BACTERIAL SPOILAGE OF FRESH MEAT IN SOME SELECTED LAGOS MARKETS

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Abstract

A study of the bacteria associated with spoilage of fresh meat was carried out. The flora causing spoilage of meat include *Alcaligenes liquefaciens, Bacillus subtilis, Clostridium perfringes, Escherichia coli, Klebsiella pneumoniae, Lactobacillus sp., Micrococcus varians, Pseudomonas aeruginosa, Sarcina sp. Serratia liquefaciens and Staphylococcus aureus. Pseudomonas aeruginosa* was the most dominant of the isolated species. It was able to utilize glucose as its primary carbon source and grew faster at 4 °C than the other meat spoilage organisms. While surface colonies were visible by the third day of incubation, strong spoilage odours were evident by the seventh day on fresh meat inoculated with isolated bacterial cultures. Greening of the fresh

1. Introduction

Meat has long been regarded as a nutritious and highly desirable food, but recently in more affluent countries the role of meat as a basic foodstuff has changed. By its very nature and origin, meat is not only highly susceptible to spoilage but also frequently implicated in the spread of food borne diseases (Jones, 1993). Because of its high water activity of 0.99, meat is an ideal culture medium for many organisms. It is rich in nitrogenous food of various degrees and complexity, plentifully supplied with minerals and accessory growth factors, as well as having some fermentable carbohydrates and a favourable pH for most micro-organisms (Frazier and Westhoff, 1978).

Bacteria are thought to be present in the tissues at the time of death or to be disseminated throughout the carcass from the intestine after death (Ingram and Dainty, 1971; Lawrie, 1974). Bacterial contamination of fresh meats, however, can occur during normal slaughter and handling procedures, although this contamination can be minimized by adherence to good hygienic practices during slaughter (Smith *et al.*, 1976; Chandran *et al.*, 1986; Gill, 1987; Dixon *et al.*, 1991) since there is considerable evidence that post-mortem invasion of carcass tissues by intestinal bacteria does not occur until at least several hours after death, when breakdown of the intestinal tissues allows release of bacteria into the body cavity (Koneman, 1970; Gill *et al.*, 1978).

Muscle foods, such as meat and poultry, are described as spoiled if organoleptic changes make them unacceptable to the consumer. These organoleptic characteristics may include changes in appearance (discolouration), the development of off odors, slime formation, changes in taste, or any other characteristic which makes the food undesirable for consumption (Jay, 1996; Jackson *et al.*, 1997). While endogenous enzymatic activity within muscle tissue postmortem can contribute to changes during storage (Koohmaraie, 1994; Jackson, *et al.*, 1997; Alomirah, *et al.*, 1998; Schreurs, 2000), it is generally accepted that detectable organoleptic spoilage is a result of decomposition and the formation of metabolites caused by the growth of microorganisms (Kakouri and Nychas, 1994; Nychas and Tassou, 1997). The organoleptic changes which take place will also vary according to the species of microflora present, the characteristics of the meat, processing methods, product composition, and the environment in which the food is stored (Jackson *et al.*, 1997).

According to Borch *et al.* (1996), Korkeala and Björkroth (1997), and Björkroth *et al.* (1998), some spoilage bacteria produce such typical changes as souring and the formation of gas, slime, and/or white liquid. Alonge (1982) reported that the quality of meat which is not just an attractive appearance includes characteristics such as tenderness, flavour, freshness, and wholesomeness which are affected by the quantity and quality of the microbial growth causing chemical changes resulting in the unacceptability of meat to the consumers due to slime, souring and putrid odours. This spoilage is commonly detected by sensory and/or microbiological analysis (Dainty, 1996) or by the measurement of chemical changes associated with the growth of specific spoilage organisms in meat and meat products (Jay, 1986; Dainty, 1996; Nychas

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et al., 1998). Among these changes, glucose, gluconic acid, D- and L- lactic acid, acetic acid and ethanol have been proposed as potential indicators of spoilage (Kakouri and Nychas, 1994; Boers *et al.*, 1994; Seymour *et al.*, 1994; Dainty, 1996; Lambropoulou *et al.*, 1996). Gill and Newton (1977) reported that moist fat will spoil more rapidly than normal muscle tissue where the bacteria must reach a cell density in excess of $10^8/cm^2$ before glucose is exhausted at the meat surface and amino acids are attacked.

From studies with meat, although low in comparison with those of protein and lipids, the concentrations of compounds such as glycogen, glucose and lactate are all sufficient to support massive microbial growth. These compounds can affect the type (e.g. saccharolytic, proteolytic) and rate of spoilage and, moreover, seem to be the principal precursors of those microbial metabolites that we perceive as spoilage (Nychas *et al.*, 1998). While Gill & Newton, (2000) and Lung *et al.*, (2000) reported that the environment to which the meat are exposed determine the type of bacteria, their rate of growth and maximum density which is attained, the significance of production of extracellular proteolytic enzymes (Ansay *et al.*, 1999), temperature, pH and oxygen, which affect the type of flora and the type of enzymes that are produced (Frazier and Westhoff, 1978) have been emphasized as factors influencing the type of bacterial degradation that occurs in a particular meat product. There appears to be more reported cases of microbial meat spoilage outside Nigeria. This study was carried out in order to identify and characterize bacteria associated with spoilage of fresh meat in retail markets in some markets in Lagos, Nigeria.

2. Materials and Methods

Different parts of beef meat such as kidney, liver, intestine and muscles were obtained from different retail markets in Lagos, South Western Nigeria during mid-morning (10.00-11.45 am). These samples were brought into the laboratory in sterile loosely tightened screw capped McCartney bottles. The isolation of bacteria was carried out by aseptically transferring 1 g of each meat sample from McCartney bottles with a sterile inoculating needle into sterile peptone water in test tubes. The test tubes were covered with cotton wool, wrapped with foil paper and then incubated at 35 °C for 24h. Growths were observed in the test tubes with changes in colour of the peptone water as well as turbidity. Using a sterilized inoculating wire loop, cultures in peptone water were streaked on different culture media, viz Nutrient Agar, Eosin Methylene Blue Agar, McConkey Agar, Potato Dextrose Agar and Chocolate Agar and then incubated at 35 °C for 24h. To obtain pure cultures of isolates, repeated subculturing of organisms from mixed cultures in original plates was done. Isolated pure cultures were kept in the refrigerator at -20 °C as stock cultures. Also, pieces of meat samples of about 1 g were each aseptically removed, shaken with 10mL of peptone water and serial dilution of the resultant suspensions were spread on Nutrient Agar. The agar plates were incubated at 35 °C for 24h. Bacterial cells numbers were determined by counting colonies on plates of suitably diluted samples. Identification of the isolates was made with the aid of Bergey's Manual of Determinative Bacteriology, (8th ed., 1974) and Singleton's Bacteria in Biology, Biotechnology and Medicine, (4th ed., 1998). While characterization of isolates were done using Gram's staining method, further identification was conducted using biochemical tests as well as their colonial cultural characteristics.

The ability of the isolated bacteria to cause spoilage was determined. Two grammes of fresh meat were obtained from the same stalls as before. These pieces of meat were washed with 70% alcohol three times and kept separately in different loosely closed sterile McCartney bottles where they were then inoculated with various already characterized bacterial isolates before being incubated at 35 °C for 24h. Those McCartney bottles containing alcohol washed meat inoculated with *Lactobacillus sp.* and *Clostridium putrefaciens* isolates were tightly closed to create an anaerobic environment. A control set up was also maintained by incubating uninoculated petri dishes under the same condition. Sterile Nutrient Agar plates were streaked with each of the samples to compare their morphological and cultural characteristics with the control experiment. All inoculations were carried out in the inoculating hood sterilized with ultraviolet light. These inoculated meat samples were kept for seven days and examined daily for physiological changes resulting from decomposition as well as odour production. After this incubation period, pieces of meat samples with bacterial growth were each aseptically removed and shaken with 10 mL of peptone water and serial dilutions of the resultant suspensions were spread on nutrient agar. The agar plates were incubated at 35 °C for 24h. Bacterial numbers were again determined by counting colonies on plates of suitably diluted samples.

3. Results

Total microbial counts on selected meat parts were determined with isolation plates incubated at 35 °C for 24 hours. The bacterial count per gram is shown in Table 1 with the intestine recording the highest, 3×10^5 bacterial count/gram. Table 2 shows colony counts resulting from inoculation and incubation of sterilized meat parts for seven days. Meat examined shortly after they were removed from the carcasses

Table 1: Bacterial count from four cow parts incubated at 35 °C for 48 hours.

s/n	Meat parts	Bacterial count/gram
1.	Flesh/tissue	1×10^3
	Liver	1×10^4
	Kidney	1×10^3
	Intestine	3×10^5
2.	Flesh/tissue	1×10^4
	Liver	1×10^4
	Kidney	50-100
	Intestine	3×10^5
3.	Flesh/tissue	1×10^3
	Liver	1×10^4
	Kidney	1×10^3
	Intestine	3×10^5

Table 2: The bacterial isolate concentration (cfu/mL) after incubation at 35 °C for 7 days

Organisms	CFU/mL
Alcaligenes spp	2×10^7
Bacillus subtilis	1×10^8
Clostridium perfringes	2×10^8
Escherichia coli	5×10^8
Klebsiella pneumoniae	2×10^6
Lactobacillus spp	4×10^6
Micrococcus varians	3×10^7
Pseudomonas aeruginosa	2×10^7
Sarcina spp	2×10^8
Serratia liquefaciens	2×10^8
Staphylococcus aureus	5×10^8

carried bacteria on the surface at a density of about 1×10^3 bacteria/gram while the bacterial load of the intestine was 3×10^5 bacteria/gram as shown in Table 1. When the freshly obtained meat parts washed in 70% alcohol were infected with the bacterial species isolated, spoilage began with the development and appearance of visible colonies on the infected surfaces. When the colonies were small, no spoilage odours were evident, but these developed as the size of the colonies increased. Surface colonies were visible by day 3 and strong spoilage odours were evident by day 7. Observation of specimens after this incubation period indicated that bacterial numbers on the surface is several times greater than the bacterial count taken shortly after they were removed from the carcasses as seen in Table 2. Green colouration was also observed on fresh meat inoculated with *Alcaligenes putrefaciens* during the incubation period. On characterizing the isolates from the contaminated freshly obtained meat parts, pure cultures were obtained and it was observed that isolated organisms were similar to those initially characterized isolates used to inoculate.

The surface flora varied considerably in composition. In eight of the meat parts investigated, including the flesh, liver, kidney and intestine, the flora were dominated by *Pseudomonas aeruginosa* (eight cases; 25.9%) followed by *Staphylococcus aureus* (six cases; 19.4%) and *Klebsiella pneumoniae* (five cases; 16.1%). Other organisms present as substantial fraction include *Escherichia coli* (four cases; 12.9%), *Alcaligenes putrefaciens* (one case; 3.2%), *Bacillus subtilis* (one case; 3.2%), *Lactobacillus spp* (one case; 3.2%), *Sarcina spp* and *Serratia liquefaciens* (one case; 3.2%). In all cases, the surface flora most frequently isolated were *Pseudomonas aeruginosa*, followed by *Staphylococcus aureus and Klebsiella pneumoniae* in that order while *Escherichia coli* was isolated from all the intestine samples collected as shown in Table 3 and Fig. 1.

4. Discussion

From this study, eleven different bacteria were isolated and identified. These are *Alcaligenes liquefaciens, Bacillus subtilis, Clostridium perfringes, Escherichia coli, Klebsiella pneumoniae, Lactobacillus sp., Micrococcus varians, Pseudomonas aeruginosa, Sarcina sp., Serratia liquefaciens and Staphylococcus aureus. Pseudomonas aeruginosa, Alcaligenes liquefaciens, Lactobacillus sp., Sarcina spp,* and *Serratia liquefaciens* have been implicated in meat spoilage (Gill and Newton, 1978; Dainty *et al.*, 1983; Hall *et al.*, 1988; and Brown and Weidemann, 2003). While the surface flora were dominated descendingly by mesophilic organisms from *Pseudomonas aeruginosa, Staphylococcus aureus* to *Klebsiella pneumoniae*, the significance of *Klebsiella pneumoniae* and *Sarcina spp* in meat spoilage require further study. However, their involvement in meat spoilage along with other isolated microorganisms was demonstrated in this study. One important factor contributing to the domination by *Pseudomonas aeruginosa* is its ability to grow faster at low temperatures than the other common meat spoilage organisms as well as utilizing glucose as its primary carbon source (Gill and Newton, 1977).

Bacterial spoilage of meat was manifested in a number of ways including off odours, slime, rancidity of fats, discolouration and phosphorescence. The spoilage becomes apparent when the surface count exceeds 10^7 organisms per gram. While the slime might be caused by species of *Pseudomonas, Alcaligenes, Streptococcus* and *Micrococcus,* rancidity of fat may be caused by lipolytic species of *Pseudomonas* and *Alcaligenes*. Many organisms associated with low-temperature spoilage, such as *Pseudomonas* and *Enterobacter,* produce malodorous compounds when degrading amino acid. According to Gill (1976), the onset of spoilage in meat at normal pH is delayed until the available glucose has been consumed while Dainty *et al.* (1983) and Brown and Weidemann (2003) observed that only then are amino acids attacked, with the production of ammonia as spoilage odours.

Table 3: List of isolated bacteria from the surface of each part of the sampled meat

Sample	Flesh	Liver	Kidney	Intestine
1	*Klebsiella pneumoniae Staphylococcus aureus Bacillus subtilis Alcaligenes spp Clostridium perfringes Pseudomonas aeruginosa*	*Pseudomonas aeruginosa Lactobacillus spp Bacillus subtilis Staphylococcus aureus*	*Staphylococcus aureus Pseudomonas aeruginosa*	*Klebsiella pneumoniae Escherichia coli Sarcina spp*
2	*Serratia liquefaciens Pseudomonas aeruginosa*	*Bacillus subtilis Klebsiella pneumoniae Staphylococcus aureus*	*Pseudomonas aeruginosa Klebsiella pneumoniae*	*Serratia liquefaciens Escherichia coli Staphylococcus aureus*
3	*Lactobacillus spp Escherichia coli Staphylococcus aureus Klebsiella pneumoniae Pseudomonas aeruginosa*	*Klebsiella pneumoniae Pseudomonas aeruginosa Micrococcus varians*	*Staphylococcus aureus Serratia liquefaciens*	*Pseudomonas aeruginosa Escherichia coli*

Note: Samples 1-3 represent the market outlets from which meat samples were obtained.

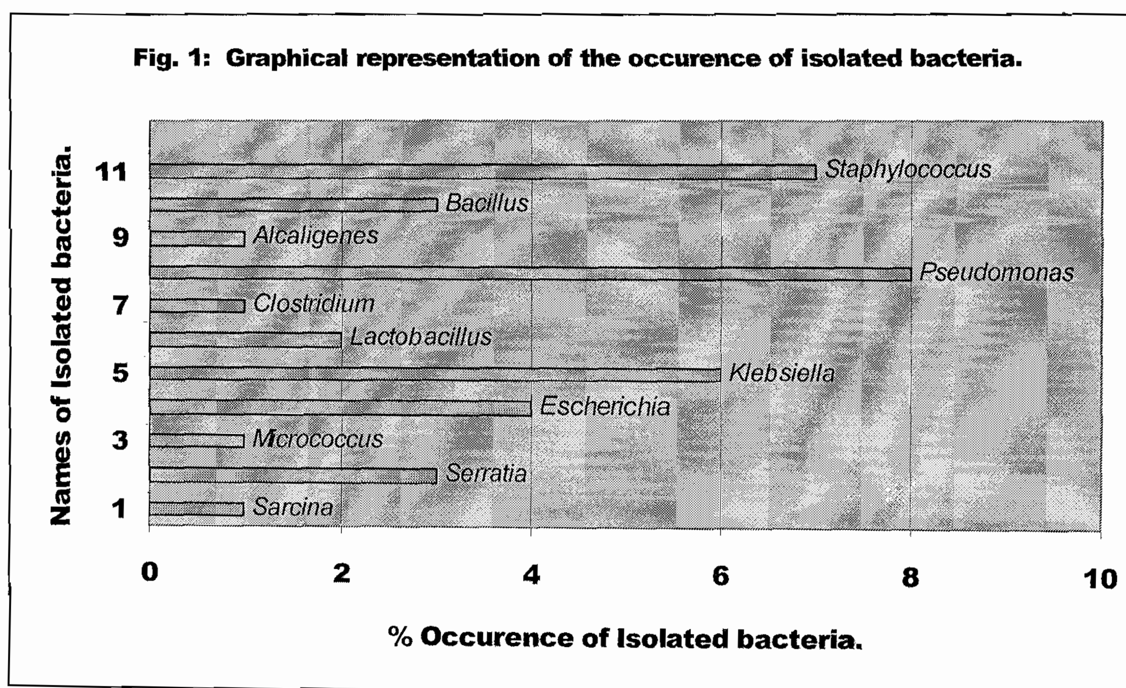

Fig. 1: Graphical representation of the occurence of isolated bacteria.

Although *Serratia liquefaciens* has been reported to be detected more frequently and in greater numbers than other members of the *Enterobacteriaceae* (Seideman *et al.*, 1976; Patterson & Gibbs, 1977; Cox *et al.*, 1979), this was not observed in this study as *Serratia liquefaciens* was not isolated from all the meat samples investigated. Rather, *Pseudomonas aeruginosa* was isolated and detected in higher percentage. During this investigation, green meat colouration was observed due to *Alcaligenes putrefaciens*. In an earlier report by Lea *et al.* (1969), the production of green colouration in treated meat was due to *Alcaligenes putrefaciens*. Their report suggested the utilization of Cystein to produce hydrogen sulphide due to protein degradation and glutathione which are rapidly oxidized under aerobic conditions, but would not occur in anaerobic environments.

The significance of the spoilage of meat by these isolates may not be underestimated. Meat, an important source of protein, susceptible to invasion by microorganisms, should be properly and carefully handled during slaughtering, dressing and cutting to avoid bacterial contamination as well as reducing

the resultant danger of food borne illness in consumers. Also, since bacteria grow more slowly on meat at a more acidic pH, washing carcasses with organic acids, trisodium phosphate or alkaline solutions will significantly decrease the pathogen levels on meat. While many of these bacteria still grow at chilling temperatures with some members of viable cells decreasing with time during cold storage, vacuum packaging of meat will prevent spoilage by *Pseudomonas aeruginosa* although facultative anaerobes such as *Lactobacillus spp* are not prevented from growing. In addition, the isolation of *Escherichia coli* which is capable of causing diarrheal disease is an indication of faecal contamination. Hence, meat contamination must be reduced to the lowest practicable level in order to have a reasonable storage life in the hands of consumers.

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