



**UNIVERSITY OF LAGOS**  
**Inaugural Lecture Series 2014**

**TOPIC:**

**UNSEEN GUESTS AT  
TABLE: THE UNSUNG  
HEROES OF OUR  
ENVIRONMENT**

By  
**PROFESSOR OLUSIMBO OLUGBO ABOABA**

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# **Unseen Guests at Table: the Unsung Heroes of our Environment**

An Inaugural Lecture Delivered at the University of Lagos  
Main Auditorium on Wednesday, 20<sup>th</sup> August, 2014

By

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## **Dedication**

This inaugural lecture is dedicated to the memory of my  
husband

**Mr. George Olumuyiwa Aboaba**

The Unseen Hero



## **Preamble**

Mr. Vice-Chancellor, Sir, man proposes but God disposes. This lecture, planned for May 2013, was not to be due to circumstances beyond human understanding. I thank God for today, the day ordained by God for the special grace to give this lecture.

This lecture is the seventh from the Department of Microbiology and the first in Food Microbiology. I seize this opportunity to salute all before me who have shared with us their contributions in other areas of microbiology in this country.

My destined journey in this interesting field of Science started when I was admitted into the Biology programme in the then School of Biological Sciences, Faculty of Science of this great University. A disappointing Higher School Certificate result shattered the dream of going to Medical School. The programme tailored me towards Microbiology as Prof. J.A. Ekundayo of blessed memory made us the pioneering set for introducing Microbiology into the Biology Curriculum. My first research publication was from my B.Sc. Project supervised by him. The NYSC Scheme took me to Ankpa in the then Benue State now Kogi State. The nearly tragic end of that service year later redefined my future and has brought me this far. The year after service was bittersweet and in September 1978, I was admitted into the M.Phil. programme in Biology with a Postgraduate scholarship from the University of Lagos. The programme was converted into a Ph.D. a year later. A life in the academia was thus born between 1978 and 1981 though my supervisor, late Prof. Ekundayo, had to move to University of Benin towards the end of the programme.

My journey as an academic teacher started with the Planning Committee of the National Open University as

the first academic staff. Fate brought me back to my alma mater on transfer when the University was suspended in 1984. I took over Dr. Femi Ogunsanya's duties in the Department as she was on leave of absence at that time. I had to teach her course: Food Microbiology. It was then an interest in that area was developed because my Ph.D. had been on Plant Pathology.

## **Key Words**

### **The Unseen Guests**

Guests refer to persons received and entertained in one's home or at the table. In some instances, they may not be invited as we will see later. The unseen guests in the context of this lecture refer to those organisms that cannot be seen with the naked eye because of their relatively small size. These are micro organisms and they represent a diverse group consisting of viruses, bacteria, algae, protozoans, yeasts and moulds. Antonnie van Leeuwenhoek (1632-1723) was the first to view living things too small to be seen by the unaided eye. It was not till the 1860s that Louis Pasteur (1822-1895) and other European investigators had proof that micro organisms were responsible for fermentation of sugar into alcohol, spoilage of foods and putrefaction of living matter. In the 1880s, Pasteur and Robert Koch (1843-1910) showed that bacteria and other micro organisms cause death in animals and man.

### **At the Table**

The dining table at mealtime will have food materials of various types. Human history can be divided into two eras depending on the methods used by man to obtain major parts of their food. These are the food gathering era and the food producing era. The second era covers the time when people started producing their own food; this period spans the last 10,000 years of our time on earth till date.



All living things, including humans, grow and reproduce only if there is continuous flow of food in usable form. The food must provide all the substances needed to produce new cells as well as obtain energy for necessary biochemical processes. This means that when food is not available, growth and life will cease. Humans depend on food which is an excellent source of nutrients for micro organisms. Man, therefore, is at risk each time he eats as there are possibilities of pathogenic organisms in the food which may cause illness or even death. This translates to the fact that death is inevitable without food and yet food may well be the bearer of death.

### **The Heroes**

Some experts have stated that life depends on micro organisms. Life will continue if all people, plants and animals were to disappear from the earth; whereas if bacteria were to disappear by some magic then the earth will disappear within 40 years. Every aspect of human evolution and everyday existence is influenced by micro organisms that surround us, live on us and live in us. Some of them cause diseases on plants, in man and his domestic animals while others are involved in biodegradation of essential components in food thus making them unfriendly. But some others are not harmful and their diverse metabolic capabilities are found useful in commercial and research applications in modern day biotechnology. Their activities have been portrayed by film makers since the earliest days of the cinema. They have been referred to as movie microbes under the microscope. Films of microscopic organisms include "Typhoid fever germs" in 1905. Food was of particular interest as seen in "The unclean world" in 1905, "The scientist's lunch" in 1905 and "A bad case" in 1909 (Kirby, 2013).

# ARE THEY UNSUNG IN OUR ENVIRONMENT?

## Introduction

### History of Microbes in Food

Man started using micro organisms to produce fermented foods about 800 years ago without knowledge of the existence of these unseen beings. Louise Pasteur's discovery of microbial biochemical processes that changed raw food into fermented foods led to the world of microbial exploitations. This gave birth to new biotechnologies, especially bioprocess technology and use of recombinant DNA technology which has greatly contributed to a variety of food and beverage products, drugs, product yields, transformations etc to the human society and the planet at large. It is clear that bioprocess technologies are at play as living cells and their enzymes are used as biocatalysts to produce a molecular product, change one molecule into another or degrade a molecule. Biocatalysts are important in sustainability as microbial cells reproduce, renew themselves and their molecular products which are the ultimate in the reuse and recycle concept. This bioprocess occurs under conditions compatible with life. It generates very few undesirable by-products and thus is less destructive to the environment. Also, the process can be improved with time as microbes can be genetically modified to give optimal results for various needs.

### Microbes and Food

The study of microbes is referred to as Microbiology while the association of microbes with food is called Food Microbiology. The interaction of microbes with plants and animals is a natural phenomenon. Plants and animals or their products make up human food supply so it is inevitable that food must contain microbes. Food serves as a source of substrate because it contains macromolecules like carbohydrates, proteins, fats and oils, and water

molecules. These are nutrients needed for growth and metabolic activities. One of the functions of microbes in nature is to reduce forms of carbon, nitrogen and sulphur in dead plants and animals to the oxidized forms required by plants which are consumed by animals. To fulfil this role in the ecosystem, microbes have the ability to use different biochemical pathways like glycolytic pathway, Entner-Duodoroff pathway, homofermentative pathway, heterofermentative pathway and the tricarboxylic acid cycle to generate energy required for growth under diverse conditions in food. They must also interact with foods in ecosystems in which the cells may exist in a variety of physical and physiological states (injured and/or viable not yet culturable cells) in which intrinsic (i.e. innate or inherent) and extrinsic (i.e. external) factors come into place. These factors are nutrients, pH, water activity, redox potential, presence of antimicrobial agents, interaction with other microbes, temperature, relative humidity, gaseous atmosphere and sometimes processing techniques. Food may be heterogeneous and so can contain several distinct microenvironments with its own associated gradients to these factors mentioned earlier.

The study of microbes in foods has close link with Bacteriology, Mycology, Virology and other important applied areas of Microbiology such as Industrial Microbiology, Medical Microbiology, Pharmaceutical Microbiology, Environmental Microbiology and Agricultural Microbiology.

### **Sources of Microbes in foods**

Raw materials for food are obtained from plants and animals so it is inevitable that food for human consumption gets contaminated with microbes from the soil, water, air and dust. Water is used in processing and it also forms an integral part of food. The processing equipment and



utensils, if not properly cleaned, can be sources of inocula during processing while the handlers are the most common vectors especially if good hygienic practices are not observed.

**Table 1: Reported isolations of potential pathogens from specific environmental sites within food preparation areas**

Environmental site	<i>Campylobacter</i> spp.	<i>Salmonella</i> spp.	<i>Y. enterocolitica</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>Bacillus</i> spp.	<i>B. cereus</i>	<i>L. monocytogenes</i>	<i>Listeria</i> spp.
Dish cloth	•	•		•	•	•		•	•
Cleaning Cloth	•	•		•	•		•		•
Wash-up sponge	•	•		•	•				•
Wash-up brush					•			•	•
Wash cloth		•						•	
Floor mop					•	•			•
Tea/hand towel				•	•	•			
Sink		•	•	•	•		•	•	•
Taps				•			•		
Refrigerator/door	•			•			•	•	•
Waste/pedal bin	•			•		•			
Chopping boards	•			•	•				
Work surfaces	•				•	•			
Floors	•				•				

Source: Griffith, 2000.

Contaminants in food may play a useful role in food transformations as in the case of spontaneous fermentation of foods. Microbes are sometimes deliberately added to food to achieve a desired product. The use of starter cultures is common in industries where microbes are used for fermentation processes. Also, microbes used as food

supplements and probiotics are produced and incorporated into foods or taken as a form of medication.

Emphasis will be placed on activities of bacteria in this lecture.

### **Detection and Enumeration of Microbes in Food**

Food Microbiologists have devised different ways to see the unseen in an effort to study them as they fulfil their roles in nature. Methods used for enumeration and detection of microbial types and their metabolic products are listed below. However, sample collection and processing may vary depending on the food material and the specific target organism.

1. **Direct Microscope Count:** This is the simplest and most rapid method used as incubation period is not required. A smear of food is prepared on a microscope slide, fixed, stained and the cells counted with a calibrated microscope.
2. **Standard Plate Count/Total Plate Count:** An aliquot from a food homogenate or same from selected ten-fold serial dilutions is inoculated on a growth medium which may be selective using the spread plate or poured plate method. The duplicate plates are incubated at an appropriate temperature and time. The isolated colonies known as colony forming units are counted from plates containing 30-300 units. In cases of injury on cells due to processing or environmental factors, a pre-enrichment procedure (to resuscitate or repair cells) in a non-selective medium and then selective enrichment (allows increased population of the target organism) steps may be necessary before use of selective or differential medium. Different organisms can be detected using specific selective media. In recent times, efforts have been made to reduce the time

spent on analysis as stomacher has replaced the blender, automated plating systems have eliminated dilutions and chromogenic media which contain specific chemicals permit the differentiation and counting of specific organisms such as *Listeria monocytogenes*. The use of colony counters has minimized errors due to manual counting.

Membrane filters may be used with liquid samples to trap bacterial cells and then placed on appropriate growth medium before incubation and colonies counted.

3. **Most Probable Number Method:** This is used to estimate the most probable number of organisms in a sample when present in low numbers. Selective agents are added to broth medium and 1ml of sample is inoculated into three or five tubes of media. The same amount of at least two other ten-fold serial dilutions are also inoculated and incubated. The numbers of positive and negative tubes based on colour change and gas production are recorded and the population obtained from a statistical table. This is useful in determining coliform counts in water and liquid foods.
4. **Metabolism-Based Methods:** Measurement of metabolic activities of micro organisms and their products can be used to identify and estimate their populations in foods. Many of the tests are now miniaturised and are the basis of rapid test kits such as API (Analytical Profile Index). Oxidation reduction indicators and dyes such as methylene blue are also used; the rate at which the indicator shows a change in colour is related to the metabolic rate of the microbial culture in the sample.
5. **Immunologically-Based Methods:** These are rapid methods based on antigen-antibody reactions. They include immunodiffusion and ELISA (Enzyme Linked



Immunosorbent Assay). They are used for pathogens such as *Listeria monocytogenes*, *E coli* 0157:H7, *Campylobacter* spp, *Salmonella* spp., and others.

6. **Molecular Methods:** These are nucleic acid-based assays for differentiation and identification of pathogens. They include Polymerase Chain Reaction (PCR), Pulse-field gel electrophoresis, ribotyping, plasmid typing, etc. The most popular of these is the PCR. Many of them are automated and kits are available. They are used successfully with *Salmonella*, *E. coli* 0157:H7, *S. aureus* and *Campylobacter* spp., etc.

### **Activities of Microbes in Foods**

The interaction of microbes and food components results in various changes which can be summarized as follows:

1. Food spoilage
2. Food intoxication and infection
3. Food production
4. Food in health promotion (Probiotics)
5. Food as nutrient supplements (Single cell protein)

### **The Bad and Good Bacteria at Work**

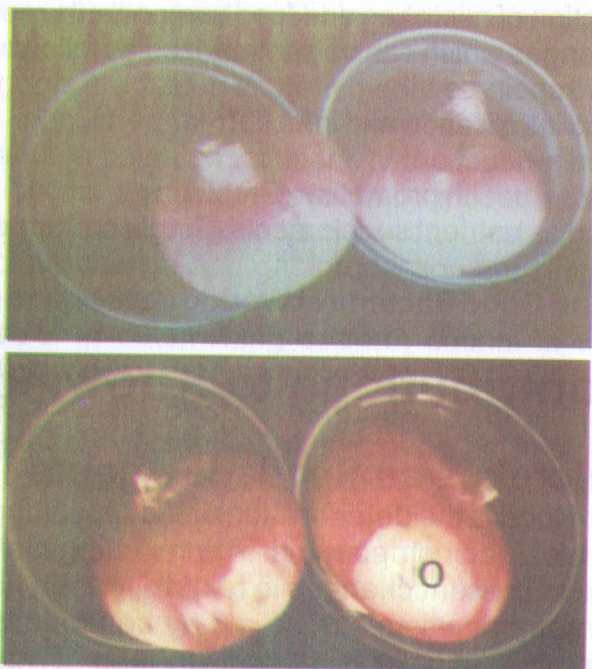
## **My Contributions to Knowledge**

### **Microbes as Spoilage Agents**

Spoilage of food is the deterioration of food resulting from breakdown of food components. The resulting materials and products present changes in texture, odour, flavour, taste and sometimes colour. This may be unpleasant if not acceptable to the consumer though it may be acceptable to others depending on culture and race. This process is biological in nature as deterioration may be due to autolysis or by activity of micro organisms.

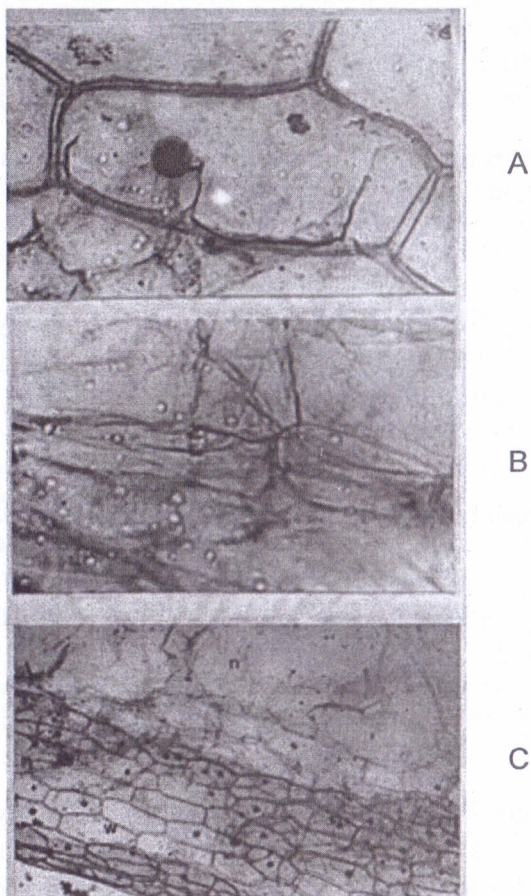
Microbes play a key role in this process as they produce a variety of degradative enzymes.

1. My research in this area examined bacterial spoilage in onions (*Allium cepa* L) and other vegetables of economic importance. In onions, *Pseudomonas fluorescens* and *Burkholderia cepacia* were found to be pathogenic on onion bulbs in storage and their virulence was high at temperature range of 28°C - 30°C and relative humidity value of 80% - 100%. This spoilage condition caused a water-soaked appearance and resulted in considerable loss in total sugars though loss in amino acid was negligible (Aboaba and Ekundayo, 2000; Aboaba, 2007).



**Plate 1: Healthy onion bulbs inoculated with sterile distilled water (A) and bulbs showing spoilage after inoculation with *Pseudomonas fluorescens* (B).**

The enzyme pectinase was produced by the pathogens during spoilage and found to be the virulence factor for infectivity (Aboaba, 2009).



**Plate 2: Photomicrographs showing intact cell (A), collapsed cell (B) and area of advancing lesion with collapsed and intact cells (C).**

2. Spoilage of melon (*Citrullus vulgaris* Schard) seeds not only caused undesired changes in texture and colour but also loss in germinability. The causative agents were *Aspergillus flavus*, *Aspergillus*



*fumigatus* and *Rhizopus* spp. The melon meal inoculated with the pathogens showed complete utilization of the total carbohydrate and a reduction in the total nitrogen and oil content. However aflatoxin was not detected in the inoculated meal (Aboaba and Amasike, 1991; Aboaba and Amasike, 1995).

**Table 2: Percentage infectivity of the pathogens on wounded melon seeds**

Infecting Agent	Percentage Infectivity Incubation Period (days)				
	1	2	3	4	5
<i>A. fumigatus</i>	50.00	60.00	80.00	100.00	100.00
<i>A. flavus</i>	80.00	100.00	100.00	100.00	100.00
<i>Rhizopus</i> spp	80.00	60.00	100.00	100.00	100.00

**Table 3: Percentage germination of infected melon seeds**

Infecting Agent	Percentage Germination Incubation Period (days)				
	1	2	3	4	5
Control	10.00	30.00	90.00	90.00	90.00
<i>A. fumigatus</i>	0.00	0.00	0.00	0.00	0.00
<i>A. flavus</i>	0.00	0.00	0.00	0.00	0.00
<i>Rhizopus</i> spp	0.00	0.00	0.00	0.00	0.00

3. The quality assessment of two marine fishes, *Liza falcipinnis* and *Pseudotolithus elongatus* was investigated. The bacterial flora of external tissue, gills and intestine consisted of *Micrococcus* spp., *Bacillus* spp., *E. coli*, *Vibrio* spp. and *Pseudomonas* spp. with population of  $10^9$ cfu/g at 28°C but only *Vibrio* spp. and *Pseudomonas* spp. with counts of  $10^4$ cfu/g were isolated at -5°C. Predicted shelf-life

was within the acceptable limit based on sensory and microbiological criteria (Okoro *et al.*, 2010).

4. The non-alcoholic beverages: Kunun-zaki, Ginger beer, Soborodo drink and Soya milk were prepared under laboratory conditions. Shelf-life studies showed they could be preserved using pasteurization and refrigeration (Osuntogun and Aboaba, 2004).

It is clear that uninvited micro organisms can be removed if measures are taken to control their survival and growth in these food commodities.

### **Waste to Wealth: the Ugly can be Good**

Micro organisms responsible for spoilage can be beneficial to mankind. Some of them were found to have potential for bioremediation of hydrocarbon-polluted sites and antimicrobial properties. In this instance they can be invited though for specific reasons.

1. Biosurfactants are naturally occurring surface active compounds produced as secondary metabolites extracellularly by a variety of yeasts, bacteria and fungi. Their hydrophobic and hydrophilic properties have found applications in industrial processes including emulsification, foaming, detergency, wetting, dispersing and solubilisation.

Food materials that have undergone deterioration and thus considered spoilt or waste from the table were examined for sources of biosurfactant-producing organisms. The spoilage organisms, *Pseudomonas* and *Bacillus* spp. isolated were found to produce biosurfactants which exhibited emulsifying ability on petroleum products and vegetable oil. This microbial product can thus be useful in bioremediation and in food processing (Aboaba and Ogunmola, 2014). This is a preliminary study which is



contained in the book of abstracts at the 3<sup>rd</sup> ISEKI Food Conference organised by the European Association for Integrating Food Science and Engineering Knowledge into the Food Chain which took place in Athens, Greece in May, 2014.

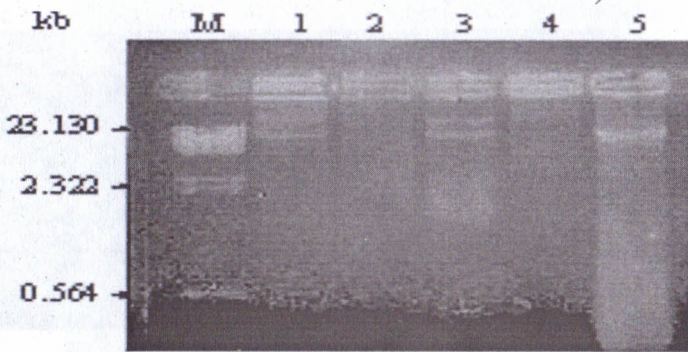
2. It was also found that effluent from fast food outlets contained microbes that caused degradation of crude oil in polluted agricultural soil. Growth studies of beans and maize seedlings confirmed the efficacy of the process. The microbes therefore have the potential to be good candidates for bioremediation (Chukwu, 2000).

## **Microbes and Food Poisoning**

The ubiquitous nature of microbes makes it possible for them to get into water, other raw materials and can be found during processing if adequate hygienic practices are not maintained. Some produce toxins in foods or their presence can cause undesirable effects on human health. Examples of such toxins include *Staphylococcus* enterotoxin and botulinum toxin causing food intoxication or presence of organisms such as *Bacillus cereus* causing food infection.

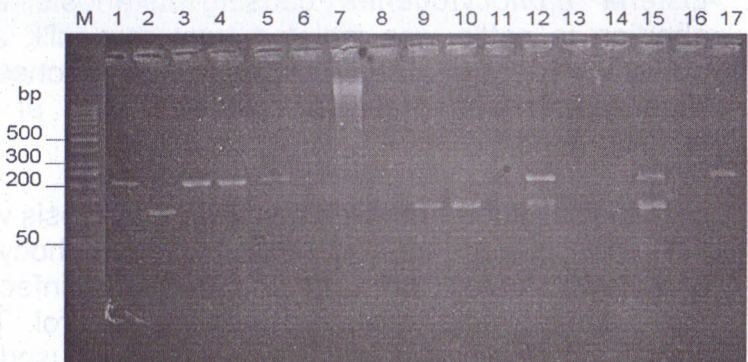
Contributions in this area focused on isolation and detection of food-borne pathogens in the environment, in foods and from the handlers. Their presence signifies health hazards to the public. This, of course, led to effective control and possible treatment as antibiotic-resistant strains are common among enteric pathogens.

1. In our laboratory, *E.coli* 0157:H7, the agent for hemorrhagic colitis was isolated from faeces of healthy animals (goats, rams, pigs and cattle). The organism exhibited multiple antibiotic resistance. It was characterised using PCR to detect the shiga toxin-producing gene (Smith *et al.*, 2003).



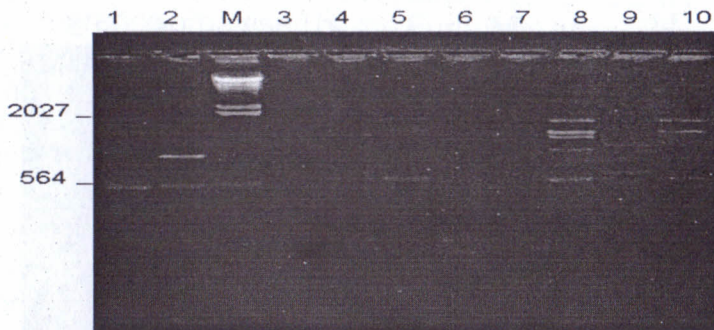
**Plate 4: Plasmid profile of *E.coli* 0157: H7 isolated from apparently healthy animals**

2. *Campylobacter jejuni*, the causative agent of campylobacteriosis was isolated from poultry droppings from poultry farms in the Lagos area. Poultry was found to serve as a reservoir for this organism (Aboaba and Smith, 2006).
3. *Salmonella typhi* causing typhoid fever, *S. enterica* serovar *Enteritidis* and *S. enteric* serovar *Typhimurium* (which is responsible for bacteraemic non-typhoidal *Salmonella* infections) were isolated from food handlers and animals (Smith *et al.*, 2010; 2011).

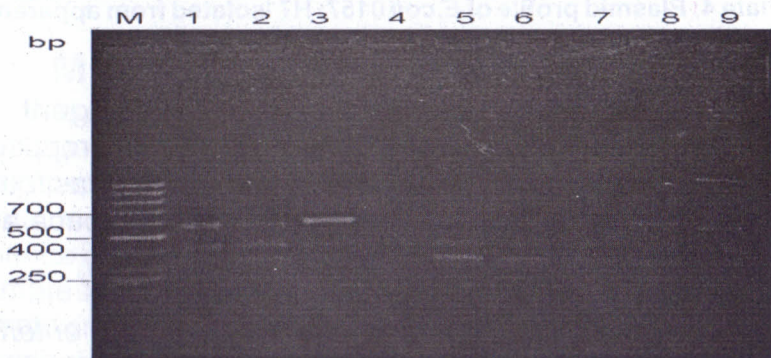


**Plate 5: Animal *Salmonella* spp.**





**Plate 6: Food handler *Salmonella* spp.**



**Plate 7: Food handler *Salmonella* spp.**

**Plates 5, 6 and 7: *Salmonella* spp from food handlers and animals identified using PCR technique.**

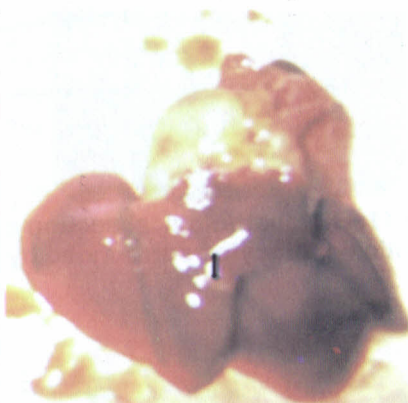
4. *Listeria monocytogenes* causing listeriosis and abortion in cattle was isolated from raw milk and abattoir effluent in all the six geographical zones of Nigeria with high prevalence rate (Enurah *et al.*, 2013c).

Histological studies showed hepatocyte necrosis with infiltration of degenerative neutrophils, lymphocytes and few plasma cells in the liver of infected experimental mice compared with the control. The strains, however, were sensitive to antibiotics used for treatment (Enurah *et al.*, 2013a; 2013b).

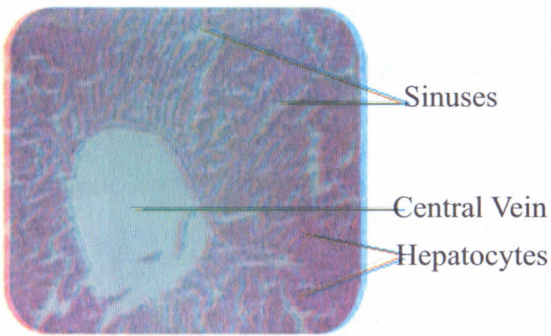




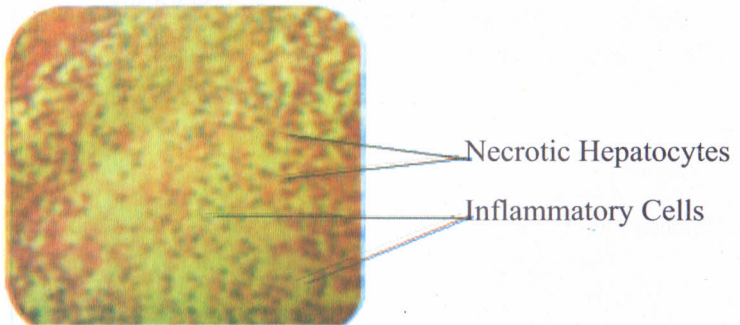
**Plate 8: Liver sample from uninfected mouse (control).**



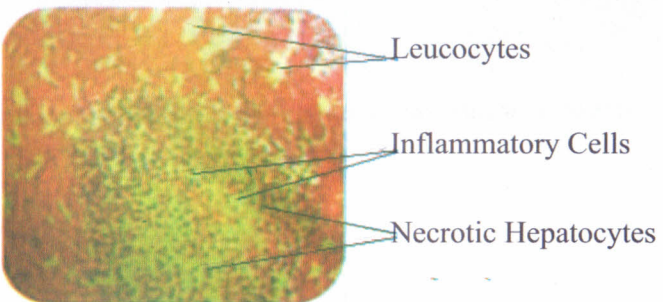
**Plate 9: Liver samples of infected mice showing hepatic abscess.**



**Plate 10: Histological section of normal mouse liver.**



**Plate 11: Histopathological section of infected liver showing necrotic hepatocytes surrounded by inflammatory cells.**

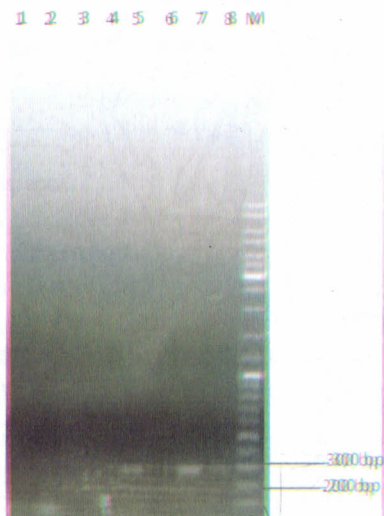


**Plate 12: Histopathological section of infected liver showing severe hepatocyte necrosis with indistinct cellular outline.**



**Plate 13: Antibiotic sensitivity pattern of *L. monocytogenes* strains.**

5. An emerging opportunistic food pathogen *Cronobacter sakazakii* causing necrotising enterocolitis in neonates and infants was isolated from infant formula retailed in our markets (Ezeh *et al.*, 2013). This is the first reported case in Nigeria and work is ongoing at the Department of Infectious Diseases, University of Houston, Houston, Texas, USA.



**Plate17: Detection of *C. sakazakii* by PCR.**

## Water as Agent for Transmission of Pathogens

Water has been considered as an integral part of foods and faecal pollution has contributed to food contamination with enteric pathogens. Egwari and Aboaba (2002) examined the bacteriological quality of domestic water supplies in Lagos metropolis. Many enteric pathogens were isolated, especially in high density areas. This was attributed to poor town planning, overcrowding, unhygienic environment along with use of old and faulty pipelines.

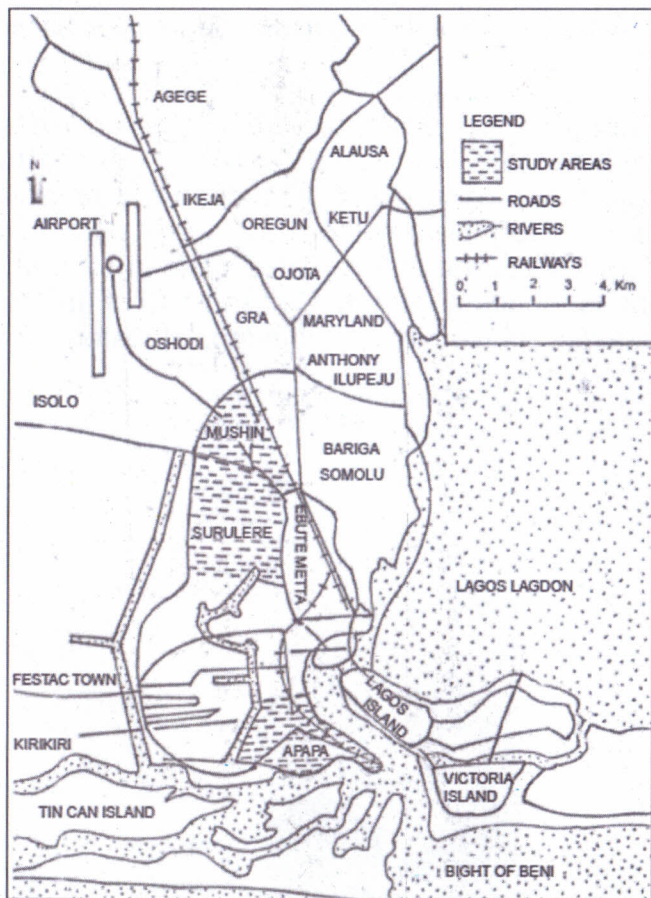
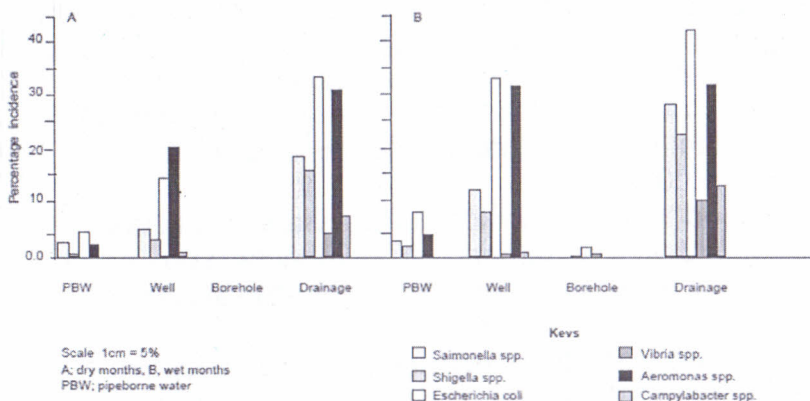


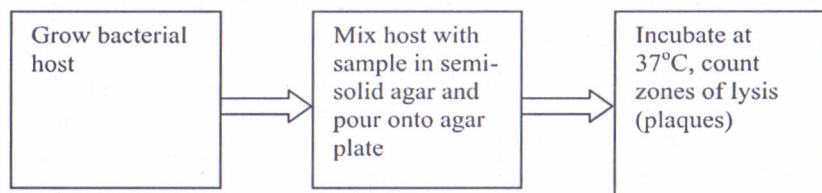
Figure 1: Map of Lagos showing sampling locations.





**Figure 2: Incidence of enteric pathogens in municipal drain water and public water samples.**

The result of this study led to a four-month study leave at the University of Brighton (Environment and Public Health Unit) to learn a technique on microbial source tracking for water pollution as a strategy for pathogen control. It is a simple methodology using phage-lysis of source specific strains of *Bacteroides fragilis* strain GB124. The Phage alert process is a three-step procedure (Ebdon *et al.*, 2007).



**Figure 3: A flow chart showing the phage alert process.** Results expressed as plaque forming units per 100ml.

### Other agents of transmission of pathogens

1. The abattoir was found to be a good source of *Listeria monocytogenes* strains (Enurah *et al.*, 2013c).
2. Raw milk was found to contain high populations of *Listeria monocytogenes* (Enurah *et al.*, 2013c).

3. The poultry farm was a good source of *Campylobacter jejuni* as high populations of cells were isolated from the poultry droppings (Aboaba and Smith, 2005).
4. *Escherichia coli* 0157:H7 was readily isolated from faecal matter of healthy domestic animals (Smith *et al.*, 2003).
5. Food handlers in local restaurants (Bukkas) were found to be carriers of *Salmonella* spp. which is responsible for typhoid fever (Smith *et al.*, 2010).

It is obvious that adequate disposal of waste from man and his domestic animals will go a long way in preventing the spread of these pathogens. Also, hygienic practices during processing and use of wholesome raw materials are important in producing safe foods. This is because the pathogens pass through the faecal oral route.

### **Emerging Pathogens as Food Safety Hazard**

One of the hazards associated with food safety is the issue of emerging pathogens. As man has found drugs and vaccine solutions to diseases, the microbes have continued to evolve alongside with us, sometimes to emerge as the cause of a new infection with greater virulence and guise (emerging pathogens). A project with the Food Agency of the UK found the possibility of saprophytic species of *Clostridium* carrying the botulinal neurotoxin gene of *Clostridium botulinum*, a very deadly food pathogen (Ghoddusi *et al.*, 2013).

### **Strategies for Safe Food**

#### **1. Predictive Modelling in Foods**

One of the measures taken to control growth of pathogens in foods was based on intensive study of them under adverse conditions found in foods such as salt concentration in the laboratory. This knowledge was used to predict their responses and thus estimate

shelf-life using predictive models. This area of Food Microbiology is called Predictive Modelling.

- a. The behaviour of *Listeria monocytogenes* was studied at different concentrations of NaCl and inoculum size making it possible for shelf-life in high salt foods to be estimated (Robinson *et al.*, 2001; Pascual *et al.*, 2001).
- b. The growth-limiting parameters for a rare strain of *Clostridium butyricum* that carries the gene encoding for botulinal type E neurotoxin was investigated and established as control strategy for this organism in the food industry (Ghoddusi *et al.*, 2013).

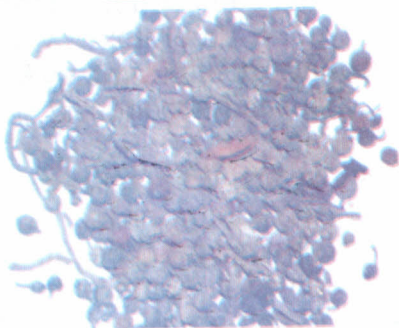
## 2. Use of edible plant extracts

Alternatives to antibiotics were also investigated because of antibiotic resistant patterns observed in some enteric pathogens.

- a. Edible plant extracts of *Entanda africana*, *Terminalia avicennoides*, *Lannae acida* and *Mitragyna stipulosa* were found to have antimicrobial activity on ten strains of *E. coli* 0157:H7. Extracts of *Entanda africana* had bacteriocidal effect while others were bacteriostatic (Aboaba *et al.*, 2006).
- b. Food spices namely *Monodora myristica*, *Piper guineense*, *Xylopi aethiopica* and *Tetrapleura tetraptera* used in flavouring of foods demonstrated antimicrobial properties on intestinal pathogens. The aqueous extract of *Piper guineense* was the most potent of all with zone of inhibition of 12-25mm compared with 10-35mm found with conventional antibiotics used for gram+positive and gram-negative bacteria. Phytochemical analysis of these plant extracts showed the presence of terpenoids, tannins, saponins, flavonoids and glycosides (Aboaba *et al.*, 2011).



**Plate 18: Seeds of *Monodora myristica* (Ehuru).**



**Plate 19: Seeds of *Piper guineense* (Uziza).**



**Plate 20: Seeds of *X. aethiopica* (Uda).**



**Plate 21: Leaves of *T. tetraptera* (Utazi).**

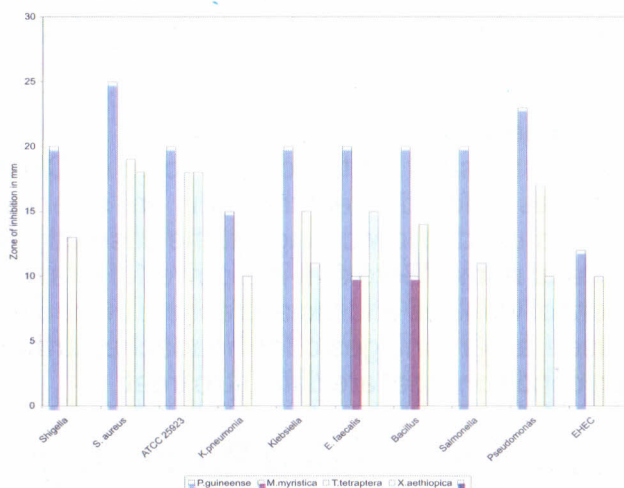




**Plate 22: Zone of inhibition of *Piper guineense* on *Bacillus* sp.**



**Plate 23: Zone of inhibition of *Piper guineense* on *S. aureus***



**Figure 4: Zones of inhibition (mm) of aqueous extracts of the four spices on test organisms.**

### Biosurfactant as an Antimicrobial Agent

It was found that biosurfactants produced from food spoilage organisms have antimicrobial property on food-borne pathogens. This can be exploited by the pharmaceutical industries as antimicrobial agents (Ogunmola, 2014). This is the first claim in Nigeria.

## **Food Safety**

The problems associated with food safety led to fundamental changes in regulatory policies and demonstrated to food processors the need for constant vigilance against food pathogens. There should be adequate control through the observation of Good Manufacturing Practices (GMP). The Hazard Analysis and Critical Control Point system (HACCP) was aimed at aiding the establishment of targets for pathogen control. Other systems include the Longitudinal Integrated Safety Assurance (LISA) which targets control from the farm to the fork i.e. food on the table. It is clear that if these pathogens are invited through inadequate control in foods, they may cause serious health risks; if uninvited, safety will be assured.

### **The bad can be good**

It is not all a sad story as the deadly toxin of *Clostridium botulinum* has offered breakthroughs in science. It is produced and used in low dosage in the cosmetic industry with the brand name "Botox". It is a powerful relaxant used in treatment of wrinkles and facial creases. In medicine, the toxin is used as an analgesic for headache, arthritis and other pain conditions that are difficult to treat.

## **Microbes in Food Production**

The bioconversion of a great variety of raw materials of plant and animal origin into products of improved nutritional quality, prolonged shelf-life and safe for human consumption is due to activities of microbes. These organisms include Lactic acid bacteria, other bacteria, yeasts and moulds. It can be said that microbes give us our daily bread.

Fermented foods play a big role in the diet in tropical developing countries like Nigeria. In the south west of Nigeria, fermented cereal gruel such as "Ogi" or "Akamu" and bread, a fermented wheat product, are very popular at the breakfast table. Fermented cassava tubers to produce "Gari", "Fufu" and "Pupuru" are dominant ingredients for lunch and sometimes dinner. Fermented soya beans, melon seeds and locust beans make tasty condiments in soups while alcoholic beverages like "Pito", "Palmwine", "Burukutu" are refreshing drinks at meal times or at celebrations.

1. Cassava-Based food:

a. Pupuru, a fermented cassava product, commonly eaten by the Ikale people in Ondo State was found to be produced by spontaneous fermentation. Organisms isolated during the production were species of *Alcaligenes*, *Leuconostoc*, *Bacillus*, *Serratia*, *Candida* and *Saccharomyces* (Aboaba *et al.*, 1988).

2. Cereal-Based Foods:

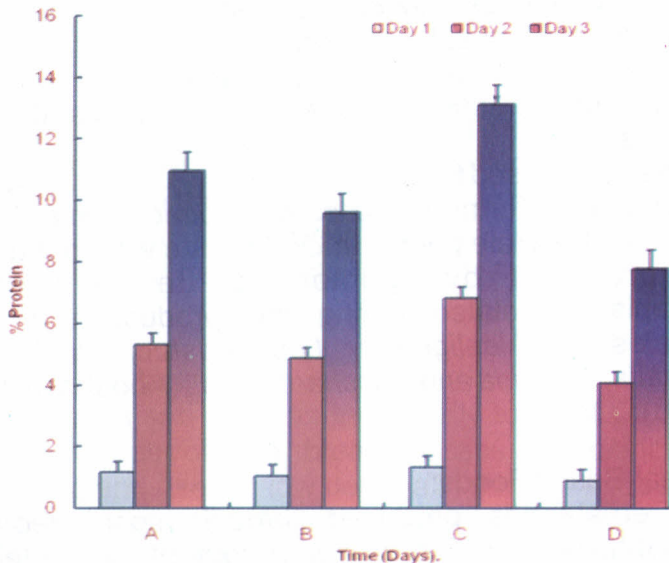
a. Cereal-foods produced through spontaneous fermentation are high in starch content, so protein enrichment is encouraged as they are used for weaning infants. Aboaba and Oyebola (1991) produced fermented cereal with increased protein content from 40mg/g to 100mg/g when *Candida mycoderma* was added to the steep water fortified with urea.

b. A fermented cereal gruel prepared from corn, millet and sorghum was produced through the action of *Lactobacillus pentosus* and *L. acidophilus* as starter cultures with improved nutritional value as protein level was increased (Opere *et al.*, 2003).

The organoleptic properties of fermented products were contributed by by-products of the fermenting organisms. Organic acids, acetoin and diacetyl were produced during fermentation of cereal gruel (Opere



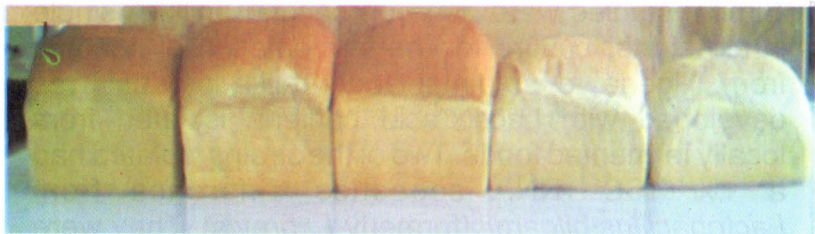
et al., 2011). The organic acids and bacteriocin produced by these organisms can inhibit undesirable organisms. This is responsible for the extended shelf-life associated with fermented foods. They are thus used for biopreservation in the food industries.



**Figure 5: Variations in average total protein content with days of fermentation in four varieties of cereals.**

A=cereals fermented with *L. acidophilus*; B=cereals fermented with *L. pentosus*; C=cereals fermented with mixed culture; D=Control.

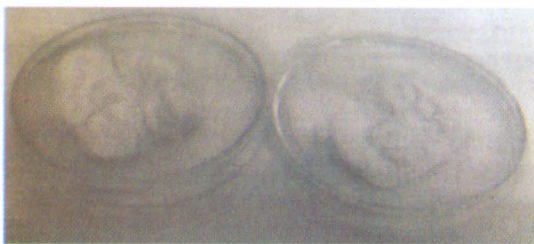
c. Investigation was done on the fermentation of composite flour consisting of wheat and cassava for bread production using *Saccharomyces cerevisiae* as the fermenting organism. Sensory evaluation studies showed the product with flour containing 20% cassava to be acceptable (Aboaba and Obakpolo, 2010).



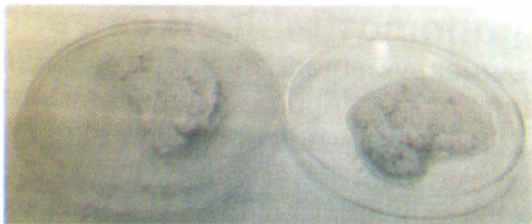
**Plate 24: Bread samples produced with composite flour of varying amounts of wheat and cassava flour.**

### 3. Milk-Based Foods:

a. Soya milk and cow's milk were allowed to ferment spontaneously and coagulation facilitated with the addition of vinegar, corn steep liquor and extract of *Calotropis procera* leaves to produce cheese-like products. Sensory evaluation showed them to be similar in texture, appearance and colour though the cow milk was more acceptable by the panelists. The organisms isolated were species of *Bacillus*, *Streptococcus*, *Lactobacillus* and *Micrococcus* (Aboaba and Adeleye, 2002). These are obviously invited guests!



A

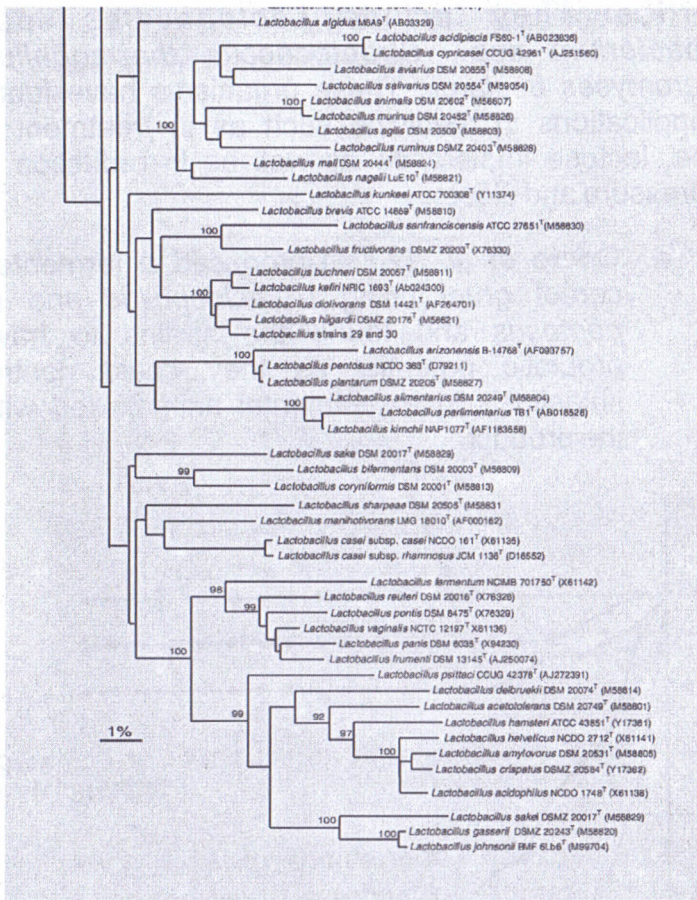


B

**Plate 25: Cheese-like products from fermented raw cow's milk (A) and soya milk (B).**

- b. Similar studies were done on coconut milk. A single strain starter culture for the fermentation of coconut from Nigeria to obtain a yoghurt-like product was developed with Lactic acid bacteria isolated from locally fermented foods. Two of the strains isolated had a 1% 16S rRNA sequence difference from *Lactobacillus hilgardii* (formerly *L. brevis*). They were tentatively named *L. lawson* (Dr. Lawson was Head of Laboratory at Institute of Food Research in Reading) and deposited at the Culture Collection Unit of University of Goteborg, Sweden as CCUG 42018 and CCUG 42019 respectively pending new name allocation. The two strains were found to give the best acid profile required for a lactic fermented product. The screened characteristics of each strain were not plasmid-mediated hence stable for the studies and as a single strain starter-culture. This research is ongoing.





**Figure 6: Phylogenetic tree showing the relatedness of novel strains isolated from locally fermented foods with known *Lactobacillus* spp.**

## Microbes as Health-Promoting Agents

The fermenting organisms themselves have been found to control intestinal pathogens and thus maintain intestinal health when ingested with food. They are called Probiotics (friendly bacteria). These are viable cells of organisms that can produce inhibitory substances, get themselves attached to intestinal mucosa among other

properties. They include *Lactobacillus* spp., *Bifidobacterium* spp., *Streptococcus thermophilus*, *Saccharomyces boulardii*. These organisms have found wide applications in medicine such as in treatment of diarrhea, lactose intolerance as well as in reduction of blood pressure and ulcers.

- a. Opere *et al.*, (2004) produced a fermented cereal gruel using *L. acidophilus* and *L. pentosus* and found the strains to have probiotic potential as they could control shigellosis in experimental animals fed with the product.

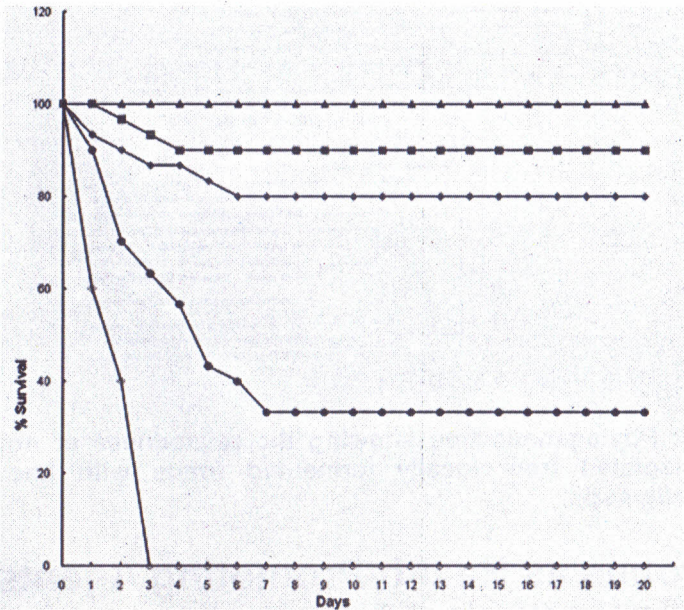
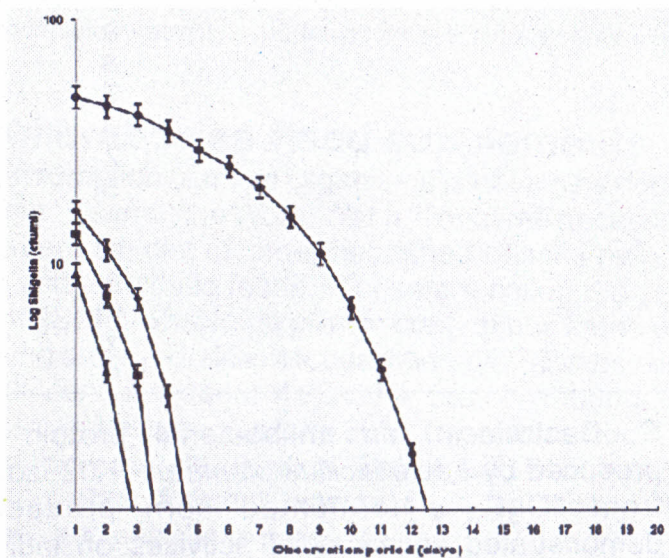


Figure 7: Survival rates of Mice after Shigella infection.

Control Mice fed with breast milk (◇), Mice fed with spontaneously fermented feed (●), Mice fed with *L. acidophilus* fermented feed (■), Mice fed with *L.*



*pentosus* fermented feed (◆), Mice fed with mixed culture fermented feed (▲).



**Figure 8: The population of *Shigella* cells in fecal samples of surviving Mice.**

Mice fed with spontaneously fermented feed (●), Mice fed with *Lactobacillus acidophilus* fermented feed (■), Mice fed with *Lactobacillus pentosus* fermented feed (◆), Mice fed with mixed culture fermented feed (▲).

b. Similarly, Lactic acid bacteria isolated from locally fermented cow milk's "Wara" showed antagonistic properties towards *E.coli* 0157:H7, an intestinal pathogen *in vitro* (Aboaba *et al.*, 2007).

**TABLE 4: Percentage zones of inhibition spectrum of strains of Lactic acid bacteria against *E. coli* 0157:H7.**

Strain	Percentage (%) zone of inhibition
M22	6
M29	9
M30	8
M27	7
M28	6
M33	7
M26	5
M13	8
M16	10
M8	8
M19	10
M31	7
M32	9

C. Bacteriocin, an antibacterial protein was produced by *Lactobacillus plantarum* K02 isolated from “Ogi”, a fermented corn product. It demonstrated antimicrobial activities on indicator organisms (Oguntoyinbo *et al.*, 2004).

**TABLE 5: Antimicrobial activities of the bacteriocin produced by *Lactobacillus plantarum* K02 against indicator strains.**

Indicator strain	Sensitivity
<i>Lactobacillus casei</i> DSM 20011	+
<i>Lactobacillus sakei</i> DSM 20017	+
<i>Lactobacillus plantarum</i> DSM 20246	+
<i>Lactobacillus fermentum</i> DSM 20052	+
<i>Enterococcus faecalis</i> DSM 20380	+
<i>Pediococcus acidilactici</i> DSM 20333	+
<i>Bacillus cereus</i> DSM 2301	- +
<i>Staphylococcus hominis</i> DSM 20328	- +
<i>Staphylococcus aureus</i> ATCC 14458	-
<i>Listeria innocua</i> DSM 20649	+
<i>Listeria monocytogenes</i> DSM 20600	+
<i>Escherichia coli</i> FIRO	-
<i>Clostridium butyricum</i> DSM 552	- +

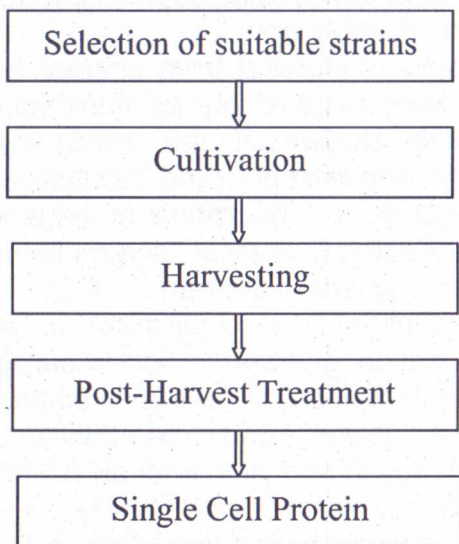
Origin of stains: ATCC, American Type Culture Collection, US., DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; FIIR, Federal Institute of Industrial Research, Oshodi, Lagos.

Key:

+ strong inhibitory activity; - no inhibitory activity; - + weak inhibitory activity.

## Microbes as Food supplements

The consumption of micro organisms is not a new concept. In Germany during the World War II, it became necessary to supplement the diet of under-nourished citizens by adding yeasts and moulds to foods. These are called Single Cell Proteins (SCP). Their high protein content has found use in improving protein-deficient foods. Microbial biomass on the average contains about 45%-53% protein though some bacteria can have as much as 80%. Organisms commonly used include yeasts, moulds and algae. SCP develops when microbes ferment wastes such as wood, straw, molasses and petroleum products.



**Figure 9: Flow chart for Single Cell Protein Production**



- a. *Candida tropicalis* was cultivated on molasses for SCP production (Kuforiji and Aboaba, 2009).
- b. The application of *Candida valida* as a protein supplement (Kuforiji and Aboaba, 2009) was also examined.

### **The good can be better**

All these organisms are good and friendly. The good organisms can be developed as starter cultures to minimise production time and to obtain better products. This is useful in product development. The probiotics can be friendlier if foods containing prebiotics are part of the diet. These are inulin, oligofructose and similar substances found in plants such as onions, garlic, chicory roots, bananas and wheat bran. They nourish the probiotics in the large intestine. The other good microbes can be improved genetically to give maximum industrial benefits.

### **My Other Research Contributions**

I have had the opportunity to co-supervise research work in related areas of Microbiology.

1. The organisms isolated from effluent from a textile industry were used singly as pure cultures in biodegradative studies of the waste water in the laboratory. The aerobes and facultative anaerobes could degrade a wide range of organic pollutants thereby reducing Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Total Dissolved Solids (TDS) (Ugoji and Aboaba, 2004).
2. The application of *Pseudomonas aeruginosa* as a bioremediating agent on crude oil polluted sites was investigated. It was found to be effective as shown by improved growth and yield in bean seedlings used to test for efficacy (Aboaba *et al.*, 2007).
3. Spoilage in water-based paint was investigated and shelf-life determined (Obidi *et al.*, 2009; 2010).

4. The use of *Trichoderma longibrachiatum* as growth-promoting and bio-control agent of cucumber (*Cucumis sativus*.L) and lettuce (*Lactuca sativa* L) was established. This research is ongoing.

## LEGACY

### ASM Bio-resource Centre

The American Society for Microbiology (ASM) is the largest single life Science Society in the world with over 40,000 global members. It aims to disseminate knowledge through state of the art journals, books, meetings and conferences. The International Affairs of the organisation is responsible for information and transfer of information to international members. It appoints ambassadors who are to reach the needs of Microbiologists in the domain. I was appointed Ambassador for West and Central Africa in 2005 and in 2008 for the West African region till 2011. This opportunity gave me the mandate to establish a Bio-resource Centre for the region which is domiciled at the Department of Microbiology and still remains the only one in West Africa. The Centre serves as a platform to foster scientific exchange. It provides ASM resources and on-site training for students, scientists, faculty, public health professionals and related disciplines. The Centre is viewed as a location to gather and connect through a shared passion for Science. It also provides cutting-edge resources and training.

### Practical Laboratory Manual

Many years of teaching have exposed me to various ways of getting information across to students using available resources at our disposal. This was put together into a manual for practical classes in Food Microbiology along with Prof. S.C.U. Nwachukwu on Industrial Microbiology.

## Contributions to Academia

I have been privileged to have taught and mentored many people who have joined me in this prestigious calling both locally and internationally. They include: Prof. B. Kuforiji, Prof. S. Ilori, Drs. S. Adebuseye, G. Oyetibo, F. Obayori, O. Omotayo, I. Nsa, others in the Faculty of Science, and Dr. Ademola Adenle, the Principal Investigator and Research Fellow at the United Nations University Institute of Advanced Studies (UNU-IAS) in Japan.

My Ph.D. graduates: Drs. B. Opere, O. Obidi, and L. Enurah, also my present Ph.D. students: Mrs. Ezech, Mr. Adeniyi, Mr. Ohai, Mr. Chukwu, Mrs. Omogolor and Mrs. Kareem.

I consider this my greatest legacy for the academic community. I remain very proud of all my past students here and abroad who are in this profession.

## CONCLUSION

The microbes existed before man, cohabit the planet with man and will remain after man has gone. It is indeed a microbial world. We are lucky to have them here with us as they form the foundation of the biosphere and we cannot exist without them. Their myriad of activities in their natural habitat is a clear manifestation that they can be our friends and heroes of our environment. The bad can become good, the ugly can be good and the good can be better. Therefore, we should appreciate them and sing their praise at all times for the future of the planet remains in their hands. They are small organisms with giant effects.

These heroes however are yet to find relevance in our environment as they remain **UNSUNG**. I look forward to seeing starter-culture produced “Gari” (fermented cassava) and “Ogi” (fermented maize or millet) on the



supermarket shelf and the baker's yeast or brewer's yeast replaced by isolates from our environment.

CREATURES OF GOD NOT SEEN BUT EVER NEAR,  
THEIR PRESENCE MAY WE FEEL.

## RECOMMENDATIONS

1. Please permit me to give a little illustration. It is common these days to receive an invitation to a social function which clearly states that the card admits. This implies that unwanted guests are not welcomed. This scenario must apply in our everyday life as we are all food handlers. Food handlers are the most common vectors of microbes in food, so if we invite them they will dine with us. The consequences may be good or bad as we have heard during the lecture. Good hygienic practices remain the only solution to be free from the unwanted UNSEEN GUESTS.
2. This same application is relevant to food vendors as eateries have found fertile ground in our environment. Their staff must have adequate training in food hygiene with full certification as proof.
3. Food industries must regularly train their staff on Good Manufacturing Practices and keep abreast of recent developments in pathogen detection and risk assessment. Quality assurance must be a priority. Universities can assist by running short courses in these areas to ensure compliance.
4. Regulatory bodies such as National Agency for Food and Drug Administration and Control (NAFDAC) and Standard Organisation of Nigeria (SON) must ensure and enforce microbiological standards in food products. These organisations can partner with Universities and Research Institutes for optimal results for consumer safety.
5. Food industries in this country must exploit the use of the friendly organisms in product development.
6. The Federal Government and all State Governments must ensure availability of potable water for all citizens and enforce adequate disposal of domestic, industrial and agricultural wastes.

## TAKE HOME MESSAGE

### HAND WASHING WITH SOAP – A “DO IT YOURSELF” VACCINE

The easiest and cheapest way to good health is to use a “do it yourself” vaccine. Washing hands with soap is that vaccine. WHY and HOW?

Human and animal faeces are the main sources of disease causing organisms (germs) responsible for diarrhea, cholera, typhoid fever, dysentery and related ailments. A small quantity (1gm) can contain millions of harmful bacteria and viruses. The domestic animals like goat, sheep, chicken, duck etc. act as reservoirs/carriers as they shed millions of cells but are not infected. Inadequate disposal of faecal materials makes it possible for pathogens to enter the environment. They are disseminated or spread by the four Fs: flies (animals), fingers (humans), fluids (water) and surfaces such as fields (environment).

The most important route of transmission is the faecal – oral route. Any form of contact with faeces such as after the use of the toilet or cleaning a child after defecation facilitate the spread of the pathogens. WHO estimates that diarrhea claims the lives of 1.87 million children under 5 years old in developing countries annually and is the second most common cause of death in children in that age bracket. Diarrhea and respiratory tract infections are the two biggest killers of children in developing countries. Hand washing helps to prevent the spread of these diseases. The ability to block routes of transmission is important as hands often act as vectors that carry disease causing pathogens from person to person through direct contact or indirectly via surfaces, food and water. Hand washing, therefore, breaks the disease cycle.

Hand washing with water alone removes only visible dirt, but water and soap form a formidable ally in efforts to combat a host of illnesses including helminthes (worms), eye infections like trachoma and skin infections such as Impetigo. Hand washing with soap is one of the most effective and least expensive ways to prevent such diseases. It reduces the rate of illness by removing bacterial pathogens found on hands and surfaces and others such as enteric viruses. The simple act of hand washing with soap can reduce the risk of diarrhea by 30-50% and respiratory illness by 21-45%.



UNICEF estimates that diarrhea kills one child every 30 seconds, thus, hand washing with soap prevents disease in a more cost effective way than any single vaccine. It, thus, presents the cornerstone of public health – an accessible “do-it-yourself” vaccine. Imbibing the habit can save more lives than any single vaccine or medical intervention. Studies showed that better hygiene such as washing hands with soap on entry into the home from outside, after using the toilet or before eating could cut the infection rate by 25%, respiratory infection in children under 5 years by 50% and skin infections by 34%. Hygienic practices can also offer solutions such as use of potable water, boiling or other treatments of raw water, care during preparation of food and adequate storage facilities for foods to prevent contamination. The isolation and safe disposal of human and animal faeces are also essential.

The way forward:

1. To promote the practice and make it a habit – Mum's advice about washing hands to be a guide.
2. The public and private sector must create effective large scale and sustainable hand washing promotion programmes as awareness strategies – MTN gives this message to their customers as there is a recent cholera epidemic in Nigeria. A study in the USA has found that 85% of adults washed their hands in public restrooms compared to 77% in 2007 as a result of an awareness campaign in the media over the years.
3. Education on good hygiene practices to those involved in child health like mothers, grandmothers, older siblings, caregivers in addition to children themselves.

Washing hands with soap and water for 20 seconds or more is a simple way to stay healthy. If you are out and about, hand sanitizers or hand wipes are good alternatives for keeping your hands clean.

The World Hand Washing Day is celebrated annually on 15<sup>th</sup> of October.

**Prof O. O. Aboaba**

Professor of Microbiology

Article culled from “By The Lagoon” December 2010.

## ACKNOWLEDGMENTS

I wish to express my appreciation first to God Almighty and to my parents who gave me life and a foundation on which my success stands. My father started as a teacher though later chose a career in printing and retired as the Acting Director of the Federal Printing Press. My mother was a teacher and retired as the Headmistress of St. Mary's Convent School, Lagos. The gene for teaching must have been acquired from them. My siblings, Chief (Mrs.) F.Y. Adegborioye, Mrs. O.T. Sobowale and Mr. B.O. Oguntuyo were part of my childhood and we were all privileged to have been given good education.

I must appreciate my secondary school classmates, most of whom have remained my lasting friends Prof. Molara Orafidiya, Drs. Mobola Ladenegan, Dayo Esan, Bola Holloway, Basirat Giwa and Tokunbo Dabiri. Also Mrs. Bola Ogunrinde, Mrs. Jumoke Araba, Mrs. Dayo Olaofe, Mrs. Ajuah Oyewole, Ms. Yele Lakeru, Mrs. Boma Doherty and Mrs. Nike Ajayi. I cannot forget my teachers in secondary school, Mrs. Abisogun Alo, Mr. Ayinde Ojemuyiwa and Mrs. Tolu Obajimi, among others.

My colleagues at the undergraduate level include Prof. Duro Ajeyalemi and Mrs. Ronke Salaam. This brings to mind Late Dr Jenmi ad Late Mrs. Uchenna Arinze, my partner in crime. My undergraduate lecturers: Professors A.W.A, Edwards, W. Odiete, T.V.I. Akpata, S. Ibe (who co-supervised my Ph.D.), S. Mabadeje, G.O. Williams, T. Okusanya and others. I must particularly mention Late Prof. J.A. Ekundayo, who supervised my B.Sc. project and Ph.D. He initiated me into academics and was a wonderful mentor and teacher. May his soul continue to rest in perfect peace, amen. My path crossed the following during my graduate studies: Drs Taiwo Odubela and Bola Opere, Mr Edward Aror, Professors Egonmwan, Chendo, Nwankwo, Omidiji

and Alo: they remain close academic associates and friends, So also other colleagues in the Biological Sciences and the entire faculty including non-teaching staff. I must appreciate the six of us that realised late Prof. J.A. Ekundayo's dream when Microbiology became a Unit under Biological Sciences. They are Professors Akpata, Amund, Ugoji, Nwachukwu and Moni Taiwo whose precious and fond memories remain with us since her exit from this world.

My sincere appreciation to the Management of the University for the award of scholarship for my Ph.D. and for making a dream come true. Special mention is made of Prof. Babatunde Sofoluwe of blessed memory who initiated the move to the professorial cadre when he was the Dean of Science. He was the Vice-Chancellor when my professorial chair was obtained. I thank the present management team under the leadership of Prof. Rahaman A. Bello and those before them for tolerating me; also at the faculty and departmental levels for a greater rate of tolerance.

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My greetings to the members of the Oguntuyo and Aboaba families here present. Of particular mention is Prof. Fola Aboaba, a retired Professor from the University of Ibadan and one time Secretary General of the Committee of Vice Chancellors; Mr. Akintade Aboaba for his support during my many trips to America. My immediate family, Olubunmi, Olusola, Afisurugbola and Oyinloluwa: for their patience and understanding during those years of Mummy's struggle in the world of academics. My latest additions, Olayinka and Morifeoluwa have added joy to my home.

The HERO of today, though an unseen guest at this gathering, is my husband, Olumuyiwa Aboaba. He made today's lecture possible as his commitment to my academic progress remained unwavering till he breathed his last. Mr. Vice-Chancellor, Sir, permit me to dedicate this lecture to his memory.

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