipsticks for identification of malaria, TB, Hepatitis C, HIV and pregnancy dipsticks. This test is rapid, accurate and easy to use [35]. Currently, in the diagnosis and treatment of diseases microarrays have become a powerful tool in contrast to the traditional DNA based tests. It is best for the study of causes of complex genetic disorders as it can identify and quantify the thousands of gene at the same time. Microarray has a great potential as it has been revolutionized the recognition and treatment of common diseases. DNA microarrays, genotypic microarrays and protein microarrays are currently used and play important role in improving the status of health [36].

#### Recombinant therapeutic proteins

Currently, five different organisms including bacteria (e.g. *Escherichia coli*, *Pseudomonas* spp. *Serratiamas cescens*, *Erweniaher bicola*, *Lactococcus lactis* and *Bacillus subtilis*), fungi (*Saccharomyces cerevisiae*), *Pichia*, plants (tobacco plant, rape and transgenic potatoes) [37], insects (*Spodoptra frugiperda*) and mammalians (Chinese hamster

Disease	Active Substance
Hepatitis C	Interferon- $\alpha$
Multiple Sclerosis	Interferon- $\beta$
Renal Cancer	Interleukin
Haemophilia	Factor 8 and 9
Diabetes	Human Insulin
Anaemia	Erythropoietin

**Table 1:** Some examples of therapeutic proteins used against different diseases (Almeida, et al. [40]).

ovary cells, baby hamster kidney cells and transgenic animals) [38] are used in biotechnological process to produce the recombinant therapeutic proteins. Increase in the production of recombinant products can be done by introducing different changes in the respective organisms through different conventional (mutagenesis, fermentation, sexual and parasexual processes) and modern techniques (recombinant DNA or the hybridoma) [39]. Biopharmaceuticals and therapeutic proteins are promising in providing the effective treatment against chronic and complex diseases [40,41], Rader [42]. In 2008, for over 100 different diseases including 254 cancers, 163 for bacterial diseases, 59 for immune diseases, and 34 for the HIV/AIDS virus 633 therapeutic products were under development according to "the pharmaceutical research and manufactures of America (PhrMA)" [43] Table 1.

In 2008, 31 noval recombinant drugs and further 12 new therapeutic proteins in 2009 are approved by FDA [43]. Now a day, these drugs are commonly used for the cure of rare diseases which are impossible to treat by conventional therapies. Therapeutic proteins may be used against asthma, different cancers, Parkinson's and Alzheimer's disease. Antibiotics, blood factors, hormones, vaccines, growth factors, enzymes and monoclonal antibodies are the different groups of biopharmaceutical or therapeutic proteins.

#### Vaccines and delivery of vaccines

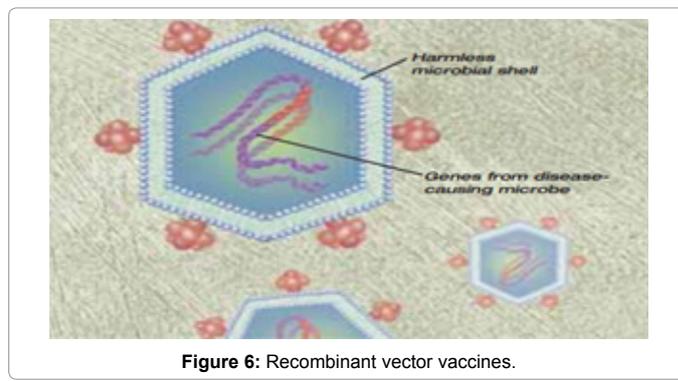
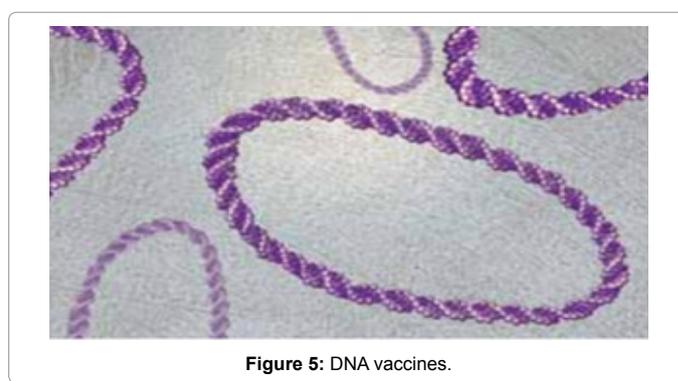
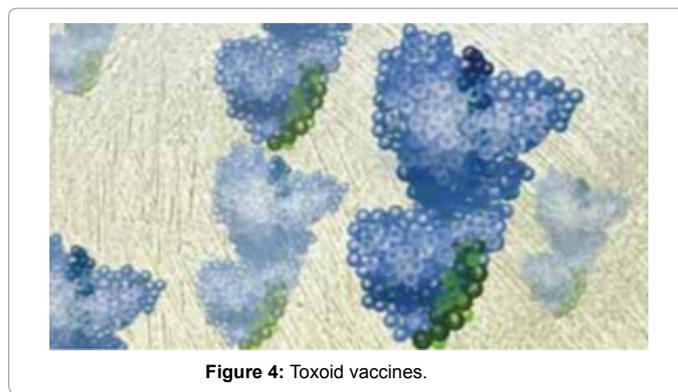
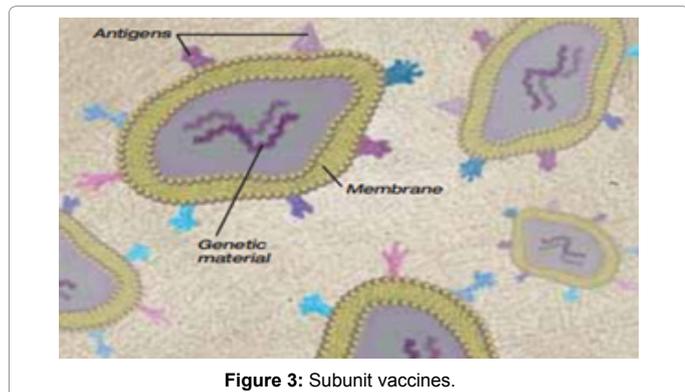
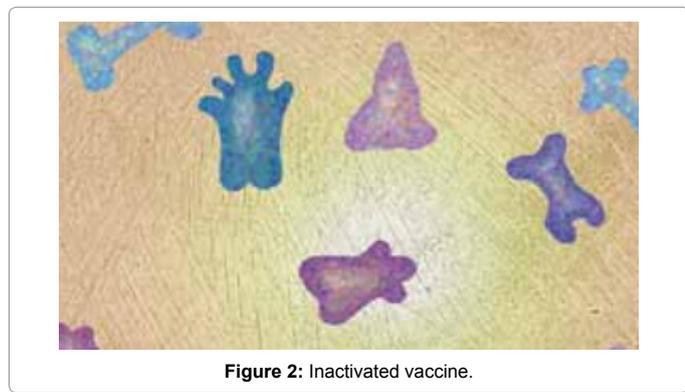
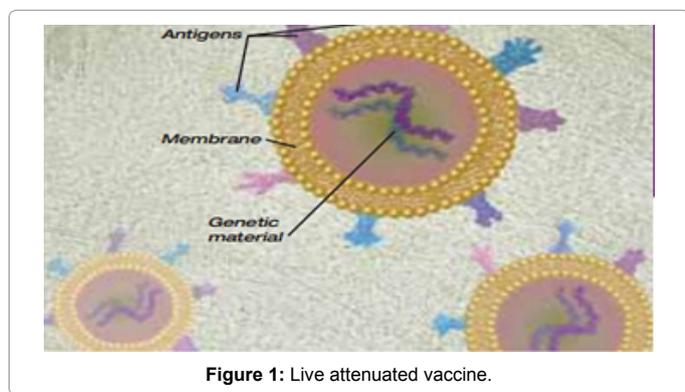
Public health is considered a major concern worldwide both in developed and developing countries. Scientist from all over the world has made a lot of contributions in preventing the infectious diseases to cure challenges regarding to human health. Global health differences are growing progressively, the vital role of science and technology for the betterment of human health cannot be ignored. Biotechnology has remarkable role to find out the problems regarding to human health and development in many developing countries [44]. At the completion of the past century, infectious diseases offered considerably higher public challenges [45]. These are relatively as a consequence of ecology fluctuation and risks related to mobility and urbanization, mismanagement of medical tools, due to environmental modification and social interruption [46]. Vaccination is a method by which a vaccine is administered. Vaccines are considered possibly the best achievement of medical advance of the form century. Small pox has completely eliminated worldwide by vaccination. It is also useful for the looming removal of polio, and offering a vivid decrease in occurrence of various transferrable infections [47]. Research in the vaccine advances is being used effectively for communicable diseases as well as non-communicable diseases for example cancer. A diversity of recombinant vaccines is being used worldwide for the treatment of human diseases. These recombinant vaccines include naked DNA vaccines, plant derived vaccines and viral vector vaccines. It has been demonstrated that a subunit vaccine RTS, S/AS02 provides protection against malarial natural infection in adults [48]. TB is another major growing concern in developing countries for which scientists are making numerous efforts to achieve a recombinant TB vaccine. Because each year, it privileges at minimum two million lives [49]. Up till now research has a subunit

vaccine having the surface antigen Mtb8.4 that is responsible for the protection of mice from the infection of tuberculosis [50].

Various types of vaccines have been made using tremendous biotechnology approaches are now being used worldwide to cure the human health challenges. The principle for the use of vaccines is very simple because they mainly stimulate the patient's immune system against the infectious agents like viruses or bacteria without causing any disease itself [49]. These vaccines include inactivated vaccines, toxoid vaccines, live attenuated vaccines, conjugate vaccines, recombinant vector vaccines, subunit vaccines and DNA vaccines (Figures 1-6).

U.S. Department of Health and Human Services. National Institutes of Health. National Institute of Allergy and Infectious Diseases. NIH Publication No. 08-4219. January (2008). www.niaid.nih.gov.

Along the development and advances in vaccines, their delivery methods also have a significant part in improving human health. Different methods are being used for different drugs and vaccine



administration. At present, the most extensively used method for applying drugs and vaccines are the use of "injections". Each year, many thousands children die with diseases that are curable with vaccine just because of expensive vaccine delivery facilities and skilled medical employees. A greater ratio of HIV/ AIDS cases, 2.3 to 4.6 million hepatitis C cases and 8 to 16 million hepatitis B cases are being reported through the misuse of needles every year (**Error! Bookmark not defined.**, Onabanjo, et al. [51], Health [52]). These problems can be avoided by the use of controlled-release delivery and injection free systems. In needle-free technologies through the use of high-speed jet of gas, vaccine is introduced inside the body. Besides these techniques, different solutions, skin patches and rubbing gels can also introduce agents into body by simple diffusion method. Nasal sprays and inhalers are also effective ways to administer drugs and vaccines through the respiratory tract. About 15% budget is required for the storage and refrigerated transport of vaccines that is the main cost in all vaccine programs [53]. So, an ideal vaccine should have high immunogenicity and long lasting immunity as well as it should be heat

Some Specific Vaccines Now in Widespread Global Use	
Cholera	Pertusis (whooping cough)
Diphtheria	Pneumococcal
Haemophilus influenzae Type B	Polio (oral and injectible vaccines)
Hepatitis B	Rabies
Human papilloma virus	Rotavirus
Influenzae A	Rubella
Influenzae B	Tuberculosis (BCG)
Japanese B encephalitis	Typhoid
Measels	Varicella (chicken pox)
Mumps	Yellow Fever

**Table 2:** Commercially available and affordable by maximum no. of people.

stable, inexpensive, commercially available and affordable by maximum no. of people (Table 2).

The role of specific vaccines around the world can be demonstrated by the following table. There is a space and greater opportunities exist in the field of molecular biology for the future prosperity and development. So, scientists from all over the world are now developing many “Edible vaccines” that will be easily immunize people against infectious diseases and will be commercially accessible in succeeding few years at lower rate [54].

### Nutrients enriched genetically modified food

In developing countries over half of the infant deaths occur due to the deficiency of essential nutrients and vitamins [52]. Malnutrition causes impaired cognitive and physical development and multiple illnesses such as anemia. Anemia is the major cause of maternal mortality caused by iron deficiency [55]. Moreover malnutrition also has adverse effects on immune system. So, to overcome these nutrients and vitamin's deficiency biotechnology enables to introduce new genes and new traits into crops more precisely than traditional breeding. These genetically modified crops have many advantages most important of them is the production of nutrients enriched food.

Vitamin A deficiency is the major cause of children death in developing countries; almost 300,000 children go blind and two third of them die every year. Vitamin A is important to improve the growth and development, to increase resistance against disease and to protect from visual impairment and blindness [52,55]. To meet vitamin A deficiency, vitamin A enriched staple food is produced. For this purpose rice enriched in beta-carotene (vitamin A precursor) are produced. These rice are known as “Golden Rice” [56,24]. Now vitamin A enriched rice and maize are being cultivated in developing countries. Researches ensure that vitamin A present in rice efficiently absorbed by human gut. To meet daily human requirement of vitamin A 300 mg of transgenic rice are enough [52]. Scientist has developed transgenic seed of rice containing ferritin, an iron storage protein. These transgenic rice seed contain twice iron content compare to non-transgenic rice [57]. Similarly, a new variety of transgenic rice contains three genes that increases iron storage in rice and its assimilation from the digestive track [58]. Bouis, Chassy, introduced phytase gene in maize and observed that maize absorbed large amount of iron. New technologies are making efforts to increase amount and bioavailability of folates, vitamin E, vitamin B5, vitamin A, vitamin C, zinc and iron [59-63].

Plant proteins mostly have less content of essential amino acids. Cereal proteins are deficient in lysine (Lys) while tuber, root, legumes and vegetables lack sulphur containing amino acids methionine (Met) and cysteine (Cys). Modified Met-rich soybean glycinin protein was expressed in rice to overcome methionine deficiency [64]. Kim et

al synthesized artificial storage protein (ASP1) containing 78.9% essential amino acids. It increased the essential amino acids supply to a large extent [65]. Different biotechnology techniques have helped to develop essential amino acids enriched transgenic crops such as staple vegetables cassava, plantain, and potato [66]. Indian researchers have developed amino acids enriched potato [67]. L-gulonolactone oxidase gene was used to increase vitamin C amount [68].

Fermented food is routinely consumed in many developing countries as a main part of food and diet ingredient; primary fermented food includes curdled milks and milk products, root crops and grains. Fermentation produces food with increased nutritional values and quality. It also modifies the starches presence in grains that protect against lethal diseases such as gastrointestinal disease and colon cancer. In South Africa consumption of fermented maize causes discrepancy in the occurrence of colon cancer among black and whites populations [69]. Different microorganism such as lactic acid bacteria in cheese, starter fermentation cultures in bakery and brewing are being introduced in food to improve their nutritional value and flavor along and also to minimize the infections by pathogenic microorganisms. Yet these are in research and development phase [70]. Modified ruminant microorganisms protect livestock from poisonous feed components [71,72]. Application of modern biotechnology has reduced the level of certain allergens and anti-nutrients in food. For example, cyanide level in Cassava roots and levels of natural glycoalkaloid toxin in potatoes have been reduced by inserting invertase gene from yeast [73-75].

Efforts are being made to produce potato with elevated level of starch so that they soak up less fat during frying [66]. Transgenic soy and canola producing oils with low level of saturated fats are essential biotechnological products. GM soybean, oilseed rape and oil palm are under R&D. Oilseed rape having high lauric acid and soy with high oleic acid have been approved in the USA [76]. Beside crops different livestock has also engineered to fulfill food requirement of world population and to meet nutritional requirement. Gene for different growth hormones have been introduced in fish [66]. New Zealand's researchers developed genetically modified cows that produce milk enriched in casein protein. Other purpose of GMO is to produce milk with reduce the lactose content so that lactose-intolerant people can enjoy milk [66, 77].

### Role of environmental biotechnology in health care

Pollution and the untreated waste are the major hazards for the human health. Direct exposure of these pollutants and the people must be avoided. Environmental biotechnology has revolutionized the safety of public health. It provides a systematic platform that submerge the knowledge of science and engineering. By using microorganisms it prevents pollution by treating and biodegrading the hazardous waste. Use of naturally occurring microbes in detoxification of harmful substances; complete destruction of waste through various biotechnological methods, are the major benefits of biotechnological treatment. Strategy of bio-treatment varies according to the physiological type of microbes used i.e. anaerobic, aerobic, aerotolerant or microaerophilic [78]. Soil surrounding the sites contaminated with industrial wastes have high concentrations of pollutant like Polynuclear aromatic hydrocarbons (PAHs) and their degradation can be enhanced through soil remediation [79]. The cost, amount of waste and the ability of microorganisms to degrade waste are major contemplations in environmental biotechnology. Following treatments commonly used:

### Biodegradation of heavy metals

Microorganisms have enzymes to reduce or oxidize the heavy metals

which helps in the biodegradation of waste containing these heavy metals. Microbial metabolites such as phosphate, H<sub>2</sub>S, CO<sub>2</sub> and organic acids promotes the precipitation of heavy metals whereas inorganic acids stimulates the metal solubilization [78]. Sulfate reducing bacteria are used to deal with the liquid waste from drainage and nuclear plants, it produces H<sub>2</sub>S gas which eliminates heavy metals and radionuclides from the drain having sulfate. The cell surface of microorganisms contain amino group (positively charged) and phosphate group (negatively charged) due to which the heavy metal adsorption depends upon the pH [80]. Precipitation is further enhanced by the organic acids produced from bacteria during anaerobic fermentation. Radionuclides such as uranium can be accumulated by fungus through biosorption. Prior to landfilling of sewage sludge, bioleaching of heavy metals is done through solubilization of metals in which the minerals in the metals are oxidized [81]. Different biotechnological strategies can be used in combination in order to biodegrade pollutants containing heavy metals.

### Bioremediation

Inappropriate disposal of waste, industrial sludge and use of pesticides in agriculture are the major sources of pollution. In contrast to the assumptions of industrial sludge as a good fertilizer, it is a potential source of contaminants, heavy metals and polynuclear aromatic hydrocarbons (PAHs). These contaminants are known to cause cancer in human beings. Long-term contact of human with lead, chromium, petroleum and pesticides can results into numerous congenital disorders and cancer. One of the most common ground water contaminant is trichloroethene (TCE) that belongs to chlorinated compounds and are most prior on the list of environmental protection agency (EPA) USA. The process of using microorganism i.e. bacteria and fungi to biodegrade, breakdown or transform the pollutants and contaminants is known as bioremediation. Contaminants are used as an energy source by the microorganisms and are then converted to less toxic form. Pollutants like chlorinated hydrocarbons are biodegraded by butane-utilizing i.e. *Pseudomonas*, *Micrococcus*, *Nocardia*, *Aureobacterium*, *Chryseobacterium*, *Comamonas*, *Rhodococcus*, *Acidovorax*, and *Variovorax* [82]. For bioremediation mainly two approaches are used; I) augment the capabilities indigenous hydrocarbon-utilizing bacteria II) introduce non-indigenous bio-degraders of hydrocarbons i.e. bio-augmentation [83]. PAHs are abundant in our environment and are considered as potential mutagens. The soil contaminated with organic compound including PAHs can be treated through bioremediation which involves the microbes, addition of nutrient, moisture and aeration [79]. In addition to bacteria, bioremediation also involves fungi in which mycelia releases extracellular enzymes to bio-degrade the contaminant and the process is called mycoremediation [84,85].

### Conclusion

From all the facts that have been discussed above biotechnology is known to influence every aspect of human health. Biotechnology has offered modern medical devices for diagnostic and preventive purposes, which include diagnostic test kits, vaccines and radio-labeled biological therapeutics used for imaging and analysis. Malnutrition mainly arises due to the lack of essential nutrients and vitamins in food and ultimately results in death. Biotechnology has play a major role in eliminating these problems by producing nutrients enriched food such as Golden Rice, Maize, potato and soybean etc. Pollutants and untreated waste are a great hazard to human health and are potential cause of cancer. Biotechnology has evolved numerous strategies to biodegrade these pollutants by making use of microorganism. Precipitation of

heavy metals and bioremediation of pollutants are the major advantages of biotechnology UNICEF and Organization [85]).

### References

1. Henderson LM (2005) Overview of marker vaccine and differential diagnostic test technology. *Biologicals* 33: 203-209.
2. Burdi DK, Qureshi S, Ghangro AB (2014) An overview of available Hypoglycemic Triterpenoids and Saponins to cure Diabetes mellitus. *Int J Adv Life Sci*.
3. Kapuscinski AR, Goodman RM, Hann SD, Jacobs LR, Pullins EE, et al. (2003) Making 'safety first' a reality for biotechnology products. *Nature Biotechnol* 21: 599-601.
4. Lafeuillade A, Stevenson M (2011) The search for a cure for persistent HIV reservoirs. *AIDS Rev* 13: 63-66.
5. Rasheed A, Ullah S, Naeem S, Zubair M, Ahmad W, et al. (2014) Occurrence of HCV genotypes in different age groups of patients from Lahore, Pakistan. *Int J Adv Life Sci* 1: 89-95.
6. Zulfiqar S, Hafeez MN, Iqbal MS, Ali Q (2015) Role of genetic studies towards solving problems of human society. *Nature Sci* 13.
7. Heim A, Ebnet C, Harste G, Pring-Akerblom P (2003) Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol* 70: 228-239.
8. Javed S, Ali M, Ali F, Anwar SS, Wajid N (2015) Status of oxidative stress in breast cancer patients in Pakistani population. *Int J Adv Life Sci* 2: 115-118.
9. Javed Z, Iqbal MZ, Latif MU, Yaqub HMF, Qadri QR (2015) Potent Implications of miRNA in Cancer Biology-A Brief Review. *Int J Adv Life Sci* 2: 106-109.
10. Gilbert SC (2001) Virus-like particles as vaccine adjuvants. *Mol biotechnol* 19: 169-177.
11. Streatfield SJ, Howard JA (2003) Plant-based vaccines. *Int j parasitol* 33: 479-493.
12. Berglund P, Smerdou C, Fleeton MN, Liljestrom P (1998) Enhancing immune responses using suicidal DNA vaccines. *Nature biotechnol* 16: 562-565.
13. Evans B (1999) The prospect for international regulatory interventions in embryo transfer and reproductive technologies in the next century. *Theriogenology* 51: 71-80.
14. Gleba Y, Klimyuk V, Marillonnet S (2007) Viral vectors for the expression of proteins in plants. *Curr Opin Biotechnol* 18: 134-141.
15. Paterson L, DeSousa P, Ritchie W, King T, Wilmut I (2003) Application of reproductive biotechnology in animals: implications and potentials: Applications of reproductive cloning. *Anim reprod sci* 79: 137-143.
16. Kochhar H, Gifford G, Kahn S (2005) Regulatory and biosafety issues in relation to transgenic animals in food and agriculture, feeds containing genetically modified organisms (GMO) and veterinary biologics. Applications of Gene-Based Technologies for Improving Animal Production and Health in Developing Countries. Springer 479-498.
17. Rehman RA, Rao AQ, Ahmed Z, Gul A (2015) Selection of potent bacterial strain for over-production of PHB by using low cost carbon source for eco-friendly bioplastics. *Adv Life Sci* 3: 29-35.
18. Seago J, Hilton L, Reid E, Doceul V, Jeyatheesan J, et al. (2007) The NPPO product of classical swine fever virus and bovine viral diarrhoea virus uses a conserved mechanism to target interferon regulatory factor-3. *J Gen virol* 88: 3002-3006.
19. Johnson IS (1983) Human insulin from recombinant DNA technology. *Science* 219: 632-637.
20. Palmiter RD, Brinster RL, Hammer RE, Trumbauer ME, Rosenfeld MG, et al. (1982) Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature* 300: 611.
21. Anderson J (1984) Use of monoclonal antibody in a blocking ELISA to detect group specific antibodies to bluetongue virus. *J immunol methods* 74: 139-149.
22. Golovan SP, Meidinger RG, Ajakaiye A, Cottrill M, Wiederkehr MZ, et al. (2001) Pigs expressing salivary phytase produce low-phosphorus manure. *Nature biotechnol* 19: 741-745.
23. Parveen S, Misra R, Sahoo SK (2012) Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. *Nanomedicine* 8: 147-166.

24. Potrykus I (2001) Golden rice and beyond. *Plant Physiol* 125: 1157-1161.
25. Quist D, Chapela IH (2001) Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 414: 541-543.
26. Kappeli O, Auberson L (1997) The science and intricacy of environmental safety evaluations. *Trends biotechnol* 15: 342-349.
27. Evan G, Furlong J (2016) *Environmental Biotechnology-Theory and Application*. University of Durham, Taurus Biotech Ltd, UK.
28. Daar AS, Thorsteinsdóttir H, Martin DK, Smith AC, Nast S, et al. (2002) Top ten biotechnologies for improving health in developing countries. *Nature genetics* 32: 229-232.
29. Nazir S, Faraz A, Shahzad N, Ali N, Khan MA, et al. (2014) Prevalence of HCV in  $\beta$ -thalassemia major patients visiting tertiary care hospitals in Lahore-Pakistan. *Adv Life Sci*.
30. Khan MT, Afzal S, Rehman AU, Zeb T (2015) Interleukin 10 (IL-10) promoter-1082 A> G polymorphism and risk of cancer: Meta-analysis. *Advancements in Life Sciences* 2: 67-73.
31. Li X, Quigg RJ, Zhou J, Gu W, Rao PN, et al. (2008) Clinical utility of microarrays: current status, existing challenges and future outlook. *Curr genomics* 9: 466-474.
32. Louie AY, Huber MM, Ahrens ET, Rothbacher U, Moats R, et al. (2000) In vivo visualization of gene expression using magnetic resonance imaging. *Nature biotechnol* 18: 321-325.
33. Harris E, Kropp G, Belli A, Rodriguez B, Agabian N (1998) Single-step multiplex PCR assay for characterization of New World *Leishmania* complexes. *J Clin Microbiol* 36: 1989-1995.
34. Park SJ, Taton TA, Mirkin CA (2002) Array-based electrical detection of DNA with nanoparticle probes. *Science* 295: 1503-1506.
35. Palmer CJ, Lindo JF, Klaskala WI, Quesada JA, Kaminsky R, et al. (1998) Evaluation of the OptiMAL test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. *J Clin Microbiol* 36: 203-206.
36. Li Z, Dong P, Ren M, Song Y, Qian X, et al. (2016) PD-L1 Expression Is Associated with Tumor FOXP3+ Regulatory T-Cell Infiltration of Breast Cancer and Poor Prognosis of Patient. *J Cancer* 7: 784.
37. Enríquez G (2010) Amazonia-Network of dermocosmetic innovations Sub-network of dermocosmetics in the Amazon from the sustainable use of its biodiversity with approaches to the productive chains of Brazil nuts and Andiroba and Copaiba oils. *Strategic Partnerships* 14: 51-118.
38. Walsh G (2003) Pharmaceutical biotechnology products approved within the European Union. *Eur J Pharm Biopharm* 55: 3-10.
39. Sarmiento MJ, Ferreira FI, de Sousa TB (1998) Tradition and change in the rural school: A case study.
40. Almeida H, Amaral MH, Lobao P (2011) Drugs obtained by biotechnology processing. *Braz J Pharm Sci* 47: 199-207.
41. Daud S, Shahzad S, Shafique M, Bhinder MA, Niaz M, et al. (2014) Optimization and validation of PCR protocol for three hypervariable regions (HVI, HVII and HVIII) in human mitochondrial DNA. *Adv Life Sci* 1: 165-170.
42. Rader RA (2008) (Re) Defining biopharmaceutical. *Nature biotechnol* 26: 743-751.
43. Baker BK (2016) Trans-pacific partnership provisions in intellectual property, transparency, and investment chapters threaten access to medicines in the US and elsewhere. *PLoS Med* 13: e1001970.
44. Singer PA, Daar AS (2001) Harnessing genomics and biotechnology to improve global health equity. *Science* 294: 87-89.
45. Morens DM, Folkers GK, Fauci AS (2004) The challenge of emerging and re-emerging infectious diseases. *Nature* 430: 242-249.
46. Weiss RA, McLean AR (2004) What have we learnt from SARS? *Philos Trans R Soc Lond B Biol Sci* 359: 1137-1140.
47. Widdus R (1999) The potential to control or eradicate infectious diseases through immunisation. *Vaccine* 17: S6-S12.
48. Bojang KA, Milligan P, Pinder M, Vigneron L, Allouche A, et al. (2001) Malaria Vaccine Trial Team: Efficacy of RTS, S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: A randomised trial. *Lancet* 358: 1927-1934.
49. Nacy CA, Sacksteder KA (2002) New tuberculosis vaccine development. *Expert Opin Biol Ther* 2: 741-749.
50. Coler RN, Neto AC, Owendale P, Day FH, Fling SP, et al. (2001) Vaccination with the T cell antigen Mtb 8.4 protects against challenge with *Mycobacterium tuberculosis*. *J Immunol* 166: 6227-6235.
51. Agbon C, Onabanjo O, Akinyemi C (2011) Micronutrient adequacy of homemade complementary foods. *Nutr Food Sci* 41: 12-19.
52. [http://www.who.int/childgrowth/standards/velocity/technical\\_report/en/](http://www.who.int/childgrowth/standards/velocity/technical_report/en/)
53. Lloyd J (2000) Technologies for vaccine delivery in the 21st century. *World Health Organization Geneva, Switzerland*.
54. Nieburg P, Marks JS, McLaren NM, Remington PL (1985) The fetal tobacco syndrome. *JAMA* 253: 2998-2999.
55. Rogo KO, Mwalali P, Ouch J (2006) Maternal Mortality.
56. Obrero An, Gonzalez-Verdejo CI, Die JV, Goomez P, Del Rio-Celestino M, et al. (2013) Carotenogenic gene expression and carotenoid accumulation in three varieties of *Cucurbita pepo* during fruit development. *J Agric Food Chem* 61: 6393-6403.
57. Gura T (1999) New genes boost rice nutrients. *Science* 285: 994-995.
58. Lucca P, Hurrell R, Potrykus I (2002) Fighting iron deficiency anemia with iron-rich rice. *J Am Coll Nutr* 21: 184S-190S.
59. Botella-Pavia P, Rodriguez-Concepcion M (2006) Carotenoid biotechnology in plants for nutritionally improved foods. *Physiologia Plantarum* 126: 369-381.
60. Chakauya E, Coxon KM, Whitney HM, Ashurst JL, Abell C, et al. (2006) Pantothenate biosynthesis in higher plants: advances and challenges. *Physiologia Plantarum* 126: 319-329.
61. DellaPenna D, Last RL (2006) Progress in the dissection and manipulation of plant vitamin E biosynthesis. *Physiologia Plantarum* 126: 356-368.
62. Ishikawa T, Dowdle J, Smirnoff N (2006) Progress in manipulating ascorbic acid biosynthesis and accumulation in plants. *Physiologia Plantarum* 126: 343-355.
63. Zimmermann MB, Hurrell RF (2002) Improving iron, zinc and vitamin A nutrition through plant biotechnology. *Curr Opin Biotechnol* 13: 142-145.
64. Katsube T, Kurisaka N, Ogawa M, Maruyama N, Ohtsuka R, et al. (1999) Accumulation of soybean glycinin and its assembly with the glutelins in rice. *Plant Physiol* 120: 1063-1074.
65. Sun SS (2008) Application of agricultural biotechnology to improve food nutrition and healthcare products. *Asia Pac J Clin Nutr* 17: 87-90.
66. Rodemeyer M (2001) Harvest on the horizon: future uses of agricultural biotechnology. *Pew Initiative on Food and Biotechnology, Washington, DC, USA*.
67. Chakraborty S, Chakraborty N, Datta A (2000) Increased nutritive value of transgenic potato by expressing a nonallergenic seed albumin gene from *Amaranthus hypochondriacus*. *PNAS* 97: 3724-3729.
68. Jain AK, Nessler CL (2000) Metabolic engineering of an alternative pathway for ascorbic acid biosynthesis in plants. *Mol Breed* 6: 73-78.
69. Ahmed R, Segal I, Hassan H (2000) Fermentation of dietary starch in humans. *Am J gastroenterol* 95: 1017-1020.
70. Fonseca MJ, Costa P, Lencastre L, Tavares F (2012) Disclosing biology teachers' beliefs about biotechnology and biotechnology education. *Teach Teach Educ* 28: 368-381.
71. Lash S, Szerszynski B, Wynne B (1996) Risk, environment and modernity: Towards a new ecology, Sage Publishers, USA.
72. Yaqoob A, Shehzad U, Ahmad Z, Naseer N, Bashir S (2015) Effective treatment strategies against Ebola virus. *Adv Life Sci* 2: 176-182.
73. Ahmed S, Nasir AI, Yaqub H, Waseem M, Tabassum B, et al. (2013) Molecular detection, phylogenetic analysis and designing of siRNA against Potato Virus X. *Adv Life Sci*.
74. Ali A, Iqbal M, Ali Q, Razzaq A, Nasir IA (2016) Gene profiling for invertase Activity: Assessment of potato varieties for resistance towards cold induced sweetening. *Adv Life Sci* 3: 63-70.
75. Buchanan B, Adamidi C, Lozano R, Yee B, Momma M, et al. (1997) Thioredoxin-linked mitigation of allergic responses to wheat. *PNAS* 94: 5372-5377.

- 
76. Wolt JD, Conlan CA, Majima K (2005) An ecological risk assessment of Cry1F maize pollen impact to pale grass blue butterfly. *Environ Biosafety Res* 4: 243-251.
  77. Dove AW (2005) Clone on the range: What animal biotech is bringing to the table. *Nature biotechnol* 23: 283-285.
  78. Ivanov V, Hung YT (2010) Applications of environmental biotechnology. *Envi Biotechnol*.
  79. Wilson SC, Jones KC (1993) Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): A review. *Environ Pollut* 81: 229-249.
  80. Moo-Young M, Anderson WA, Chakrabarty AM (2013) *Environmental biotechnology: Principles and applications*. Springer Science Business Media, USA.
  81. Ito A, Takachi T, Aizawa J, Umita T (2001) Chemical and biological removal of arsenic from sewage sludge. *Water Sci Technol* 44: 59-64.
  82. Morse WR, Brochu WJ, Carter SR, Gibbs JA, Orchard Park NY, et al. (1997) National petrochemical & refiners association 1899 L Street, NW, suite 1000 Washington, DC 20036, USA.
  83. Rosenberg E, Ron EZ (1996) Bioremediation of petroleum contamination. *Biotechnol Res Series* 6: 100-124.
  84. Nazir S, Faraz A, Shahzad N, Ali N, Khan MA, et al. (2014) Prevalence of HCV in  $\beta$ -thalassemia major patients visiting tertiary care hospitals in Lahore-Pakistan. *Adv Life Sci* 1: 197-201.
  85. [http://apps.who.int/iris/bitstream/10665/44110/1/9789241598019\\_eng.pdf](http://apps.who.int/iris/bitstream/10665/44110/1/9789241598019_eng.pdf)