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Review

Re-energized Interest of medical and veterinary Biochemists to DNA technology

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The illumination of the structure, function and metabolism of Deoxyribonucleic acid (DNA) has led to the current global revolution in the recombinant DNA technology, with the possibility to modify these molecules in many ways for the benefit of man and animals. In this review, we considered the basic principles of genetic engineering (gene cloning), bioinformatics, and its applications in medical and veterinary sciences. The issue of ethical questions and fears about biotechnology is also discussed. The ultimate goal of this paper is to re-energized the interest of medical and veterinary personnels, biochemists and related scientists to this technology, which is well able to have an effect on the way all the biosciences will be practiced in this new millennium.

Key words: Biotechnology, medical, veterinary research, DNA technology, biochemist

INTRODUCTION

The discovery and elucidation of the structure and function of DNA by Watson and Crick in the early 1950s led to the present day recombinant DNA technology (Kwaga and Kabir, 1999).

Biotechnology is the technical applications of biological systems for the production of natural substances (biogas, antibiotics, enzymes organic acids etc) it involves the use of living organisms deliberately to carry out defined chemical processes and exploitatio n of biological pro-cesses for man's use (Olasupo, 2005). Modern biotechnology is hinged on the tools of recombinant technology also called gene manipulation or genetic engineering.

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Importance and applications of molecular biology to veterinary medicine and medical sciences

Recombinant DNA technology offers a rational approach to the understanding of the molecular basis of a number of diseases e.g. sickle cell disease, cystic fibrosis etc.

Human proteins can be produced in abundance for therapeutic purposes e.g. insulin, growth hormone, recombinant factor VIII etc. Proteins for vaccines (e.g. hepatitis B) and for diagnostic tests (e.g. AIDS Test) can be obtained. It's used to diagnose existing diseases

Table 1. Human Proteins Synthesized By Recombinant DNA Technology

Protein	Therapeutic Use
Insulin	diabetes
Somatostatin	growth disorders
Somatotropin	growth disorders
Factor VIII	haemophilia
Factor IX	Christmas disease
Interferon	leukaemia and other cancers
Interferon	cancers, AIDS
Interferon y	cancers, rheumatoid arthritis
Interleukins	cancers, immune disorders
Granulocyte colony Stimulating factor	cancers
Tumour necrosis factor	cancers
Epidermal growth factor	ulcers
Fibroblats growth factor	ulcers
Erythropoietin	anaemia
Tissue plasminogen activator	Heart attack
Superoxide dismutase	Free radical damage in kidney transplants
Lung surfactant protein	respiratory distress
1-antiytypsin	emphysema
Serum, albumin	used as a plasma supplement
Relaxin	used to aid child birth

and predict the risk of developing a given disease.

Special techniques have led to remarkable advances in forensic medicine.

Gene therapy for sickle cell disease, the thalassaemias, adenosine deaminase deficiency and other diseases may be devised (Murray et al., 2000).

Gene cloning

Gene cloning is the technique whereby multiple copies of a plasmid or other cloning vehicles are produced by inserting the plasmid into a suitable host capable of producing multiple copies and growing in a bulk culture. The bacterium *Escherichia coli* is often used as the host organism for this purpose (Coombs,1992).The word gene cloning is often used in place of genetic engineering (Soetan, 2007).The basic steps in a gene cloning experiment has been reported by Brown (1998).

The list of human proteins synthesized by recombinant DNA technology is fast growing rapidly. (Table 1)

Production of recombinant vaccines

Development of vaccines for protozoan and helminths parasites of livestock has not been successful. This is because of difficulties encountered in identifying antigens which induce protective immune responses and in obtaining sufficient quantities of vaccine trials (Gamble and Zarlenga, 1986). The use of monoclonal antibodies and genetic engineering technologies could provide the essential tools to help overcome these difficulties (Soetan, 2007).

African swine fever, a disease for which there is no effective prophylaxis nor vaccine, could be effectively conquered with the application of biotechnology techniques used in the production of vaccines through the use of monoclonal antibodies and genetic engineering (Soetan, 2007).

Molecular biology has brought more light into the principle and causes of ageing in mammals and this offers the prospect for both understanding and control of ageing (Brash et al., 1979). Reverse transcriptase polymerase chain reactions (RT-PCR) has brought about new ideas on the detection of RNA Viruses in tissues and body fluids. RNA viruses can now be detected at a high level of sensitivity in infected materials. (Wambura, 2006).

The detection of Newcastle disease virus (NDV) in infective allantoic fluids using PCR were first discovered by Jestin and Jestin, 1991. Later, several RT -PCR methods were developed and used in molecular studies of NDV (Belak and Ballagi-Pordany, 1993; Kant et al., 1997; Cavanagh 2001; Wang et al., 2001; Aldous and Alexan-

der, 2001). Successful extraction and purification of RNA is very important and is the initial step in RT-PCR for successful detection of infectious agents by this technique (Wambura, 2006). Rinderpest (RP), a highly contagious disease of cattle, buffaloes and some wild animals is caused by the rinderpest virus (RPV) and is characterized by a very high mortality (Scott, 1964; Plowright, 1968) and is an economically important disease in Africa, Asia and the middle-East (Couacy-Hyman et al., 2006). Peste-des- petits ruminant (PPR), a highly contagious disease of sheep and goats, similar to rinderpest, is also characterized by a very high mortality and it is caused by PPR virus (PPRV).

There is a close relationship between the RPV and PPRV and they both belong to the morbilly virus genus of the paramyxoviridae family (Gibbs et al., 1979). In infected animals, both diseases are clinically very similar, thus making it very difficult to differentiate them on the field. Molecular biology techniques have been developed to differentiate RPV from PPRV. Diallo et al. (1989), Pandey et al. (1992) and Libeau et al. (1994) have all developed different molecular techniques to differentiate RPV from PPRV. Couacy-Hyman et al., 2006, describe the use of a reverse transcription –PCR (RT-PCR) technique for the specific diagnosis of RPV and also report the use of NP gene of RPV to distinguish all RPV strains from those of PPRV and the use of the technique in epidemiological surveys for rinderpest.

DNA fingerprinting, also called DNA profiling is a DNA identification technique that is based on similarity investtigation of two nucleotide sequences. This is a molecular biology method that has application in Agriculture and the medical sciences (Iwalokun, 2005).The use of DNA fingerprinting technique is now regarded as a milestone in diagnosis and surgical pathology (Persing, 1993).

Gene therapy in medicine and veterinary medicine

This is another application of gene cloning in medicine and veterinary medicine. Gene therapy is the name given to methods that aim to cure an inherited disease by providing the patient with a correct copy of the defective gene. Gene therapy has been successful with experimental animals and clinical trials with humans have been approved by the relevant regulatory agencies (Brown, 1998).

Gene therapy is a therapeutic technique in which a functioning gene is inserted into a cell to correct a metabolic abnormality or to introduce a new function is one of the outcomes of breakthroughs in molecular biology. Gene therapy is a promising approach to the treatment of cancer and other genetic diseases in human and animals.

Polymerase chain reaction (PCR)

The PCR is a very sensitive and specific in-vitro technique for the amplification of a DNA or RNA segment, through a succession of incubation steps at different temperature, making use of a thermostable DNA polymerase, two primers unique to and which flank the particular segment and the four deoxynucleoside triphosphate. The PCR is one of the most versatile techniques in molecular genetics with a wide range of applications in the medical and veterinary sciences (Saiki et al., 1988; Erlich et al., 1991).

Applications of PCR

PCR has multiple applications in gene cloning, biodiversity studies, diagnostics, forensics etc. Biotechnology has great potentials for harnessing the genetic potential of animals and for enhancing their genetic performance. Animal breeding techniques like embryo transfer have been used in safely propagating animals of similar genotype and phenotype. Recent DNA based techniques have facilitated the identification of specific gene sequences, called genetic markers, that labels desirable or undesirable traits in animals (Nyira, 1995).

A gene marker differentiating Bos taurus from Bos indicus cattle was recently identified using PCR amplification of pooled DNA and RFLP (Restriction fragment length polymorphism). This marker is potentially useful for the breeding of high yielding temperate animals for resistance to trypanosomosis (Kemp and Teale, 1994).

Markers are also employed to determine the genetic relatedness in animals (Grunder et al., 1994; Medjugorac et al., 1994) using RFLP and other DNA fingerprinting methods, for purposes of trade, litigation or phylogenetic studies.

DNA finger printing can also be used accurately to trace offsprings to parents or genetic source.

Bioinformatics and its application in medicine and veterinary medicine

Bioinformatics is the use of Information Technology (IT) in biotechnology for data storage, data warehousing and DNA sequence analysis. It is the comprehensive application of mathematics (e.g. probability and statistics), science (e.g. biochemistry) and a core-set of problem-solving methods (e.g. computer algorithms) to the understanding of living systems (Iwalokun, 2006) . The knowledge gained from the study of bioinformatics is crucial for the understanding of the code and evolution of life as well as applications in other areas of life like Agriculture, Health, Environment, Energy etc. (Iwalokun, 2006).

Bioinformatics is one of the latest additions to the scientific world. The word suggests a bridge between biology and information technology. Biochemistry was the first to serve as a bridge between physical and biological sciences. Bioinformatics is also known as computational molecular biology. The origin of bioinformatics can be traced to the development by Sanger and Coulson (1973). Bioinformatics is essentially a theoretical disci-

pline which attempts to make predictions about biological functions from sequence data. It is a powerful tool in experimental design. Kaikabo and Kalshingi (2007) reported that bioinformatics has advanced the course of research and future veterinary vaccines development because it has provided new tools for identification of vaccine targets from sequenced biological data of organisms. Bewaji (2003) defined bioinformatics as application of information technology to the domain of biology. The aims of bioinformatics are:

1. To organise data in a way that allows researchers to access existing information and submits new entries as they are produced (Bernstein et al., 1997; Beran et al., 2000).
2. To develop tools are sources that aid in the analysis of data (Pearson and Lipman, 1988; Altschul et al., 1990; Thompson et al., 1994).
3. To use tool(s) to analyze the data and interpret the results in a biologically meaningful manner.

According to Kaikabo and Kalshingi (2007), in veterinary research, bioinformatics tools could be used to generate biological data for research (Pongor and Landsman, 1999), retrieve and analyze biological data, predict and identify protein(s) in a sequence and also has laboratory application

In veterinary research, bioinformatics tools were used in the detection of new castle diseases (NDV). Alfonso et al. (2006) used bioinformatics to examine the genome of NDV to determine which sequence mismatches have the potential to produce false negative results. Similarly, Kumar (2003) used bioinformatics approach to identify antigenic epitopes from rabies virus glycoprotein G, which could be used to develop antirabies sub-unit vaccine. All the above examples reveal how bioinformatics may be used to identify diagnostic problems and to generate novel solutions for the continued improvement and development of molecular diagnostics (Kiakabo and Kalshingi, 2007).

Berge et al. (2004) reported the use of antibiotic susceptibility Patterns and Pulsed –field Gel Electro-phoresis (PFGE) to compare historic and contemporary isolates of muilti-drug resistant *Salmonella enterica* sub-spenterica serovar newport.

Chen et al. (2004) reported the characterization of multiple antimicrobial resistant salmonella serovars isolated from retail meats purchased in the United States and the peoples Republic of China. A better understanding of the molecular mechanisms by which antimicrobial resistance emerges and spreads should enable us in the future to design intervention strategies to reduce its progression (Chen et al., 2004). Because antimicrobial resistant bacteria may be transferred to humans through the food chain (Threlfall and Ward, 2001; Witten, 1998), selection of novel antimicrobial resistance mechanisms in Salmonella in animals (Threlfall and Ward, 2001), which specify resistance to antibiotics used in humans is troubling (Chen et al., 2004).

Aminov et al. (2001) reported the molecular ecology of tetracycline resistance: Development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. The report by Aminov et al. (2001) was the first demonstration of the applicability of molecular ecology techniques to estimation of the gene pool and the flux of antibiotic resistance genes in production.

Adah (2007) reported that in Africa rotavirus gastroentesitis remains a major cause of high mortality among human infants and young animals. Rotavirus infection in young animals could also constitute a meaningful threat to the control of the disease in humans. Therefore, the application of a safe and effective rotavirus vaccine which incorporates the diversity of circulating strains in the target population will expectedly reduce the disease prevalence. Adah (2007) also reported that the application of advanced molecular techniques of nucleic acid probe hybridization, restriction endonuclease analysis of PCR, generated cDNA copies, PCR and sequencing analysis to the characterization of African rotavirus strains has changed the epidemiology of rotavirus diversity in the African continent.

Ducatez et al. (2007) reported the molecular and antigenic evolution and geographical spread of H5NI highly pathogenic avian influenza viruses in Western Africa (Ducatez et al., 2006b; Igbokwe et al., 1996; Owoade et al., 2006, 2004 a, b). Owoade et al., 2007, reported the cross reactivities of rabbit anti-chicken horse radish peroxidase conjugate with sera of chic-kens, ducks, geese, guinea fowl, hawks, pigeons and turkeys in indirect enzyme linked immunosorbent assay (ELISA) system. Owoade et al. (2006) reported the seroprevalence of avian influenza virus (AIV), infectious bronchitis virus (IBV), Reovirus, Avian Pneumovirus (APV), infectious laryngotracheitis virus (ILTV) and avian leukosis virus in Nigerian poultry. Their report was the first report of serological evidence of the above viruses in West Africa. In the study, they made use of ELISA, a molecular biology technique.

In recent years, various approaches such as mutational analyses and biochemical and pharmacological characterization have yielded significant information about the relationship of structure and function of P-glycoprotein (Ambudkar et al., 1999). Molecular biology has helped to advance the management of cancer in humans and animals with the discovery and knowledge of P-glycoprotein.

Cadmus et al. (2006) reported the molecular analysis of Human and Bovine Tubercle bacilli from a local setting in Nigeria. Their study was the first molecular analysis of M. tuberculosis complex strains circulating among humans and cattle in Nigeria and the results have significant implications for disease control. Oluwayelu et al. (2005) reported the isolation and characterization of

Table 2a. Examples of international movement of embryos

Purpose	Product
Improved dairy breeds	North American Holsteins
Improve beef breeds	European cattle
High milk production	North American Holsteins
Rapid growth rates in cattle	Large European breed
Rapid growth rates in swine	North American or Danish swine

Table 2b.

Heat tolerance	Bos indicus breeds from Latin America and India
Disease resistance	N'dama cattle from Africa
More hair production	Angora goats from Australia
High ovulation rate in sheep	Booroola Merinos form Australia
High ovulation rate in swine	Prolific Chinese breeds.
Animals for game farming	Elk from North America

of chicken anaemia virus (CAV) from chickens in Nigeria. This was the first time CAV was isolated from the Nigerian chicken population. In a similar study, Oluwayelu (2006) reported a molecular analysis study of chicken anaemia virus (CAV) in backyard chickens in Nigeria using molecular cloning and sequence analysis to characterize chicken anaemia virus strains obtained from commercial chickens and Nigerian backyard chickens.
Luther et al., 2007, reported the use of PCR to detect the genome of African swine fever virus (ASFV) from natural infection in a Nigerian baby warthog (Phacochoereus aethiopicus) . They stated that application of PCR for the detection of organs of ASFV genomic DNA presents a sensitive and specific method of identifying the virus (Saiki et al., 1985, 1988). Their communication is reported to be the first documented report of the detection of ASFV from a Nigerian warthog reported hitherto only in eastern southern African countries (Scott, 1965; Plowright et al., 1969; Lither et al., 2001).

Embryo transfer

Embryo transfer is a technique by which embryos are collected from a donor female and transferred to a recipient female which serves as a surrogate mother for the remainder of pregnancy. Such techniques have been applied to nearly every specie of domestic animals, of wild life and exotic animals as well as to humans and other primates. Embryo transfer is used in buffalo and dromedary camel (Camelus dromedaries) (Musa, 1992). (Table 2a and 2b)

Embryo transfer is useful for rapidly increasing numbers of an imported breed or line animals. There are many applications of embryo transfer. They are training and research, testing for deleterious recessive genes, management of disease, conservation of native breeds, exotic and wild animals (Kuzan and Seidel, 1986).

Ethical questions raised by gene therapy and genetic engineering in general.

Morals are the norms or values considered or judged as being good or bad, right or wrong. Morals are societal values and may differ between societies or cultures. Ethics deals with morals and moral rules. Ethical conduct is that perceived to be morally right by the society Osuntoki, (2005).

Every area of human endeavor generates unique ethical challenges. In biotechnology, ethical conducts are based on four key factors:

1. Beneficence
2. Risk prevention
3. Fundamental principles of respect for persons
4. Justice (Osuntoki, 2005).

Advances in medical biotechnology raise some ethical questions. For example;
1. Should gene therapy be used to cure human disease!
2. Is it right to manipulate genes that generations unborn may inherit!
3. Does the embryo have the moral status to prescribe its genetic alteration or exploitation regardless of the potential benefit!
4. Is it right to generate genetic information on humans which could have negative socio-psychological impacts!
5. Is it right to screen children and teens for adult onset genetic pathologies!
6. Can genetic information about individuals be used for unethical things!
7. Do we have a right to ''play God''!
8. Who defines normality!
9. Why alter what is not fully known! (Osuntoki, 2005).

It is very important to exploit ways of resolving these ethical and moral issues. However, ethical dilemmas are minimized when mechanisms that sustain a solid foundation of trust and safety are incorporated into research design and implementation and also in the biotechnological production processes (Osuntoki, 2005).

Dr. Norman Borlaug, who was awarded the Nobel Peace Prize for his development of study, high-yielding cereal grains for use in developing countries had this to say about biotechnology:

''----- Too many opponents of biotechnology too easily dismiss the many safety and regulatory checks that govern whether a new agricultural product brought to the market- place is worthless. Unfortunately, they willfully choose to emphasize highly potential risks rather than recognize the years of experienced research and regulatory oversight that govern the safe use of these new technologies''.

The immediate past Vatican was quoted as making this statement regarding biotechnology:

''---- We are increasingly encouraged that the advantages of genetic engineering of plants and animals are greater than the risks. The risks should be carefully followed through openness, analysis and controls, but without a sense of alarm''.

Finally, former President Jimmy Carter of the United States of America had this to say:

''------Responsible biotechnology is not the enemy; starvetion is. Without adequate food supplies at affordable prices, we can't expect world health and peace''.

Conclusion

In conclusion, Recombinant DNA technology has provided highly sensitive and specific tools for the diagnosis, prognosis and disease surveillance in humans and animals. In the future, preventive human and veterinary medicine will focus on genetically engineered animals resistant to specific endemic diseases. Gene's confering resistance to specific diseases may be copied, sequenced and propagated using PCR, and inserted into the genes of recipient humans and animals (Kwaga and Kabir, 1999).

RECOMMENDATIONS

1. An urgent, serious and strong Government commitment is essential for the success of biotechnology project.
2. The introduction of Biotechnology to the Nigerian Environment has a unique strategic significance, in that it can contribute a lot considerably to improve the quality of life of Nigerians by providing solutions to survival problems such as diseases, foods, fertilizers, fuels etc.
3. There is need for bioinformatics training in Nigeria. This should be introduced into the curriculum of the Nigerian universities, as this will aid rapid research and development in the country.
4. Establishment of small software groups, biotechnology groups and companies should be encouraged.

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