Advances in Meat Research

Meat and Poultry Microbiology

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Preface

Fresh meat and poultry not only provide ideal media for the growth of spoilage microorganisms but they are frequently involved in foodborne illnesses. The role of meat and poultry products in the transmission of pathogenic microorganisms to human beings has been better elucidated in recent years, leading to improved methods for controlling their growth on these important foods. Consequently, the present volume is devoted to providing the most up-to-date information on the microbiology of these products to researchers in universities, governmental agencies, and industry in order to minimize both meat spoilage and the illnesses transmitted to human beings.

Topics covered include the whole gamut of microorganisms found on meat and poultry products, with emphasis on their sources and methods for controlling their growth. In view of the importance of certain groups of microorganisms, they have received special emphasis, i.e., sporeforming bacteria, salmonellae, psychrotrophs, and staphylococci. Chapters are also devoted to parasitic organisms, miscellaneous pathogenic bacteria, fungi and mycotoxins, and to viruses in meat and poultry products.

Two other important topics discussed are the microbiology of ready-to-eat and fermented meat and poultry products. These two chapters not only cover the best method of controlling growth of microorganisms in processed products, but in the latter case demonstrate how certain species of microorganisms can be utilized to produce special products that are relatively shelf stable. The use of desirable microorganisms as an aid to preservation is an interesting and virtually untouched area

for future research. This brings us to the last chapter, which emphasizes perspectives for future research that can be useful in improving the shelf life and in preventing meat and poultry food-borne illnesses.

A. M. Pearson
T. R. Dutson

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Microbial Ecology of Meat and Poultry

F.H. Grau[1]

INTRODUCTION

Meat animals may be regarded as a source of edible tissue sandwiched between two regions that are heavily contaminated with microorganisms. These two regions are the external layer of skin, hair, wool or feathers and the internal intestinal tract and its contents. The first aim of the abattoir is to harvest the edible tissue (meat) from between these highly contaminated layers with as little contamination as possible. Second, care is needed to ensure that contamination of dressed carcasses and edible offals from sources within the abattoir itself is kept to a minimum. Subsequent procedures for the handling of meat through chilling, freezing, cooking, packaging, and distribution to the consumer are aimed at reducing or preventing increases in the microbial content that may occur either by growth or by further contamination. The only time that the growth of particular organisms is encouraged is when it is desired to manufacture a fermented product (e.g., salami).

The relative importance of the wide variety of microorganisms that may contaminate meat during its production is dependent on the type of microorganism and the subsequent treatment of the meat.

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GENERAL BACKGROUND

Classification of Microorganisms

Viruses are noncellular entities composed of nucleic acid, either deoxyribonucleic or ribonucleic, wrapped in a protein coat. They are obligate parasites having to grow within an appropriate host cell (Chapter 12). Viruses are not considered further here.

Bacteria are typically unicellular, lack a nuclear membrane, reproduce predominantly by binary fission, and are spherical, rodlike or spiral in shape. Gram-positive bacteria have a single-layered thick cell wall. They retain crystal violet during the gram stain reaction and appear deep purple-violet in color when examined after this procedure. Gram-negative bacteria have a triple-layered thinner cell wall, lose crystal violet during the gram stain reaction, and when conterstained with safranine appear red. Molds and yeasts have a nuclear membrane and internally are structurally more complex than are bacteria. Molds are generally filamentous and yeasts generally unicellular. Of these three categories of microorganisms, bacteria have received the most attention as contaminants of meat.

Microorganisms can also be classified or grouped, by their temperature and water requirements for growth, and by their metabolic response to oxygen.

Temperature Requirements

Psychrotrophic microorganisms are able to grow at normal refrigeration temperatures (Eddy 1960). Psychrotrophic pseudomonads are able to grow down to −3°C (Barnes 1976), and some psychrotrophic molds down to about −5° to −7°C (Barnes 1976; Gill and Lowry 1982). The temperature for optimum growth of psychrotrophs is at or above 20°C, but many psychrotrophs will not grow at 35°–37°C. Psychrophiles are also able to grow at refrigeration temperatures but have an optimum temperature for growth at 15°C or less and a maximum temperature for growth at about 20°C (Morita 1975). The microorganisms that grow on chilled meat appear to be psychrotrophs and not psychrophiles. Mesophilic microorganisms are not able to sustain growth below about 5°–7°C and grow optimally at 30°–40°C. Thermophiles have even higher minimum and optimum temperatures for growth, often failing to grow below about 35°–40°C and being able to grow up to 80°C and above. Facultative thermophiles, or thermotrophs, have minimum and optimum growth temperatures that fall between those for mesophiles and thermophiles (Mossel 1977). The distinction between these groups is not sharp since there is a gradual merging of the minimum, optimum, and maximum temperatures for growth of different microorganisms.

Water Requirements

Microorganisms cannot grow without water. The water requirements for microbial growth are best specified in terms of water activity (Scott, 1957, 1962). Water activity (a_w) is the chemical potential of the water in a food or solution relative to that of pure water at the same temperature and pressure. The a_w of a food is thus equal to p/p_0, where p and p_0 are the water vapor pressures of the food and of pure water, respectively, at the same temperature. Thus pure water has an a_w of 1.00. Freezing point, boiling point, equilibrium relative humidity, osmotic pressure and a_w are all related to the colligative properties of the number of molecules and ions in solution.

As aqueous solutions are cooled below 0°C, pure ice will form at the freezing point and the solutes remain in the liquid phase. The ice and the liquid phase are in equilibrium and both have the same vapor pressure. The vapor pressure of the ice depends only on the temperature of the ice. The ratio vapor pressure of pure ice at the freezing point to that of pure water at the same temperature is the a_w. Lean beef has an a_w of 0.993 and will begin to freeze at about −0.8°C. As the temperature of frozen meat decreases the remaining unfrozen solution will have a lower a_w.Thus, at −5°C the unfrozen liquid in meat will have an a_w of 0.953 (vapor pressure of ice at −5° ÷ vapor pressure of water at −5°C). For an organism to be able to grow on frozen meat at −5°C, the organism will have to be able to grow not only at −5°C but also at an a_w of 0.953. Similarly, at −10°C, the organism would need to be able to grow at an a_w of 0.907 (Scott 1962). Such a combination of temperature and water activity must be sufficient to prevent microbial growth since microbial growth does not occur in frozen foods below about −10°C.

The most rapid growth of many microorganisms, including pathogenic bacteria, is at an a_w in the range 0.995–0.980. The approximate minimum values of a_w allowing growth of some microorganisms when other growth conditions are at or near the optimum are given in Table 1.1. A more detailed list is given by Troller and Christian (1978). Halophilic bacteria require sodium ions and often need substantial amounts of sodium chloride for growth. The optimum sodium chloride concentrations for the growth of some halophiles corresponds to an a_w of 0.80 to 0.85 (Scott 1962; Christian 1980). Halophilic bacteria are common in the cover brine used in the manufacture of Wiltshire-style bacon (Gardiner 1982) and halophilic *Vibrio* spp. can cause spoilage of bacon (Gardiner 1980–1981). The term osmophilic is usually applied to yeasts that are able to grow in high concentrations of sugars. Pitt (1975) defined xerophilic fungi as those yeasts and molds able to grow at a_w values below 0.85, thus including osmophilic yeasts in his definition.

As the water activity of meat decreases, either by drying (e.g., during chilling of carcasses and the production of fermented sausages) or by the

TABLE 1.1. Approximate Minimal a_w for Growth of Some Microorganisms Found on Meats

Organism	a_w	Reference
Bacteria		
Pseudomonas fluorescens	0.97	Wodzinski and Frazier (1960)
Enterobacter aerogenes	0.95	Wodzinski and Frazier (1961A)
Escherichia coli	0.95	Burcik (1950)
Salmonella spp.	0.95	Christian and Scott (1953)
Lactobacillus brevis	0.95	Lanigan (1963)
Clostridium perfringens	0.95	Troller and Christian (1978)
Clostridium botulinum A	0.95	Troller and Christian (1978)
Clostridium botulinum B	0.94	Troller and Christian (1978)
Brochothrix thermosphacta	0.94	Brownlie (1966)
Lactobacillus viridescens	0.94	Wodzinski and Frazier (1961B)
Pediococcus cerevisiae	0.94	Lanigan (1963)
Lactobacillus plantarum	0.93	Lanigan (1963)
Micrococci	0.75–0.94	Christian and Waltho (1962)
Staphylococcus aureus	0.86	Scott (1953)
Vibrio costicolus	0.86	Troller and Christian (1978)
Yeasts		
Trichosporon pullulans	0.89	Scott (1957)
Candida spp.	0.88–0.90	Bem and Leistner (1970)
Debaryomyces spp.	0.87	Bem and Leistner (1970)
Molds		
Mucor sp.	0.93	Leistner and Rödel (1975)
Rhizopus sp.	0.93	Leistner and Rödel (1975)
Cladosporium cladosporoides	0.86–0.92	Gill and Lowry (1982)
Penicillium spp.	0.79–0.83	Pitt (1975)

addition of salt, the growth of gram-negative nonhalophilic bacteria is repressed first. Gram-positive lactic acid bacteria and micrococci then tend to be the principle flora capable of fastest growth. For example, the addition of 2 to 3% sodium chloride to fresh meat (a_w, 0.98–0.975) is sufficient to reduce the growth rate of pseudomonads but still allow rapid growth of lactobacilli. Under conditions of even lower a_w, for example, below about 0.93, micrococci, yeasts, and molds tend to dominate the flora (e.g., on the surface of dried sausages).

Oxygen Requirements

Aerobic microorganisms (e.g., pseudomonads) couple the oxidation of a substrate to the reduction of oxygen by means of a respiratory chain containing cytochromes. The flow of electrons through the respiratory chain to oxygen results in the formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and phosphate. ATP provides energy for growth and metabolic processes. Some aerobic organisms are also able to synthesize a modified respiratory chain that is able to use nitrate (or nitrite) instead of oxygen as the terminal electron acceptor. Microaerophilic organisms are aerobes which grow best at oxygen concentrations less than that found in air [e.g., *Campylobacter jejuni (coli)*]. The

concentration of oxygen in air may be bacteriostatic or bacteriocidal for such organisms.

Obligately anaerobic organisms are not able to synthesize the components of an oxygen (or nitrate)-linked respiratory chain. Such organisms obtain energy for growth by fermentation in which a variety of organic compounds, carbon dioxide, hydrogen, and sulfur compounds are involved in oxidation-reduction reactions. The yield of ATP, and therefore of cells, per mole of substrate metabolized is less than that obtained by aerobes. Many anaerobes cannot tolerate oxygen and are killed by exposure to air. Such strict anaerobes lack both catalase and superoxide dismutase activity needed to metabolize potentially lethal hydrogen peroxide and the superoxide radical 0_2^- formed by the oxidation of electron carriers such as reduced flavins, quinones, and iron-sulfur proteins by oxygen. The mere absence of oxygen is often not sufficient to ensure growth of many strict anaerobes. A low redox potential is also needed (e.g., for *Bacteroides* spp.).

Facultative anaerobes (e.g., Enterobacteriaceae) are able to grow aerobically using a respiratory chain coupled to oxygen, and anaerobically using fermentation reactions for the generation of energy.

Importance of Microorganisms

Microorganisms on meat are important for three reasons: (1) some organisms may be pathogenic (able to produce disease in man); (2) some may cause spoilage of meat; and (3) some may be used as indicator organisms.

Food Poisoning. The principal pathogenic organisms of concern on meats are those able to cause food poisoning. Food poisoning is caused by two different mechanisms. In food-borne infections, the organisms are ingested with the food and grow in the intestinal tract causing diarrhea, vomiting, nausea and/or abdominal pain. Members of the genus *Salmonella, Yersinia entercolitica, Clostridium perfringens,* and *C. jejuni (coli)* all cause infective food poisoning. While growth of these organisms is usually necessary to produce sufficient viable cells to form an infective dose, it is unlikely that this applies to *C. jejuni* on meats. An infective dose of *C. jejuni* can be only a few hundred cells (Robinson 1981). Growth of *C. jejuni* requires a temperature between 32° and 45°C (Skirrow and Benjamin 1980; Doyle and Roman 1981), and an atmosphere higher in carbon dioxide and lower in oxygen than occurs in air, i.e., it is microaerophilic (Doyle 1981). In the second type of food poisoning, the microorganisms grow in the food and excrete a toxin which causes intoxication when the food is ingested (e.g., *Cl. botulinum, Staphylococcus aureus, Bacillus cereus*). The lower limit of temperature for growth of nonproteolytic type B and the aquatic type E *Cl. botulinum* is 3°–4°C. *Yersinia enterocolitica* is able to grow at refrigeration temperatures, and incubation temperatures of 4°C are used in its isolation. The other food-poisoning bacteria are either mesophilic or facultative thermophiles and do not grow below about 7°C.

Spoilage. Spoilage of chilled, raw meats in air is caused mainly by the growth and metabolic activities of psychrotrophic aerobic organisms such as pseudomonads, moraxellas, and altermonads. When raw meats are vacuum-packaged, the principle microflora that develops during chilled storage consists of lactic acid bacteria together with a varying population of *Brocothrix thermosphacta,* psychrotrophic Enterobacteriaceae, *Aeromonas* spp. and *Altermonas* spp. The extent of the population of these latter groups of organisms on vacuum-packaged meat is dependent on the pH of the tissue, oxygen permeability of the packaging film, and temperature of storage. All of these organisms can play some role in the deterioration of vacuum-packed fresh meat.

On cooked cured meats, lactic acid bacteria, *Br. thermosphacta,* micrococci, yeasts, and molds can cause spoilage.

Indicator Organisms. Frequently estimates of the numbers of aerobic mesophiles, Enterobacterieaceae, coliforms, fecal coliforms, and *Escherichia coli* are made on carcasses at slaughter. These counts can be regarded as counts of indicator organisms.

The number of mesophiles on carcasses is usually taken as an indicator of the degree of care taken in sanitation during slaughter and dressing (Johnston and Elliot 1976; Roberts *et al.* 1980) even though very few of the organisms will actually grow on meat. Because of differences in the processes of slaughtering and dressing of the animal species used for meat, the significance of the mesophilic count will not be the same for all meats. In the production of pig and poultry carcasses, the skin is not removed so that the number of organisms on the skin is a reflection of the destruction of organisms by scalding (and singeing) and of recontamination in the abattoir. On sheep and cattle carcasses, the number of mesophiles is a consequence of contamination of a surface which was sterile before removal of the skin or viscera. The number of mesophiles detected on carcasses will vary with the temperature of incubation used during their enumeration. Incubation temperatures of 35°–37°C give somewhat lower counts than are found at 20°–30°C (Barnes 1976; Ingram and Roberts 1976; Roberts *et al.* 1980), but the high temperatures have the advantage in that the estimate is obtained more quickly.

In general, procedures in slaughtering which reduce the mesophilic count on carcasses would be expected to reduce the count of psychrotrophs. For instance, scalding reduces the numbers of both mesophiles and psychrotrophs on poultry skin (Notermans *et al.* 1977). However, during later stages of poultry processing the count of mesophiles on the carcass appears to bear little relationship to the psychrotrophic count. In the stages after evisceration, when there may be no significant increase in mesophiles, considerable increases in psychrotrophs occur. While there was no significant difference in the mean count of mesophiles on carcasses produced at two abattoirs, the mean counts of psychrotrophs differed by more than 100-fold (Notermans *et al.* 1977).

The count of psychrotrophs also varies in relation to the count of mesophiles for carcasses of other animal species. For example, psychrotrophs have been found to range from 32% to less than 0.03% of the count of mesophiles (20°C) obtained from one site on sheep carcasses at one abattoir over a 2-week period (F.H. Grau unpublished). Nevertheless, on average there is a correlation between the counts of mesophiles and psychrotrophs for cattle and sheep carcasses. When pooled samples of five sites from each of 63 sheep carcasses were examined over a 3-month period, the correlation coefficient between counts of meophiles and psychrotrophs was 0.50 (F.H. Grau unpublished). While this is not a high correlation, it is statistically significant. The ratio of psychrotrophs to mesophiles on beef carcasses is lower in summer and in warmer climates since this ratio shows a negative correlation with ambient temperature (Empey and Scott 1939). For instance, the mean count of psychrotrophs expressed as a percentage of the mean count of mesophiles on sheep carcasses is 2.7 to 10% in the United Kingdom (Roberts 1980; Roberts *et al.* 1980), 0.3 to 4.7% in New Zealand in spring-summer (Newton *et al.* 1978), and about 0.2% in Southeastern Queensland (Australia) in spring-summer (F. H. Grau, unpublished). The count of mesophiles can be a useful, although necessarily crude, indicator of psychrotrophic contamination on beef and sheep carcasses at the completion of slaughter and dressing. A better indicator of the keeping quality of meat which is to be stored under aerobic, chilled conditions is a direct count of psychrotrophs.

In general, the majority of aerobic mesophiles that contaminate carcasses do not originate from the intestinal tract but from the outer surfaces of the animals and from equipment in the abattoir. There may be little significant change in the count of mesophiles as a result of evisceration but contamination with organisms from the intestinal tract can occur (Grau 1979; Gerats *et al.* 1981). There appears to be no clear relationship between the count of mesophiles on carcasses and the numbers of Enterobacteriaceae, coliforms or fecal streptococci (Roberts *et al.* 1980). Thus, the count of mesophiles does not appear to be a particularly good indicator of possible contamination of the carcass with organisms, including pathogens, which may have arisen from the intestinal tract.

Counts of Enterobacteriaceae, coliforms, fecal coliforms, and *E. coli* have been used as indicators of contamination of carcasses with organisms associated with intestinal tract contents. The detection of such organisms on carcasses does not a priori indicate direct contamination from the intestinal tract during slaughter since these organisms, along with salmonellae, are frequently found on the outside surfaces of animals (Hess and Lott 1970; Smith and Grau 1973; Notermans *et al.* 1977; Grau 1979; McBride *et al.* 1980). Although there is usually not a very large difference between counts of Enterbacteriaceae, coliforms, and *E.coli* obtained from intestinal tract contents (Dickinson and Mocquot, 1961; Grau 1979), Enterobacteriaceae on the outside surfaces of animals are often 100- to 1000-fold more numerous than *E.coli* (Notermans *et al.* 1977; Grau 1979). Both the