21 Biotechnology and genetic modification

Focus

Earlier in this book we looked at how cells grow and divide to form new cells, and what information is stored in them. The information is encoded in DNA in the form of genes and scientists have learned how to read this code. This chapter investigates how scientists can change or add to the genetic code to give the organism different qualities. How can our food crops be improved now that we have this new knowledge and skills? Is it safe to change the DNA of a living thing? Genetic modification raises a number of ethical issues which we will consider here.

Biotechnology and genetic modification

FOCUS POINT

- How are bacteria useful in biotechnology and genetic modification?

- Why are bacteria useful in biotechnology and genetic modification?

Biotechnology is the application of biological organisms, systems or processes to manufacturing and service industries. **Genetic modification** involves the transfer of genes from one organism to (usually) an unrelated species.

Both processes often make use of bacteria because they can make complex molecules (proteins, for example) and have a rapid reproduction rate.

Use of bacteria in biotechnology and genetic modification

Bacteria are useful in biotechnology and genetic modification because they can be grown and manipulated without raising ethical concerns. They have a genetic code that is the same as all other organisms, so scientists can transfer genes from other animals or plants into bacterial DNA. They are especially useful because they multiply so fast (up to three times an hour).

Bacterial DNA is in the form of a circular strand and also small circular pieces called plasmids. Scientists have developed techniques to cut open these plasmids and insert sections of DNA from other organisms into them. When the bacterium divides, the DNA in the modified plasmid is copied, including the extra DNA. This may contain a gene to make a protein like insulin, which can be extracted and used as a medicine to treat diabetes.

Biotechnology

FOCUS POINT

- What is the role of anaerobic respiration in yeast during the production of biofuels and in bread-making?
- What is the role of pectinase in fruit juice production?
- What is the role of biological washing powders that contain enzymes?
- What is the role of lactase in lactose-free milk production?

- How are fermenters used in the large-scale production of useful products?
- What conditions need to be controlled in a fermenter?

Although biotechnology appears to be one of the latest discoveries in science, we have been making use of it for hundreds of years. The baking of bread, wine-making, the brewing of beer and the production of cheese all depend on fermentation processes brought about by yeasts, other fungi and bacteria, or enzymes from these organisms.

Antibiotics, like penicillin, are produced by mould fungi or bacteria. The production of industrial chemicals like citric acid or lactic acid needs bacteria or fungi to bring about essential chemical changes.

Sewage disposal depends on bacteria in the filter beds to form the basis of the food chain that purifies the effluent.

Biotechnology is not only concerned with the use of microorganisms. Cell cultures and enzymes are also involved in modern developments.

Biofuels

Fermentation includes a wide range of reactions under the influence of enzymes or microorganisms such as yeast. In Chapter 12, the anaerobic respiration of glucose to alcohol was described. This is a form of fermentation.

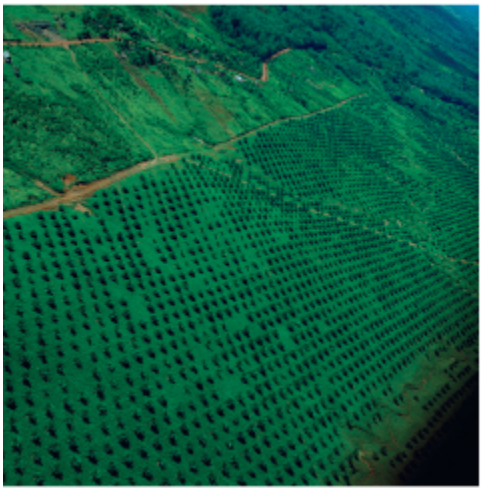
Microorganisms involved in fermentation are using the chemical reaction to release energy, which they need for their living processes.

In ‘Conservation’ in Chapter 20, it is pointed out that ethanol (alcohol) can be produced from fermented sugar or spare grain. This could replace, or at least supplement, petrol.

Brazil, Zimbabwe and the USA produce ethanol as a renewable source of energy for cars. Since 2013, 94% of new cars in Brazil use a mixture of petrol and ethanol. As well as being a renewable resource, ethanol produces less pollution than petrol.

However, biofuels are not yet economical to produce. For example, the energy used to grow, fertilise, harvest and transport sugar cane, plus the cost of extracting the sugar and converting it to ethanol, uses more energy than the ethanol releases when burned.

There are also environmental costs, some of which were outlined in Chapter 20. Forests are being destroyed to plant soy beans or oil palms (Figure 21.1), removing the habitats of thousands of organisms, some of which, like the orang-utan, are on the verge of extinction.



▲ **Figure 21.1** A new palm oil plantation, replacing a rain forest

Another biofuel, oil from rapeseed or sunflower seed, can, with suitable treatment, replace diesel fuel. It is less polluting than diesel but more expensive to produce.

Bread

Yeast is the microorganism used in bread-making, but the only fermentation product needed is carbon dioxide. The carbon dioxide makes bubbles in the bread dough. These bubbles make the bread light in texture.

Flour, water, salt, oil and yeast are mixed to make a dough. Yeast has no enzymes for digesting the starch in flour, but adding water activates the amylases already present in flour. The amylases digest some of the starch to sugar. With highly refined white flour, adding sugar to the dough helps the process. The yeast then ferments the sugar to alcohol and carbon dioxide.

A protein called gluten gives the dough a sticky, elastic texture, which holds the bubbles of gas. The dough is repeatedly folded and stretched (kneaded) either by hand, in the home, or mechanically in the bakery. The dough is then left for an hour or two at a temperature of about 27°C while the yeast uses sugar for respiration. The carbon dioxide bubbles build up, making the dough rise to about double its volume (Figure 21.2). The dough may then be kneaded again or put straight into baking tins and into an oven at about 200°C. This temperature makes the bubbles expand more, kills the yeast and evaporates the small quantities of alcohol while the bread bakes.

▲ **Figure 21.2** Carbon dioxide produced by the yeast has caused the dough to rise

Fruit juice production

Pectinases are enzymes used to separate the juices from fruit like apples. The enzymes can be extracted from fungi (e.g. *Aspergillus niger*). They work by breaking down pectin, the jelly-like substance that sticks plant cell walls to each other. The enzymes can also be used to make fruit juice more transparent. During the breakdown process several different polysaccharides are released, which make the juice cloudy. Pectinases break these down to make the juice clearer. The sugars produced also make the juice sweeter.

Biological washing powders

Most commercial enzyme production involves protein-digesting enzymes (proteases) and fat-digesting enzymes (lipases) for use in the food and textile industries. When combined in washing powders they remove stains in clothes caused by proteins (e.g. blood or egg) and fats (e.g. grease and oil). Protein and fat molecules tend to be large and

insoluble. When they have been digested the products are small, soluble molecules, which can pass out of the cloth.

Biological washing powders save energy because they can be used to wash clothes at lower temperatures, so there is no need to boil water. However, if they are put in water at higher temperatures the enzymes become denatured (see Chapter 5) and so they are not as effective.

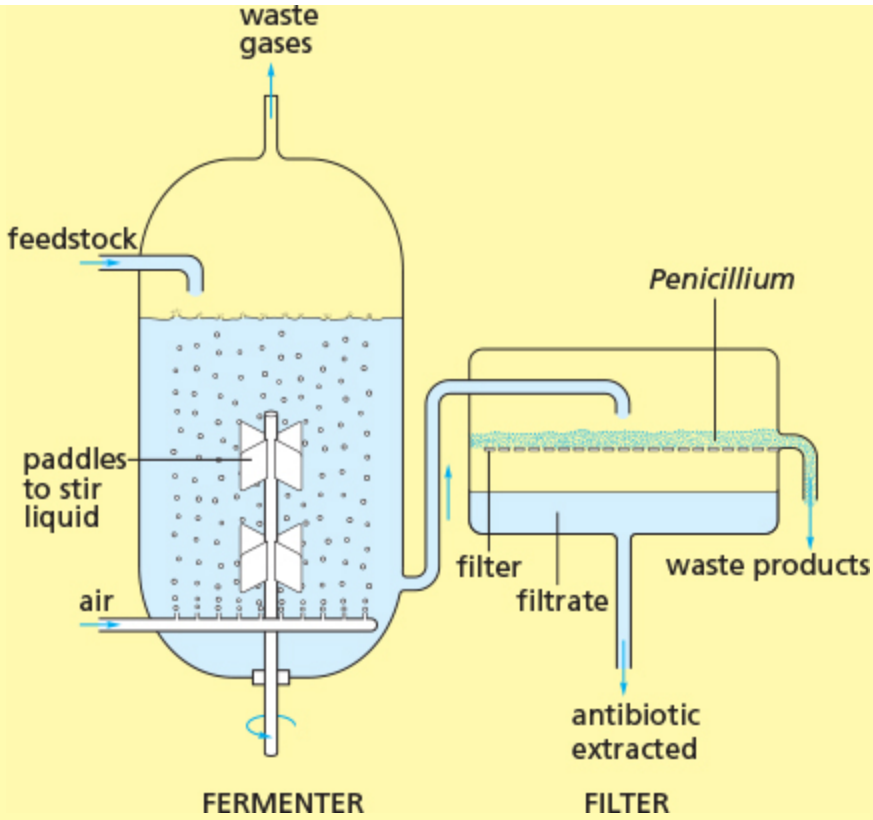
Lactose-free milk

Lactose is a type of disaccharide sugar found in milk and dairy products. Some people have problems with **lactose intolerance**. This is a digestive problem where the body does not produce enough of the enzyme lactase. As a result, the lactose stays in the gut, where it is fermented by bacteria. This causes symptoms like flatulence (wind), diarrhoea and stomach pains. Many foods contain dairy products, so people with lactose intolerance cannot eat them, or experience the symptoms described above. However, lactose-free milk is now produced using the enzyme lactase.

The lactase can be produced on a large scale by fermenting yeasts (e.g. *Kluyveromyces fragilis*) or fungi (e.g. *Aspergillus niger*). The fermentation process is shown in Figure 21.3.

A simple way to make lactose-free milk is to add lactase to milk. The enzyme breaks down lactose sugar into two monosaccharide sugars: glucose and galactose. Both can be absorbed by the intestine.

An alternative, large-scale method is to immobilise lactase on the surface of beads. The milk is then passed over the beads and the lactose sugar is effectively removed. This method avoids having the enzyme molecules in the milk because they stay on the beads.



▲ **Figure 21.3** Principles of antibiotic production using a fermenter

Going further

The role of bacteria in yoghurt production The food industry uses lactase in the production of milk products like yoghurt: it speeds up the process and makes the yoghurt taste sweeter.

The two main species of bacteria used as starter cultures in the production of yoghurt are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The bacterial culture is added to pasteurised milk at 42°C. The function of the bacteria is to ferment lactose, the sugar present in milk. One of the products of the reaction is lactic acid, which gradually increases the acidity of the fermented milk to a pH of 4.5. The acidity causes the milk to clot, forming a soft gel (yoghurt) and gives the product its characteristic natural sharp flavour. Yoghurt is produced commercially in large **fermenters** (Figure 21.4).

▲ **Figure 21.4** Large-scale production of yoghurt in fermenters

Antibiotics

When microorganisms are used to produce antibiotics, it is not their fermentation products that are wanted but complex organic compounds, called antibiotics, that they synthesise (Figure 21.5).

Most of the antibiotics we use come from bacteria or fungi that live in the soil.

Perhaps the best known antibiotic is penicillin, which is produced by the mould fungus *Penicillium* and was discovered by Sir Alexander Fleming in 1928 (see Chapter 15).

▲ **Figure 21.5** A laboratory fermenter for antibiotic production, which will eventually be scaled up to 10 000-litre fermentation vessels

Going further

Antibiotics

One of the richest sources of antibiotics is *Actinomycetes*. These are filamentous bacteria that look like microscopic mould fungi. The

actinomycete *Streptomyces* produces the antibiotic streptomycin.

Penicillin is still an important antibiotic, but it is produced by mutant forms of a different species of *Penicillium* from the fungus studied by Fleming. The different mutant forms of the fungus produce different types of penicillin.

The penicillin types are chemically altered in the laboratory to make them more effective and to make them suitable for use with different diseases. Ampicillin, methicillin and oxacillin are examples.

Antibiotics attack bacteria in a variety of ways. Some of them upset the production of the cell wall. This stops the bacteria from reproducing or even causes them to burst open. Some affect protein synthesis, stopping bacterial growth.

Animal cells do not have cell walls, and the cell structures involved in protein production are different. As a result, antibiotics do not damage human cells, although they may produce some side-effects like allergic reactions.

Commercial production of insulin

Insulin can be produced in large quantities using fermenters. Bacteria are **genetically modified** to carry the human insulin gene (insulin is a protein). Bacteria respire aerobically, so air is pumped into the fermenter. Other conditions such as nutrient levels, temperature, pH and moisture are maintained at optimum levels so that the bacteria grow and reproduce rapidly. The nutrients are then reduced, and the bacteria begin to produce the insulin.

Penicillin

Antibiotics are produced in giant fermenting tanks, up to 100 000 litres in capacity. The tanks are filled with a nutrient solution. For penicillin production, the carbohydrate source is sugar, mainly lactose or corn-steep liquor, which is a by-product of the manufacture of cornflour and maize starch. It contains amino acids as well as sugars. Mineral salts are added and the pH is adjusted to between 5 and 6. The temperature is maintained at about 26°C, air is blown through the liquid and it is stirred.

The main features of industrial fermentation are shown in Figure 21.3.

A culture of the appropriate microorganism is added to the nutrient liquid and is allowed to grow for 24 to 48 hours. Sterile conditions are essential. If other bacteria or fungi get into the system, they can completely upset the process. As the nutrient supply reduces, the microorganisms begin to secrete their antibiotics into the medium.

The nutrient fluid containing the antibiotic is filtered off and the antibiotic extracted by crystallisation or other methods.

Mycoprotein

Mycoprotein is a protein-rich meat substitute extracted from fungi. The filamentous fungus, *Fusarium venenatum*, is found in soil. Mycoprotein is becoming more popular because it contains no cholesterol and is lower in saturated fats than protein in meat products. It is suitable as part of a vegan diet (which contains no animal products), partly because of its high protein content. Its manufacture has been developed so it can be made commercially. It is fermented in a similar way to antibiotics and enzymes, using glucose and salts as the feedstock. One mycoprotein product is called *Quorn* (Figure 21.6, also see Chapter 7 – 'Vegetarian and vegan diets', and Tables 7.2 and 7.3).

▲ **Figure 21.6** Food made with pieces of Quorn

Conditions that need to be controlled in a fermenter

These have been described in the section on the commercial production of penicillin, opposite.

Table 21.1 summarises these.

▼ **Table 21.1** Conditions that need to be controlled in a fermenter.

condition	details
temperature	maintained at around 26°C. Heat is generated during fermentation, so the mixture needs to be cooled
pH	slightly acidic – 5 to 6
oxygen	sterilised air is blown into the mixture through air pipes and the mixture is stirred to aerate it
nutrient supply	depends on what is being manufactured, but for penicillin the feedstock is molasses or corn-steep liquor
waste products	depends on what is being manufactured, but for penicillin they are the waste nutrient fluid with bacterial residue. These are quite hazardous because of the presence of traces of antibiotic. Gases given off may include carbon dioxide.

Test yourself

1. Outline the biology involved in making bread.
2. State how DNA in a bacterium is different from DNA in an animal cell.
3. Give two reasons why bacteria are more suitable for use in genetic modification than, for example, mammals.

4 Some people are lactose intolerant. Explain how biotechnology can be used to allow people with this condition to eat milk products.

Genetic modification

FOCUS POINT

- What is genetic modification and when is it used?

- How is genetic modification achieved?
- What are the advantages and disadvantages of genetically modifying crops?

Key definitions

Genetic modification is changing the genetic material of an organism by removing, changing or inserting individual genes.

Use of bacteria and restriction enzymes in genetic modification

To understand the principles of genetic modification you need to know something about bacteria (Figure 2.9) and **restriction enzymes**.

Bacteria are microscopic single-celled organisms with cytoplasm, cell membranes and cell walls, but without a proper nucleus. Genetic control in a bacterium is carried out by a double strand of deoxyribonucleic acid (DNA) in the form of a circle, but not enclosed in a nuclear membrane. This circular DNA strand carries the genes that control bacterial metabolism.

Also, a number of small, circular pieces of DNA called plasmids are present in the cytoplasm. The plasmids often carry genes that give the bacterium resistance to particular antibiotics like tetracycline and ampicillin.

Restriction enzymes are produced by bacteria. They cut DNA molecules at specific sites, for example, between the A and the T in the sequence GAA–TTC. For example, the gene for insulin can be cut from a human DNA molecule. Restriction enzymes can be removed from bacteria and purified. By using a selected restriction enzyme, DNA

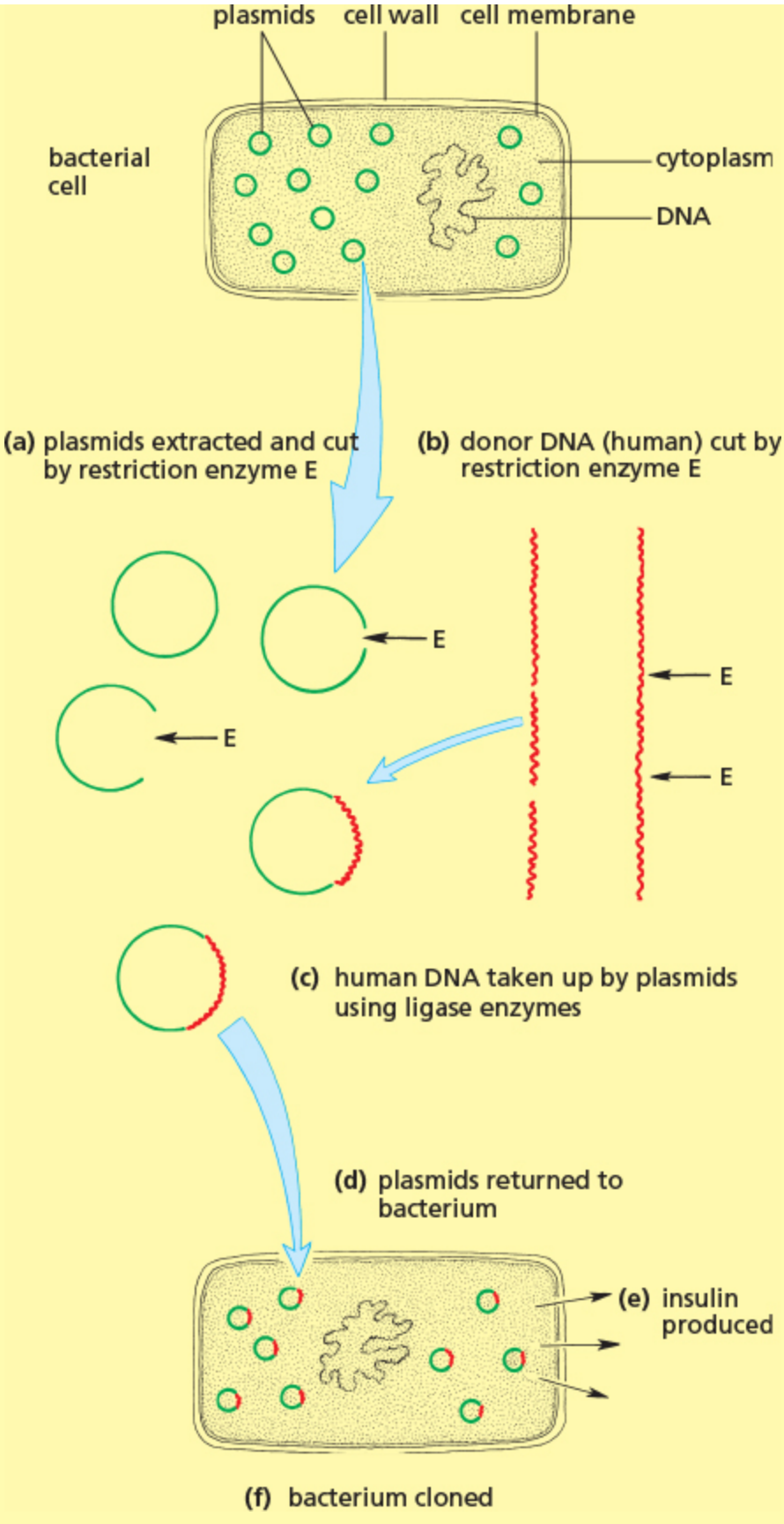
molecules removed from different organisms can be cut at predictable sites and made to produce lengths of DNA that contain specific genes.

DNA from human cells can be removed and restriction enzymes used to cut out a sequence of DNA that includes a gene, for example, the gene for production of insulin (Figure 21.7). These lengths have sticky ends. Plasmids are removed from bacteria and cut open with the same restriction enzyme. When the human DNA is added to a suspension of the plasmids, some of the human DNA attaches to some of the plasmids by their sticky ends. When exposed to suitable enzymes like **ligase**, the plasmids close up again. These plasmids are called **recombinant plasmids**.

The bacteria can be stimulated to take up the plasmids. Scientists have devised a way of selecting only the bacteria that contain the recombinant DNA. Given suitable nutrient solutions, these bacteria containing the recombinant DNA reproduce rapidly by mitosis (Chapter 17) to make millions of cells. Each daughter bacterium will contain the same DNA and the same plasmids as the parent.

The human DNA in the plasmids continues to produce the same protein as it did in the human cells. In the example mentioned, this would be the protein insulin (Chapter 14). When bacteria are modified so they can produce a human protein like insulin, it is described as **expression**.

The bacteria are cultured in special vessels called fermenters (Figure 21.3) and the insulin that they produce can be extracted from the culture medium and purified for use in treating diabetes (Chapter 14).


plasmids
cell wall
cell membrane
bacterial cell
cytoplasm
DNA
(a) plasmids extracted and cut by restriction enzyme E
(b) donor DNA (human) cut by restriction enzyme E
E
E
E
E
(c) human DNA taken up by plasmids using ligase enzymes
(d) plasmids returned to bacterium
(e) insulin produced
(f) bacterium cloned

▲ **Figure 21.7** The principles of genetic modification

Going further

Variations in genetic modification

The plasmids are said to be the *vectors* that carry the human DNA into the bacteria and the technique is sometimes called *gene-splicing*. Using plasmids is only one type of genetic modification. The vector may be a virus rather than a plasmid; the DNA may be inserted directly, without a vector; the donor DNA may be synthesised from nucleotides rather than extracted from cells; yeast may be used instead of bacteria. The outcome, however, is the same. DNA from one species is inserted into a different species and made to produce its normal proteins.

In the example shown in Figure 21.7, the gene product, insulin, is harvested and used to treat diabetes. In other cases, genes are inserted into organisms to promote changes that may be beneficial. Bacteria or viruses are used as vectors to deliver the genes. For example, a bacterium is used to deliver a gene for herbicide resistance in crop plants.

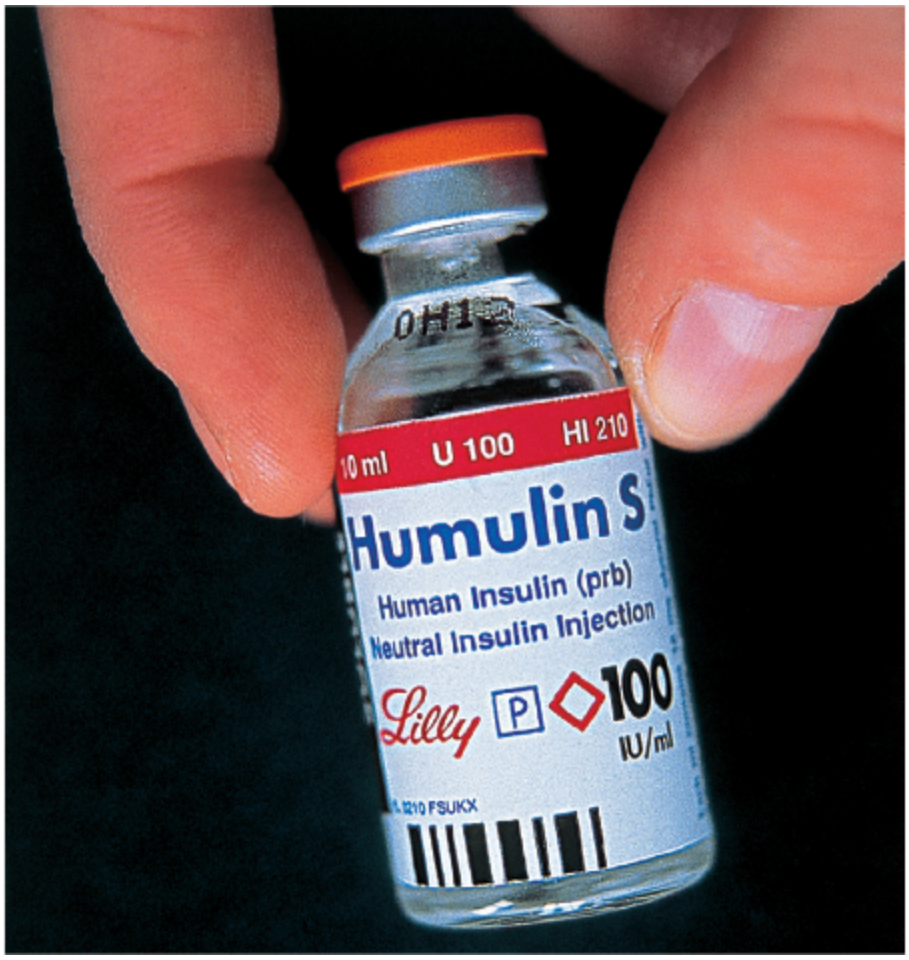
Applications of genetic modification

The following section gives only a few examples of genetic modification, a rapidly advancing process. Some products, like insulin, are in full-scale production. A few genetically modified (GM) crops (e.g. maize and soya bean) are being grown on a large scale in the USA. Many other projects are still at the experimental stage, undergoing trials, awaiting approval by regulatory bodies or simply on a wish list.

Production of human proteins

Human proteins such as insulin can be produced by genetically modified bacteria, and it has been in use since 1982. The human insulin gene is inserted into bacteria, which then secrete human insulin. The human insulin produced in this way (Figure 21.8) is purer than insulin prepared from pigs or cattle, which sometimes triggers allergic reactions because of traces of foreign protein. The

GM insulin is acceptable to people with a range of religious beliefs who may not be allowed to use insulin from cows or pigs.



▲ **Figure 21.8** Human insulin prepared from genetically modified bacteria. Though free from foreign proteins, it does not suit all patients

Going further

Hepatitis B vaccine

The gene for the protein coat of the hepatitis virus is inserted into yeast cells. When these are cultured they produce a protein that acts as an antigen (a vaccine, Chapter 10) and promotes the production of antibodies to the disease.

Transgenic plants have been modified to produce vaccines that can be taken effectively by mouth. These include vaccines against rabies and cholera.

Several species of plant have been used, including the banana, which is cheap and widespread in the tropics, can be eaten without cooking and does not produce seeds (Figure 21.9).

▲ **Figure 21.9** It is important to ensure that plants modified to produce drugs and vaccines cannot find their way, by chance, into the human food chain. Strict control measures must be applied

GM crops

Genetic modification has huge potential benefits in agriculture but, apart from a relatively small range of crop plants, most developments are in the experimental or trial stages. In the USA, 94% of the soya bean crop and 92% of the maize harvest consist of genetically modified plants, which are resistant to herbicides and insect pests.

In many countries, GM crops are grown only on a trial basis and there is resistance to their growth and the presence of GM products in food.

Pest resistance

The bacterium, *Bacillus thuringiensis*, produces a toxin that kills caterpillars and other insect larvae. The toxin has been in use for some years as an insecticide. The gene for the toxin has been successfully introduced into some plant species such as maize, cotton and soybean using a bacterial vector. The plants produce the toxin and show increased

resistance to attack by insect larvae. The gene is also passed on to the plant's offspring. Unfortunately, there are signs that insects are developing immunity to the toxin.

Most American GM maize carries a pesticide gene, which reduces the damage caused by the stem-boring larva of a moth (Figure 21.10).

▲ **Figure 21.10** The maize stem borer can cause considerable losses by killing young plants

Herbicide resistance

Some of the most effective herbicides are those, like glyphosate, which kill any green plant but become harmless as soon as they reach the soil. These herbicides cannot be used on crops because they kill the crop plants as well as the weeds. A gene for an enzyme that breaks down glyphosate is introduced into a plant cell culture (Chapter 16). Most American GM maize has a herbicide-resistant gene. This should lead to a reduced use of herbicides. Some scientists suspect that glyphosate is carcinogenic, and it is being banned from use in some countries.

Modifying plant products

Traditionally, vitamins and minerals have been added to food to boost their nutritional value or given in tablet form

to help people avoid deficiency diseases. The development of GM technology is now allowing scientists to study other ways of helping populations to achieve a balanced diet. Examples include:

- Golden rice (see Figure 21.11), which has a gene inserted to increase vitamin A levels. Vitamin A deficiency can lead to blindness and affects millions of children across the world.
- Maize, with a gene inserted to increase iron uptake. Shortage of iron in the diet leads to anaemia.
- Wheat with a gene inserted to make it free of gluten, which is a major cause of a food allergy.
- Vegetables with a gene inserted to boost vitamin E levels to help fight heart disease.
- Cassava with genes inserted to increase root iron uptake and increase protein levels. Cassava is a staple food in many African countries. Although it is high in carbohydrates it is a poor supplier of protein and some vitamins and minerals.

Advantages and disadvantages of genetically modifying crops

Although GM crops show increased yields, one of the objections is that only the farmers and the chemical companies in the industrialised world have benefited. So far, genetic modification has done little to improve yields or quality of crops in the newly industrialising world, except perhaps in China. However, there are many trials in progress, which have hopes of doing just that. Here are just a few examples.

Inadequate intake of iron is one of the major dietary deficiencies (Chapter 7) worldwide. An enzyme in some plant roots enables them to extract more iron from the

soil. The gene for this enzyme can be transferred to plants, like rice, enabling them to take in iron from iron-deficient soils.

Over 100 million children in the world are deficient in vitamin A. This deficiency often leads to blindness. A gene for beta-carotene, a precursor of vitamin A, can be inserted into plants such as rice to lessen this widespread deficiency. This is not the only way to increase vitamin A availability, but it could make a significant contribution.

Some acid soils contain levels of aluminium that reduce yields of maize by up to 8%. About 40% of soils in tropical and subtropical regions have this problem. A gene introduced into maize produces citrate, which binds the aluminium in the soil and releases phosphate ions. After 15 years of trials, the GM maize was made available to farmers, but pressure from environmental groups has blocked its adoption. The USA, Brazil and Argentina are the only countries that grow GM maize at present.

As a result of irrigation, much agricultural land has become salty and unproductive. Transferring a gene for salt tolerance from, for example, mangrove plants to crop plants could bring these regions back into production.

If the gene, or genes, for nitrogen fixation (Chapter 19) from bacteria or leguminous plants could be introduced to cereal crops, yields could be increased without the need to add fertilisers.

Similarly, genes for drought resistance would make dry areas available for growing crops.

Genes coding for human vaccines have been introduced into plants.

In conventional cross-breeding, the genes transferred come from the same, or a closely related, species. However, in cross-breeding the whole range of genes is transferred and this has sometimes had bad results when

genes other than the target genes have combined to produce harmful products. Genetic modification offers the advantage of transferring only those genes that are required.

The differences between the genetic make-up of different organisms is not as great as we tend to think. Plants and animals share 60% of their genes and humans have 50% of their genes in common with fruit flies. Not all genetic modification involves transfer of alien genes. In some cases, it is the plant's own genes that are modified to improve its success in the field.

One of the possible harmful effects of planting GM crops is that their modified genes might get into wild plants. If a gene for herbicide resistance found its way, via pollination, into a weed plant, this plant might become resistant to herbicides and so become a super weed. The purpose of field trials is to assess the likelihood of this happening. Until it is certain that this is a tiny risk, licences to grow GM crops will not be issued.

To prevent the transfer of pollen from GM plants, other genes can be introduced. These genes would stop the plant from producing pollen and stimulate the seeds and fruits to develop without fertilisation. This is a process that occurs naturally in many cultivated and wild plants.

GM food

This is food prepared from GM crops. Most genetic modifications are aimed at increasing yields rather than changing the quality of food. However, it is possible to improve the protein, mineral or vitamin content of food (Figure 21.11) and the shelf life of some products.

Possible hazards of GM food

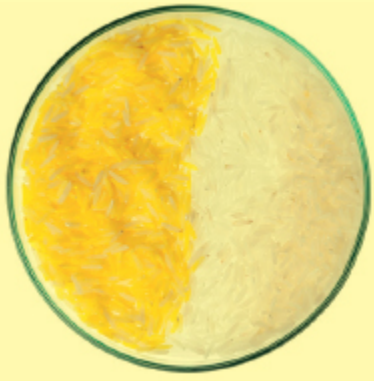
One of the worries is that the bacteria for delivering recombinant DNA contain genes for antibiotic resistance.

The antibiotic-resistant properties are used to select only those bacteria that have taken up the new DNA. If, in the intestine, the DNA managed to get into potentially harmful bacteria, it might make them resistant to antibiotic drugs.

Although there is no evidence to suggest this happens in experimental animals, the main biotech companies are trying to find methods of selecting bacterial vectors without using antibiotics.

Another concern is that GM food could contain pesticide residues or substances that cause allergies (allergens). However, all GM products are rigorously tested for toxins and allergens over many years, far more so than any products from conventional cross-breeding. The GM products must be passed by a series of regulatory and advisory bodies before they are released onto the market. In fact, only a handful of GM foods are available. One of these is soya, which is included, in one form or another, in 60% of processed foods.

Golden rice (Figure 21.11) carries a gene that is responsible for making beta-carotene, a precursor of vitamin A.



▲ **Figure 21.11** Samples of golden rice and non-GM rice

However, some argue that there is a danger of the precursor changing into other, toxic chemicals once eaten.

So far, only the Philippines and Bangladesh allow the GM golden rice to be grown.

There were also concerns about a reduction in biodiversity as a result of the introduction of GM species. Subsistence farmers could also be tied to large agricultural suppliers who may then manipulate seed prices.

Apart from specific hazards, there is also a sense of unease about introducing genes from one species into a totally different species. This is something that does not happen in nature and so the long-term effects are not known.

At least some of the protests against GM crops may be ill-judged (Figure 21.12).

▲ **Figure 21.12** Ill-judged protest. These vandalised poplars carried a gene that softened the cell walls, reducing the need for environmentally damaging chemicals used in paper making. They were also all female plants so no pollen could have been produced

Test yourself

5 Outline how genetic modification is carried out for each of the following uses:

5 to produce human proteins
6 to make crop plants resistant to insect pests
7 to improve the nutritional qualities of crop plants.

6 Make a table to outline the advantages and disadvantages of GM crops.
7 Describe how genetic modification can be used to solve major worldwide dietary deficiencies like vitamin and mineral deficiencies.

Practical work

For safe experiments/demonstrations which are related to this chapter, please refer to the *Biology Practical Skills Workbook* that is also part of this series.

Safety

- Eye protection must be worn.
- Wipe up any spillages immediately and rinse the cloth thoroughly with water. Do not allow spillages to dry up.

Investigating the use of pectinase in fruit juice production

- Make 100 cm^3 of apple purée using a liquidiser, or use a tin of apple purée.
- Transfer the purée to a 250 cm^3 beaker.
- Using a syringe or small measuring cylinder, add 5 cm^3 of 50% pectinase, stir the mixture and leave it for about 5 minutes.
- Place a funnel in the top of a 100 cm^3 measuring cylinder and line the funnel with a folded filter paper.
- Transfer the purée into the filter funnel and leave it in a warm place for up to 24 hours.
- Set up other measuring cylinders in the same way, with purée left to stand at different temperatures to compare the success of juice extraction.

Result

Juice is extracted from the purée. It collects in the measuring cylinder and is transparent (it has been clarified).

Interpretation

Pectinase breaks down the apple tissue, releasing sugars in solution. More juice collects in the measuring cylinder when the purée has been kept in warm conditions; colder temperatures slow down the process.

Further investigation

If other enzymes are available, try comparing cellulase and amylase with pectinase. Combinations of these could be used to find out which is the most effective in extracting the juice. Remember to control variables to make a fair comparison.

2 Investigating the use of biological washing powder

- Break an egg into a plastic beaker and whisk it with a fork, spatula or stirring rod until thoroughly mixed.
- Cut up four pieces of white cloth to make squares 10 cm × 10 cm, smear egg evenly onto each of them and leave to dry.
- Set up four 250 cm^3 beakers as follows:
  1. 100 cm^3 warm water with no washing powder.
  2. 5 cm^3 (1 level teaspoon) of non-biological washing powder dissolved in 100 cm^3 warm water.
  3. 5 cm^3 (1 level teaspoon) of biological washing powder dissolved in 100 cm^3 warm water.
  4. 5 cm^3 (1 level teaspoon) of biological washing powder dissolved in 100 cm^3 water and boiled for 5 minutes, then left to cool until warm.

- Place a piece of egg-stained cloth in each beaker and leave for 30 minutes.
- Remove the pieces of cloth and compare the effectiveness of each washing process.

Result

The piece of cloth in beaker **C** is most effectively cleaned, followed by **B** and then **D**. The cloth in **A** is largely unchanged.

Interpretation

The enzymes in the biological washing powder break down the proteins and fats in the egg stain to amino acids and fatty acids and glycerol. These are smaller, soluble molecules, which can escape from the cloth and dissolve in the water. Non-biological washing powder is less effective because it does not contain enzymes. Boiled biological washing powder is not very effective because the enzymes in it have been denatured. Beaker A was a control, with no active detergent or enzymes. Soaking the cloth in warm water alone does not remove the stain.

3 Action of lactase

This investigation uses glucose test strips (diastix). They are used by people with diabetes to test for glucose in their urine (see ‘Homeostasis’ in Chapter 14 for details of diabetes). The strips do not react to the presence of other sugars (lactose, sucrose, etc.)

- Pour 25 cm^3 warm, fresh milk into a 100 cm^3 beaker.
- Test the milk for glucose with a glucose test strip.
- Measure out 2 cm^3 of 2% lactase using a syringe or pipette and add this to the milk.
- Stir the mixture and leave for a few minutes.

Result

Milk gives a negative result for glucose, but milk exposed to lactase gives a positive result.

Interpretation

Lactase breaks down the lactose in milk, as shown in the equation below.

$$\text{lactose} \xrightarrow{\text{lactase}} \text{glucose} + \text{galactose}$$

Note: milk sometimes contains traces of glucose. If the milk gives a positive result with the glucose test strip, an alternative method would be to use a solution of lactose instead of milk. However, the amount of glucose in the milk, as indicated by the colour change on the test strip, should increase after treatment with lactase.

Practical work questions

1. In the modification of the experiment to study the effect of temperature on fruit juice production, suggest two factors that should be controlled to make sure the results are reliable.
2. In practical 2, investigating the use of biological washing powder, the results are based on a ***qualitative*** analysis (you were looking at the appearances of the stains after washing). Describe how you could modify the method to carry out the same investigation but collect ***quantitative*** results (results you can measure).

3. In experiment 3, Diastix testing strips were used to test for the presence of glucose.
   - **a** If those test strips were not available, describe how you could test for glucose.
   - **b** A statement in the method suggested that milk sometimes contains traces of glucose. Describe how your test could distinguish between a sample with a trace of glucose and a sample with a lot of glucose present.

Revision checklist

After studying Chapter 21 you should know and understand the following:

- Bacteria are useful in biotechnology and genetic modification because of their ability to make complex molecules and their rapid reproduction.
- Bacteria are useful in biotechnology and genetic modification because of lack of ethical concerns over their manipulation and growth.
- The genetic code in bacteria is shared with all other organisms.
- Bacteria contain DNA in the form of plasmids, which can be cut open to insert genes.
- Biotechnology is the application of living organisms, systems or processes in industry, often using microorganisms in processes called fermentations.
- The required product of biotechnology may be the organism itself (e.g. mycoprotein) or one of its products (e.g. ethanol).
- Yeasts can be used to make biofuel and for bread-making.
- Pectinase can be used to extract fruit juices.
- Lipase and protease enzymes are used in biological washing powders to remove fat and protein stains.
- Lactase is used to produce lactose-free milk.

✔ Fermenters are used in the production of antibiotics like penicillin on an industrial scale.

- Genetic modification is changing the genetic material of an organism by removing, changing or inserting individual genes.
- Examples of genetic modification include

– the insertion of human genes into bacteria to produce human proteins (e.g. insulin) – the insertion of genes into crop plants to confer resistance to herbicides or insect pests – the insertion of genes into crop plants to improve nutritional qualities.

- ✔ In the process of genetic modification, – human gene DNA is isolated using restriction enzymes, forming sticky ends.

  – bacterial plasmid DNA is cut with the same restriction enzymes, forming matching sticky ends.

  – human gene DNA is inserted into the bacterial plasmid DNA using DNA ligase to form a **recombinant plasmid**.

  – the plasmid is inserted into bacteria.

  – the bacteria containing the recombinant plasmid are replicated.

  – they make a human protein as they express the gene.

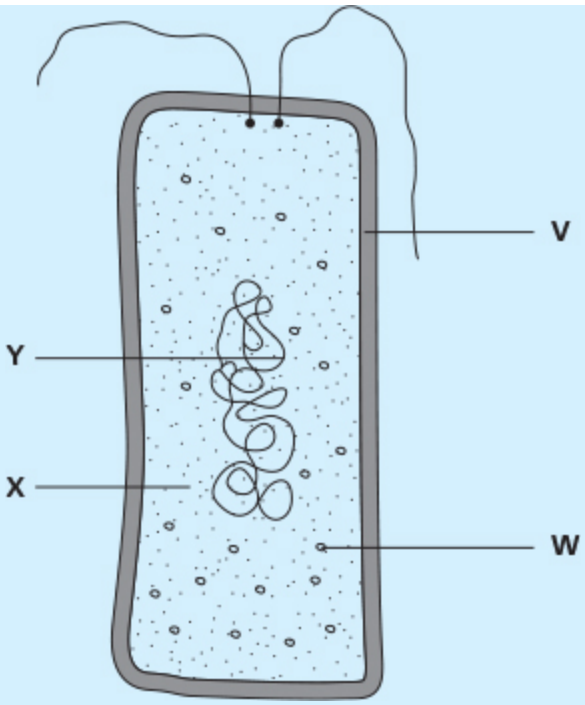
  – plasmids and viruses are vectors used to deliver the genes.

- Crop plants can be genetically modified to resist insect pests and herbicides.

- ✔ There are advantages of genetically modifying crops, such as giving them resistance to pests and herbicides and increasing their nutritional qualities.
- ✔ There is concern that the genes introduced into crop plants might spread to wild plants.

Exam-style questions

1. The diagram shows a bacterial cell.



1. Identify the labelled parts. [4]
2. Explain why bacterial cells are very useful organisms in the process of genetic modification.

2. 1. Name the waste product made by yeast which is used [2]

   i) as a biofuel

   ii) [2]

   in baking.

   2. Explain why biological washing powders can be more effective in removing stains than non-biological washing powders. [4]

3. Copy and complete the table, using ticks ( ) and crosses (x), to compare the structure of a bacterial cell with that of an animal cell. [5]

	Present in	
cell part	**bacterial cell**	**animal cell**
cell wall		
membrane		
nucleus		
plasmid		
cytoplasm		

4 Match the terms to their meanings. [6]

term	meaning
ligase	DNA in a plasmid into which a gene has been added
plasmid	end of a section of DNA that will attach to a cut plasmid
recombinant DNA	enzyme used to close up a plasmid
restriction enzyme	a means of transferring DNA from one species into the cells of another species
sticky end	cuts open a plasmid or DNA molecule at a specific site
vector	circular piece of DNA

5 A fermenter is used in the large-scale production of human insulin.

a State the part played by each of the following in this production:

a air

b feedstock

c paddles

d thermometer

e filter. [4]

6 Suggest why

4 the air being blown into the fermenter is sterilised [1]

5 the waste products of the fermentation of antibiotics are considered to be hazardous [2]

6 the temperature of the liquid may rise. [3]

- 2. Define the term genetic modification. [2]

i) Name two enzymes used in the process of genetic modification. [2]

ii) Outline the roles of these two enzymes in the process. [3]

- Outline two advantages and two disadvantages of genetically modified crops. [4]