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BIOTECHNOLOGY: ORIGINS AND DEVELOPMENT IN THE CARIBBEAN

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Table of Contents

List of Tables

List of Figures

Glossary

AFLP - Amplified Fragment Length Polymorphism; a molecular marker generated by a combination of restriction digestion and selective PCR amplification

Chromosome - a cellular structure comprised of a long, folded DNA molecule and protein.

DNA - deoxyribonucleic acid, the substance within cells that carries the "recipe" for the organism and is inherited by offspring from parents.

DNA fingerprinting - cutting the DNA of an organism with restriction enzymes, separating the pieces by electrophoresis and identifying hypervariable fragments using probes thus generating a unique pattern, or "fingerprint" for each species, breed or hybrid. DNA fingerprints can also be generated by amplification of one or more hypervariable DNA sequences found within the DNA of organisms using PCR technology followed by electrophoresis of fragments.

Electrophoresis - a lab technique for determining DNA fragment sizes by separating them in a gel placed in an electric field.

Eukaryotic Cell – A cell that contains membrane-bound compartments in which specific metabolic activities take place

Gene - a functional unit of DNA, one "word" in the DNA recipe.

Genetic code - the information contained in DNA molecules that scientists describe on the basis of a 4-letter alphabet (A, C, G, and T).

Genetic engineering - the process of transferring DNA from one organism into another using the recombinant-DNA technology that results in a genetic modification; the modified organisms are referred to as genetically modified organisms (GMOs) living modified organisms (LMOs) or transgenic organisms.

Genetic map - the locations of specific genes along a chromosome marked with probes.

Genome - the entire DNA "recipe" for an organism, found in every cell of that organism.

Km - the Michaelis-Menten constant.

Isozyme - a molecular marker system based on the staining of proteins with identical function, but different electrophoretic mobilities.

Mutation - change/s to the DNA "recipe" of an organism caused by chemicals, ultraviolet light, X-rays, or natural processes.

PCR - polymerase chain reaction is a method that allows rapid duplication of specific DNA molecules in response to temperature changes in a computer-controlled heater.

Plasmid - a small, circular DNA that is used to transfer genes from one organism into another.

Probe - a very short piece of labeled DNA used to find a specific sequence of "letters" in a very long piece of DNA from a chromosome or genome.

RAPD - Randomly Amplified Polymorphic DNA; a molecular marker based on the differential PCR amplification of a sample of DNA segments from the genome using short oligonucleotide sequences as primers.

Recombinant DNA (rDNA) - DNA formed by joining pieces of DNA from two or more organisms.

RFLP - restriction fragment length polymorphism, which describes the patterns of different (polymorphism) sizes of DNA (fragment length) that result from cutting with restriction enzymes (restriction). See DNA fingerprinting above.

Sequence - the order of "letters" in the DNA "recipe." The DNA sequence is the chemical structure that contains information.

Transformation - a procedure to transfer DNA into the cells of an organism. Can be done with Agrobacterium (most dicots), physical (freeze-thaw), chemical (bacteria), electroporation (any organism), or the particle gun (any organism).

Transgenic - an organism that has been modified by genetic engineering to contain DNA from an external source.

Vmax - Vmax is the limiting velocity of an enzyme as substrate concentrations get very large. Vmax (and V) are expressed in units of product formed per time.

Vector - any DNA molecule capable of self replication that is used to transfer DNA into an organism; most commonly used are plasmid DNA vectors or viruses.

Abstract

The biotechnology movement in the Caribbean is a fledgling industry that has tremendous potential for development. It focuses on the use of fermentation and enzyme technologies, tissue culture and recombinant DNA (rDNA) technology and is more greatly applied to plant varieties rather than animal species. Tissue culture is by far the most developed type of technology but increasing attention is being paid to rDNA technology. There are also notable efforts on improved disease diagnostic and genetic resource management services and marker-assisted selection for economic traits, including disease resistance. Main areas include application in the agriculture sector but the use in medicine, biology and forensics is also being promoted.

Greater application of biotechnology in the Caribbean would benefit from the development of a regional policy with accompanying legislation and regulations and the expansion and improvement of well-equipped institutions that are adequately staffed. Collaboration in development of the biotechnology movement would be enhanced through the formation of public-private partnerships in the interest of promoting a unified agenda. Public awareness and education, both informally and formally, would indeed enhance the region's potential for development of a biotechnology industry but this should be complemented with the appropriate communication strategies to ensure that information needs would be met.

The biotechnology revolution heralds a new race in which the Caribbean is already behind. It is a critical area that must be developed to achieve the region's development goals in a sustainable manner and for the region to function in an increasingly competitive global economy. Biotechnology development requires a coherent framework, a focused strategic approach, the right policies and fiscal environment including tax regime, resource provision structure, capacity-building, joint university-private sector projects, venture capital and multidisciplinary highly skilled teams. There are several successful models, which can be evaluated and adapted to meet the needs of the Caribbean. The biotechnology revolution provides the opportunity to develop the region's strengths, conquer its weaknesses and grasp the opportunity presented by the greatest challenge yet to confront mankind.

Introduction

What is biotechnology?

Contrary to its name, biotechnology is not a single technology. It is, however, a group of technologies that share two (common) characteristics, working with living cells and their molecules and having a wide range of practice uses that can improve our lives1.

In its purest form, the term "biotechnology" refers to the use of living organisms or their products to modify human health and the human environment (Peters 1993) for commercial purposes (Betsch 1994). The term brings to mind many different things. Some think of developing new types of animals while others anticipate almost unlimited sources of human therapeutic drugs. Still others envision the possibility of growing crops that are more nutritious and naturally pest-resistant to feed a rapidly growing world population.

A narrower and more specific definition of biotechnology is "the commercial application of living organisms or their products, which involves the deliberate manipulation of their DNA molecules" (Betsch 1994). This definition implies a set of laboratory techniques developed within the last 20 years that have been responsible for the tremendous scientific and commercial interest in biotechnology, the founding of many new companies, and the redirection of research efforts and financial resources among established companies and universities. These laboratory techniques provide scientists with a spectacular vision of the design and function of living organisms, and provide technologists in many fields with the tools to implement exciting commercial applications.

Biotechnology in one form or another has flourished since prehistoric times. When the first human beings realized that they could plant their own crops and breed their own animals, they learned to use biotechnology. The discovery that fruit juices fermented into wine or that milk could be converted into cheese or yogurt, or that beer could be made by fermenting solutions of malt and hops began the study of biotechnology. When the first bakers found that they could make soft, spongy bread rather than a firm, thin cracker, they were acting as fledgling biotechnologists. The first animal breeders, realizing that different physical traits could be either magnified or lost by mating appropriate pairs of animals, engaged in the manipulations of biotechnology.

Throughout human history, a great deal has been learnt about the different organisms that our ancestors used so effectively. The marked increase in our understanding of these organisms and their cell products gains us the ability to control the many functions of various cells and organisms. Using the techniques of gene splicing and recombinant DNA technology, it is now possible to combine the genetic elements of two or more living cells. Functioning lengths of DNA can be taken from one organism and placed into the cells of another organism. As a result, for example, bacterial cells can be manipulated to produce human molecules. Cows can produce more milk for the same amount of feed, and therapeutic molecules that have never before existed can be synthesized.

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[1] http://www.ces.ncsu.edu/depts/foodsci/ext/pubs/bioapp.html

History of the biotechnology movement

According to the University of California Digital Library the biotechnology movement may be traced back to 1652 when Robert Hooke used a microscope to label the walled cavities in plant tissue as "cells". In 1830, the Scottish physician, Robert Brown, named the opaque spot in plant cells the "nucleus" and in 1857, biologists first observed small rodlike bodies in dividing cells, which, 23 years later, were named chromosomes after being found to absorb certain dyes. This was followed in 1869, by the isolation of nuclein from cells of pus collected in bandages by Swiss biologist Fredrich Miescher which was later named deoxyribonucleic acid (DNA). DNA was identified in 1871 in sperm of trout from the Rhine River although its exact role in heredity remained unclear. In 1879, the German biochemist Albrecht Kossel found that nucleic acids are composed of combinations of the five bases adenine, thymine, cytosine, guanine and uracil. In 1882 Belgian biologist Eduard Van Beneden discovered that every species has a characteristic number of chromosomes.

The 1900s was an important stage in the biotechnology movement when Gregor Mendel's classic experiments in plant genetics and heredity, published 35 years earlier, were rediscovered and verified, sparking renewed interest in the field. This was followed in 1902 by the work of Walter S. Sutton who studied grasshopper sperm and in so doing, discovered that each chromosome pairs with another physically similar one and that they separate during meiotic cell division with one member of each pair going into a different cell. The development of tissue culture techniques in 1902 by an American pathologist was followed, in 1909, by the coining of the word "genes" for the still misunderstood elements of heredity by the Danish botanist, Wilhelm Ludwig Johannsen.

In 1919, Thomas Hunt Morgan identified the XY male and XX female chromosomes and suggested that some traits may be sex-linked. He later showed how genes can mutate or change. Another major beneficial legacy of early twentieth century biotechnology was the discovery by Alexander Fleming, in 1928, of penicillin, an antibiotic derived from the mould Penicillium. Large-scale production of penicillin was achieved in the 1940s. However, the revolution in understanding the chemical basis of cell function that stemmed from the post-war emergence of molecular biology was still to come. It was this exciting phase of bioscience that led to the recent explosive development of biotechnology.

The invention of the electron microscope in 1930 enabled researchers to see viruses and with the advent of better contrast and staining agents, macromolecules such as DNA were eventually defined. This was complemented by the invention of electrophoresis (use of an electric field to move charged particles dispersed in liquid) in 1937 that quickly contributed to development of the disciplines of immunology, biochemistry, and microbiology.

In 1944, the Rockefeller University researcher, Oswald T. Avery, and colleagues demonstrated that DNA was indeed the carrier of genetic information and also discovered the bacteria that cause bacterial pneumonia. This was followed in 1949, by the discovery of the sequence of DNA molecules that was found to control cell growth via some sort of code. During

[2] http://www.library.ucsf.edu/collres/archives/bio/chron4.html

this period, and using electrophoresis, Linus Pauling separated normal haemoglobin from sickle cell anaemia hemoglobin, proving that genetic disease can be understood in molecular terms.

In 1950, Alfred Hershey and Martha Chase used radioactively labelled Escherisha coli to prove that DNA and not proteins had penetrated the interior of cells and was the true carrier of heredity. This was followed in 1951 by the discovery of Maurice Wilkins and Rosalind Franklin who determined the DNA molecule "outline" using crystallography and X-ray diffraction. It was in 1953 that James Watson and Francis Crick proposed a double helix as the molecular structure of DNA, a discovery that later won them the Nobel Prize. Following this and in 1958 American researchers, Matthew Meselson and Franklin Stahl, showed that old strands of DNA act as templates for formation of new strands and that synthesis of new DNA occurs while old chromosomes are in the process of uncoiling. During this period, Americans George W. Beadle and Edward L. Tatum working with bread mould developed the hypothesis of "one gene, one enzyme."

The 1960s realized significant discoveries in the functioning of genes by the French molecular biologists, Francois Jacob and Jacques Monod, who proposed that differences in the structure and function of cells result from the selective expression and repression of certain genes. This was followed by the cracking of the genetic code by Marshall Nirenburg in 1964.

It was not until the 1970s that the application of biotechnology was realized. This began in 1970 with isolation of the first restriction enzyme, a specialized protein used to cut DNA strands precisely at specific locations. In 1972, Paul Berg and Stanford colleagues synthesized the first recombinant DNA molecule by linking different DNA fragments in a test tube and in 1973, Herbert Boyer and Stanford's Stanley Cohen of the University of California, San Francisco (UCSF) performed the first genetic engineering experiment by splicing toad genes into E. coli bacteria. In 1976, UCSF's J. Michael Bishop and Harold Varmus discovered genes, called oncogenes, that can lead to cancer and 13 years later, they shared the Nobel Prize in Medicine for that discovery. At this time, Yuet Wai Kan pioneered molecular techniques that allowed the first foetal test to identify sickle cell anaemia, a hereditary blood disease that affects people of African origin. In 1977, using rDNA techniques, K. Itakura and colleagues cloned the first human gene which produced the growth inhibiting hormone somatostatin. William Rutter and UCSF colleagues isolated the gene for rat insulin and transplanted it into bacteria. Five years later, genetically engineered human insulin was commercialised.

During this period, the development of rapid DNA-sequencing techniques fuelled the growth of scientific inquiry. In 1979, the biotechnology company, Genentech Incorporated, in collaboration with UCSF, developed synthetic human growth hormone. Using gene mapping techniques, UCSF's Y.W. Kan and Judy Chang discovered the single genetic mutation responsible for beta thalassemia, the most common form of life-shortening blood disease. This discovery led to prenatal tests for scores of different regional mutations. John Baxter and Howard Goodman were the first to clone the gene for human growth hormone, which became the second genetically engineered product to receive government approval.

The 1980s began with cloning of the virus-fighting interferon and was followed in 1981 by production of the first transgenic mice and transgenic fruit flies. They soon became standard

ways for studying mutations, gene expression and human disease. Seven years later, a cancer prone transgenic mouse became the first patented life form. In 1983, Barbara McClintock won the Nobel Prize in Physiology for her discovery that some genes can "jump around" from one chromosome to another and in 1987, biochemist William Rutter and his Emeryville Chiron Corporation produced the first genetically engineered vaccine against hepatitis b to be put on the market.

In 1990, the National Institute of Health in the United States approved the use of gene therapy on a four-year-old girl afflicted with a rare immune system disorder. This era also realized the start of America's Human Genome Project, its goal being to map and clone every gene in the human body which was achieved in 2003.

In 1992, Agenda 21, the work programme adopted by the United Nations Conference on Environment and Development (UNCED) asserted that biotechnology:

> *"promises to make a significant contribution in enabling the development of for example, better health care, enhanced food security through sustainable agricultural practices, improved supplies of potable water, more efficient industrial development processes for transforming raw materials, support for sustainable methods of afforestation and reafforestation and detoxification of hazardous wasters (UN, 1992)".*

The decade following the adoption of these commitments has shown little progress in the application of biotechnology in the developing world despite visions of a promising future. Instead, the international community has devoted considerable resources to managing perceptions of biotechnology risks rather than exploring new opportunities for its use (Pardo et al. 2002). It is now more than a decade since world leaders signed Agenda 21. Since then, three major developments have occurred. Firstly, the institutions of globalization that were being crafted at the time of the adoption of Agenda 21 are now in place and their influence on the international trading system has become a subject of considerable debate. Secondly, biotechnology products have made their debut on the international market and it is now possible to assess the performance of biotechnology in the global economy. Thirdly, advances in biology, especially molecular biology, signal the prospect of a new generation of products and services that were not conceivable a decade ago (Juma and Konde 2005).

I. USING BIOTECHNOLOGY TO MODIFY PLANTS AND ANIMALS

This section describes three specific areas of biotechnology namely:

- Industrial biotechnology - fermentation and enzyme technology;
- Tissue culture and plant biotechnology; and
- Molecular biotechnology - recombinant DNA technology and molecular markers.

A. Industrial biotechnology

1. Fermentation technology

Fermentation technology is the oldest of all biotechnological processes. It is based on fermentation which is a process of chemical change caused by organisms (table 1) or their products, usually producing effervescence and heat. Microbiologists consider fermentation as "any process for the production of a process by means of mass culture of micro-organisms" and this is the definition that is widely accepted by biotechnologists.

Table 1: Micro-organisms used in Fermentation Processes

Prokaryotic	Unicellular – bacteria and cyanobacteria Multicellular - cyanobacteria
Eukaryotic	Unicellular – yeast and algae Multicellular – fungi and algae

Fermentation technology began with sweet substances (vegetable or animal) in different parts of the world. The process of fermentation was probably discovered by observing the changes in the juices of several fruits and other substances that had been kept for a day or more. It appears that fermentation technology started simultaneously with settled agriculture during the Neolithic period.

2. Enzyme technology

Enzyme technology is best described as the technology associated with the application of enzymes as the tools of industry, agriculture and medicine. Although the earliest reports concerning exploitation of enzymes were documented in the late 1800s, true industrial application of enzymes only began in earnest in the 1960s. The majority of enzymes used in industrial/biotechnological applications are derived from particular fungi (Aspergillus) and bacteria (Bacillus). Safe organisms must be used for consumer-related applications.

Enzymes are proteins and are nature's own biocatalysts. They are produced by living systems to accelerate and sustain the myriad of chemical reactions necessary to sustain life with more than 3000 enzymes catalyzing a wide array of reactions being known to exist. The disintegration of foodstuffs to amino acids, sugars and lipids is normally accomplished within

three to six hours depending on the amount and type of food. In the absence of enzymes, hydrolysis by digestive enzymes would take more than 30 years.

Enzymes have many advantages over their chemical counterparts in that they are more specific and generally possess high catalytic properties. Enzymes can be immobilized, that is, enzyme can be linked to an inert support material without loss of activity which facilitates reuse and recycling of the enzyme. Enzymes can also be encapsulated or entrapped.

a) Process of enzyme technology

The identification of a microbial source of an enzyme is necessary to start the process of enzyme technology. The properties of the enzyme must be determined as follows:

- Temperature for optimum productivity;
- Temperature stability profile;
- pH (hydrogen ion concentration) optimum and stability;
- Kinetic constants (Km, Vmax);
- Whether or not there is substrate or product inhibition;
- Whether or not they have the ability to withstand components of the expected feedstock other than substrate.

If any of these parameters is unsatisfactory, the screen must continue until improved enzymes are located.

Once an enzyme with suitable properties has been located, various decisions must be made concerning the acceptability of the organism containing the enzyme to the regulatory authorities, the productivity of the organism, and the way in which the enzyme is to be isolated, utilised (free or immobilised) and, if necessary, purified. If the organism is unacceptable from a regulatory viewpoint, two options exist: to eliminate that organism altogether and continue the screening operation, or to clone the enzyme into an acceptable organism. The latter approach is becoming increasingly attractive especially as cloning could also be used to increase the productivity of the fermentation process. Cloning may also be attractive when the organism originally producing the enzyme is acceptable from the health and safety point of view but whose productivity is unacceptable. However, cloning is not yet routine and invariably successful so there is still an excellent case to be made for applying conventional mutation and isolation techniques for the selection of improved strains.

Screening for new enzymes is expensive so that the intellectual property generated must be protected against copying by competitors. This is usually done by patenting the enzyme or its production method or, most usefully, the process in which it is to be used. Patenting is initiated as soon as there is evidence that an innovative discovery has been made.

Clearly defined media are usually out of the question for large-scale use on cost grounds but may be perfectly acceptable when enzymes are to be produced for high value uses, such as analysis or medical therapy where very pure preparations are essential. Often the enzyme may be

purified several hundred-fold but the yield of the enzyme may be very poor, frequently below 10% of the activity of the original material. In contrast, industrial enzymes will be purified as little as possible, only other enzymes and material likely to interfere with the process which the enzyme is to catalyse, will be removed. Unnecessary purification will be avoided as each additional stage is costly in terms of equipment, manpower and loss of enzyme activity. As a result, some commercial enzyme preparations consist essentially of concentrated fermentation broth, plus additives to stabilise the enzyme's activity. The enzymes may then be available for sale.

Table 2: List of enzymes by name/class and their traditional applications

Enzyme	Application
Proteases	Detergents Chill proofing of beer Leather baiting and tendering Digestive aids Clotting and manufacture of cheese Flavor control and production Biomedical applications
Cholesterol esterase and oxidase	Monitoring serum cholesterol levels
Glucose Isomerase	Manufacture of high-fructose syrups as “high sweeteners”
Glucose oxidase	Analysis of blood glucose levels (monitoring serum levels in diabetic patients)
Pectinases	Juice/Wine clarification Coffee bean fermentation
Glucanases	Beer making Degradation of haze polysaccharides
Hemicellulases	Baking Brewing Animal feedstuffs Neutraceutics
Amylases	Production of glucose from starch Digestive aids Brewing

Source: http://www.odofin.com/english/enzyme%20technology.htm

Novel applications and future uses of enzyme technology will include:

(a) The exploitation of enzymes as electrocatalysts (specific biosensors);

(b) Enzymes as analytical tools to measure specific compounds, for the regeneration of specific metabolites;

(c) Enyzme utilization in the synthesis of bulk organic materials and the production of fragrances and cosmetics;

(d) Enzyme utilisation in formation of food flavours and aroma compounds;

(e) The use of enzymes as tools for the detoxification of pesticide residues;

(f) Enzymes as monitors of toxic chemical levels in food and water.

Biomedical applications of Enzyme Technology will include:

(a) The synthesis of new anti-microbial compounds;

(b) Enzyme replacement therapy;

(c) Enzymes in the treatment of cancer;

(d) Enzyme graft and dermatological applications;

(e) Enzymes as activators of precursor biomolecules;

(f) Enzyme technology in the prevention of dental cavities.

B. Plant biotechnology and tissue culture

Plant biotechnology includes tissue culture/micropropagation as well as the use of growth-promoting bacteria to improve plant growth and confer disease resistance. It also includes plant breeding using molecular tools.

Tissue culture is a method of biological research in which fragments of tissue from an animal or plant are transferred to an artificial environment in which they can continue to survive and function. The cultured tissue may consist of a single cell, a population of cells, or a whole or part of an organ. This is typically facilitated via use of a liquid, semi-solid, or solid growth media, such as broth or agar. Tissue culture commonly refers to the culture of animal cells and tissues, while the more specific term plant tissue culture is used for plants. In modern usage, "tissue culture" generally refers to the growth of eukaryotic cells in vitro. It is often used interchangeably with cell culture to specifically describe the in vitro culturing of sperm donor cells. Cells in culture may multiply; change size, form, or function; exhibit specialized activity (muscle cells, for example, may contract); or interact with other cells. However, "tissue culture" can also be used to refer to the culturing of tissue pieces, i.e. explant culture or whole organs, i.e. organ culture. It is a tool for the study of animal cell biology in vitro model of cell growth to allow a highly selective environment which is easily manipulated (used to optimise cell signalling pathways).

1. The process of tissue culture

Modern plant tissue culture is performed under aseptic conditions under filtered air. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilisation of starting materials (explants) in chemical solutions (usually alcohol or bleach) is required. Explants are then usually placed on the surface of a solid culture medium, but are sometimes placed directly into a liquid medium, particularly when cell suspension cultures are desired.

Figure 1: Plants placed in a growth medium

Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of a gelling agent, usually purified agar. The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explant. For example, an excess of auxin will often result in a proliferation of roots, while an excess of cytokinin may yield shoots. A balance of both auxin and cytokinin will often produce an unorganised growth of cells, or callus, but the morphology of the outgrowth will depend on the plant species as well as the medium composition. As cultures grow, pieces are typically sliced off and transferred to new media (subcultured) to allow for growth or to alter the morphology of the culture. The skill and experience of the tissue culturist are important in judging which pieces to culture and which to discard.

As shoots emerge from a culture, they may be sliced off and rooted with auxin to produce plantlets which, when mature, can be transferred to potting soil for further growth in the greenhouse as normal plants. These plants could now be used for extraction of substances of interest.

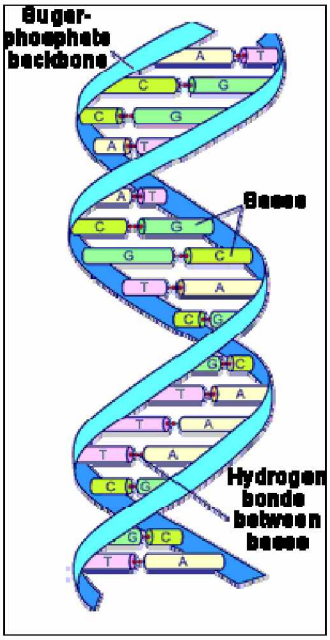
Figure 2: Plantlets replanted to produce fruit

C. Recombinant DNA technology and molecular markers

1. The structure of DNA

DNA is the heredity material of a cell that contains all the information needed to recreate an organism. All DNA is made up of a base consisting of sugar, phosphate and one nitrogen base. There are four nitrogen bases: adenine (A), thymine (T), guanine (G), and cytosine (C). The nitrogen bases are found in pairs, with A & T and G & C paired together. The sequence of the nitrogen bases can be arranged in an infinite number of ways, and their structure is known as the famous "double helix" (Figure 3).

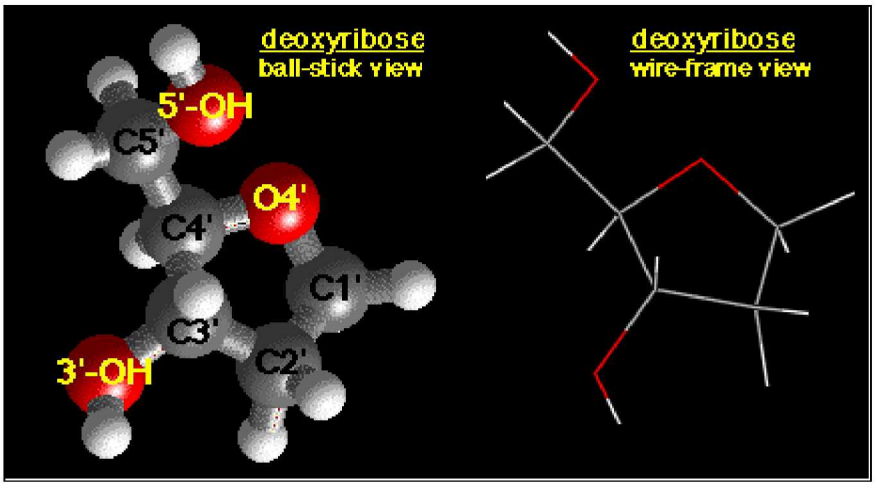
Figure 3: Molecular structure of DNA



Source: (Matthew Kuure-Kinsey and Beth McCooey for Biochemical Engineering Fall 2000)3

The sugar used in DNA is deoxyribose which is essentially a monosaccharide containing five carbon atoms, and including an aldehyde functional group in its linear structure. It is a derived from the pentose sugar ribose by the replacement of the hydroxyl (OH) at the 2 position with hydrogen, leading to the net loss of an oxygen atom (Figure 4).

Figure 4: Molecular Structure of a Deoxyribose Sugar

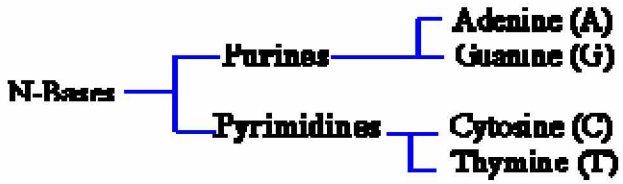


Source: Hallick (1995)

[3] http://www.rpi.edu/dept/chem-eng/Biotech-Environ/Projects00/rdna/rdna.html

The four nitrogen bases are the same for all organisms (Figure 5).

Figure 5: The nitrogen-containing organic bases



The number of bases and their specific sequence form the basis of diversity. DNA does not actually make the organism but it makes proteins of which organisms are composed. The DNA is transcribed into messenger ribonucleic acid (mRNA) and mRNA is translated into protein which then forms the organism. The way in which a protein is formed is determined by the specific DNA sequence. This leads to either a different protein, or an inactive protein.

2. Recombinant DNA (rDNA)

Combining DNA, referred to as rDNA, from different existing organisms such as plants, animals, insects and bacteria results in modified organisms with a combination of traits from the parents. The sharing of DNA information takes place naturally through sexual reproduction and has been exploited in plant and animal breeding programmes for many years.

However, sexual reproduction can occur only between individuals of the same species. A Holstein cow can be mated with a Hereford bull because the two animals are different breeds of the same species, cattle. But trying to mate a cow with a horse, a different species of animal, would not be successful.

Since 1972 scientists have been able to identify the specific DNA genes for many desirable traits and transfer only those genes, usually carried on a plasmid or virus, into another organism. This process is called genetic engineering and the transfer of DNA is accomplished using either direct injection or the agrobacterium, electroporation, or particle gun transformation techniques. These techniques provide methods of transferring DNA between any living cells (plant, animal, insect, bacterial). Virtually any desirable trait found in nature can, in principle, be transferred into any chosen organism. An organism modified by genetic engineering is called transgenic.

a) Process of forming Recombinant DNA

In order to form rDNA, the DNA from both organisms of interest needs to be cut or restricted. This is achieved through the use of an enzyme referred to as a restriction endonuclease that recognizes a specific sequence of bases on both DNA molecules such as the following:

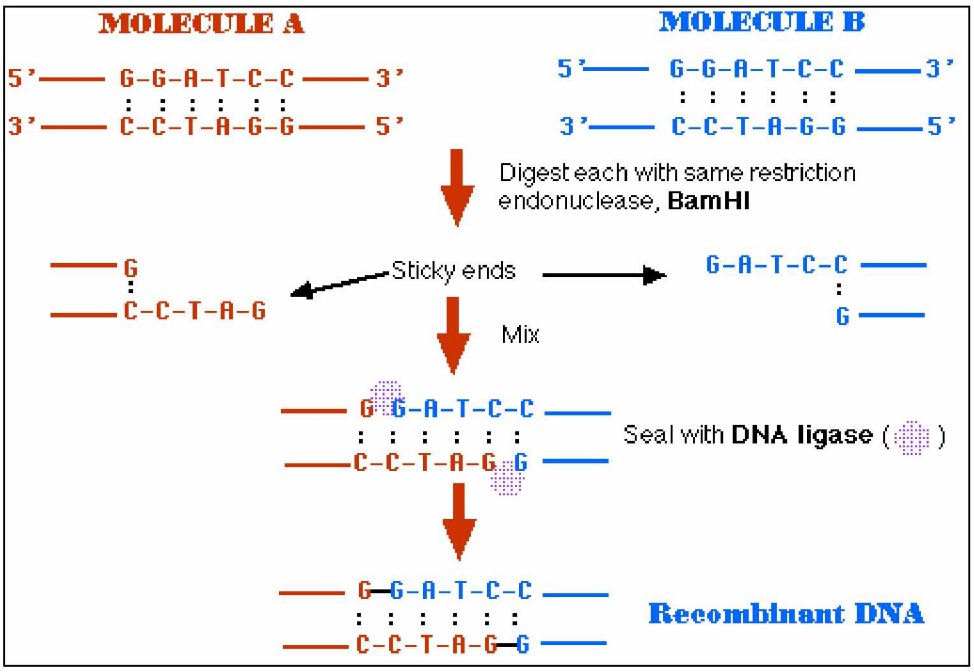
5' GGATCC 3'
3' CCTAGG 5'

The restriction endonuclease Bam HI cuts the same site on each molecule at the position indicated by the arrows.

5' G↓GATCC 3'
3' CCTAG↑G 5'

This results in each cut DNA molecule having an overhanging piece of single-stranded DNA which are referred to as "sticky ends" because they are able to base pair with any DNA molecule containing the complementary sticky end. In this case, both DNA preparations have complementary sticky ends and thus can pair with each other when mixed. The cut sequence from each molecule may then be linked by a covalent bond using the enzyme DNA ligase into a molecule of recombinant DNA (Figure 3).

Figure 6: Example of the process involved in forming recombinant DNA.



Source: http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/R/RecombinantDNA.html

To be useful, the recombinant molecule must be replicated many times to provide material for analysis. Producing many identical copies of the same recombinant molecule is called cloning. Cloning can be done in vitro, by a process called the polymerase chain reaction (PCR) whereby minute amounts of rDNA are subjected to enzymatic replication in the presence of a polymerase enzyme at the desired temperature. It may also be achieved in vivo using unicellular microbes such as Escherichia coli, unicellular eukaryotes such as yeast and in mammalian cells grown in cell culture.

In every case, the rDNA must be taken up by the cell in a form in which it can be replicated and expressed. This is achieved by incorporating the DNA in a host cell. The recombinant DNA is created by placing a DNA fragment into a vector (plasmid or virus), which is then transformed or transfected into bacterial, yeast or mammalian cells. Depending on the type of vector used one can either clone or express or modify the inserted DNA sequence. A number of viruses (both bacterial and of mammalian cells) can serve as vectors.

3. Molecular markers

A molecular marker is any kind of landmark along the DNA molecules of organisms. Molecular markers are used in molecular biology and biotechnology experiments to identify a particular sequence of DNA along the chromosomes. The three most common types of markers used today are:

- Restriction Fragment Length Polymorphism (RFLP);
- Amplified Fragment Length Polymorphism (AFLP);
- Single Nucleotide Polymorphism (SNR);
- Single Sequence Repeat (SSR)
- Randomly Amplified Polymorphic DNA (RAPD); and
- Isozymes.

Of the six marker types, the most common molecular markers today are SSR and SNPs and to some extent AFLPs.

4. Classification of biotechnology

For ease of reference, several branches of biotechnology have been derived as follows[4]:

- Red biotechnology is applied to medical processes. Some examples are the designing of organisms to produce antibiotics, and the engineering of genetic cures through genomic manipulation. For example, since the early 1980s Cuba has invested in red biotechnology and has become an important producer of biotechnology derived medicines, vaccines and diagnostic kits (CTA 2005). The foreign exchange generated through sales of these products is an important contribution to the country's Gross Domestic Product;

[4] http://en.wikipedia.org/wiki/Biotechnology

- Green biotechnology is biotechnology applied to agricultural processes. An example would be the selection of plants via micropropagation. Another example is the designing of transgenic plants to grow under specific disease pressure environmental constraint or in the presence (or absence) of certain agricultural chemicals such as herbicides. An example of this is the engineering of a plant to express a pesticide, thereby eliminating the need for external application of pesticides. An example of this would be the Bt corn. Roundup-ready soybean similarly allows the use of the herbicide to clear weeds without affecting the soy crop. Whether or not green biotechnology products such as this are ultimately more environmentally friendly is a topic of considerable debate.

- White biotechnology, also known as industrial biotechnology, is biotechnology applied to industrial processes. An example is the designing of an organism to produce a useful chemical. Another example is the using of enzymes as industrial catalysts to either produce valuable chemicals or destroy hazardous/polluting chemicals. White biotechnology tends to consume less in resources than traditional processes used to produce industrial goods.

- Blue biotechnology is a term that has been used to describe the marine and aquatic applications of biotechnology, but its use is relatively rare.

- The investments and economic output of all of these types of applied biotechnologies form what has been described as the bioeconomy.

II. APPLICATIONS OF BIOTECHNOLOGY

Advances in biotechnology-related fields such as genomics, genetic engineering, chemical engineering and cell technology are transforming the industrial process and management landscape. Functional genomics, a relatively new area of research, aims to determine patterns of gene expression and interaction in the genome, based on the knowledge of extensive or complete genomic sequence of an organism. It can provide an understanding of how microorganisms respond to environmental influences at the genetic level (i.e. by expressing specific genes) in different situations or ecologies, and should therefore allow adaptation of conditions to improve technological processes. For a range of microorganisms, it is now possible to observe the expression of many genes simultaneously, even those with unknown biological functions, as they are switched on and off during normal development or while an organism attempts to cope with pathogens or changing environmental conditions.

A. Agricultural biotechnology

1. Disease resistance

Genetic engineering is used in the development of improved plants that are resistant to insects, weeds, and plant diseases. For example, an "insect protection" gene (Bt) has been inserted into several crops - corn, cotton, and potatoes - to give farmers new tools for integrated pest management. Bt corn is resistant to European corn borer. This inherent resistance thus reduces pesticide use for controlling European corn borer, and in turn requires less application of chemicals and potentially improves yield. Advances in biotechnology are having particularly far-reaching impacts on the chemical industry. Micro-organisms, enzymes or their products are replacing processes that depended heavily on chemicals, many of which are implicated in environmental damage. However, much discussion on biotechnology currently focuses on agricultural applications and, to some extent, biomedical uses.

2. Crop improvement

Although plant science is a relatively modern discipline, its fundamental techniques have been applied throughout human history. When early man went through the crucial transition from nomadic hunter to settled farmer, cultivated crops became vital for survival. These primitive farmers, although ignorant of the natural principles at work, found that they could increase the yield and improve the taste of crops by selecting seeds from particularly desirable plants.

Farmers long ago noted that they could improve each succeeding year's harvest by using seed from only the best plants of the current crop. Plants that, for example, gave the highest yield, stayed the healthiest during periods of drought or disease, or were easiest to harvest tended to produce future generations with these same characteristics. Through several years of careful seed selection, farmers could maintain and strengthen such desirable traits.

The possibilities for improving plants expanded as a result of Gregor Mendel's investigations in the mid-1860s of hereditary traits in peas. Once the genetic basis of heredity was understood, the benefits of cross-breeding, or hybridization, became apparent: plants with different desirable traits could be used to cultivate a later generation that combined these characteristics.

An understanding of the scientific principles behind fermentation and crop improvement practices has come only in the last hundred years. But the early, crude techniques, even without the benefit of sophisticated laboratories and automated equipment, were a true practice of biotechnology guiding natural processes to improve man's physical and economic well-being.

More recently, using cross-breeding techniques, transgenic plants are being obtained. These plants are more tolerant of herbicides, resistant to insect or viral pests, or express modified versions of fruit or flowers have been grown and tested in outdoor test plots since 1987. The genes for these traits have been delivered to the plants from other unrelated plants, bacteria, or viruses by genetic engineering techniques. This is but one biotechnological application in crop improvement. The process of plant breeding has been improved through improved biotechnological processes that allow the specific identification of heterotic groups, molecular approaches to screening, molecular marker assisted selection, embryo rescue, haploid breeding, exploitation of somaclonal variation and through site directed mutagenesis or gene silencing approaches.

Crop improvement could also be achieved through tissue culture, improved diagnostics and other conventional biotechnological approaches such as N-fixation and biopesticides.

B. Industrial biotechnology

Industrial biotechnology applies the techniques of modern molecular biology to improve the efficiency and reduce the environmental impacts of industrial processes like textile, paper and pulp, and chemical manufacturing. For example, industrial biotechnology companies develop biocatalysts, such as enzymes to synthesize chemicals. Using biotechnology, the desired enzyme can be manufactured in commercial quantities.

Commodity chemicals such as polymer-grade acrylamide and specialty chemicals can be produced using biotechnology applications. Traditional chemical synthesis involves large amounts of energy and often-undesirable products, such as hydrochloric acid (HCl). Using biocatalysts, the same chemicals can be produced more economically and more environmentally friendly. An example would be the substitution of protease in detergents for other cleaning compounds. Detergent proteases, which remove protein impurities, are essential components of modern detergents. They are used to break down protein, starch, and fatty acids present on items being washed. Protease production results in a biomass that in turn yields a useful byproduct- an organic fertilizer. Biotechnology is also used in the textile industry for the finishing of fabrics and garments. Biotechnology also produces biotech-derived cotton that is warmer, stronger, has improved dye uptake and retention, enhanced absorbency, and wrinkle- and shrink-resistance.

Some agricultural crops, such as corn, can be used in place of petroleum to produce chemicals. The crop's sugar can be fermented to acid, which can be then used as an intermediate to produce other chemical feedstocks for various products. It has been projected that 30% of the world's chemical and fuel needs could be supplied by such renewable resources in the first half of the next century. It has been demonstrated, at test scale, that biopulping reduces the electrical energy required for wood pulping process by 30%.

However, the perspectives of, and existing constraints on the development of modern industrial biotechnology are located primarily in advanced industrialized nations while the performance of Caribbean countries remains marginal (Dembo et al. 1989; Goldstein 1991).

C. Environmental biotechnology

Environmental biotechnology is used in waste treatment and pollution prevention and applications of this type of biotechnology can more efficiently clean up many wastes than conventional methods and greatly reduce our dependence on methods for land-based disposal.

Every organism ingests nutrients to live and produces by-products as a result. Different organisms need different types of nutrients. Some bacteria thrive on the chemical components of waste products. Environmental engineers use bioremediation, the broadest application of environmental biotechnology, in two basic ways. They introduce nutrients to stimulate the activity of bacteria already present in the soil at a waste site, or add new bacteria to the soil. The bacteria digest the waste at the site and turn it into harmless byproducts. After the bacteria consume the waste materials, they die off or return to their normal population levels in the environment.

Bioremediation, is an area of increasing interest. Through application of biotechnical methods, the development of enzyme bioreactors that will pre-treat some industrial waste and food waste components and allow their removal through the sewage system rather than through solid waste disposal mechanisms has been realised. Waste can also be converted to biofuel to run generators. Microbes can be induced to produce enzymes needed to convert plant and vegetable materials into building blocks for biodegradable plastics.

In some cases, the byproducts of the pollution-fighting micro-organisms are themselves useful. For example, methane can be derived from a form of bacteria that degrades sulfur liquor, a waste product of paper manufacturing. This methane can then be used as a fuel or in other industrial processes.

D. Human applications of biotechnology

This section presents some examples of how biotechnology products may be applied in areas such as medicine and forensics.

1. Pharmaceuticals

Many human genes have been cloned in E. coli or in yeast. This has made it possible, for the first time, to produce unlimited amounts of human proteins in vitro. Cultured cells (E. coli, yeast, mammalian cells) transformed with a human gene are being used to manufacture more than 100 products for human therapy. Human drugs such as insulin for diabetics have been produced through cloning of vectors that now carry the chosen gene. Other uses include:

- Production of the human growth hormone (HGH) for individuals with pituitary dwarfism;
- The factor VIII for males afflicted with haemophilia A, factor IX used in treatment of haemophilia B;
- Erythropoietin (EPO) for treating anaemia;
- Several types of interferons that play an important role in the first line of defense against viral infections;
- Interleukins that are particularly important in stimulating immune responses, such as inflammation;
- Granulocyte-macrophage colony-stimulating factor (GM – CSF) for stimulating bone marrow after a bone marrow transplant;
- Granulocyte colony - stimulating factor (G – CSF) for stimulating neutrophil production, for example, after chemotherapy and for mobilizing hematopoietic stem cells from the bone marrow into the blood;
- Tissue plasminogen activator (TPA) for dissolving blood clots and therefore useful for heart attack victims;
- Adenosine deaminase (ADA) for treating some forms of severe combined immunodeficiency (SCID);
- Parathyroid hormone which is the most important endocrine regulator of calcium and phosphorus concentration in extracellular fluid;

- Several monoclonal antibodies that are the most widely used form of cancer immunotherapy at this time;

- Hepatitis B surface antigen (HBsAg) used in the preparation of vaccinations against the hepatitis B virus;

- C1 inhibitor (C1INH) that is used to treat hereditary angioneurotic oedema (HANE);

In addition, animal drugs such as the growth hormones, bovine or porcine somatotropin, are being produced by the fermentation of transgenic bacteria that have received the appropriate human, cow, or pig gene.

2. Medical diagnostic applications

a) Gene therapy

The first clinical gene therapy is underway to correct an enzyme deficiency called Adenosine Deaminase Deficiency (ADA) in children. Bone marrow cells are removed, defective DNA in bone marrow cells is supplemented with a copy of normal DNA, and the repaired cells are then returned to the patient's body.

b) Diagnosing infectious diseases and genetic disorders

Diagnosis of infectious diseases is a profound application of the new DNA technology. Tuberculosis, AIDS, papillomavirus, and many other infectious diseases, in addition to the inherited disorders like cystic fibrosis or sickle cell anemia, are diagnosed within hours by the PCR technique rather than days or weeks by traditional methods. The greatly increased sensitivity and speed of the PCR technique, as compared with traditional methods, allows earlier intervention and treatment. PCR assays will soon be available to diagnose diseases of crops and livestock.

c) Harnessing microbes for health

In 1897, the German scientist, Buchner made the vital discovery that enzymes extracted from yeast are effective in converting sugar into alcohol. Major outbreaks of disease in overcrowded industrial cities led eventually to the introduction, in the early years of the present century, of large-scale sewage purification systems based on microbial activity. By this time it had proved possible to generate certain key industrial chemicals such as glycerol, acetone, and butanol using bacteria.

Another major beneficial legacy of early twentieth century biotechnology was the discovery by Alexander Fleming, in 1928, of penicillin, an antibiotic derived from the mould Penicillium. Large-scale production of penicillin was achieved in the 1940s. However, the revolution in understanding the chemical basis of cell function that stemmed from the post-war emergence of molecular biology was still to come.

d) Forensics

Since each living creature is unique, each has a unique DNA recipe. Individuals within any given species, breed, or hybrid line can usually be identified by minor differences in their DNA sequences - as few as one difference in a million letters can be detected. Using the techniques of DNA fingerprinting and PCR, scientists can diagnose viral, bacterial, or fungal infections, distinguish between closely related individuals, or map the locations of specific genes along the vast length of the DNA molecules in the cells.

DNA fingerprinting is becoming a common practice in forensics. Similar techniques were used recently to identify the bones of the last Czar of Russia and several members of his family. Immunoassays are used not only in medicine for drug level and pregnancy testing, but also by farmers to aid in detection of unsafe levels of pesticides, herbicides, and toxins on crops and in animal products. These assays also provide rapid field tests for industrial chemicals in ground water, sediment, and soil.

3. DNA fingerprinting

a) Identifying organisms

By using Restriction Fragment Length Polymorphism (RFLP) technology, DNA fingerprints can be generated. Any individual organism can be uniquely identified by its DNA fingerprint. Consequently, this fingerprint can be used to determine family relationships in paternity litigation, match organ donors with recipients in transplant programs, connect suspects with DNA evidence left at the scene of a crime (in the form of hair or body fluids), or serve as a pedigree for seed or livestock breeds.

b) Identifying genes

One important aspect of genetic engineering projects is to identify the DNA gene that controls a particular trait. In the same way that a visitor might use the state, city, street, and house number to locate a friend's house, genetic engineers use genetic "maps" to locate genes. The genetic maps are generated by statistical analyses, PCR, RFLP, and DNA sequencing. Maps are being developed for humans, mice, swine, cattle, corn, wheat, and other plants or animals with commercial or research importance.

A current agricultural controversy involves the tomato. A recent article in the New Yorker magazine compared the discovery of the edible tomato that came about by early biotechnology with the new “Flavr-Savr” tomato brought about through modern techniques. In the very near future, the opportunity to bite into the Flavr-Savr tomato, the first food created by the use of rDNA technology ever to go on sale would be provided.

III. BIOTECHNOLOGY IN THE CARIBBEAN

A large number of island communities in the developing world lie in rural or semi-urban areas where agriculture and aquaculture are the main economic activities. The application of biotechnology to these two scientific "cultures" would to a very large extent help revolutionise traditional practices into self-sustaining market ventures that could generate badly needed capital (DaSilva 1998). Contemporary research in Asia, Latin America and Africa has already led to the widespread use of the techniques of tissue culture and genetic engineering in maintaining export markets and creating new ones. Current efforts in the Caribbean region are indicative of this growing trend.

The Caribbean nations consist of more than 25 island States with varying populations that depend on a rich diversity of plant genetic resources for their nutrition, health and well-being. The plan "Caribbean Development to the Year 2000: Challenges, Prospects and Policies" emphasised the general absence of innovation in Caribbean industry. The acquisition of appropriate technology from abroad and the development of a well-defined infrastructure to sustain such technology are identified as effective strategies in responding to these inherent weaknesses. Still again, many Caribbean countries with their island economies based on terrestrial and marine resources are encouraged to reconcile the sustained and judicious development of these resources with climatic and environmental changes. To meet these challenges, and to capitalise on the potential wealth of their resources, several Caribbean island countries have resorted to the systematic application of biotechnology.

One such step has been the establishment of a Caribbean Biotechnology Network, within the framework of the United Nations Educational, Scientific and Cultural Organization (UNESCO) global network of Microbial Resources Centres (MIRCENs) which resulted from political commitment when, a decade ago, the Grenada National Commission for UNESCO, representing the general agreement of Caribbean ministers responsible for UNESCO Affairs and the member States in the Caribbean subregion, resolved that "an extension of the international network of resources centres be created in the Caribbean region".

Since then, the Caribbean region has maintained a positive attitude towards biotechnology. Since 1975, the Caribbean Agricultural and Research Institute (CARDI) has led the region's development of agricultural biotechnology. However, no comprehensive legal or regulatory frameworks that address the major issues relevant to biotechnology have been implemented in any of the Caribbean Community (CARICOM) Countries. For the most part, existing legal and administrative mechanisms within the Caribbean Region are not adequately designed to address modern biotechnology issues such as research and development, trade, biosafety, food safety and labelling. However, in 2003, CARICOM Ministers mandated CARDI to develop a regional policy on biosafety and in 2005, the CARICOM Minister with portfolio responsibility for Science and Technology provided an Endorsement Statement to support the commercialization of biotechnology in the Caribbean region.

In the run-up to the year 2000 and beyond, several island countries, especially in the Caribbean region, are confronted by the challenges and threats of globalisation. In response, several island countries are initiating self-reliant strategies aimed at national and regional

endogenous development. Amongst these strategies, the potential of biotechnology for economic development and technological growth is being tapped.

However, in 2002, Barbados established a National Biosafety/Biotechnology Committee. In 2003, the National Biosafety Council was developed in Grenada to assume administrative functions in relation to issues outlined in the Cartagena Protocol on Biosafety and Grenada has recently developed legal apparatus relevant to genetically modified food imports (Hoffman 2005).

The Dominican Republic established the Biotechnology and Biodiversity Centre (Centro de Biotecnologia y Biodiversidad – CIBIO) in 2000 to serve as the national and scientific base for finding biotechnology solutions to the main problems affecting the agriculture, forestry and fisheries sectors (Eneas 2005). This Centre serves as the base for exploiting modern biotechnologies. Specialized laboratories which focus on tissue culture, molecular biology, molecular diagnostics, germplasm management, industrial biotechnology and nutraceuticals have been established. Emphasis is also being given to human resource development; a new Masters Degree programme has been set-up and protocols have been developed for a wide variety of biotechnology products (biopesticides, medicinal extracts, diagnostic methods, scale-up of tissue culture protocols).

At the national level, since the early 1980s and consistently over the last 20 years, Cuba has invested in red biotechnology and has become an important producer of biotechnology-derived medicines, vaccines and diagnostic kits. The foreign exchange generated through sales of these products is an important contribution to the country's Gross Domestic Product (GDP). Since the 1990s Cuba has placed special emphasis on biotechnology applications to increase agricultural productivity and development of their aquaculture sector (Eneas 2005).

Despite these initiatives and owing to the lack of regulatory frameworks concerning modern biotechnology, there are no biotechnology products currently approved for direct consumption, processing or for animal feed. Furthermore, there is no information currently available for the field testing of biotechnology crops in the area, or the Region's policy on co-existence between biotechnology and non-biotechnology crops. However, research in different biotechnologies continues to progress especially at academic institutions and these are presented below.

A. Industrial biotechnology

1. Fermentation technology

The use of fermentation technology to make industrial products is very much underutilized in the Caribbean with Cuba and Puerto Rico making use of this technology (Mitchell et al. 2004). To date, the most common application of biotechnology in the Caribbean islands has been in the fermentation of rum and beer. In addition, research in the sugar industry has focused on the utilisation of products and by products of the sugar industry for the production of value-added products which can decrease foreign exchange expenditure through

import substitution, as well as increase revenue through non-traditional exports (P. Umaharan5, pers. comm.).

A novel process of the bacterial production from molasses, of xanthum gum, a raw material utilized in the pharmaceutical and chemical industries, and which has applications in enhanced oil recovery in the petroleum industry, has been developed and patented (Mitchell et al. 2004).

Biomethanation programmes in the island States of the Caribbean region have been supported by the Caribbean Development Bank (CDB). Jamaica, with funding from the Latin American Energy Organization (OLADE) has been particularly active in experimenting with a variety of biodigester designs and models (Clancy and Hulscher 1994).

In Trinidad and Tobago a fermentation process, in which methane from natural gas is used as a carbon source for a bacterial culture, found in the natural habitat, can be utilized in an industrial production of a highly rich animal protein product. The only other raw materials are oxygen, ammonia salts, and a few other minerals. This product is at least nutritionally equivalent to high quality fishmeal and furthermore free from dioxin and heavy metals[6].

Other applications as articulated by DaSilva and Taylor 1998 include the following:

- Fermentation of sorghum for food and feed;
- Uses of waster yeast from the fermentation industry;
- Identification and characterization of local food pathogens with a view to increasing regional food safety;
- Production of citric acid from molasses;
- Use of the beneficial fungus, VesicularArbuscular Mycorrhyzae to increase vegetable and legume crop yield in red kidney beans (Phaseolus vulgaris), Winged bean (Psophocarpus tetragonolobus), and moth bean (Vigna aconitifolia);
- Development of legume inoculant;
- Recycling agricultural wastes for the production of animal feed and organic fertilizer;
- Use of microbes in animal feed as a substitute for antibiotics.

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[6] http://www.unibio.dk/

2. Enzyme technology

In the Caribbean, work with immobilized Protein A or Protein G revealed that the yield of antibodies purified using affinity chromatography was increased by 500% by including kosmotropic salts in the binding buffer. This is due to the inclusion of strongly hydrated anions (citrate, sulphate and phosphate) and weakly hydrated cations such as ammonium and potassium (Ngo and Narinesingh 2008).

Several other studies that have investigated the properties and activities of enzymes have been conducted (Wu et al. 1999, Mankasingh et al. 2000, Brahim et al. 2001, Brahim et al. 2002, Ngo et al. 2005).

B. Plant biotechnology and tissue culture

Most of the tissue culture work on all crops was initiated at UWI in the early 1980s at its St. Augustine campus. Protocols for a number of tropical crops including banana, plantain, pineapple, aloe, breadfruit, jackfruit, sweetpotato, cocoa, anthuriums, tropical orchids, carambola were developed. An in vitro breeding method for fusarium resistance in banana as well as an in vitro hardening method for banana was developed. Protocols for developing yam varieties free of brown spot virus was developed in CARDI headquarters in Trinidad and Tobago. They worked on germplasm storage as well as commercial micropropagation of anthurium.

More recently, tissue culture has been used as an economical tool for the micropropagation of many food and ornamental plants (Table 3).

Table 3. Examples of more recent application of tissue culture to the agricultural sector

Country	Application
Bahamas	The Ministry of Agriculture supports the bulk production of citrus fruits and root crops as well as conservation and production of native orchids and floristic ornamentals.
Barbados	Since 1979, the White Lisbon cultivar of the yam species, Discorea alata has been improved through elimination of the viral disease, internal brown spot. Distribution of plantlets of the improved variety has led to 40% increase in crop yield; Development of a resistant strains of hot pepper and tomato to bacterial spot disease caused by Xanthomonas campestris var. vesicatoria.
Dominica	Leaf burning disease caused by the fungus, Pythium myriotylum in cocoyam (tannia) has been eliminated through biocontrol systems.
Grenada	The spread of Moko disease caused by Pseudomonas solanacearium in bananas has been controlled.
Guyana	National Agricultural Research Institute (NARI) and FAO are engaged in the production of shoots from dormant axillary buds of pineapple (Ananas comosus L. Marr.).
Jamaica	Development of virus-free planting material for the Irish potato, Solanum tuberosum Production of yam plantlets of the variety Dioscorea cayensis, D. rotunda, D. alata and D. Trifida; Development of the mushroom industry using oyster mushrooms, Pleurotus sajor-caju; Development of strains of the hot pepper (Capsicum chinense) that are resistant to potyviruses; Tissue protocols for the following have been developed: - sweet potato – Ipomoea batatas - cassava – Manihot esculenta - dasheen – Colocasia esculenta - plaintains – Musa spp. - breadfruit – Artocarpus altilis - jackfruit – A. heterophyllus - carambola – Avevrhoa carambola - yam bean – Pachyrhizus erosus - cacao – Theobroma cacao - pineapple – Ananas comosus - sugar cane – Saccharum officinarum
St. Kitts & Nevis	Production of tuber crops
St. Vincent & the Grenadines	Yam tissue cultures introduced by the Chinese
Trinidad & Tobago	UWI developing protocols and appropriate biocontrol and quarantine measures targeted at improving yields of yams, sweet potatoes, cassava and plaintain species.
CARDI	Repository for virus-free yam material and germplasm storage of selected crop species of economic significance.

Sources: DaSilva and Taylor 1998; Hoffman 2005; Lawrence et al. 2005.

Another recent development is research into methods for food preservation and extension of the shelf-life of plant products. The Mona and St. Augustine campuses of UWI are involved in joint ventures in tissue culture applications. Also, the Jamaican Government collaborates with industry in the production of white potatoes, ginger, banana, and yam. In Trinidad, gingerlilies, roses and orchids are produced for local markets. Recently, the technique of embryo transfer has being used to boost meat and milk production (DaSilva and Taylor 1998).

Specifically, at the Mona Campus, extracts from a number of plants have been identified as having potential in the field of medicine (Table 4).

Table 4: Examples of the potential of plant extracts in medicine

Plant	Usage
Unripe tamarind	Antibacterial activity
Leaf-of-life leaves (Bryophyllum pinnatum)	Antibacterial activity
Spirit weed (Eryngium foetidium)	Anti-convulsion properties
Breadfruit (Artocarpus altilis)	Anti-flammatory potential
Freeze-dried noni (Morinda citrifolia)	Anti-flammatory potential
Abutilon trisulcatum (choline-rich plant)	Treatment of memory disorders
Ginger (Zingiber officinale)	Treatment of rhematoid arthritis
Neem	Disinfectant potential
Microbes in animal feed	Antibiotics
Guinea hen weed	Bioactive anti-cancer chemical
Turmeric (Curcuma longa)	Anti-inflammatory
Aloe (Aloe vera)	enhances immune function
Sarsaparilla (Smilax regelii)	Prevents or relieves rheumatism

Source: Mitchell et al. 2008; Bone 2003.

C. Recombinant DNA technology and molecular markers

1. Recombinant DNA

Recombinant DNA technology is being successfully used in development of the anthurium industry. This is a commercial venture that is being undertaken at UWI, St. Augustine and seeks to broaden the range of colours of the indigenous anthurium, Anthurium andraeanum, through the introduction of genes that code for new colours from other species (Elibox and Umaharan 2008). It is expected that these unique colour schemes would greatly enhance the marketability for the species.

Within the same species, research into the development of resistant varieties using a bioengineered strain of Xanthomonas axonopodis pv dieffenbachiae has resulted in the first bacterial blight resistant varieties of Anthurium andraeanum Hort., which are presently being commercially exploited. Further research on this species is addressing the development of nematode-resistant varieties (Elibox and Umaharan 2007).

At the Mona Campus of UWI, research has been conducted into developing varieties of papaya resistant to ring spot disease using transfer of resistant genes that have been obtained from local varieties (P. Tennant7, pers. comm.). Results have indicated that 40 - 50 % of these transgenic plants are free of the virus and research using rDNA techniques to ensure 100 % success continues (Cai et al. 1999). Research using rDNA techniques is also being conducted into producing virus-resistant varieties of the sweet orange and gragefruit.

2. Molecular markers

a) Molecular markers in genetic resource management

Molecular markers, namely RFLPs and RAPDs have been used to determine the stock structure of the four-wing flyingfish, Hirundichthys affinis, in the central western Atlantic (Gomes et al. 1998, 1999). This research confirmed that the Caribbean stock (inclusive of the Netherlands Antilles and Aruba) is indeed genetically separate from the South American stock and, as such, should be managed separately.

Microsatellite markers were used to determine the Barbados Blackbelly population structure through the of microsatellite loci to include or exclude individuals as belonging to this population. Polymerase chain reaction genotype analysis using dinucleotide microsatellite markers were used to examine the genetic diversity present in the Barbados population as compared with populations of West African origin, Virgin Island White and Blackbelly from the United States. Results suggested that the observed heterozygosity prevailing in the Barbados Blackbelly and populations from the United States were genotypically different (Roberts and Thomas 2003).

Molecular fingerprinting of all cocoa genetic resources available at the International Cocoa Genebank in Trinidad using multiplex SSR markers has allowed the identification of mislabeled accession. The information has also been used to develop a core collection representing the cocoa genepool and having desirable characteristics.

i. Molecular markers in crop improvement

Molecular markers have been used to identify heterotic groups within hotpepper (Capsicum chinense Jacq.) (Moses and Umaharan, 2008). This information is being used in heterosis breeding to improve the yield in hotpepper (Umaharan, P. pers. Comm.).

Molecular markers based on AFLP have been used to identify markers for sugar and fibre content in Sugarcane, which can be used to improve yield. Similarly, SSR markers associated with disease resistance, butterfat content and other agronomically important traits in cocoa (Theobroma cacao) is being developed using a Refractario population using association mapping. Such markers will have widespread usage beyond individual crosses. Molecular markers linked to bacterial blight resistance in anthurium are also being pursued in collaboration with researchers in Hawaii.

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ii. Molecular markers in diagnostics

There are numerous begomoviruses that affect tomato production in the Caribbean. Work is ongoing at the Mona and St. Augustine campuses of UWI as well as in Cuba and Guadeloupe. In a European Union-funded regional project, viz., BETOCARIB involving Cuba (Domican Republic (ISA), Trinidad and Tobago (UWI), France (INRA, CIRAD) and the United Kingdom (NRI), molecular markers were developed to characterize the various begomoviruses and strains affecting tomato within the Caribbean region. Tomato varieties were identified with resistant to these viruses for deployment. Similarly Phytophthora palmivora and Crinipellis perniciosa strains were characterized to determine relationship with aggressiveness.

There is ongoing work on characterizing the bacterial wilt strains in the family Solonaceae and Musaceae (Ralstonia solanacearum) throughout the Caribbean (UWI, St. Augustine; CIRAD, Guadeloupe), pepper viruses, coconut diseases (UWI, Mona; Coconut Research, Jamaica), Citrus tristeza virus in citrus and bacterial and fungal diseases in a number of vegetables including cabbage and pumpkin. The improved precision with which pathogens can be diagnosed is allowing appropriate deployment of resistance; appropriate quarantine measures to be developed by the Council for Trade and Economic Development (COTED) and providing a tool for epidemiological studies aimed at integrated pest management.

IV. CONCLUSIONS AND RECOMMENDATIONS

The biotechnology movement in the Caribbean is a fledgling industry that has tremendous potential for development and this would be facilitated through the development of policies complete with the required legislation and regulations to support them. A coherent regional biotechnology policy, within a broader science, technology and innovation policy is critical for creating an enabling environment that can foster biotechnology development. However, no regional policy exists and since each country has the responsibility for policies and regulations in their own islands, the situation posed by the geographical and political fragmentation of the Caribbean Region poses a great challenge for development of such a policy. Notwithstanding the challenge, a regional policy is desirable towards not only providing a vision and identify priority areas for development but also to encourage a culture of innovation through funding mechanisms that would promote collaboration in research and industry development in the identified priority areas. Such a policy should also provide fiscal incentives for funding research/ technology development initiatives as well as mechanisms to promote technology transfer and foreign direct investments. In addition, they should support enabling systems dealing with intellectual property rights, biosafety and rewards.

Recognising the importance of a regional policy CARICOM has established a working group with a mandate to develop a Caribbean Biotechnology and Biosafety Policy. In the absence of a regional policy, Barbados and Jamaica have developed national biotechnology policies and Trinidad and Tobago is in the process of developing a draft policy (P. Umaharan8 pers. comm.) having already developed some enabling policies and legislation on intellectual properties (Mitchell et al. 2004). In the Greater Caribbean, Cuba and the Dominican Republic also have well developed biotechnology and biosafety policies.

At the regional level, the Inter-American Institute for Co-operation on Agriculture (IICA) has developed a biotechnology strategy document entitled "Caribbean Programme on Biotechnology and Biosafety", which includes four strategic elements including research and development, information management, capacity building and commercialisation of biotech products. The Caribbean programme for biotechnology and biosafety dovetails into a hemispheric programme on biotechnology and biosafety developed by IICA. It is recommended that the region take the initiative to develop such a policy and to ensure the institutionalisation of the appropriate supporting agencies and furnishing with necessary human resource and technical capacity.

The development of a regional policy and the successes gained by countries that have invested in science and technology and, more specifically, biotechnology are largely attributed, first and foremost to visionary leadership and strong political commitment. Such commitment would be manifested in the development of appropriate policies in a participatory manner that would guide development and commercialization of the industry.

The development of key partnerships among public research (universities, national research institutions and centres of excellence), the private sector (industry) and the government

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are necessary in order to promote the biotechnology agenda. Public and private investment in science and technology infrastructure and biotechnology programmes would promote research in biotechnology resulting in the production of useful products which could then be commercialised. However, there is a disconnect between research and the industrial activities of the private sector. Many national institutions that were created to serve as the link between the University and the industry have largely failed (P. Umaharan pers. comm9.). The new developmental paradigm requires that the university work alongside the private sector in industrial parks so that there is a close direct link between research and enterprise development. However, such ventures would require venture capital. It is recommended that the appropriate environment should be created for the private sector and venture capitalists to become critical partners in biotechnology efforts of the country.

In this regard, organizations such as the National Commission on Science and Technology (NCST) in Jamaica could have a primary role to play, because it is a body that can facilitate linkages, bridge the existing gaps and ensure best results. The Commission's National Biotechnology Coordination Committee is well positioned to play a critical role in monitoring, coordinating and providing advice for biotechnology development at a national level. Governments are also well poised to establish commonly agreed biotechnology research and development targets among key organizations in agreed priority areas. In this regard, research at the university level will be linked to industry needs and special efforts will be given to linking science faculties and their centres to businesses locally and overseas. Governments could also support and encourage the transfer of the technology ensuring that research results are moved to commercialization. In order to further promote the development of the biotechnology/industry interphase, scientists should be offered special training courses in business and marketing to augment a business/entrepreneurial culture[10].

Given the current global food crisis the promotion of agricultural research that includes a higher biotechnology component is recommended. In this regard, governments could encourage the establishment and development of Centres of Excellence in biotechnology and bio-engineering. Also, the introduction of formal/university training programmes in biotechnology can also assist in the development of capacity. This would provide the necessary enabling environment for research and development in biotechnology and allied fields and could be promoted through the development of infrastructure, incentives and regulatory frameworks. Priority should be given to agro-biotechnological research, particularly to increasing agricultural productivity and food security in the region. To date, more than half of the Caribbean islands are using biotechnology in agricultural production but there is little commercialization of the products except for tissue culture and the micropropagation of plants. The biotechnology centres and research programmes that are located in Barbados, Grenada, Jamaica and Trinidad and the researchers are anxious to promote research and generate positive biotechnology policy initiatives.

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[10] Biotechnology for Socio-Economic Development: A Policy for Jamaica. National Commission on Science and Technology, Draft 5. September 2006.

Further to this it is recommended that support and encouragement for the formation of clusters of high value-added manufacturers in the supply chain should be provided[11]. As such, biotechnology should result in increasing productivity and improving quality to meet market needs. The promotion of entrepreneurship and provision of rewards for innovators would support investment in biotechnology approaches.

The creation of an enabling environment for science, technology and innovation and more specifically for biotechnology to thrive is considered necessary. This would include laboratory structures complete with relevant facilities for diagnostic applications. Of importance here is the establishment of a commercial tissue culture facility that would allow the marketing of plantlets to farmers (S. Mitchell[12], pers. comm.). This is already in existence in Jamaica in the Manchester area where the Christiana Potato Growers Association has set up a facility that has resulted in the production of sweet and irish potato and ginger plantlets (M. Blair[13], pers. comm.). These facilities should be equipped with an efficient internet system, complete with state of the art computers to facilitate ease of communication and instructional activities.

The appropriate technical expertise to operate these facilities would be needed (NIHERST/CCST 2004). The lack of a critical mass of scientists in academic and research institutions has been identified as a constraint to biotechnology development. This can be overcome by capacity-building and by enhancing collaboration among players within the region. The universities in the region should aim at recruiting graduates from a broad disciplinary base (biologists, chemists, information technologists, social scientists, agriculturists, engineers, medical scientists and clinicians) into postgraduate programmes that provide a flexible training environment that will provide the requisite grounding in a specific area and the team skills to function in a multi-disciplinary unit that can support biotechnology industry development (P. Umaharan[14], pers. comm.).

Although many efforts were made in the past, these efforts could not be sustained. The only successful collaborations have been through the implementation of joint regional research projects, which helped to build trust among partners and led to other successful projects. The regional efforts should therefore focus on establishing competitive funding mechanisms with the goal of bringing Caribbean biotechnologists together to achieve regional development goals. Teams established through such mechanisms will develop a level of expertise, coherence and impetus that should enable them to attract independent funding. A consultative group of Caribbean biotechnologists can form the basis of developing the regional agenda[15].

Development and implementation of a public education strategy addressing the key elements of biotechnology, and focusing in general on methodologies and more specifically on opportunities and risks would assist in creating appropriate attitudes to the technology and promoting support for it. Such a strategy could be included in the curriculum of the Caribbean

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[11] Biotechnology for Socio-Economic Development: A Policy for Jamaica. National Commission on Science and Technology, Draft 5. September 2006

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Examinations Council (CXC)and adapted to meet the needs of young people of primary school age. In conjunction with this strategy would be the development of an efficient electronic system where information on biotechnology would be available and easily accessible.

Public education should be pursued in conjunction with a public awareness strategy which should result in acceptance of the value and potential of biotechnology applications. Ongoing public education and awareness campaigns as well as participatory systems of development are required to ensure that there is no discord between the development goals of the region and the public interest. Particular emphasis will be placed on internships, beginning at the secondary school level, for teachers and students. Also, in an effort to develop national capability in biotechnology, at the highest international standard, a specific programme could be implemented to provide training of top students identified at the tertiary level, through the award of scholarships and fellowships to support training at reputable overseas institutions.

In terms of the research agenda, more specific recommendations would include the following:

- Development of Biological Control and Pest Management Strategies. This would encompass the conduct of specialized research towards the development and production of biocontrol agents for pest management to significantly reduce the use of chemicals;

- Biopesticides and genetic approaches also provide a more environment friendly approach to managing pest and disease problems. Cuba is the forerunner in biopesticides research and development and the Caribbean region could use this model in developing a range of products that can be marketed throughout the Region. There is a growing impetus in the region for developing genetic resistance to biotic stresses in the region, in many islands. A co-operative approach can considerably strengthen this effort supported by molecular tools to develop it into an industry to supply the region with tropically adapted germplasm. Patenting the tools developed can provide global leverage and economic returns;

- Pests and disease problems in the agricultural sector are of tremendous concern as the movement of pests and the spread of diseases among the islands of the Caribbean increases the need for effective quarantine measures16. In this regard, biotechnology provides tools to diagnose diseases rapidly and monitor their evolution and spread. These tools can greatly assist quarantine systems in the region which are already operating at full capacity;

- The medical branch of biotechnology extends beyond the genomic principles and is closely related to physics and mathematics through the development of diagnostic equipment. As such it would be important to provide support for the

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development of sub-surface systems that have multiple uses in medicine and petrochemical excavation;

- Research into the promotion of athlete-enhancing potential models through biotech advancements within the context of the limitations to human performance, could provide an advantage. Some of these include legal metabolic enhancers possibly herbal sources and athletic wear17.

- Development of telecommunication systems would greatly enhance the delivery of healthcare services such as Telemedicine;

- The conservation of genetic resources becomes even more urgent in small island States, where alternate use of land is leading to rapid genetic erosion. In this regard, biotechnology can provide novel tools such as microchip arrays that can be used to rapidly screen for genes and proteins, and methods to isolate and engineer genes and mass produce novel products in bioreactors18. It also provides tools for genetic resource monitoring and sustainable use and development of efficient strategies for the in situ conservation of bioresources. Focus should therefore be on exploiting the enormous marine and land biodiversity as well as the indigenous knowledge available in the region;

- It is important to also develop strategic partnerships with global leaders in each area to commercialize the products from these bioresources, so that pharmaceuticals, neutraceuticals and other industrial products can be developed and countries can benefit;

- In order to maximize the application of products that have been successfully developed, the ability to store, manipulate, manage and decipher information from very large genome-based or protein-based data sets or molecular marker data (bioinformatics) is imperative to the functioning of a biotechnology enterprise. In this regard, it would be useful to obtain the necessary equipment and expertise to ensure that this information is secure and could be sorted.

Protection of Intellectual Property Rights (IPRs). Governments are well positioned to play a proactive role in creating awareness of the importance of IPRs in biotechnology research and innovation through the development of databases and assistance to scientists and entrepreneurs through the establishment of appropriate IPR Offices. In Jamaica, such an office has been established and in Trinidad and Tobago a committee to oversee the protection of IPRs has recently been established. However it is recommended that harmonization of IPR legislation to include issues related to access and benefit sharing given the various treaties and agreements that Caribbean countries have signed and/or ratified (the World Trade Organization/Trade-related aspects of Intellectual Property Rights (WTO/TRIPS) Agreement, Convention on

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Biological Diversity and its Cartagena Protocol and the International Treaty on Plant Genetic Resources) should be pursued (CTA 2005).

The technological gap between the developed and developing world has been identified as the single most important constraint to biotechnology development in the developing countries and it is continuing to widen, as new biotechnologies are developed at a rapid rate. The United Nations Industrial Development Organization (UNIDO), recognizing the weakness of developing countries in this area, established two international centres (Trieste, Italy; New Delhi, India) for genetic engineering and biotechnology with the objective of transferring technologies to member countries. These should be fully utilized in the Region. In addition, many biodiversity-rich developing countries have established bilateral collaborative programmes with technology-rich developed countries to allow access to biodiversity in exchange for technology. Others have established collaborative agreements with institutes in the United States where local researchers work with their counterparts, towards developing local products and processes, which will benefit the local economy. Each country needs to define an appropriate strategy which also takes into account (IPR) issues to overcome this hurdle of the ever-expanding technology gap (Umaharan 2005).

The development of marketing strategies for commercial use of developed crop varieties would greatly enhance distribution and help to bring economic returns. In this regard, indigenous crop genetic diversity should be systematically exploited for developing novel ornamental, medicinal or other crop varieties for commercial purposes. Such initiatives may result in the development of niche agricultural industries in the region. For example, ornamental crops which require high capital inputs and intensive production systems are ideally suited for the small farm holdings in the Caribbean. Linkage of the ornamental plant industry to the dynamic tourism sector in the Caribbean together with the geographical proximity of the Caribbean region to the vast North American market could well provide opportunities for market expansion. Biotechnology also has the potential for providing opportunities for developing specialty foods based on indigenous crops (Umaharan 2005).

The role of government in the development of biotechnology is to induce the creation of national innovation systems, which can provide the resources and capabilities needed to master and develop the technologies involved, with particular emphasis on their engineering, technological and commercial aspects. Such scientific capabilities are the basis for later industrial applications. Agricultural biotechnology has developed in a limited and uneven way in the Caribbean. The advances that have occurred are the results of individual actions taken by scientists, research managers and companies. Governments have frequently supported or complemented the initiatives of scientists and research institutes to create or strengthen scientific capabilities through special biotechnology development programmes. Changes in intellectual property protection, expanding it to include biological materials and living beings, are too recent to have had an effect on biotechnology. The relatively more advanced countries in the region are ready to implement programmes targeted on specific industries.

In this regard and given the cross-cutting and interdisciplinary nature of biotechnology as well as the policy and trade-related issues, it would be necessary to coordinate the activities of key ministries such as environment, agriculture, health, industry, planning, finance, trade,

education and foreign affairs, administrations, industries, legal bodies and research institutions (CTA 2005).

However, it is posited that in the face of growing privatization of the biotechnology industry, the industry would never be a primary source in the developing world (Schatzmayr and Bernades 1994). The onus is therefore on governments to develop the appropriate policies to promote the application of biotechnology to industry thereby providing competition to the private sector in promoting a viable biotechnology opportunity in the Caribbean. Along with this, a cooperative approach can consistently strengthen this effort supported by molecular tools to develop it into an industry to supply the Caribbean region with tropically adapted germplasm. To date, only Cuba has had a national strategy of creating a biotechnology-based industry, as part of its ambitious export plans. Patenting the tools developed can provide global leverage and economic returns.

The biotechnology revolution heralds a new race in which the Caribbean is already behind. It is a critical area that must be developed to achieve the region's development goals in a sustainable manner and for the region to function in an increasingly competitive global economy. Biotechnology development requires a coherent framework, a focused strategic approach, the appropriate policies and fiscal environment including tax regime, resource provision structure, capacity building, joint university-private sector projects, venture capital and multi-disciplinary highly skilled teams. There are several successful models, which can be evaluated and adapted to meet the needs of the Caribbean. The biotechnology revolution provides the opportunity to develop the region's strengths, conquer its weaknesses and grasp the opportunity presented by the greatest challenge yet to confront mankind.

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