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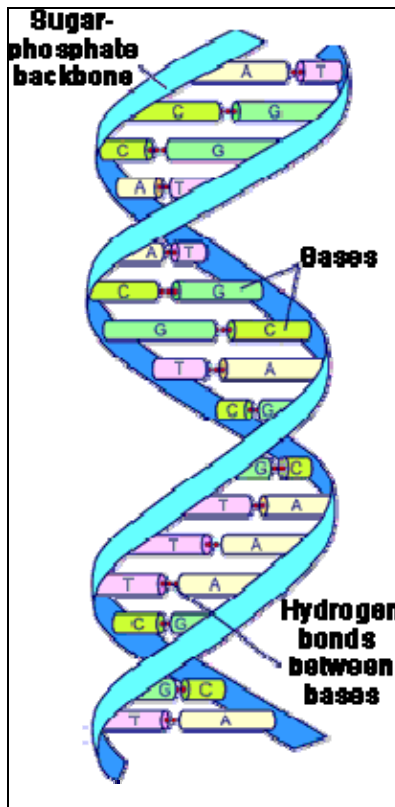


SIXTY YEARS WITH LATIN AMERICA AND THE CARIBBEAN

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## POLICY BRIEF

### BIOTECHNOLOGY WITH SPECIAL REFERENCE TO THE CARIBBEAN



Molecular structure of DNA



## INTRODUCTION

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. Main areas include application in the agriculture sector but the use in medicine and biology are also being promoted.

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 for commercial purposes. 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.

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, we have learned a great deal 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, we can now actually 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, we can cause bacterial cells to produce human molecules. Cows can produce more milk for the same amount of feed. And we can synthesize therapeutic molecules that have never before existed.

Three specific areas of biotechnology may be identified:

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

## Industrial biotechnology

### 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	<ul style="list-style-type: none"> <li>• Unicellular – bacteria and cyanobacteria</li> <li>• Multicellular - cyanobacteria</li> </ul>
Eukaryotic	<ul style="list-style-type: none"> <li>• Unicellular – yeast and algae</li> <li>• Multicellular – fungi and algae</li> </ul>

**Figure 1**  
**Products of Fermentation Technology**



Beer



Wine

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.

The use of fermentation technology to make industrial products is very much underutilized in the Caribbean with Cuba and Puerto Rico making the most 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 utilization 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. Umaharan<sup>1</sup>, *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 Organisation (OLADE) has been particularly active in experimenting with a variety of biodigester designs and models (Clancy and Hulscher 1994).

Other applications 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, Vesicular Arbuscular Mycorrhizae 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.

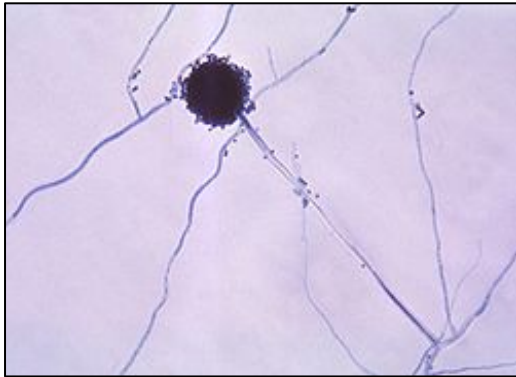
### Enzyme technology

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 such as *Aspergillus* and bacteria such as *Bacillus* (figure 2). Safe organisms must be used for consumer-related applications.

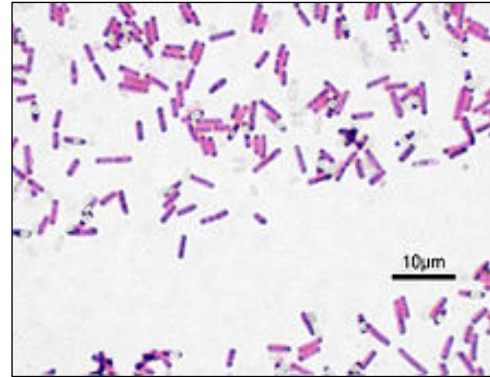
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<sup>1</sup> Professor Pathmanathan Umaharan, Professor of Biotechnology, University of the West Indies, St. Augustine Campus, St. Augustine, Trinidad and Tobago

**Figure 2**  
*Aspergillus niger* and *Bacillus subtilis* used in enzyme technology



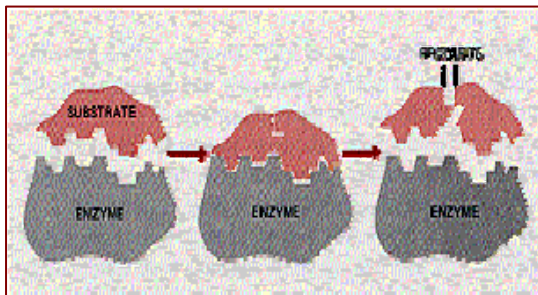
Spore head of *Aspergillus niger*



*Bacillus subtilis*

Source: <http://en.wikipedia.org/wiki/Aspergillus>; <http://en.wikipedia.org/wiki/Bacillus>;

**Figure 3**  
**Enzyme-substrate reaction**



Source: <http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/E/Enzymes.html>

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 (figure 3). Competitive inhibitors bind reversibly to the enzyme, preventing the binding of substrate. On the other hand, binding of substrate prevents binding of the inhibitor. Substrate and inhibitor compete for the enzyme.

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, enzymes 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.

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

Enzyme	Application
Proteases	<ul style="list-style-type: none"> <li>• Detergents</li> <li>• Chill proofing of beer</li> <li>• Leather baiting and tendering</li> <li>• Digestive aids</li> <li>• Clotting and manufacture of cheese</li> <li>• Flavor control and production</li> <li>• Biomedical applications</li> </ul>
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	<ul style="list-style-type: none"> <li>• Juice/Wine clarification</li> <li>• Coffee bean fermentation</li> </ul>
Glucanases	<ul style="list-style-type: none"> <li>• Beer making</li> <li>• Degradation of haze polysaccharides</li> </ul>
Hemicellulases	<ul style="list-style-type: none"> <li>• Baking</li> <li>• Brewing</li> <li>• Animal feedstuffs</li> <li>• Nutraceuticals</li> </ul>
Amylases	<ul style="list-style-type: none"> <li>• Production of glucose from starch</li> <li>• Digestive aids</li> <li>• Brewing</li> </ul>

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

### **Tissue culture and plant biotechnology**

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 (figure 4).

Tissue culture commonly refers to the culture of animal cells and tissues, while the more specific term plant tissue culture is used for plants.

**Figure 4**  
**Tissue culture of a plant**



Source: [www.gemination.com/Images/TissueCultureContainer.jpg](http://www.gemination.com/Images/TissueCultureContainer.jpg)

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).

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

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 the University of the West Indies (UWI) are involved in joint ventures in tissue culture applications. Also, the Government of Jamaica collaborates with industry in the production of white potatoes, ginger, banana and yam. In Trinidad and Tobago, gingerlilies, roses and orchids are produced for local markets. Recently, the technique of embryo transfer has been used to boost meat and milk production (DaSilva and Taylor 1998).



**Table 3**  
**Examples of application of tissue culture to the agricultural sector in the Caribbean**

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	<ul style="list-style-type: none"> <li>• Since 1979, the White Lisbon cultivar of the yam species, <i>Dioscorea alata</i> 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;</li> <li>• Development of resistant strains of hot pepper and tomato to bacterial spot disease caused by <i>Xanthomonas campestris</i> var. <i>vesicatoria</i>.</li> </ul>
Dominica	Leaf burning disease caused by the fungus, <i>Pythium myriotylum</i> in cocoyam (tannia) has been eliminated through biocontrol systems.
Grenada	The spread of Moko disease caused by <i>Pseudomonas solanacearum</i> 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 ( <i>Ananas comosus</i> L. Marr.).
Jamaica	<ul style="list-style-type: none"> <li>• Development of virus-free planting material for the Irish potato, <i>Solanum tuberosum</i></li> <li>• Production of yam plantlets of the variety <i>Dioscorea cayensis</i>, <i>D. rotunda</i>, <i>D. alata</i> and <i>D. Trifida</i>;</li> <li>• Development of the mushroom industry using oyster mushrooms, <i>Pleurotus sajor-caju</i>;</li> <li>• Development of strains of the hot pepper (<i>Capsicum chinense</i>) that are resistant to potyviruses;</li> <li>• Tissue protocols for the following have been developed: <ul style="list-style-type: none"> <li>- sweet potato – <i>Ipomoea batatas</i></li> <li>- cassava – <i>Manihot esculenta</i></li> <li>- dasheen – <i>Colocasia esculenta</i></li> <li>- plaintains – <i>Musa spp.</i></li> <li>- breadfruit – <i>Artocarpus altilis</i></li> <li>- jackfruit – <i>A. heterophyllus</i></li> <li>- carambola – <i>Avevrhoa carambola</i></li> <li>- yam bean – <i>Pachyrhizus erosus</i></li> <li>- cacao – <i>Theobroma cacao</i></li> <li>- pineapple – <i>Ananas comosus</i></li> <li>- sugar cane – <i>Saccharum officinarum</i></li> </ul> </li> </ul>
St. Kitts and Nevis	Production of tuber crops
St. Vincent and the Grenadines	Yam tissue cultures introduced by the Chinese
Trinidad and 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.

Specifically, at the Mona Campus of the UWI, 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**  
*(S. Mitchell<sup>2</sup>, pers. comm.)*

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

## Recombinant DNA technology and molecular markers

### Recombinant 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 5).

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 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.

The four nitrogen bases are the same for all organisms. 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.

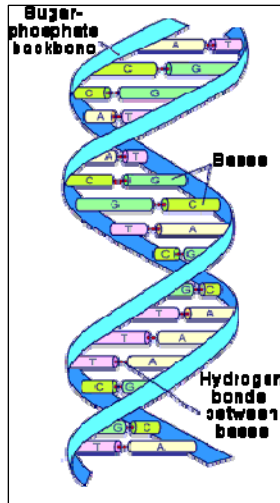
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<sup>2</sup> Dr. Sylvia Mitchell, Medicinal Plant research Group, Biotechnology Centre, University of the West Indies, Mona, Jamaica.

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.

Combining DNA, referred to as recombinant DNA, 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.

**Figure 5**  
**Structure of DNA**  
**showing the double helix**



Source: (Matthew Kuure-Kinsey and Beth McCooley for Biochemical Engineering Fall 2000)<sup>1</sup>

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.

At the UWI St. Augustine Campus, recombinant DNA technology is being successfully used in development of the anthurium industry (P. Umaharan<sup>3</sup>, *pers. comm.*). This is a commercial venture that 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. 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 has been undertaken and these have used varieties from the Netherlands and Hawaii as feed stock. Further research on this species would address the development of nematode-resistant varieties.

At the UWI Mona Campus, research has been conducted into developing varieties of papaya resistant to ring spot disease using transfer of resistant genes that have been obtained

<sup>3</sup> Professor Pathmanathan Umaharan, Professor of Biotechnology, University of the West Indies, St. Augustine Campus, St. Augustine, Trinidad and Tobago

from local varieties (P. Tennant<sup>4</sup>, *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. Research using rDNA techniques is also being conducted into producing virus-resistant varieties of the sweet orange and grapefruit.

### Molecular markers

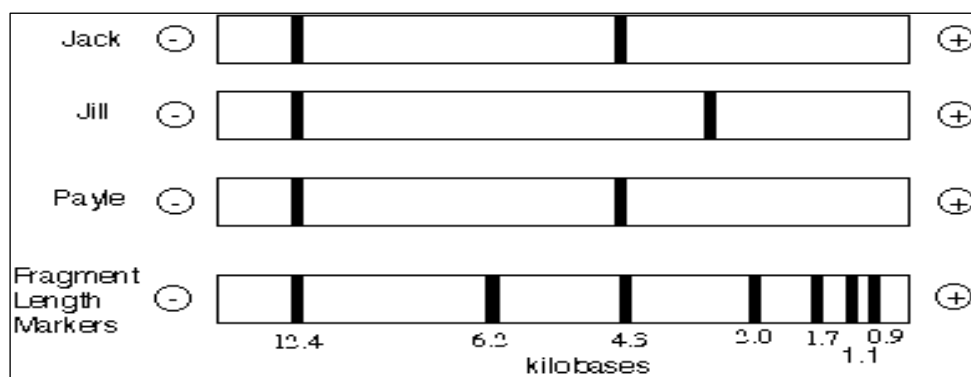
A molecular marker is any kind of molecule indicating the existence of a chemical or physical process. Molecular markers are used in molecular biology and biotechnology experiments to identify a particular sequence of DNA. As the DNA sequences are very highly specific, they can be identified with the help of the known molecular markers which can find out a particular sequence of DNA from a group of unknown sequences. The three most common types of markers used today are:

- Restriction Fragment Length Polymorphism (RFLP);
- Randomly Amplified Polymorphic DNA (RAPD); and
- Isozymes.

Of the three marker types, RFLPs have been used the most extensively (Figure 6). RFLP markers have several advantages in comparison with the RAPD and isozyme markers: (a) they are codominant and unaffected by the environment; (b) any source DNA can be used for the analysis; and (c) many markers can be mapped in a population that is not stressed by the effects of phenotypic mutations. The primary drawback to RAPD markers is that they are dominant and do not permit the scoring of heterozygous individuals. The weakness of isozyme markers is that each of the proteins that are being scored may not be expressed in the same tissue and at the same time in development. Therefore, several samplings of the genetic population need to be made.

**Figure 6**  
**Paternity test results**

*(Jack is the father; Jill is the mother. Payle may be the offspring since she shares the 12.4 kb band)*



Source : <http://www.bio.davidson.edu/COURSES/genomics/method/RFLP.html>

<sup>4</sup> Dr. P. Tennant, Lecturer, Medicinal Plant research Group, Biotechnology Centre, University of the West Indies, Mona, Jamaica.

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 of America. Results suggested that the observed heterozygosity prevailing in the Barbados Blackbelly and populations from the United States were genotypically different (C. Roberts<sup>5</sup>, *pers. comm.*).

## CONCLUSION

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 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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<sup>5</sup> Dr. Cyril Roberts, CARDI, Barbados.