Applications of Biotechnology

Superbug, edible vaccine,hgh,humulin

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Role of transgenics in Bioremediation(Superbug)

Bioremediation-(Definition and introduction)The use of either naturally occuring or deliberately introduced microorganisms to consume and break down environmental pollutants, in order to clean a polluted site.

Nature has its own way of cleaning the environment by removing **xenobiotics(xenobiotics** are **chemicals found in the environment** which **are not produced** by the **organisms.These** are mostly produced by human activities and excite public awareness due to their ability to interact with the living environment.**)**to maintain a **perfect balance**. but in this era of industrialization the rate of **xenobiotics discharges** has crossed **the tolerance limit of the nature**. Therefore, there is a **need to find out** the method of **remediating xenobiotics** from the environment. **Microbial remediation of xenobiotics** has proved the **effectiveness and low cost technology** but there are **several limitations in using microbes**.

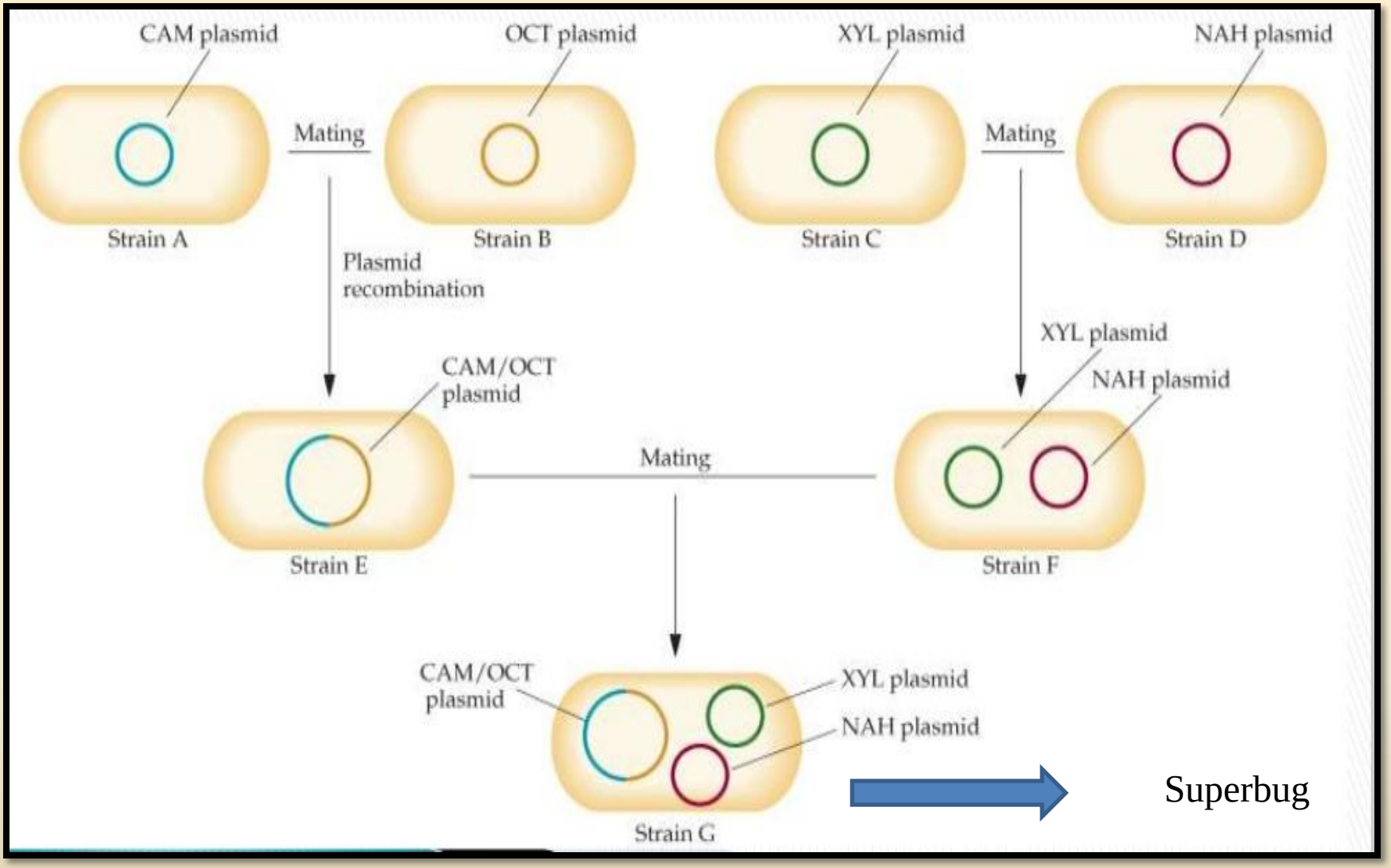
Thus, **genetic engineering approaches** are used by **genetic engineers** to **construct new strains of microbes (Genetically engineered microorganisms, GEMs or transgenic microorganisms)** that have **the unique characteristics** compared to **the wild type** and **broad spectrum of catabolic potential for bioremediation** of **xenobiotics.**

Oil spill occurs due release of **petroleum hydrocarbons** of any form into the **environment due to human activity**. The spill can originate from **oil tankers,off shore platforms,oil rigs** etc. The effect of this on **marine ecosystem is terrifying**.

Prof. Ananda mohan chakraborty et al. (1970) developed and patented a **“superbug”** that was made to **degrade oil** and **hence can clean up this oil spillover from the sea.**

- ***Pseudomonas putida*** was transformed with plasmids derived from four different bacteria containing **camphor, octane, xylene and napthalene degrading plasmids to make it into a superbug.**

So, now the **superbug** can **degrade all the four components alone** unlike it’s sources which could break down any one of these four and can be **effectively used as an agent to clean the oil spills in the ocean.**



How superbug was created

SUPERBUG SPECIES

- The organism is ***Pseudomonas putida***.
- It is Gram negative, Rod shaped, Saprotrophic, Soil bacteria.
- It is for wild type.
- Size 0.7-1.1 / 2.4 µm

Degradation of plastic-*Pseudomonas putida* degrades plastic as well because some strains utilize **polyethylene** as a **sole source** of **carbon and nitrogen.**
The diverse metabolism of wild-type strains of ***P. Putida*** may be exploited for **bioremediation;** for example, it has been shown in the laboratory to function as a **soil inoculant** to **remedy naphthalene-contaminated soils**. ***P. Putida*** is capable of converting **styrene oil** into the **biodegradable plastic PHA**. This may be of used in the **effective recycling of polystyrene** foam, otherwise thought to be not biodegradable.

Other examples of bioremediation by microorganisms-Scientists have developed ***Anabaena sp***. and ***Nostoc Ellipsosporum*** by the insertion of **linA (from *P. paucimobilus*) and fcbABC (from *Arthrobacter globiformis*)** respectively. The **gene linA** controls the **biodegradation of lindane (γ-hexachlorocyclohexane**), and **fcbABC** confers the ability to **biodegrade halobenzoates** and can be used to remediate these pollutants from water sources.

There are several developments in **GEMs** which **overcome** the **limits of using wild type microbes**. Microbes are confined to **aerobic catabolic and co-metabolic pathway**s and therefore **cannot be applied to anaerobic environment**. **GEMs** are developed by **inserting genes for oxygenases** make it **possible** to **use them** in **anaerobic environmental** conditions. One must consider **all the xenobiotics present in multi-contaminated sites** before **applying microbes for bioremediation.** GEMs **offer the properties of many microbes due to the insertion of genes in a single microorganism**. Therefore, GEMs can be used **successfully for bioremediation purpose.**

Edible Vaccines-

Vaccines definition- Infectious diseases account for more than 54% of total mortality in developing countries, where vaccines are the most effective means of prevention. **Vaccine is a substance used to stimulate the production of antibodies and provide immunity against one or several diseases, prepared from the causative agent of a disease, it's products, or a synthetic substitute, treated to act as an antigen without subjecting the individual to the risk of true infection or disease.** Diseases such as **cholera, typhoid fever, tuberculosis, and poliomyelitis** have been controlled by massive vaccination campaigns.

Limitations of traditional vaccination-

Despite the advantages of vaccination, **limitations** restricting the use of vaccines remain.

1)**Not all** pathogenic agents **can be cultivated** in an **exogenous medium** and, due to their **highly pathogenic features**, the cultivation of some **agents demands biosecurity** and **biosafety infrastructures** that are **difficult** for some countries to afford. Consequently, the production of **certain vaccines** remains **costly** and **restricted** in **numerous countries**, thus generating an undesirable dependence on hygiene.

2) Another **restrictive factor** is that, although the **attenuation of bacteria or viruses involves** very controlled processes, the possibility that **these pathogens could revert** to their **original pathogenic form** must be considered.

3)Additionally, the **highly specified expiration time** and **refrigeration requirements** inherent to nearly all commercial vaccines demand constant attention to the pathogen contained in such vaccines, thus increasing **control, storage, and distribution costs**.

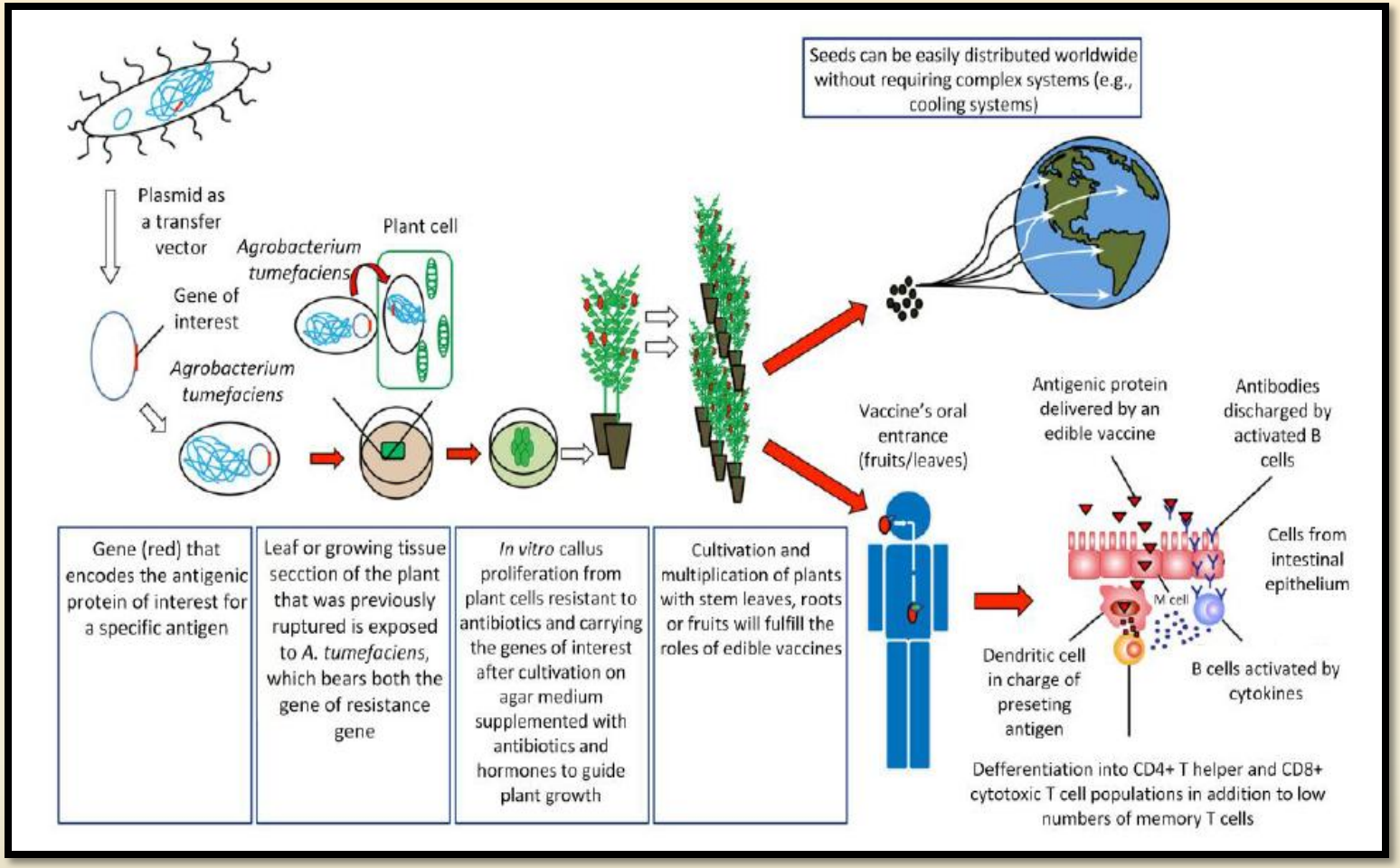
4)Vaccine **degradation after acid digestion in the stomach** is another **concern.**

Edible vaccine- definition, introdution and concept

Recognizing these limitations and exploiting advances in recombinant DNA technology, **Mason et al.** succeeded in **expressing a surface antigen from hepatitis B in tobacco plants**. This finding immediately suggested that **plants were potentially effective vectors** for the **production of vaccines to prevent diseases**, giving rise to the concept of **"edible vaccines," Edible vaccines(definition)**; as the name suggests, **are foods that provide nourishment in terms of vitamins, proteins, and other nutritional qualities that also act as vaccines to immunize the consumer against a certain disease.** These are of interest as **alternative methods of vaccination.** Edible vaccines include all vaccines that are produced in a **type of edible format** (i.e., **part of a plant, its fruit, or subproducts derived from that plan**t) that, upon **oral ingestion, stimulate the immune system**. It is worth mentioning that edible does not necessarily mean tasty, or organoleptically pleasing, since edible vaccines need **only be safe (non-toxic) for human consumption**.

To create an **edible vaccine**, the information **necessary** to produce an **antigenic protein** must be **introduced into the plant** of interest by **genetic engineering techniques**. Once an individual consumes an **edible vaccine,** the **outer wall of plant cells protects** the **antigens** from **degradation** by **gastric secretion**, allowing the **antigens to be delivered** to the **intestinal mucosal surfaces**, where they are **absorbed by different mechanisms** in order to **stimulate a strong and specific immune response**.

How edible vaccines are developed



Procedures involved in obtaining an edible vaccine and an immune response. Edible vaccine development begins with the identification of the gene encoding the antigenic protein and its introduction into the plant that will process the food (edible vaccines), which can then potentially be distributed globally. After an edible vaccine has been consumed, and the subsequent passage of the antigenic protein through the M cells specialized in the delivery of antigens to dendritic cells, the individual's immune system triggers a response involving B cells and T helper cells as the main factors.

How Are Edible Vaccines Developed?

The mechanisms of edible vaccines involve a series of general principles. The first step consists of **the identification, isolation, and characterization of the antigen** of a particular disease. This antigen **must elicit a strong specific immune response**.

If the antigen elicits a strong specific immune response, the **gene encoding for this antigen must be cloned** into a **transfer vector** carrying an **antibiotic-resistance gene**, followed by **transformation of the plant of interest**.

Plant **viral vectors** appear to be the **most promising** for expressing **foreign proteins** in plants. Plant transformation is attained by different methodologies. One of the most commonly used methods for efficiently transferring **recombinant DNA into plant cells** involves the **bacterium *Agrobacterium tumefaciens*.**

An *Agrobacterium* strain has been designed to eliminate virulent genes that produce a tumor growing at the base of plants while retaining the genes involved in efficient DNA transfer. The tumor DNA (T-DNA) is contained in a plasmid called the **Ti plasmid.**

The sequence **of interest (pathogen) is then inserted into T-DNA to** produce the antigenic protein. Once the **transgene (T-DNA + antigen DNA) is integrated into the plant genome, the sequence should be expressed and inherited in a typical Mendelian fashion**, following **permanent or temporary (transient) expression of the antigen of interest in the plant or fruit**.

Later, this genetic line may be propagated by **vegetative methods(cutting)** or **seeds arising from asexual reproduction**.

Examples-

Year	Plant	Disease or Infectious Agent	Antigen	References
1998	Potato	Enteritis produced by *Escherichia col*	-	[103]
1998	Potato	Norwalk virus capsid	-	[104]
1998	Potato	Non-toxic subunit (CT-B) of *Vibrio cholerae* enterotoxin	-	[27]
1998	Potato	Rabbit hemorrhagic	Protein VP60	[106]
2003	Algae	Foot-and-mouth disease virus	Viral structural protein VP1	[29]
2003	Cherry tomatillo	Hepatitis B	HBsAg (surface protein of Hepatitis B)	[138]
2003	Pea	Rinderpest virus	Hemagglutinin protein (H)	[98,136]
2004	Alfalfa	Hog rotavirus (BVR)	Antigen eBRV4	[129]
2005	Banana	Hepatitis B	HBsAg (surface protein of Hepatitis B)	[134,135]
2005	Lettuce	Hog pest virus	Glycoprotein E2	[72]
2005	Potato	Hepatitis B	-	[72]
2005	Tomato	Coronavirus	-	[23]
2006	Tomato	Norwalk virus	Surface protein	[50,115]
2007	Algae	Swine fever (CSFV) disease	Surface protein E2	[102,145]
2007	Papaya	Cysticercosis caused by *Taenia solium*	Synthetic peptides	[80]
2007	Rice	Infectious bursitis	VP2 protein	[86]
2007	Tomato	*Vibrio cholerae* B toxin	CT-B protein	[28]
2007	Tomato	Hepatitis B	HBsAg (surface protein of Hepatitis B)	[50,116,117]
2007	Tobacco *	Chicken infectious anemia	Virus VP1 protein	[109]
2008	Rice	Hepatitis B	HBsAg (surface protein of Hepatitis B)	[122,123]
2010	Carrot	*Helicobacter pylori*	Subunidad UreB	[126]
2010	Corn	Rabies virus	Antigen glycoproteins	[13,85]
2012	Tobacco *	Avian flu virus	HPAIV H5N1	[112,113]
2012	Quinoa	Infectious bursitis virus	VP2 protein	[133]
2014	Algae	Diabetes	Glutamic acid decarboxylase	[102]
2014	Algae	Human Papilloma Virus	E7 protein	[102]
2014	Algae	Hepatitis B	HBsAg (surface protein of Hepatitis B)	[102]

* Although the tobacco plant is not a food, we have included it because it has been demonstrated that it can serve as a pharma plant.

Edible vaccines represent a valuable solution to treating certain diseases **whose control and**
prevention is restricted by the **inherent limitations of traditional vaccines**, such as their **production costs, storage requirements, and expensive logistics**. **Sixteen foods are already producing antigens** to counter **human and animal diseases**. Bacterial surface protein such as ***Vibrio cholerae*** **B subunit** has been synthesized in **transgenic tobacco,tomato and rice plants** and shown to **elicit an anti cholera immune response** when **leaves fruits or seeds** are **fed** to **mice**. Attempts are also being made to **engineer plants** that express a **variety of vaccines**, so that **immunity against a range of diseases** can be acquired.

Advantages- 1. The **ease with which plants can be grown and harvested** means that this will be **applicable for developing countries of** the world where more expensive approaches of recombinant protein production are **difficult to sustain**.

2.This is a very **simple and cheap way of carrying out mass vaccination programme.**

Disadvantages-1.The amount of recombinant protein synthesized by the plant is often **insufficient to stimulate complete immunity against a target disease**.(only 0.5% instead of the desired 8-10%)

2.Solution to this problem is to place the cloned gene in the chloroplast genome, but this again creates another problem, because **proteins made in chloroplast are not glycosylated so the vaccines that require post translational modification cannot be made this way.**

3.**Biosafety issue** is another concern.

Genetically engineered products- Human growth hormone, Humulin

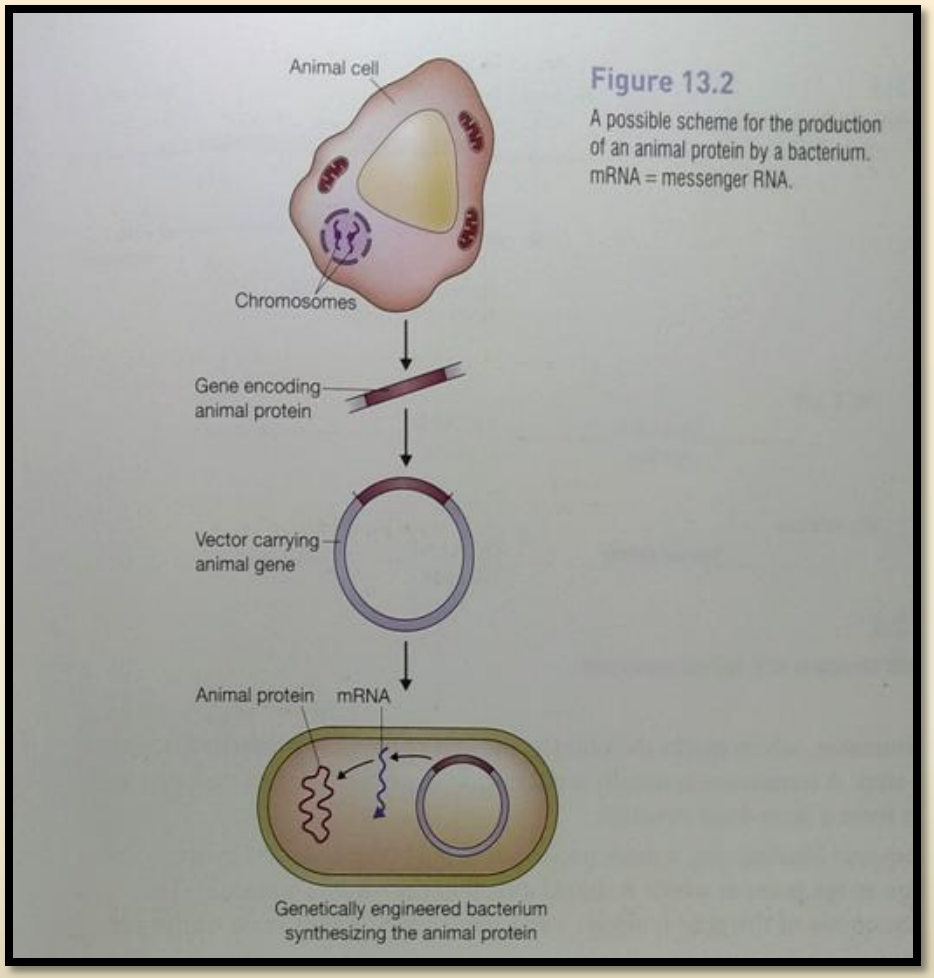


Figure 13.2
A possible scheme for the production of an animal protein by a bacterium. mRNA = messenger RNA.

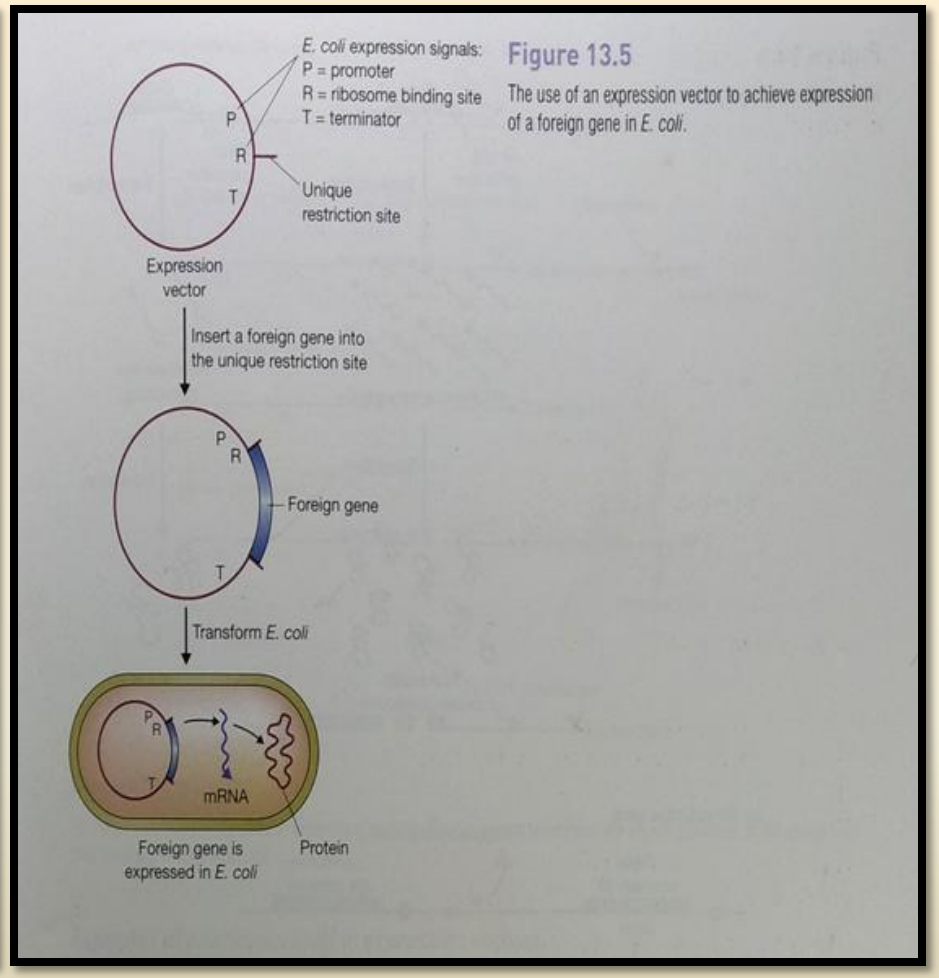


Figure 13.5
The use of an expression vector to achieve expression of a foreign gene in *E. coli*.

Essential sequences to express an eukaryotic gene in a prokaryote system

If a foreign (i.e., non-bacterial) gene is simply ligated into a standard vector and cloned in *E. coli*, it is **very unlikely that a significant amount of recombinant protein** will be **synthesized**. This is because expression is dependent on the gene being surrounded by a collection of signals that can be recognized by the bacterium. These signals, which are short sequences of nucleotides, advertise the presence of the gene and provide instruction tions for the transcriptional and translational apparatus of the cell. The three most important signals for *E. coli* genes are as follows-

The promoter, which **marks the point at which transcription of the gene should start**. In *E. coli*, the promoter is recognized by **the sigma subunit** of the transcribing enzyme **RNA polymerase.**

The terminator, which **marks the point at the end of the gene where transcription should stop.** A terminator is usually a nucleotide sequence that can base pair with itself to form a stem-loop structure.

The ribosome binding site, a **short nucleotide sequence recognized by the ribosome as the point at which it should attach to the mRNA molecule.** The initiation codon of the gene is always a few nucleotides downstream of this site.

The genes of higher organisms are also surrounded by expression signals, but their nucleotide sequences are not the same as the *E. coli* versions. There are similarities, but it is unlikely that an *E. coli* RNA polymerase would be able to attach to a human promoter. A foreign gene is **inactive** in *E. coli*, simply because the bacteria **does not recognize its expression signals**.

A solution to this problem would be to **insert the foreign gene into the vector in such a way that it is placed under control of a set of *E. coli* expression signals**. If this can be **achieved**, then the **gene should be transcribed and translated**. Cloning vectors that provide these signals, and can therefore be used in **the production of recombinant protein**, are called **expression vectors.**

Humulin or human insulin

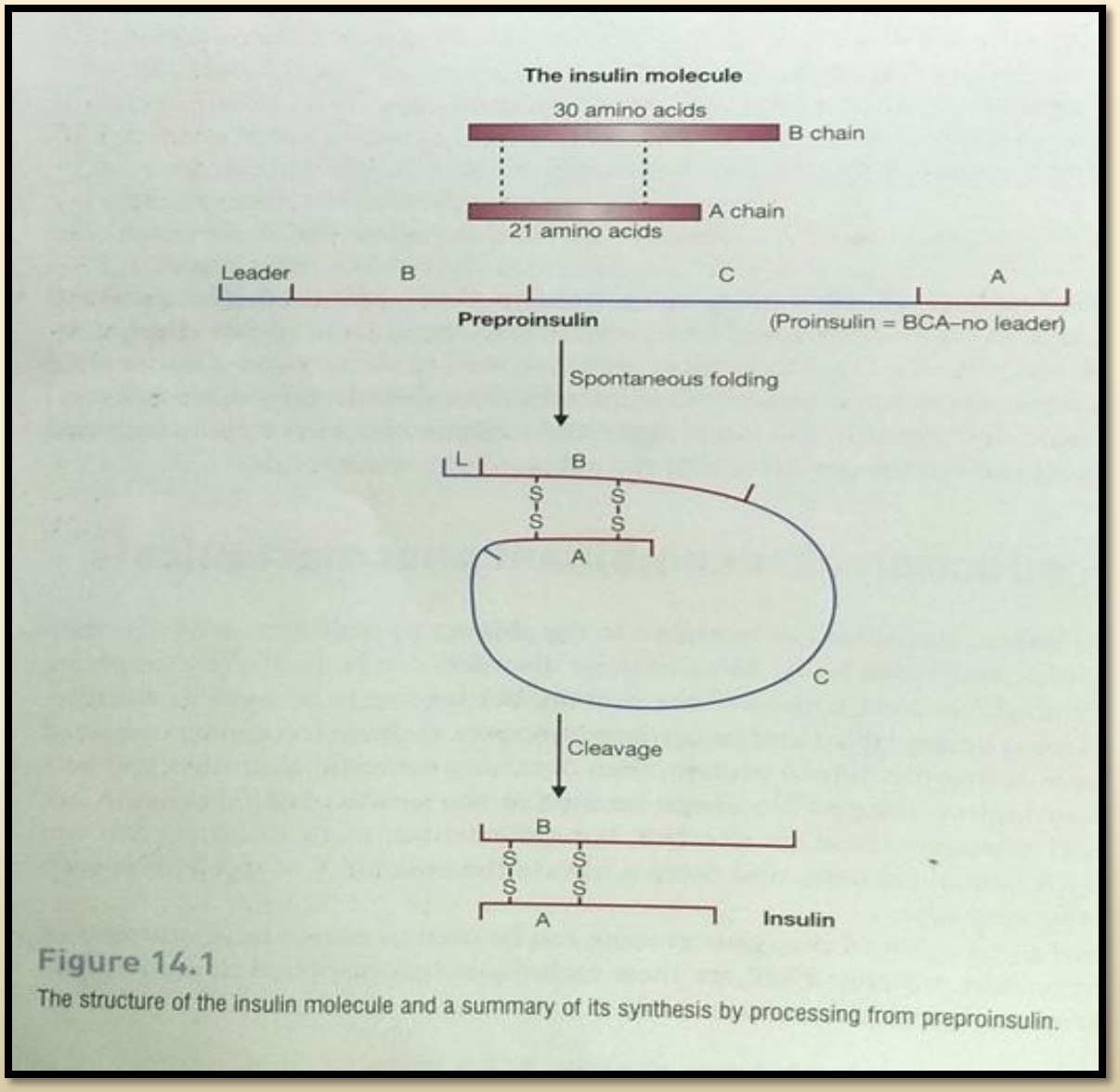


Figure 14.1

The structure of the insulin molecule and a summary of its synthesis by processing from preproinsulin.

Insulin, synthesized by the **B-cells of the islets of Langerhans in the pancreas**, controls the **level** of **glucose in the blood**. An **insulin deficiency** manifests itself as **diabetes mellitus**, a complex of symptoms which may lead to death if untreated. Fortunately, many forms of **diabetes can be alleviated by a continuing program of insulin injections**, thereby **supplementing** the **limited amount of hormone synthesized by the patient's pancreas**.

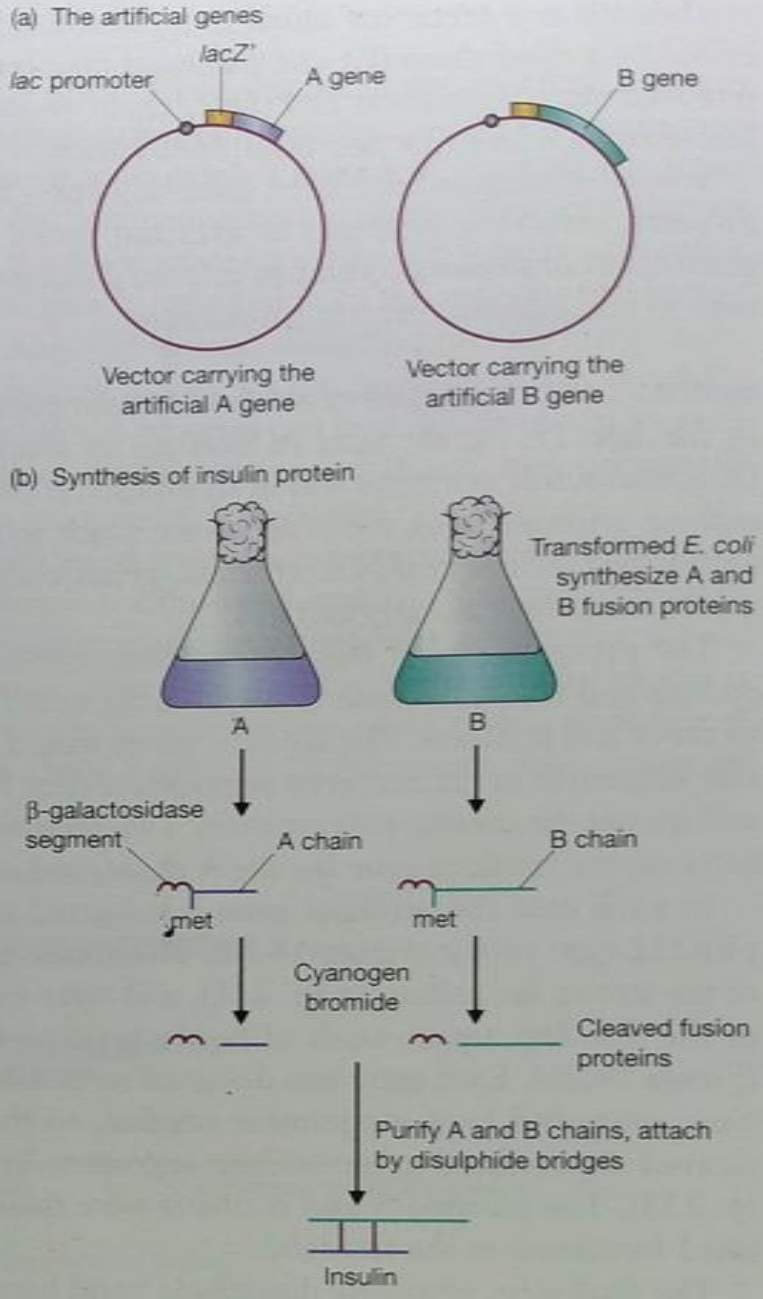
Insulin **displays two features** that **facilitate** its **production by recombinant DNA techniques**. **The first** is that **the human protein is not modified** after **translation by the addition of sugar** molecules, **recombinant insulin** synthesized by a **bacterium** should therefore **be active**.

The second advantage concerns the **size of the molecule**. Insulin is **a relatively small protein**, comprising two polypeptides, one **of 21 amino acids (the A chain**) and one of **30 amino acids (the Bchain).** In humans these **chains synthesized as a precursor** called **preproinsulin**, which contains the **A and B segments linked by a third chain (C) and preceded by a leader sequence.(refer to fig in previous slide). The leader sequence is removed** after translation and the **C chain excised**, leaving the **A and B polypeptides linked to each other by two disulfide bonds.**

Several strategies have been used to obtain recombinant insulin. One of the first projects, involving synthesis of artificial genes for the A and B chains followed by production of fusion proteins in E. coli, illustrates a number of the general techniques used in recombinant protein production.

Figure 14.2

The synthesis of recombinant insulin from artificial A and B chain genes.



Synthesis and expression of artificial insulin genes

Genes coding for the A and B chains of insulinwere synthesized as early as 1978.

The procedure used was to **synthesize trinucleotides representing all the possible codons and then join these together in the order dictated by the amino acid sequences of the A and B chains.** The artificial genes would not necessarily have the same nucleo tide sequences as the real gene segments coding for the A and B chains, but **they would still specify the correct polypeptides.**

Two Recombinant plasmids were constructed, one carrying the **artificial gene for the A chain**, and **one the gene for the B chain**. In **each case the artificial gene was ligated to a lacZ' reading frame present in a PBR322-type vector.** The **insulin genes** were therefore **under the control of the strong lac promoter**, and were **expressed as fusion proteins**, consisting of the **first few amino acids of B-galactosidase followed by the A or B polypeptides**. (refer to fig in previous slide)

Each gene was **designed** so that its **B-galactosidase** and **insulin segments** were **separated by a methionine** residue, so that the **insulin polypeptides** could be **cleaved from the B-galactosidase segments by treatment** with **cyanogen bromide**.

The **purified A and B chains were** then **attached to each other by disulphide bond formation** in the test tube. The final step, involving disulphide bond formation, is actually rather inefficient.

A **subsequent improvement** was to synthesize not the individual **A and B genes**, but the entire **proinsulin reading frame**, **specifying B chain-C chain-A chain** (Although this is a more daunting proposition in terms of DNA synthesis, **the prohormone has the big advantage of folding spontaneously into the correct disulphide-bonded structure.** The **chain segment** can then be **excised** relatively easily by **proteolytic cleavage**.(refer to figure in slide 15)

Human growth hormone

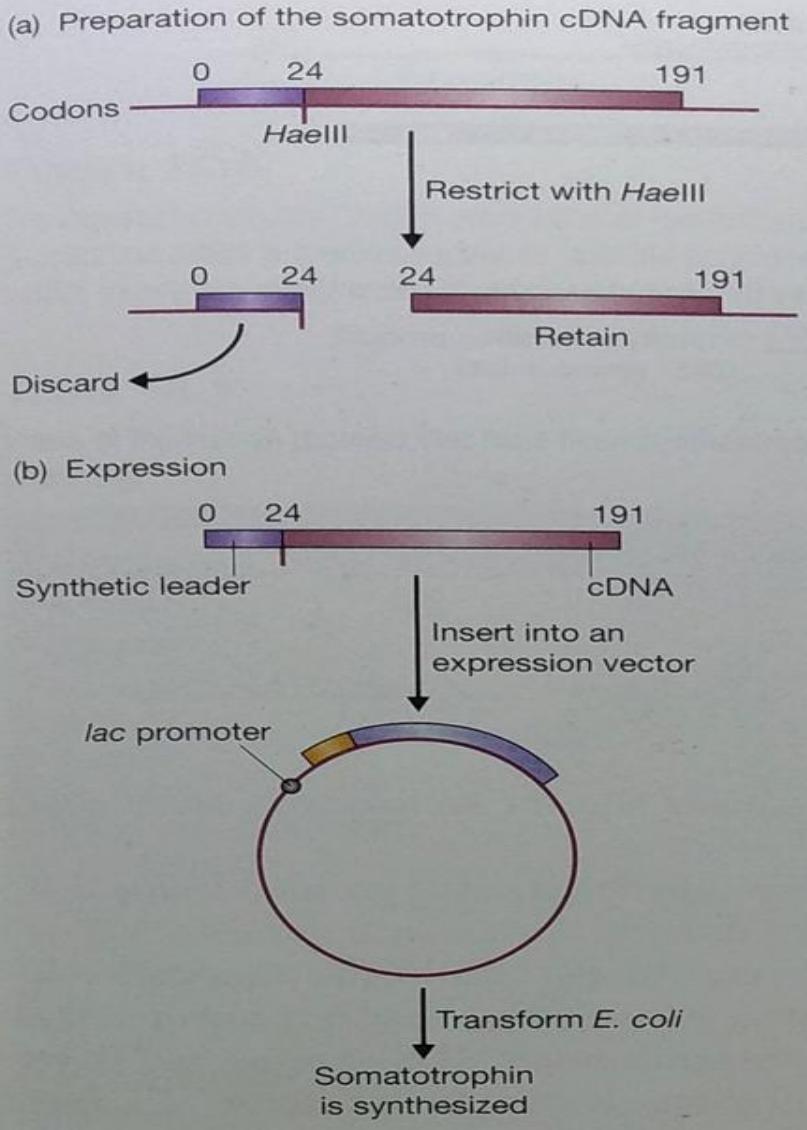


Figure 14.4

Production of recombinant somatotrophin.

Synthesis of human growth hormones in *E.coli*

At about the same time that recombinant insulin was first being made in *E. coli*, other researchers were working on similar projects with the **human growth hormones Somatostatin and Somatotropin**. These two proteins **act in conjunction to control growth processes** in the human body, their **malfunction leading to painful and disabling disorders Such as acromegaly (uncontrolled bone growth) and dwarfism**.

Somatostatin was the **first human protein** to be **synthesized in *E. coli***. Being a very short protein, **only 14 amino acids in length**, it was ideally suited for **artificial gene synthesis.** The strategy used was the **same** as described for **recombinant insulin**, involving **insertion of the artificial gene** into a **lacZ' vector** synthesis of a **fusion protein**, and **cleavage with cyanogen bromide**.

Somatotropin presented a more difficult problem. This protein is **191 amino acids in length**, equivalent to **almost 600 bp**, an **impossible prospect** for the **DNA synthesis capabilities** of the late 1970s.

In fact, **a combination of artificial gene synthesis and complementary DNA (DNA) cloning was used to obtain a somatotropin-producing *E. coli* strain**. **Messenger RNA** was obtained from the **pituitary,** the gland that **produces somatotropin** in the human body and a **cDNA library prepared.** The **somatropin DNA** contained a **single site for the restriction endonuclease HaeIII,** which therefore cuts **the cDNA into two segments**. The **longer segment, consisting of codons 24-191**. was **retained** for use in **construction of the recombinant plasmid.** The **smaller segment** was **replaced by an artificial DNA molecule** that **reproduced** the **start of the somatotropin gene** and provided the **correct signals for translation in** *E, coli*. The **modified gene was then ligated** into an **expression vector** carrying the **lac promoter**.(refer to figure in the previous slide).

Biosafety concerns-

A)Bioterrorism-

One of the main problems related to the generation of biotechnological tools is their perception as **destructive and non-productive**, as in the case of **bioterrorism,** which is defined **as the threat of use or use of a biological agent by individuals or groups based on political, religious, ecological, or ideological goals. Terrorism** using **biological agents** that are **harmful** to **humans,** like intentional release of **viruses,bacteria or other germs that can sicken or kill people.**

The **inappropriate management of a modified plant or fruit that contains a vaccine, whether due to negligence or ill-intentioned purposes (bioterrorism), would present serious challenges to public health and global safety.** Moreover, because edible vaccines represent a **very powerful technological tool,** their possible use by terrorists has not been discounted, and it remains **difficult to eliminate** such ill-intentioned use.

Such vaccines will continue to be regarded as **genetically modified organisms**, a term that **alarms** the **majority** of the **population**.

Although genetically modified organism development has been under stringent control with regard to the acceptance of production and distribution, edible vaccines still require more regulation.

However**, edible vaccines** could be used not only in an undesirable way but also as a **solution to counter bioterrorism**. An example is the **edible vaccine developed in 2014** to combat **anthrax (carbuncle),** based on a tobacco **plant expressing the PA**; the antigen was tested using murine models that exhibited a **high serum content of IgA and IgG.**

Correspondingly, the possible **beneficial** or **harmful** use of current biological tools will depend on the people who develop them and the manner in which their use is **regulated**. Moreover, despite the future promise of edible vaccines, both veterinary and human medicine studies are lacking to promote their use within these areas and to restrict their ill-intentioned use.

2)Safety concerns with selectable markers

One of the main areas of concern to emerge from the debate over genetically modified tomatoes is the **possible harmful effects of the marker genes** used with **plant cloning vectors**. Most **plant vectors** carry a copy of a gene for **kanamycin resistance**, enabling transformed plants to be identified during the cloning process. The **kan gene,**

is bacterial in origin and codes for the enzyme **neomycin phosphotransferase II**. This gene and its enzyme product are present in all cells of an engineered plant. The fear that neomycin phosphotransferase might be toxic to humans has been analysed by tests with animal models, but two other safety issues remain:

Could the **kan gene** contained in a **genetically modified foodstuff** be **passed** to **bacteria in the human gut**, making these **resistant to kanamycin** and **related antibiotics**?

Could the kan gene be **passed** to **other organisms** in the environment, and would this result in **damage to the ecosystem**? Neither question can be fully answered with our current knowledge. It can be argued that digestive processes would destroy all the kan genes in a genetically modified food before they could reach the bacterial flora of the gut, and that, even if a gene did avoid destruction, the chances of it being transferred to a bacterium would be very small. Nevertheless, the risk factor is not zero.

3)Possible harmful effect on the environment

Another area of concern **regarding genetically modified plants** is that their **new gene combinations might harm the environment** in some way. These concerns have to be **addressed individually for each type of GM crop**, as different engineered genes might **have different impacts.**

We will see the work that has been carried out to assess whether it is possible that herbicide resistant plants, can have a harmful effect.

As these are the most widely grown GM crops, they have been subject to some of the most comprehensive environmental studies. In particular, in 1999, the UK Government commissioned an independent investigation into how herbicide resistant crops can affect the environment.

The study involved 273 field trials throughout England, Wales, and Scotland, and included **glyphosate resistant sugar beet** as well as **maize and spring rape engineered** for **resistance to a second herbicide glufosinate-ammonium.**

The team found that there **were differences** in the **abundance of wildlife between GM crop fields and conventional crop fields.** The results of this study suggest that **growing such GM crops could have implications for wider farmland biodiversity. But** this area needs further research.

References-

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