THE TEENY WEENY THINGS IN OUR LIFE: THE NEEDS AND POTENTIALS

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BY

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1.0 INTRODUCTION

The Vice Chancellor, distinguished colleagues, invited guests, great students of Lagos State University, ladies and gentlemen. It is with a heart of gratitude that I stand before you today to present to you an inaugural lecture on a subject that has been very dear to me. The inaugural lecturer standing before you today will not bother you with the history of what position he is occupying among the list of former inaugural lecturers either in his Department, Faculty or University. I am a Professor of Microbiology and not that of history, so I will leave out the history aspect of inaugural lectures in the University. Many people think that an inaugural lecture is an opportunity for celebration; this is not so. Rather, it is an opportunity to showcase one's academic achievements in terms of knowledge and defend one's intellectual capability for occupying that highly cherished and revered academic position of 'Professor'.

In the light of this, I, Nurudeen Ayoade Olasupo present myself to the University community to give my inaugural lecture entitled, "The Teeny Weeny Things in Our Life: The Needs and Potentials". The lecture will take us through a journey of academic endeavours including the following:

- What is Microbiology?
- Does the field do any good at all to mankind, apart from causing havoc?
- What is the contribution of the lecturer to the development of knowledge in Microbiology?
- Conclusion
- Acknowledgements

It is my hope that by the time the above-listed questions are answered, the integrity of today's lecturer would have been determined. I must also state that this lecturer will not bore you, as the one-hour time schedule will be strictly adhered to.

2.0 WHAT IS MICROBIOLOGY?

Microbiology is the study of large diverse groups of microscopic organisms (called micro-organisms) that exist as single cells or cell clusters. It also includes the viruses, which are microscopic but not cellular. Microscopic cells are thus distinct from the cells of animals and plants, which are unable to live alone in nature and can exist only as parts of multicellular organisms. A single microbial cell is generally able to carry out its life processes of growth, energy generation and reproduction, independently of other cells either of the same kind or of a different kind.

The next question is "What then is Microbiology all about? It can be simply presented that microbiology is about:

- 1. Micro-organisms which are livings cells and how they work.
- 2. Microbial diversity and evolution. That is, about how different kinds of micro-organisms arose and why?
- 3. What micro-organisms do in the world at large, in human society, in our own bodies and in the bodies of animals and plants
- 4. The central role that microbiology plays as a basic biological science and how an understanding of microbiology helps in understanding the biology of higher organisms including humans.

2.1 A BRIEF HISTORY OF MICROBIOLOGY

Although the existence of organisms too tiny to be seen with the naked eye had long been suspected, their discovery was linked to the invention of the microscope. Robert Hooke in 1664 described the fruiting structure of moulds (eukaryotic cells), but the first person to see micro-organisms in any detail was the Dutch amateur microscope builder Anton van Leeuwenhoek (in 1684), who used a simple microscope that he constructed.

Leeuwenhoek's microscope was extremely crude by today's standards, but by careful manipulation and focusing, he was able to see organisms as small as bacteria. Fig 1 shows the preliminary phases of development in the construction of the microscope.



FIG 1. PHASES OF DEVELOPMENT IN THE CONSTRUCTION OF THE MICROSCOPE

He reported his findings in a series of letters to the Royal Society of London, which published them in English. Drawings of some of Leeuwenhoek's *"wee animalcules"* (as he referred to them) are shown in Fig 2.



Fig 2. Leeuwenhoek's Drawing Of Bacteria, Published In 1684. Even From These Crude Drawings We Can Recognize Several Morphological Types Of Common Bacteria. A, C, F, and G, Rod – Shaped Or Coccus – Shaped; H, Cocci Packets

The observation of Leeuwenhoek was confirmed by other workers, but progress in understanding the nature and relevance of these tiny organisms came slowly. It was not until the 19th century that improved microscopes became available and widely distributed. This led to improved knowledge about the details of the cells. Microbiology as a science did not develop until the latter part of the 19th century. The long delay occurred because in addition to microscopy, certain basic techniques for the study of micro-organisms needed to be developed. In the same 19th century, investigation of two critical questions led to the development of these techniques and laid the foundation of microbiology. The two central questions are:

- Does spontaneous generation exist?
- What is the nature of contagious diseases?

By the end of the 19th century both questions had been answered and the science of microbiology was firmly established as a distinct and growing field.

Louis Pasteur's work on spontaneous generation led to the development of methods for control of the growth of micro-organisms. By means of his famous goose-necked flask experiment, Louis Pasteur, a French chemist-turnedmicrobiologist, was able to prove that the putrefaction or fermentation of food and broth was not a spontaneous process, arising from a non-living origin, but rather due to living entities contracted from air and the environment. This was a build-up on the work of earlier scientists such as the Italians Franscesco Redi (1668) and Lazzaro Spallanzani (1776) who had laboured arduously to show, albeit by crude experimentation, that life (maggot) could not originate spontaneously, no matter the circumstances, from decaying organic matter. Robert Koch developed a set of criteria that provided an experience framework for the study of infectious micro-organisms and developed the first methods for the growth of pure cultures of micro-organisms. In the 20th century, basic and applied aspects of microbiology had worked hand in hand to yield a number of important practical advances and a revolution to molecular biology.

Table 1 shows the contributions of different workers (founding fathers) to the development of microbiology.

YEAR	INVESTIGATOR(S)	DISCOVERY			
1684	Anton van Leeuwenhoek	Discovery of bacteria			
1798	Edward Jenner	Smallpox vaccination			
1857	Louis Pasteur	Microbiology of the lactic acid			
		fermentation			
1860	Louis Pasteur	Role of yeast in alcoholic			
		fermentation			
1864	Louis Pasteur	Settled spontaneous generation			
		controversy			
1867	Robert Lister	Antiseptic principles in surgery			
1881	Robert Koch	Methods for study of bacteria in pure			
		culture			
1882	Robert Koch	Discovery of cause of tuberculosis			
1884	Robert Koch	Koch's postulates			
1884	Robert Koch	Discovery of cause of cholera			
1884	Christian Gram	Gram-staining method			
1889	Sergei Winogradsky	Concept of chemotherapy			
1889	Martinus Beijerinck	Concept of a virus			
1890	Sergei Winogradsky	Autotrophic growth of			
		chemolithotrophs			
1901	Martinus Beijerinck	Enrichment culture method			
1901	Karl Landsteiner	Human blood groups			
1908	Paul Ehrlich	Chemotherapeutic agents			
1928	Frederick Griffith	Discovery of pneumococcus			

TABLE 1. THREE HUNDRED YEARS OF MICROBIOLOGY: Some key discoveries in Microbiology,

		transformation
1929	Alexander Fleming	Discovery of penicillin
1944	Oswald Avery, Colin	Explanation of Griffith's work - DNA
	Macleod, Maclyn McCarty.	is genetic material
1944	Selman Waksman	Discovery of streptomycin
1953	James Watson, Francis	Structure of DNA
	Crick	
1959	Arthur Pardee, Francois	Gene regulation by a repressor
	Jacob, Jacques Monod	protein
1959	Rodney Porter	Immunoglobulin structure
1959	F. Macfarlane Burnet	Clonal selection theory
1960	Francois Jacob, David	Concept of an operon
	Perrin, Carmon Sanchez	
	Jacques Monod	
1975	Georges Kohler, Cesar	Monoclonal antibodies
	Milstein	
1976	Susumu Tonegawa	Rearrangement of immunoglobulin
		genes
1977	Fred Sanger, Steven	Methods for sequencing DNA
	Niklen, Alen Coulson	
1983	Luc Montagnier	Discovery of HIV, the cause of AIDS

3.0 DOES THE FIELD OF MICROBIOLOGY DO ANY GOOD AT ALL TO MANKIND, APART FROM CAUSING HAVOC?

3.1 MICRO-ORGANISMS AS DISEASE AGENTS

Today the healthcare sector is the area where the role of micro-organisms and that of the microbiologist stand out glaringly even to the layman. The role of micro-organisms as agents of human diseases having been well elucidated over the past century, it is indeed commonplace, especially among averagely educated folks, to see all "bacteria" as agents or cause of diseases and all diseases as effects of "bacteria" and "viruses". Recent viral pandemics such as the HIV-AIDS (Human Immune Deficiency Virus - Acquired Immune Deficiency Syndrome) and Severe Acute Respiratory Syndrome (SARS) have further lent credence to such half-witted assertions. AIDS, which is today the world's most dreaded disease, was only first described as a disease entity some 25 years ago (1981). HIV, the causative agent, was identified two years later (1983), and by

1984 the first confirmed African case was reported in Nairobi, Kenya. By 1986 the first evidence of AIDS was reported in Nigeria by Nasidi and Harry. Such was the severity with which the scourge of HIV-AIDS impressed itself on mankind that today people now accept that AIDS is real, has no cure but can be well managed.

However, man has not always associated diseases with micro-organisms. In ancient times, causes ranging from sin and witchcraft to "poisonous vapour" were some of the "postulated" causes of human diseases. In medieval Europe "scientists and philosophers" had put forward three rather contradicting notions of disease causation, namely that:

- (a) Disease was a divine punishment for individual or societal transgression
- (b) Disease was the result of "miasma" or stench of decay
- (c) Disease was the result of person to person contagion.

The proof that micro-organisms could cause disease (i.e. the germ theory of disease) became established in 1876 when the German scientist, Robert Koch, put forward his now famous four-point postulate, called Koch's postulates. According to Koch's postulates, for an organism to be named as the agent of any disease, it must fulfil certain conditions, namely,

- i. The organism must be constantly associated with the disease irrespective of location and time.
- ii. The organism must grow in pure culture outside the diseased host.
- iii. When such pure culture is inoculated into a healthy susceptible host the symptoms of the disease must be produced.
- iv. The organism must be reisolated from the disease so induced and found identical to the original isolate.

Koch's postulates laid to rest all superstitious beliefs and laid the basis for the revolutionization of man's knowledge of disease such that today there is hardly any infectious disease whose causative agent is not known. The most central role in designing strategies for disease treatment and control falls within the purview of the microbiologists. Antibiotic and vaccine production and administration programmes (local, national and global) as well as the tracking of emerging infectious agents are some of the areas in which microbiologists have become indispensable.

However, just as medical microbiologists and other health workers seem to have, over the years, acquired boundless resources for tracking diseases, microbes seem to be relentlessly poised to maintain a head-start in this unending combat (Albinu *et al.,* 2004). Nosocomial (or hospital acquired infections) and virulent strains of previously unpathogenic species (*E. coli* 0157: H7) are some of the new challenges in this area.

Though micro-organisms constitute a serious threat to human existence, many are not harmful to humans; instead they are actually beneficial, carrying out processes that are of immense value to human society. In fact less than 5% of bacterial species are pathogenic.

3.2 MICROBIOLOGY AND AGRICULTURE

It is ancient wisdom that in order to increase yield and save mankind from the scourge of famine and bankruptcy, the farmer must devise a means of controlling or circumventing diseases associated with his crops. The several blights, rots, smuts and swollen shoots in history leading to famines and shifts in human population and migration have been largely due to micro-organisms: from the potato blight which displaced more than 2 million Irish men in the 1840s to the avian influenza which blossomed in the 1900s and has now assumed the level of a pandemic; from the foot-and-mouth disease caused by a virus-like proteinaceous agent (prion) in cow to the mad cow disease which threatened the beef market in Europe in the year 2000; the list of diseases caused by microorganisms is indeed endless. Thus the microbiologist is greatly challenged in agriculture not only to discover the causative agents of the myriad diseases, but also to design appropriate control measures. Vaccine production, and application in animal husbandry, production of pesticides such as fungicides, insecticides, antibiotics and tests of their efficacy are all within the purview of the microbiologists.

The microbiologist is also interested in the use of micro-organisms as agents of biological control of plant pests and diseases. Certain pseudomonads have been found to be useful in the control of fungal plants disease. *Bacillus thuringiensis* is a good insecticide, while certain viruses have also been found to be useful as agents of control of rodents.

Furthermore, micro-organisms are useful tools in the development of high yielding strains of agricultural materials through genetic engineering to improve photosynthesis, resistance to herbicides, resistance to disease and optimized nitrogen fixation. Their plasmids and enzymes are important tools in this regard.

In modern day agriculture there is much emphasis on the use of chemical fertilizers to improve yield. The consequence of this is an unhealthy accumulation of these inorganic minerals (nitrates, phosphates and ammonium) in soils and surrounding water bodies with negative impact on the environment, including algal bloom and global warming, as a result of the excessive release of CO₂ and green house gases which create "holes" in the protective ozone layer. The monitoring of bacterial community diversity and populations in agricultural land is therefore very important. Also the use of nitrogen fixing cyanobacteria has been found to increase rice yield to the tune of 15–25 % in India. Table 2 shows some diseases caused by micro-organisms in agriculture.

Organism	Disease
Cassava mosaic virus	Cassava mosaic disease
Maize streak virus	Maize streak disease
Tobacco mosaic virus	Tobacco mosaic disease
Erwinia carotovora	Tomato and Irish potato rot
Verticillium theobromae	Banana cigar-end rot
Rhizopus stolonifer	Mango rot
Macrophomina phaseolina	Yam rot
Phythophthora palmivora	Black pod of cocoa
Alternaria radicina	Carrot rot
Choanephora cucurbitarum	Cowpea pod rot
Alternaria citri	Orange rot
Botryodiplodia theobromae	Kola nut rot
Fusarium solani	Onion bulb rot
Oidium caricae	Pawpaw powdery mildew

TABLE 2.SOME DISEASES OF PLANTS

3.3 MICRO-ORGANISMS AND THE ENVIRONMENT

Micro-organisms play a very important role in defining and reshaping the human environment. It has been recently postulated that the first organisms to colonize the face of the earth were a group of hardy bacteria known as "archae" (a group of phylogenetically related prokaryotes distinct from bacteria). They are a peculiar group that can tolerate extreme conditions such as high salt concentration, extreme temperature and high acidity. Early earth environment was harsh and only such hardy organisms could have survived it. The most important role of micro-organisms in nature is in the recycling of nutrients such as nitrogen, carbon, phosphorus, iron, silicon and manganese. Cyanobacteria, for instance, are instrumental to replenishing the atmospheric oxygen and removing excess carbon dioxide during photosynthesis. Important in this regard are species of *Oscillatoria*, *Nostoc*, *and Anabaena* etc.

Although nitrogen makes up about 79 percent of atmospheric air, only 0.03 percent of earth's nitrogen is in readily available form. Bacteria such as species of *Rhizobium, Frankia, Azotobacter* and *Azospirillum* are able to trap and fix such nitrogen, converting it to ammonia which is a readily available form.

Many bacteria are heterotrophs which are unable to manufacture their own food but depend on already manufactured food for their survival, hence their special role in the removal of pollutants from the environment through biodegradation. Thus cellulose, domestic wastes and industrial effluents are readily degraded by a wide range of micro-organisms, an ability which has found application in modern waste management techniques including landfills, septic tanks, oxidation ponds, trickling filter (or its modified form called biodiscs) and anaerobic digestor.

The active ingredients of many pesticides, herbicides, fungicides and other agrochemicals are derivatives of hydrocarbon which have the tendency to accumulate in the environment and in the tissues of living things, with consequent toxicity, mutagenicity, carcinogenicity and teratogenicity (adverse effect on reproductive organs).

A unique pollutant in Nigeria is oil arising from spillages from oil prospecting, exploration, exploitation and transportation. The removal of such toxic waste from the environment depends on the activities of a very few well-adapted micro-organisms, particularly the *Pseudomonads* and *Actinomycetes* such as *Mycobacterium sp* and *Rhodococcus sp*.

The application of the ability of micro-organisms "to eat up" pollutants for rapid removal of these pollutants from the environment is called bioremediation and this has recently gained ascendancy over other methods such as chemical and physical methods because it is more environment friendly.

3.4 MICROBIOLOGY AND INDUSTRIAL PRODUCTION

The application of micro-organisms in industrial production predated the actual conceptualization of the science of microbiology in the 19th century. Beer making was thought to have been begun by the Egyptians between 6000 and 5000 BC.

The Chinese were already using lactic acid bacteria for yoghurt making at about 4000 B.C while the Egyptians and Babylonians independently discovered leavening of bread by use of yeast about 1800BC.

However, modern biotechnology was born when Louis Pasteur discovered the role of yeast in alcoholic fermentation in 1856. By combining the right organism, an inexpensive substrate, and the proper environment, fermentation has become the saving grace in several industrial production processes. The range of such industrial products from micro-organisms includes enzymes, antibiotics, vaccines, fuel and plastic. Fermentation enables the production of these metabolites in a more environmental friendly way and in most cases more economical than extraction by chemical methods.

(a) <u>Food industry</u>

The role of the microbiologist in these processes has grown from that of strain selection, monitoring and quality control and maintenance of standard production procedures in the early 20th Century to that of application of molecular biology and genetic engineering to develop desired strains of fermenting micro-organisms and cheap raw materials. The use of polymerase chain reaction and biodetection for detection of toxins and pathogens in food industries is one of the latest developments that have revolutionized quality control and quality assurance in the last quarter of the century.

Over the past fifty years, much has also been done in the area of discerning the process microbiology of many African fermented foods, including their shelf life and safety qualities (Table 3). What indeed remains to be done in most cases is the application of this knowledge in the development of cottage industries and large scale production through appropriate optimization and scale-up designs.

Food	Substrate	Microorganisms Involved
Kunu zaki Millet or Sorghum		Enterococcus faecalis Pediococcus
		pentosaceus
		Lactobacillus fermentum
Wara	Milk	Lb. fermentum Lactococcuss
		lactis Lactobacillus sp.
Nono	Milk	Pediococcus pentosaceus
		Lactobacillus fermentum
Iru	African locust bean	Lactobacillus fermentum

TABLE 3: MICROBIOLOGY OF SOME INDIGENOUS FERMENTEDFOODS

		Bacillus sp.
Agadagidi	Plantain	Yeast
(plantain wine)		Lactic and bacteria
Ogi	Cereals: Maize Millet or	Lactobacillus sp.
0	Sorghum	Yeast
Fufu	Cassava	Lactic acid bacteria

(Olasupo *et al.*, 2001 and Odunfa 1985)

Another interesting role of micro-organisms in foods is their contribution to carbonated soft drinks. The major sugar in many soft drinks is fructose, produced from corn starch via microbial activity. In diet soft drinks, the artificial sweetener aspartame is a combination of two amino acids (aspartic acid and phenylalanine). Both are produced microbiologically. Finally the citric acid added to many soft drinks to give them tang and bite is produced in a large scale industrial process using a fungus.

(b) <u>Mining Industry</u>

The application of micro-organisms in the mining industry has helped tremendously not only to improve the overall quality of purified ores but also in transforming hitherto economically unviable ores deposits into virtual goldmines. Iron ore, copper, gold and many other metals can now be released from poor quality ores by exploiting the sulfur oxidizing property of sulphate producing bacteria such as *Acidithiobacillus ferroxidans*, which is a gram-negative bacterium able to grow in conditions of high acidity (pH< 2.5) and commonly found in acid mines. This process is known as bioleaching and strains of this organism have already been patented in the United States and have become, in Brazil, the saving grace of many mining companies which were on the brink of bankruptcy.

(c) <u>Petroleum Industry</u>

In the petroleum industry micro-organisms find application in exploration as indicators of availability of oil, in exploitation and production for enhanced recovery of oil trapped in shales, particularly in tertiary recovery, and also in clean-up of spills resulting from production, refining, transportation and use of crude oil. The fate of oil slicks could be tracked by monitoring the shift in community diversity, a process which relies largely on the application of molecular biology techniques divested of actual culturing and counting of organisms. Bioremediation which is the application of micro-organisms or other biological entities for the removal of pollutants from the environment with the aim of restoring the environment to its pristine condition is the hallmark of petroleum microbiology because it dovetails towards environment friendly measures to control and curtail anthropogenic pressures on the biosphere, as against inadequate physical methods and the tangential problems engendering chemical methods.

(d) <u>Energy Supply</u>

Our country today is suffering from erratic power supply and this negatively affects our complex industrial society which is mainly energy driven. Here also micro-organisms play major roles. Natural gas, mostly methane, is a product of bacterial action, arising from the activities of methanogenic bacteria. The Federal Institute of Industrial Research, Oshodi (FIIRO) has developed a prototype for biogas production in Nigeria and this result-oriented research is visible at Oko-Oba in Agege area of Lagos State. Biogas is harvested world wide in vast amounts as a primary fuel. Examples of countries where biogas usage is very popular are India and China. There is also an interesting relationship between micro-organisms and the petroleum industry. Crude oil is subject to microbial attack and therefore drilling, recovery and storage of crude oil all have to be done under conditions that minimize microbial attack.

Because human activity will result in the complete consumption of available fossil fuels during the next century, it is important to seek new ways to supply the energy needs of society. In the future, microorganisms may provide major alternative energy sources. Phototrophic bacteria can harvest light energy for the production of biomass energy stored in living organisms. Microbial biomass and existing waste materials such as domestic refuse, surplus grains and animal wastes can be converted to "biofuels" such as methane and ethanol by other micro-organisms.

4.0 MY RESEARCH CONTRIBUTIONS

Mr. Vice-Chancellor, please permit me to say that among Nigerian microbiologists, I am one of the highly favoured in terms of good and sound international exposure having won several fellowship awards from Deutscher Akademischer Austauschdienst (DAAD, Germany), International Centre for Genetic Engineering and Biotechnology (ICGEB, Italy), United Nations Educational, Scientific and Cultural Organisation (UNESCO, Paris), Republic of Greece State Scholarships Foundation (IKY, Greece), Alexander von Humboldt Stiftung (AvH, Germany) and The Royal Society (United Kingdom). These awards have taken me to different European countries on several occasions. The

support given by these organizations has made possible the research described below:

(A) SELECTION OF INDIGENOUS YEASTS FOR INDUSTRIAL USE

Due to economic imperative of developing local raw materials for the brewing industry in Nigeria, the brewery industries could use locally available raw materials such as sorghum and maize in place of barley malt for beer production. However, sorghum and maize do not possess (Olaniyi and Akinrele, 1987) endogenous adequate amylase enzymes for the malting process and thus the industries have had to rely on expensive commercial amylase. This problem of the brewing industries can be overcome by the use of industrial yeast that is capable of producing amylase enzymes. Such industrial yeast may be obtained either through genetic transfer of the gene for amylase production from an amylase- producing organism such as *Bacillus* or through natural selection.

Some of the characteristics of industrial yeasts are high resistance to heavy metals such as copper, good fermentation ability, maltase activity, high flocculation rate, ethanol production and genetic stability (Welch *et al.*, 1983; Olasupo and Scott-Emuakpor, 1991). In an attempt to select yeast strains for the brewing industry in Nigeria, effort was made to isolate, identify and screen local yeast isolates from different sources, namely soil, palm wine, nectar of flowers, sugar cane and tomatos.

The local yeasts were screened using the industrial brewer's yeasts as the standard for comparison. The studies revealed that local yeast strains have promising potential, even better than the industrial brewer's yeast, as they showed higher resistance to copper, grew and flocculated better and produced more alcohol than the industrial yeasts. Fig 3 shows the flocculation pattern of selected industrial and local yeast isolates.



FIG 3. FLOCCULATION PATTERN OF SELECTED INDUSTRIAL AND LOCAL YEAST ISOLATES

- IY 2 (Saccharomyces carlsbergensis)
- □ IY3 (S. carlsbergensis)
- × N1 (S. carlsbergensis)
- o N2 (S. carlsbergensis)

- ∎ PW1 (S. cerevisiae)
- Δ T1 (S. carlsbergensis)
- ▲ S3 (S. carlsbergensis)

The findings of these works was reported in *Acta Biotechnologica* (Olasupo and Scott-Emuakpor, 1991), *Folia Microbiologica* (Olasupo *et al.*, 1993a), and *Journal of Scientific Research and Development* (Olasupo *et al.*, 1993b). The results of the studies clearly indicate that the local yeast isolates have a great potential for industrial use in Nigeria as a way of saving foreign exchange through utilization of locally sourced materials.

In a study by Olasupo *et al.* (1996a), an amylase-producing yeast, *Saccharomyces cerevisiae*, was isolated from yam tuber. The enzyme showed optimum activity at pH 5.0 and temperature 60°C. In another related study, amylolytic *Lactobacillus* species were isolated from Nigerian fermented foods (Olasupo *et al.*, 1996b). The amylase enzymes from these studies will be optimized for possible industrial use.

In further pursuance of my research on yeasts. I did a collaborative study with the National Centre for Yeast Collection in the United Kingdom on the genetic identification method (Using 26S ribosomal DNA sequencing) to identify yeast strains isolated from Nigerian sugar-cane peels. The results of the work was published in the *Journal of Basic Microbiology* (Olasupo *et al.,* 2003a).

(B) NATION'S CURRENCY RESEARCH

In 1990, some researchers and I initiated a study on the microbiological evaluation of dirty Naira notes. The work was prompted by the unhygienic way in which Nigerians handle the Naira, which calls for great concern as this may contribute to health hazard due to the presence of microorganisms. This study sought to know (a) whether the notes harbour microorganisms, (b) what types of microorganisms they carry and (c) whether there is a possibility that those notes can transmit diseases. The result of the study, which was published in the Nigerian Medical Practitioner (Olasupo et al., 1992) showed that a total of ten different bacterial species were identified from the dirty Naira notes comprising eight species of Bacillus namely: B. cereus, B. polymyxa, B. licheniformis, B. circulans, B. alvei, B. macerans, B. subtilis, B. pumilus, and two species of Staphylococcus, namely, S. aureus and S. epidemidis. Other bacteria found were Pseudomonas aeruginosa and Escherichia coli. Some moulds were also isolated but were not as numerous and diversified as the bacteria. It is worth noting that *B. cereus* and *S.* aureus were identified in all the sources of the collection considered (samples from beggars, bus conductors, markets, petrol-stations and restaurants). The identification of bacteria such as B. cereus, S. aureus, E. coli and P. aeruginosa suggests the capability of their acting as vehicles for food poisoning and other infections

I am happy to say that this work provided some baseline information on the microbial flora of dirty Naira notes in this country and was the first of such study to be published in any scientific journal. The study has up till today stimulated public and government interest on the need to handle the naira properly and hygienically. Today, jingles on the need to handle the naira properly and hygienically are being aired on television stations such as AIT and NTA almost daily.

(C) STUDIES ON DISEASE MANAGEMENT

In different collaborative studies, I have extensively investigated how causative agents of diseases can be controlled with the right choice of antibiotics. Genetic bases of resistance of micro-organisms to antibiotics have also been well documented, as well as the use of indigenous herbs in the management of diseases.

Staphylococcus aureus is known to produce a wide spectrum of diseases ranging from superficial skin lesions to serious systemic infections (Shanson, 1986). The endemic strains are commonly resistant to many antibiotics and these multi-resistant strains cause nosocomial infections. *S aureus* strains have been known to

be resistant to as many as 20 antimicrobial agents including disinfectants and antiseptics, thus making the choice of appropriate therapy difficult. The spread of *Staphylococcus* infections requires careful monitoring of isolates by microbiologists. Our various studies with Dr. Olukoya of the Nigerian Institute of Medical Research (Olukoya *et al.*, 1995; Olukoya *et al.*, 1997; Olukoya and Olusupo, 1997; Olasupo *et al.*, 1999a) have shown the role of microbiologists in determining the drug of choice in the management of many microbial borne diseases.

Many cases of resistance to antibiotics by microorganisms have been reported to be due to the presence of plasmids in the microorganisms (Olukoya and Olasupo, 1997). The high incidence of drug resistance makes it necessary to determine sensitivity pattern before initiating antibiotic therapy.

In another study by Olasupo *et al.* (2003) effort was made to evaluate the effect of Nigerian indigenous chewing sticks on oral bacterial flora. Table 4 shows chewing sticks used in the study. The result indicated the effectiveness of our indigenous chewing sticks (*Pako*) in the maintenance of good oral hygiene (Fig 4)

TABLE 4: TEN SELECTED CHEWING STICKS COMMONLY USED IN THE "YORUBA'S LOCALITY" IN NIGERIA

Botanical Name	Local Name	Plant Part
Anogeissus leiocarpus	Pako Dudu	Root
Citrus aurantigolia	Pako Ayin	Root and Twig
Fagara		
zamthoexyloides	Orin Ata	Root and Stem
Garcinia kola	Pako Orogbo	Twig and Root
Azadirachta indica	Pako Dangoyaro	Twig
Massularia accuminata	Pako Ijebu	Stem
Nauclea latifolia	Egbo Egbesi	Root
Serindeia warnekei	Pako Meyinro	Stem
Terminalia glaucescens	Pako Pupa	Root
Vernonia amugdalina	Pako Ewuro	Stem and Root

FIG 4: MEAN ORAL BACTERIAL LOAD BEFORE AND AFTER USING DIFFERENT CHEWING STICKS



(D) AFRICAN FERMENTED FOODS RESEARCH

Fermented foods are foods of animal and plant tissue subjected to the action of micro-organisms and or enzymes to give desirable biochemical changes and significant modification of food quality (Odunfa, 1985; Olasupo, 2004a). Extensive research studies have been carried out on the microbiology of African fermented foods namely, *gari, fufu, iru, ugba, kunun-zaki, wara* and *nono*. Today scientific data are widely available in literature attesting to the safety status of many traditional fermented foods in Africa. This has led to improved consumption and acceptance of African fermented foods by the populace. Many of these foods are now commercially available in the form of *gari, fufu, maggi cube (dawadawa)* and palmwine (*emu)*, to mention a few.

Mr. Vice-Chancellor sir, I am happy to say that my doctoral supervisor, Professor Sunday Ayodele Odunfa could be regarded as the founding father of this area of research. However, my research work on bacteriocins and starter cultures development opened an entirely new research area on this subject matter and I am proud to say it loud and clear that "Olasupo N.A" is the most cited author in both local and international journals in the area of bacteriocins research among African scientists and compares favourably well with foreign scientists.

Bacteriocins are proteinaceous substances usually produced by lactic acid bacteria that inhibit closely related organisms (Holzapfel *et al., 1995*). The use of bacteriocins or bacteriocin-producing cultures as protential biopreservatives, and possibly for replacing chemical preservatives has also been widely studied by me and my research collaborators in different parts of the world.

Several reports have been made (Franz *et al.,* 1998; Holzapfel *et al.,* 1998; Olasupo *et al.,* 1994a,b; 1999b,c;) on the production of bacteriocins by lactics isolated from

different African fermented foods. These include enterocin NA, plantaricin NA and nisin Z. The *bacteriocins* inhibited notable pathogens such as *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens* (Fig 5).



FIG. 5: THE EFFECT OF DIFFERENT Lactococcus lactis BACTERIOCINS ON Bacillus cereus DSM 2301

Bacteriocins or producing cultures have also been demonstrated to be a potential biopreservative of African fermented foods (Odunfa *et al.*, 1996.). As part of a programme to develop foods with potential in the control of diarrhoeal diseases, an improved *ogi* named "Dogik" was prepared using *Lactobacillus* starters with antimicrobial activity against diarrhoeagenic bacteria (which include *Escherichia coli, Vibrio cholerae, Salmonella* Typhimurium, *Shigella flexneri*) and also possessing amylolytic activity. The result as indicated in Fig 6 showed non detection of all the diarrhoeagenic bacteria in Dogik after 6hrs whereas in the locally fermented ogi, *Salmonella, Escherichia coli and Shigella sp* survived for 24hrs or more but showed a sharp decrease in numbers, while *Vibrio cholerae* survived for 12hrs. (Olukoya *et al.*, 1994). The *ogi* with the starter also had better shelf-life.



Fig. 6: Comparison Of The Survival Of Diarrhoeagenic Bacteria In Ogi Produced By Natural Fermentation (A) And That Produced By The Use Of Starter Cultures (B)

In another study bacteriocins-producing *Lactobacillus* strain was used as starter in the production of *ogi* to make *agidi* (Olasupo *et al.*, 1997b). Fig 7 shows the comparison of the degree of spoilage in *agidi* prepared from naturally fermented *ogi* and that fermented with a bacteriocin producing starter. The use of the starter organism extended the shelf life of *agidi* from 7 days to 11 days at a prevailing temperature in hawking and home storage.



Fig 7. Comparison Of The Degree Of Spoilage In Agidi (Eko) Prepared From *Ogi* Fermented With Bacteriocin Producing Strain 012 (A) And *Ogi* Fermented Naturally (B)

In a collaborative study (Kimaryo *et al.*, 2000), we evaluated the use of starter cultures on the reduction of the cyanogenic glucoside in the production of *kivunde*, a traditional Tanzanian cassava food product. Our specially selected lactic starters drastically reduced the cyanogenic glucoside during cassava fermentation to values below the maximum value of 10mg/kg recommended by Codex/ FAO for cassava flour (Table 5).

TABLE 5: EFFECT OF FERMENTATION ON THE CYANOGENIC CONTENT (MG/KG OF DRY WEIGHT) OF CASSAVA DURING PROCESSING INTO KIVUNDE INTRIAL 3 (CONDUCTED AT TRIDO, Dar-es-salaam, Tanzanian), as compared to trails 1 and 2 (conducted at IHT, BFE, Karlsruhe, Germany; in each trial, the traditional procedure of submerged cassava fermentation of *kivunde* was followed.

FERMENT		FERMENTATION TYPE							
ATION	SPONTANEOUS			BACK-		STARTER			
PERIOD				SL	SLOPPING		0	CULTURE	
(DAY)									
	3a	1b	2c	3a	1b	2c	3a	1b	2c
0(fresh	175.	126.	164.	176.	124.	164.	176.	127.	164.8
cassava)	9	5	8	8	5	2	3	1	
1	94.6	73.2	Nd	132.	96.4	Nd	144.	63.9	Nd
				6			7		
2	67.2	21.6	39.7	89.3	32.6	69.6	62.5	36.2	63.0
3	45.9	19.8	38.1	62.4	26.4	64.4	38.9	28.7	18.5
4	43.5	10.7	23.7	47.7	17.9	56.3	12.6	9.2	14.3
5	39.1	Nd	24.0	32.9	Nd	38.0	8.2	8.9	9.0
Dry	17.8	9.3	4.4	26.5	Nd	8.1	6.3	5.5	6.3
Kivunde									

a, trail 3 conducted in Tanzania (Tirdo, Dar es Salaam)

b, trial 1 conducted in Germany (IHT, Karlsruhe).

C, trial 2 conducted in Germany (IHT, Karlsruhe

Nd, no data available because of spoilage or experimental error.

Bacteriocins called nisin produced by *Lactococcus lactis* was also evaluated in the preservation of *nono*, a dairy product. Sensory and shelf life studies revealed that nisin can be recommended to produce a *nono* having good sensory properties

with a shelf life of 10 days at 25°C and 25 days at 8°C (Olasupo *et al.*, 1996c). Ordinary *nono* has a shelf-life of 24 hours.

Malnutrition remains a major problem in the developing countries of the world. It is estimated that more than 460 million people in the world are severely malnourished (NAS, 1975) The reasons advanced for this situation include higher population density, poor socio-economic status of the people and insufficient quantity and quality of food. Although these factors are closely interrelated, major food sources, dietary habits and the processing methods used in the preparation of food significantly influence nutritional status.

In a programme aimed at controlling malnutrition in Nigeria, we (Olukoya, Smith, Olasupo, Ogunjimni, Abaelu, Apena and Iyanda) formulated a new cereal-based food called *ogi*, with improved nutritional qualities using *Lactotacillus acidophilus* (DK 52) and *Lactobacillus pentosus* (DK 77) as starter cultures (Olukoya *et al.*, 2000). The reducing sugars, protein content and essential amino acids were significantly higher in the newly developed *ogi* than in the locally prepared brand (Table 6)

TABLE 6: ESTIMATION OF PROTEIN AND FREE SUGAR IN OGI FERMENTED NATURALLY AND THAT FERMENTED WITH STARTER CULTURES (Olukoya *et al.*, 2000)

	CONCENTRATION OF (mg/ml)*			
Sample	Protein	Reducing Sugar		
Control	1.84 ± 0.017	0.41 ± 0.01		
DK 52	2.63 ± 0.035	5.23 ± 0.53		
DK 77	2.28 ± 0.08	5.70 ± 0.083		
DK 52/77	2.83 ± 0.28	4.02 ± 0.17		

Control: naturally fermented *ogi* without the use of starter DK52: *ogi* fermented with Lactobacillus acidophilus DK77: *ogi* fermented with Lactobacillus pentosus DK52/77: *ogi* fermented with mixture of two starters * Mean value ± SD In an on-going attempt to develop starter cultures for improving the safety and quality of traditional foods in Africa, lactic acid bacteria isolated from Nigerian fermented food were screened for some technological properties useful in food processing. The technological properties include growth at different temperatures and pH, bacteriocin production, bile salt hydrolysis, phytic acid degradation, enzymes profiles and production of biogenic amines. The results of this work highlighted the relevance of technological features of starter cultures in food processing in the African environment (Olasupo *et al.*, 2000; Tamang *et al.*, 2000).

Furthermore, in a study to select appropriate starter cultures for cheese making, the utilization of amino acids and peptides by *Lactococcus lactis* was investigated. It is established that certain amino acids and peptides have taste profiles or taste-enhancing effects, as well as serving as substrates for other reactions leading to the formation of compounds which are capable of influencing the flavour profile of cheese. The result of the study which was confirmed by sensory evaluation, showed that the organism had the capability to improve cheese flavour (Olasupo and Roussis, 1999; Roussis *et al.*, 2000). Knowledge from this investigation has direct application in improving the flavour quality of our indigenous cheese called *wara*.

(E) RESEARCH ON BIOTECHNOLGY OF MICRO-ORGANISMS

Many works have been carried out on the detection of plasmids in various microorganisms (Olukoya *et al.*, 1995 & 1997; Olukoya and Olasupo, 1997; Olasupo *et al.*, 1994). Plasmids are extra pieces of DNA, "mini chromosome" which can replicate independently of and coexist with the host chromosome. Plasmids are essential tools in gene cloning and transfer of traits from one organism to another.

In further pursuance of my research on biotechnology of micro-organisms, molecular methods (such as DNA hybridization and ribotyping) were used by me and my colleagues across the globe to ensure correct species identification of microorganisms (Olasupo *et al.*, 2000, 2003a; Tamang *et al.*, 2000). Using this method the identification of some micro-organisms, including lactic acid bacteria and yeasts were redefined. Experience has shown that the use of traditional methods (biochemical and phenotypic features) alone could not be regarded as reliable for proper identification. Incorrect species identification of micro-organisms, apart from giving confusing microbial identity and safety status, can

also lead to problems in developing appropriate control measures in cases of outbreaks of food borne diseases.

For the first time by any African scientist, I and fellow other scientists in the UK and Germany sequenced a bacteriocin (nisin Z) gene (Fig 8) and our findings were reported in the *International Journal of Food Microbiology* (Olasupo *et al.,* 1999.).

gene isolated from *Lactococcus lactis* BFE 1500. The anino acid sequence is shown below the coding sequence. The nucleotide in the nis Z sequence that differs with that in the nisin A gene sequence is indicated by a bold letter. The nucleotides above the sequence with asterisks indicate differences in the nisin A operon (Dodd *et al.*, 1990).

Fig 8

The research is novel considering the fact that up till date only nisin has practical application in food processing. The nisin has been well tested and confirmed as non-toxic when consumed orally and has proved to be a safe food preservative (Delves-Broughton, 1990). Based on my international recognition in the area of biotechnology, I was selected to contribute a chapter in a book entitled *Food Biotechnology*. The book has 1982 pages with 126 contributors selected worldwide and I am happy to tell you that I am the only contributor selected from Africa. The book is published by CRC Taylor and Francis, New York (Olasupo, 2006).

(F) NATURAL ORGANIC COMPOUNDS AS ANTIMICROBIALS IN FOOD

Many food preservation procedures, such as heat treatment and addition of chemical preservatives, are used to reduce the risk of outbreaks of bacterial food poisoning and food spoilage (Periago and Moezelaar, 2001). However, some of these systems can have undesired effects which are against the demands of the food industry and consumers who desire fresh, additive-free and more natural tasting food products while maintaining microbiological safety and stability (Gould, 1996). There has been an increasing interest in the development of effective natural antimicrobials as food preservatives.

This concern prompted a new research area in the use of natural organic compounds, namely, diacetyl, and the essential oil components (*thymol, transcinnamic acid, eugenol and carvocrol*) in the control of food spoilage organisms. The study was done using the natural organic compound alone and in combination with nisin. The results showed the potential of natural organic compounds in the inhibition of notable food spoilage organisms, such as *Bacillus subtilis, Listeria innocua, Escherichia coli and Salmonella enterica serovar* Typhimurium (Table 7a & b) (Olasupo *et al.*, 2003b, 2004a)

TABLE 7A.	MINIMUM	INHIBIT	ORY	CON	ICENTRAT	ION	(MIC	C) OF	THE
NATURAL	ANTIMICRO	DBIALS	AGAIN	IST	Escherichia	coli	and	Salmo	nella
Typhimuriu	m								

	MIC (mmol ⁻¹)		
Test	Salm.	E. coli	
Compound	Typhinurin		
Nisin	No effect	No effect	
Carvacrol	1.0	1.5	
Cinnamic acid	7.5	5.0	
Diacetyl	12.5	7.5	
Eugenol	3.0	2.5	
Thymol	1.0	1.2	

	MIC (mM)			
Inhibitory compound	Bacillus subtilis	Listeria innocua		
Nisin	0.001	0.002		
Carvacrol	1.0	1.5		
Cinnamic acid	3.0	10.0		
Diacetyl	10.0	15.0		
Eugenol	1.5	2.5		
Thymol	0.8	1.2		

Table 7b. Mics Of Nisin And Some Natural Organic Compounds AgainstSelected Organisms

In a related study, the combined effect of pasteurization and addition of sodium benzoate (a preservative with GRAS label) was used to prolong the shelf life of *kunun-zaki* for up to 6 months at room temperature storage. This project was done in collaboration with the Federal Institute of Industrial Research, Oshodi, (FIIRO), and was published in *Acta Alimentaria*, an International Journal of Food Science in 2000 (Olasupo *et al.*, 2000b)

The African environment is currently undergoing a developmental phase in which there is a strong emphasis on the quality and safety of traditional fermented foods.

This awareness has transformed into general interest in the assessment of microbiological status of fermented foods. The needs for control over foods remain firm. Increasingly we appear to be paying for lapses in our vigilance over food control with outbreaks of serious food poisoining in different regions particularly in Africa (Campbell–Platt, 1997). For many years food laws have been founded on the principle that all pathogenic micro-organisms of public health significance should be absent from all foods (Qvist, 1996). This concept of zero tolerance, however, does not reflect scientific and technical reality especially in the developing world. In the African environment, laws and standards to control the safety and hygiene of traditionally fermented foods are not available, much less implemented. We are thankful to the current leadership of NAFDAC for taking strong action in ensuring compliance with standards by food and

pharmaceutical industries. This prompted the work on the microbial status of traditionally fermented foods in the Nigerian environment, which provided the first of such information in the literature published in the *Journal of Food Safety* (Olasupo *et al.,* 2002). The study showed the presence of various presumable pathogens and baseline microbial count in the tested foods (Table 8).

Table 8:	Bacterial Association And Microbial Counts In Some Nigerian
	Fermented Foods.

Fermented food	Identity	AMC	YMC	VC	ETC
Ogi	Escherichia coli (1) Bacillus subtilis (2) Klebsiella sp. (2) Enterococus faecalis (1)	3.5 x 10 ⁶	1.0 x 10 ⁵	0	4.0 x 10 ⁵
Kunun-zaki	Bacillus subtilis (3) Enterococus faecalis (2) Staphylococus aurens (1)	2.6 x 10 ⁶	2.0 x 10 ⁵	0	1.2 x 10 ⁶
Wara	Staphylococus aurens (4) Klebsiella sp. (2)	5.0 x 10⁵	1.0 x 10 ⁵	0	4.5 x 10⁵
Nono	Salmonella sp. (2) Klebsiella sp. (1) Escherichia coli (2)	1.5 x 10 ⁷	3.3 x 10 ⁷	0	1.8 x 10 ⁷

O, detection limit < 10 cfu/ml or g; Data represent means of 5 samples of each food products. AMC, Aerobic mesophilic count, YMC, yeast and mould count; VC, Vibrio count and ETC, enterobacteriaceae

The study revealed that the consumption of foods containing high antibiotic resistant strains could serve as a means of spreading résistance to susceptible microbes among the consumers and further promote the perennial problem of disease treatment by antibiotics.

(G) IMPROVED PRODUCTION OF INDIGENOUS ALCOHOLIC PRODUCT I and a colleague in the Department of Microbiology of our great university have developed strategies involving the modification of the traditional methods for *ogogoro* (ethanol) production from palm wine. This has resulted in *ogogoro* with improved alcoholic content of 55% (Olasupo and Obayori, 2003). The alcohol (ethanol) content of traditionally produced *ogogoro* ranges between 26.8 and 39.9% (Odeyemi, 1977). The modification adopted in our study includes temperature monitoring during distillation, air tightness of the production system and improved cooling system for better condensation and product delivery (Fig 9).



FIG. 9: CROSS SECTION OF A MODEL FOR LOCAL PRODUCTION OF OGOGORO

The high alcoholic content of 55% is remarkable as the product can be diluted to improve the volume yield of *ogogoro*, or upgrade its usage as a possible source of fuel, as in countries such as USA and Brazil. It might be of interest to note that ethanol is presently used in Brazil and USA as fuel supplement for car engines. While Brazil obtained her own ethanol from sugar cane, the USA used corn to produce ethanol.

As a further work, *ogogoro* was aromatized using anise seeds (*Pimpanella anisum*). Anise seeds are flavouring agents with good international acceptance in alcoholic drinks. It is also used for medical and therapeutic purposes such as digestive expectorant and natural sedatives (Karsali and Basoglu, 1995). This is aimed at improving the local and international acceptability of *ogogoro* (Olasupo *et al.*, 2004b)

5.0 CONCLUSION

Many are called but few are chosen. I thank Almighty Allah for making me among the favoured in my chosen career and profession. In Britain, U.S.A, Canada and Germany, microbiology is a professional course and highly favoured but in Nigeria it is usually considered when candidates fail to be admitted for medicine. Perhaps this led to the government's attitude of not recognizing microbiology as a profession. I use this opportunity to appeal to governments at all levels to accord microbiology the status of a professional course, so that degree holders in microbiology will not require any licence or belong to a body other than the Nigerian Society for Microbiology before they can open their own laboratories for practice.

It is usually said that any nation that refuses to plan for the future of its youth is probably on the road to self destruction and extinction. In most of the academic departments in Nigerian universities, many young and old colleagues are yet to obtain their Ph.D. degree after spending many years. Many of them have limited or no facilities to work with and there are no research grants available to them. May I raise the alarm that something urgent should be done so that Ph.D. holders in our universities should not be allowed to go into oblivion; they should be supported with research funds to encourage and motivate them in their research. It is important to note that failure to urgently address this issue in our universities will lead to difficulties in replacing the experienced hands that are leaving the system. There is also a strong need to improve the laboratory facilities in our universities to ensure the production of quality graduates in the sciences. A situation where the supply of electricity and water is a luxury in our ivory towers is not the best for our integrity and productivity.

Food preservation is crucial if adequate food supply to the populace is to be guaranteed. This is because a lot of food is usually wasted due to microbial attack. Refrigeration which is one the ways to preserve food at the household level still looks like a dream due to erratic power supply. The Government of Nigeria is implored to please do something urgently to improve the electricity supply in the country. Africa is presently passing through a developmental phase in which there is a strong emphasis on local sourcing of raw materials. This awareness has given rise to a general interest in commercial processing of indigenous foods. One major problem we are still facing today is the upgrading of laboratory researches to acceptable standards in which investors/industries will be interested. A way out of this is the establishment of pilot plants in the universities and research institutes across the country. There is the need for governments at the various levels to embrace small and medium scale enterprises (SME). I wish to recognize the on-going effort of His Excellency, Asiwaju Bola Ahmed Tinubu towards the establishment of Research Incubation Centres across Lagos State through the watchful eyes of the Commissioner for Science and Technology, Dr. Femi Hamzat.

Traditional small scale fermentation technology offers considerable potentials for stimulating development in the food industry of African countries in the light of their low cost, scalability contribution to food safety and nutrition, minimal energy and infrastructural requirements and the wide acceptance of fermented products in these countries. The introduction of appropriate starter culture techniques may contribute one major step towards improved safety, quality and security of traditional small scale fermentation.

In the developed countries, many of the fermented foods are produced on an industrial scale. The contrary is the case with fermented foods in Nigeria, as many are still being produced only at the household level. There is the need to upgrade the production of our fermented foods in order to improve their supply and possibly save some from going into extinction.

Food-borne microbial diseases cause considerable morbidity and mortality throughout the world, Preventive measures such as good manufacturing practices (GMP), supplemented by the Hazard Analysis Critical Control Point (HACCP) system, should be introduced as a means of ensuring the production of safe food for human consumption.

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