

Genetically Modified Bacteria from Natural and Artificial Habitats

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ABSTRACT

The genetic modification of bacteria from natural and managed habitats will impact on the management of agricultural and environmental settings. The potential applications include crop production and protection, degradation of environmental pollutants, extraction of metals from ores, industrial fermentation and productions of enzymes, diagnostics and chemicals. The applications of this technology will ultimately include the release of beneficial agents in the environment. If safely deployed, genetically modified bacteria should be able to provide significant benefits in management of environmental systems and in the development of new environmental control processes.

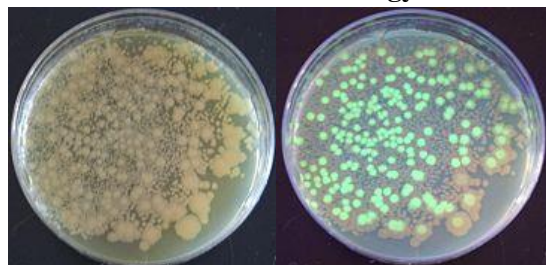
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INTRODUCTION

Many distinct forms of bacteria exist in nature each with potentially useful or detrimental attributes. The several strategies can be used to modify bacteria for useful purposes. There are also circumstances when the survival of useful microorganisms may be improved by single gene transfer or by genetic selection to toxic substances. Although the adaptations that enable bacteria to colonize or survive in specific habitats are generally unknown, it is likely that many characteristics collectively determine survival. Thus it is presently difficult or impossible to transfer all the genetic determinants enabling a bacterium to survive in a habitat to which it is not already adapted. However, certain traits that may be

desirable to have expressed in a given environment are conferred by single genes or gene clusters which can be transferred and expressed in a bacterial strain indigenous to that environment. This review will focus on the modification of some bacteria that affect important natural and industrial processes.

Research Technology



Bacteria transformed with p_GLO under ambient and ultraviolet light

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The genetically modified bacteria were the first organisms to be modified in the laboratory due to their simple genetics. These organisms are now used for several purposes and are particularly important in producing large amounts of pure human proteins for use in medicine (Melo et al., 2007).

This made them important tools for the creation of other genetically modified organisms. Genes and other genetic information from a wide range of organisms can be added to a plasmid and inserted into bacteria for storage and modification. Bacteria are cheap, easy to grow, clonal, multiply quickly, are relatively easy to transform, and can be stored at -80 °C almost indefinitely. Once a gene is isolated it can be stored inside the bacteria providing an unlimited supply for research. The large number of custom plasmids make manipulating DNA excised from bacteria relatively easy (Fan et al., 2005). Their ease of use has made them great tools for scientists looking to study gene function and evolution. The DNA manipulation takes place within bacterial plasmids before being transferred to another host. Bacteria are the simplest model organism and most of our early understanding of molecular biology comes from studying *Escherichia coli*. Scientists can easily manipulate and combine genes within the bacteria to create novel or disrupted proteins and observe the effect this has on various molecular systems. Researchers have combined the genes from bacteria and archaea, leading to insights on how these two diverged in the past. In the field of synthetic biology, they have been used to test various synthetic approaches, from synthesizing genomes to creating novel nucleotides (Malyshev et al., 2014).

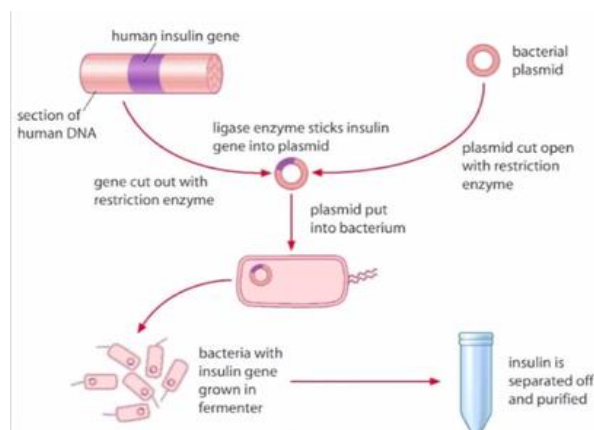
Food Technology

Bacteria have been used in the production of food for a very long time, and specific strains have been developed and selected for that

work on an industrial scale. They can be used to produce enzymes, amino acids, flavourings, and other compounds used in food production. The advent of genetic engineering, new genetic changes can easily be introduced into these bacteria. Most food-producing bacteria are lactic acid bacteria, and this is where the majority of research into genetically engineering food-producing bacteria has gone. The bacteria can be modified to operate more efficiently, reduce toxic by product production, increase output, create improved compounds, and remove unnecessary pathways (Kärenlampi & von Wright, 2016). The food products from genetically modified bacteria include alpha amylase, which converts starch to simple sugars, chymosin, which clots milk protein for cheese making, and pectin esterase, which improves fruit juice clarity.

The chymosin is an enzyme found in a calf's stomach. This helps the calf break down the milk to digest. Chymosin is necessary in order to make cheese. It turns the milk into cheese. Scientists have found a way to alter yeast to grow chymosin enzymes for making cheese. This process is much more efficient because previously calves had to be slaughtered in order to extract the chymosin from the inner lining of the stomach. Also this offers a vegetarian friendly way to make cheese. It depends on what and how the bacteria does the process. Genetically modified bacteria are used to produce large amounts of proteins for industrial use. Generally the bacteria are grown to a large volume before the gene encoding the protein is activated. The bacteria are then harvested and the desired protein purified from them (Jumba, 2009). The high cost of extraction and purification has meant that only high value products have been produced at an industrial scale.

Pharmaceutical Production

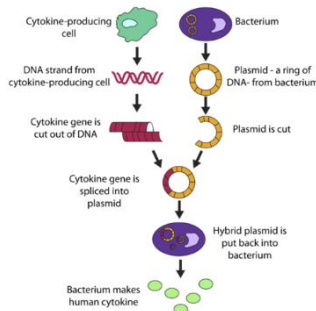


Production of insulin

The majority of the industrial products from bacteria are human proteins for use in medicine (Leader et al., 2008). Many of these proteins are impossible or difficult to obtain via natural methods and they are less likely to be contaminated with pathogens, making them safer. Prior to recombinant protein products, several treatments were derived from cadavers or other donated body fluids and could transmit diseases. Indeed, transfusion of blood products had previously led to unintentional infection of haemophiliacs with HIV or hepatitis-C. The first medicinal use of genetically modified bacteria was to

produce the protein insulin to treat diabetes (Walsh, 2005). The other medicines produced include clotting factors to treat haemophilia, human growth hormone to treat various forms of dwarfism, interferon to treat some cancers, erythropoietin for anaemic patients, and tissue plasminogen activator which dissolves blood clots. Outside of medicine they have been used to produce biofuels. There is interest in developing an extracellular expression system within the bacteria to reduce costs and make the production of more products economical (Zhou et al., 2018).

Health Technology



Modified bacteria for cytokinin

The greater understanding of the role that the microbiome plays in human health, there is the potential to treat diseases by genetically altering the bacteria to themselves be therapeutic agents. The ideas include altering gut bacteria so they destroy harmful bacteria, or using bacteria to replace or increase deficient enzymes or proteins. One research focus is to modify *Lactobacillus*, bacteria that

naturally provide some protection against HIV, with genes that will further enhance this protection (Reardon, 2018). The bacteria which generally cause tooth decay have been engineered to no longer produce tooth-corroding lactic acid. These transgenic bacteria, if allowed to colonize a person's mouth, could perhaps reduce the formation of cavities. Transgenic microbes

have also been used in recent research to kill or hinder tumours, and to fight crohn's disease (Baat et al., 2006).

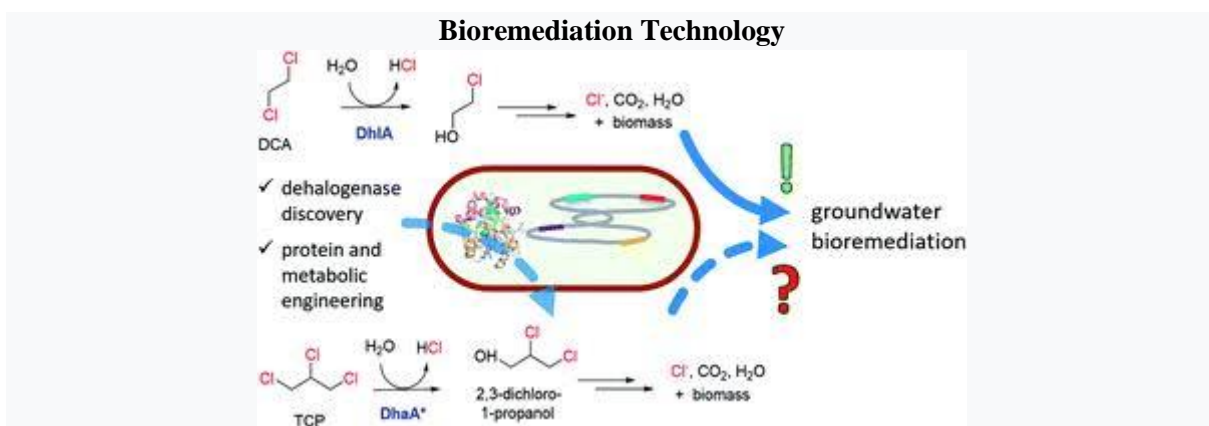
If the bacteria do not form colonies inside the patient, the person must repeatedly ingest the modified bacteria in order to get the required doses. Enabling the bacteria to form a colony could provide a more long-term solution, but could also raise safety concerns as interactions between bacteria and the human body are less well understood than with traditional drugs.

The example of such an intermediate, which only forms short-term colonies in the gastrointestinal tract, may be *Lactobacillus acidophilus* MPH734. This is used as a specific in the treatment of lactose intolerance. This genetically modified version of *Lactobacillus acidophilus* bacteria produces a missing enzyme called lactase which is used for the digestion of lactose found in dairy products or, more commonly, in food prepared with dairy products. The short-term colony is induced over a one-week, 21-pill treatment regimen, after which, the temporary colony can produce lactase for three months or more before it is removed from the body by a natural process. The induction regimen can be repeated as often as necessary to maintain

protection from the symptoms of lactose intolerance, or discontinued with no consequences, except the return of the original symptoms.

Agriculture Production

For over a century bacterium have been used in agriculture. The crops have been inoculated with *Rhizobia* sp. to increase their production or to allow them to be grown outside their original habitat. The application of *Bacillus thuringiensis* (Bt) and other bacteria can help protect crops from insect infestation and plant diseases. With advances in genetic engineering, these bacteria have been manipulated for increased efficiency and expanded host range. Markers have also been added to aid in tracing the spread of the bacteria. The bacteria that naturally colonise certain crops have also been modified, in some cases to express the Bt genes responsible for pest resistance. *Pseudomonas* strains of bacteria cause frost damage by nucleating water into ice crystals around themselves. This led to the development of ice-minus bacteria, that have the ice-forming genes removed. When applied to crops they can compete with the ice-plus bacteria and confer some frost resistance (Amarger, 2002).



The uses for genetically modified bacteria include bioremediation, where it used to convert pollutants into a less toxic form. The genetic engineering can increase the levels of the enzymes used to degrade a toxin or to make the bacteria more stable under

environmental conditions (Sharma et al., 2018). The genetically modified bacteria have also been developed to leach copper from ore clean up mercury pollution and detect arsenic in drinking water (McBride et al., 2002) In the 1980s artist Joe davis and

geneticist dana boyd converted the germanic symbol for femininity into binary code and then into a DNA sequence, which was then expressed in *Escherichia coli*. This was taken a step further when a whole book was encoded onto DNA. Paintings have also been produced using bacteria transformed with fluorescent proteins.

The tools now exist to genetically modify bacteria and to detect, disable, or measure cell activity in natural environments, a consensus has yet to be reached on what constitutes a safe release. There is need for better integration of research both on the ecology and molecular biology of bacteria and better focus on relevant questions that can be addressed by scientific methods. The modified bacteria, when properly applied, can become an important component of our environmental protection strategies in the future.

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