Chapter 11

Biotechnology and Its Applications

- Biotechnological Applications in Agriculture
- Biotechnological Applications in Medicine
  - Genetically Engineered Insulin
  - Gene Therapy
  - Molecular Diagnosis
- Transgenic Animals
- Ethical Issues

BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE

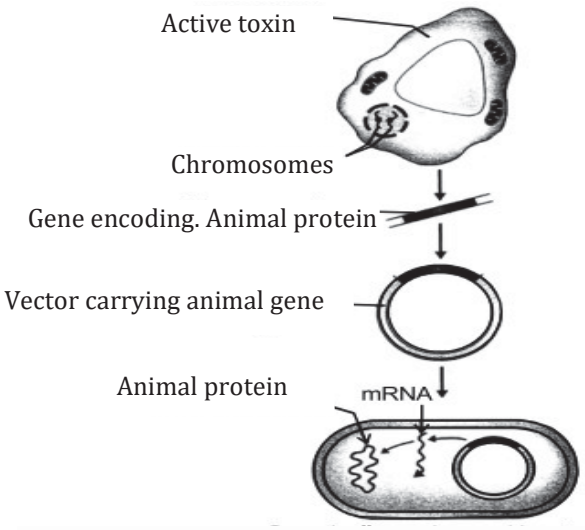
Biotechnology primarily focuses on the industrial-scale production of biopharmaceuticals and biological products utilizing genetically modified microbes, fungi, plants, and animals. Its applications span across various fields including medicine, therapeutics, diagnostics, bioremediation, agriculture, waste treatment, processed food, and energy production.

Key aspects of biotechnology include:

1. Enhancement of organisms, primarily microbes or pure enzymes, to serve as efficient catalysts.
2. Engineering of optimal conditions to facilitate the action of these catalysts.
3. Utilization of downstream processing technologies to purify proteins or organic compounds.

This chapter elaborates on the applications of gene cloning, PCR, and other DNA analysis techniques in biotechnology, medicine, and agriculture. Additionally, it addresses the ethical considerations surrounding the manipulation of genomes in microbes, plants, and animals.

Biotechnology is defined as the utilization of biological processes in industry and technology. It has garnered significant attention over the past three decades, largely due to gene cloning. While many useful products have been derived from microbial cultures, the scope was previously restricted to compounds naturally synthesized by microorganisms. However, the advent of gene cloning has broadened possibilities by allowing the insertion of genes for important animal or plant proteins into cloning vectors, which can then be introduced into bacteria, as illustrated in the figure.



Genetically engineered bacterium synthesizing the animal protein

Fig.: A possible scheme for the production of an animal protein by a bacterium

If manipulations are executed accurately, the gene will be activated, leading to the synthesis of the recombinant protein by the bacterial cell. Subsequently, it might be feasible to acquire substantial quantities of the protein via batch cultures or continuous cultures.

During the past century, comprehensive advancements across various sectors have notably enhanced the quality of life, consequently leading to a significant surge in population growth. Such escalated growth rates pose a risk of absolute scarcity of essential necessities like food. Many individuals foresaw this impending crisis and endeavored to seek solutions.

Three alternatives were considered to bolster food production:

(i) Agrochemical-based Agriculture: This approach involves the utilization of agrochemicals, encompassing a broad spectrum of chemical products applied in agriculture. These include various pesticides such as insecticides, herbicides, and fungicides, along with synthetic fertilizers, hormones, chemical growth agents, and concentrated sources of raw animal manure.

(ii) Organic Agriculture/Farming: In organic farming, farmers employ manure, biofertilizers, biopesticides, and biocontrol's to augment crop production, rather than relying on artificial fertilizers and pesticides. Organic farming emphasizes harmonizing with nature, utilizing techniques to achieve robust crop yields without causing harm to the natural environment or to the individuals residing and working within it.

(iii) Genetically Engineered Crop-based Agriculture: Among the forerunners of these initiatives was Norman E. Borlaug, widely recognized as the father of the Green Revolution.

What is the Green Revolution?

The Green Revolution refers to a period during the 20th century marked by a substantial increase in agricultural productivity, particularly in grains such as wheat and rice. This surge in productivity resulted from several factors:

(i) Introduction of improved crop varieties, known as high-yielding varieties.

(ii) Implementation of enhanced management practices, including irrigation, mechanization, and soil conservation techniques.

(iii) Utilization of agrochemicals such as fertilizers and pesticides.

Its initial remarkable success was observed in regions like Mexico and the Indian subcontinent. Despite tripling the food supply, the Green Revolution still fell short in adequately nourishing the expanding human population.

Challenges

The Green Revolution faced obstacles in developing countries due to various reasons:

- Agrochemicals often proved too costly for farmers in these regions.
- Further increasing yields with existing varieties became challenging through conventional breeding methods.
- The new varieties required substantial amounts of fertilizers and pesticides to achieve high yields, raising concerns about cost and potential adverse effects.
- There was a need to minimize the use of fertilizers and chemicals to mitigate their harmful environmental impacts.

To address these challenges, the use of genetically modified crops emerged as a potential solution. Gene cloning introduces a new dimension to crop breeding by enabling targeted alterations to the genotype of a plant, circumventing the random processes inherent in conventional breeding methods. Two primary strategies have been employed:

(a) Gene addition: This involves using cloning to modify a plant's characteristics by introducing one or more new genes.

(b) Gene subtraction: Genetic engineering techniques are utilized to deactivate one or more existing genes in the plant.

Plants, bacteria, fungi, and animals whose genes have been manipulated through such techniques are referred to as genetically modified organisms (GMOs) or transgenic organisms.

Transgenic plants have proven to be highly beneficial in various aspects, contributing to:

(i) Increased tolerance of crops to abiotic stresses such as cold, drought, salt, and heat.
(ii) Reduced dependency on chemical pesticides through the development of pest-resistant crops.
(iii) Mitigation of post-harvest losses.
(iv) Enhanced efficiency in the utilization of minerals by plants, thus preventing premature depletion of soil fertility.
(v) Augmentation of the nutritional value of food, exemplified by Vitamin A-enriched rice.

Golden Rice

Golden Rice, developed by Ingo Potrykus and Peter Beyer, addresses vitamin A deficiency by accumulating higher levels of β-carotene. This transgenic variety of rice (Oryza sativa) contains significant amounts of β-carotene, which serves as a precursor to vitamin A. The yellow coloration of the rice grains, attributed to β-carotene, has earned it the moniker "golden rice."

Plants equipped with their own insecticides

Plants face threats from a variety of organisms including viruses, bacteria, fungi, and animals, but in agricultural settings, insects pose the most significant challenge. To mitigate losses, crops often undergo regular spraying with insecticides. However, the application of biotechnology has led to the development of pest-resistant plants, thereby reducing the reliance on pesticides, as discussed below:

Utilizing Bacillus thuringiensis endotoxins

Insects not only consume plants but also occasionally include bacteria in their diet. In response, various bacteria have evolved defense mechanisms against insect predation, with Bacillus thuringiensis (Bt) being a prime example. During sporulation, B. thuringiensis forms intracellular crystalline bodies containing an insecticidal protein known as endotoxin.

The endotoxin present in the bacterium initially exists as an inactive precursor. Upon ingestion by the insect, this protoxin is cleaved by proteases in the alkaline conditions of the gut, resulting in shorter protein versions exhibiting toxic activity. These proteins bind to the interior of the insect's midgut, damaging the surface epithelium by creating pores that induce swelling and lysis. Consequently, the insect becomes unable to feed, leading to starvation and eventual death.

This toxin, referred to as Bt toxin, produced by Bacillus thuringiensis, has been cloned in bacteria and expressed in plants to confer resistance to insects, obviating the need for insecticides and effectively creating a bio-pesticide.

Examples include Bt cotton, corn, rice, tomato, potato, and soybean, among others.

Specific Bt toxin genes isolated from Bacillus thuringiensis have been incorporated into various crop plants, such as cotton, based on the crop and the targeted pest, as most Bt toxins exhibit specificity towards particular insect groups. These genes, often denoted as cry genes, are effective against a range of insects, including Lepidopterans (e.g., tobacco budworm, armyworm), Coleopterans (beetles), and Dipterans (flies and mosquitoes).

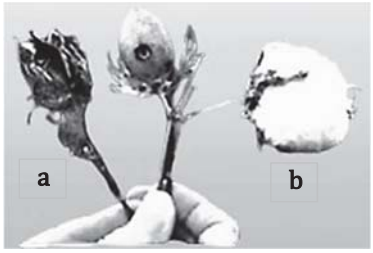


Fig.: Cotton Boll: (a) Damage caused by bollworms to a cotton boll (b) A fully mature cotton boll

Several such genes exist; for instance, proteins encoded by genes cry IAc and cry IIAb control cotton bollworm, while cry IAb targets corn borer.

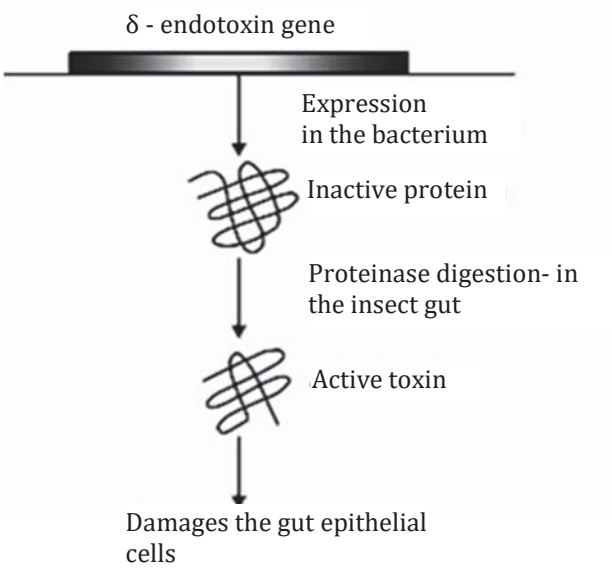


Fig.: Mode of action of a δ-endotoxin

Recent advancements in gene transfer technology have enabled the incorporation of Bt proteins into corn plants. However, this achievement necessitates significant modifications to the Bt genes, including the synthesis of synthetic versions of these genes, rather than relying on the microbial Bt gene directly.

Gene Subtraction

Gene subtraction is another technique for altering a plant's genotype, involving the inactivation or knockout of specific genes. Several strategies exist for inactivating targeted genes in living plants, with the most practical and successful method to date being the use of anti-sense RNA.

Utilization of Anti-sense RNA in Developing Pest-Resistant Plants

Numerous nematodes parasitize a wide range of plants and animals, including humans, posing a threat to various food and fiber crops by invading plant roots. These nematodes, commonly referred to as root-knot nematodes, feed on root cells, causing the formation of large galls or knots, thereby damaging crops and reducing yields.

One such nematode, Meloidogyne incognita, severely affects tobacco plant roots, resulting in significant yield reductions. To effectively manage this damage, bioengineered resistant plants have been developed to prevent nematode infestation. The strategy employed to prevent this infestation is based on RNA interference (RNAi).

RNAi is a natural mechanism that leads to the "silencing" of genes, preventing the synthesis of specific proteins. This mechanism is utilized for the regulation of specific genes and serves as a defense against viruses in nature. In research, RNAi has been employed for loss-of-function studies, where genes responsible for parasitism are selectively silenced. This process occurs in all eukaryotic organisms as a cellular defense mechanism.

RNAi involves the silencing of specific mRNA molecules through the formation of double-stranded RNA (dsRNA) molecules. These dsRNA molecules are formed by the binding of complementary RNA molecules, known as anti-sense RNA, to the original mRNA, thereby preventing translation of the original mRNA (silencing). The source of this complementary RNA could be from viruses with RNA genomes or mobile genetic elements (transposons) that replicate via an RNA intermediate.

To introduce nematode-specific genes responsible for parasitism into the host plant, Agrobacterium vectors are utilized. The introduction of DNA is such that it produces both sense and anti-sense RNA

in the host cells, forming dsRNA. These two RNAs, being complementary to each other, form dsRNA that is taken up by the parasitic nematode, initiating RNAi and silencing the specific mRNA of the nematode. As a result, the parasite cannot survive in a transgenic host expressing specific interfering RNA, thereby protecting the transgenic plant from infestation.

Utilizing Agrobacterium vectors, genes specific to nematode parasitism were introduced into the host plant. This DNA introduction was designed to generate both sense and anti-sense RNA in the host cells, leading to the formation of double-stranded RNA (dsRNA). These complementary RNA strands formed a dsRNA molecule, which was then absorbed by the parasitic nematode, initiating RNA interference (RNAi). As a result, the specific mRNA of the nematode was silenced. Consequently, the parasite was unable to survive in a transgenic host that expressed specific interfering RNA. Thus, the transgenic plant acquired protection from the parasite.

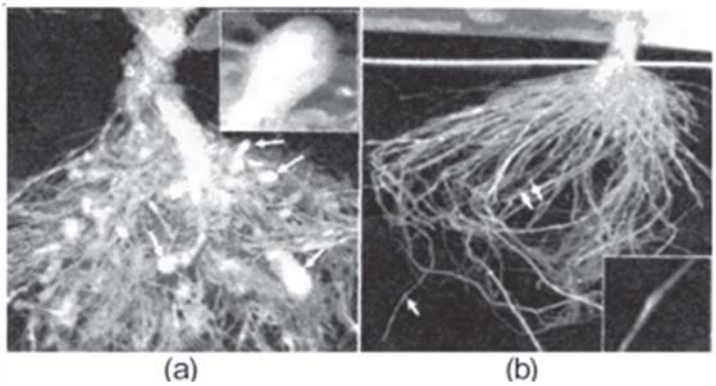


Figure: The novel mechanism of protection against nematode infestation triggered by host plant-generated dsRNA: (a) Illustration of roots from typical control plants; (b) Image depicting transgenic plant roots five days after deliberate nematode infection, yet showing protection through a novel mechanism.