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Lactic acid bacteria of meat and meat products

AUBREY F. EGAN

CSIRO Division of Food Research, Meat Research Laboratory, P.O. Box 12, Cannon Hill, Queensland, 4170, Australia

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When the growth of aerobic spoilage bacteria is inhibited, lactic acid bacteria may become the dominant component of the microbial flora of meats. This occurs with cured meats and with meats packaged in films of low gas permeability. The presence of a flora of psychrotrophic lactic acid bacteria on vacuum-packaged fresh chilled meats usually ensures that shelf-life is maximal. When these organisms spoil meats it is generally by causing souring, however other specific types of spoilage do occur. Some strains cause slime formation and greening of cured meats, and others may produce hydrogen sulphide during growth on vacuum-packaged beef. The safety and stability of fermented sausages depends upon fermentation caused by lactic acid bacteria. Overall the presence on meats of lactic acid bacteria is more desirable than that of the types of bacteria they have replaced.

INTRODUCTION

Fresh meat stored in air spoils rapidly due to the growth of gram-negative bacteria which cause putrefaction. A number of methods of preserving meats are known including the traditional ones of salting and curing. When the bacterial flora of such meats is examined it is commonly found that lactic acid bacteria are a major component. They are non-putrefactive and when spoilage does occur it is usually due to souring.

Lactic acid bacteria are able to grow under a variety of conditions which prevent the growth of gram-negative aerobes such as pseudomonads. Lactic acid bacteria do not require oxygen for growth, are resistant to inhibition by carbon dioxide, nitrite and smoke and are able to grow at relatively high salt concentrations. Further, they tolerate lower pH values than the gram-negative bacteria

commonly found on meats, especially under anaerobic conditions. Hence conditions favourable for the growth of these organisms occur in cured meats and meats packaged in films of low gas permeability.

This paper examines the occurrence, properties and origins of the lactic acid bacteria of meats. The mechanisms by which they cause spoilage and their significance as spoilage organisms are discussed. For a detailed treatment of the literature readers are referred to the articles of Sharpe (1962), Kitchell and Shaw (1975) and Reuter (1975), which, however, mainly discuss manufactured meats. The present paper emphasizes recent studies with packaged fresh meats.

Media

Many strains of lactic acid bacteria isolated from meats form only pin-point colonies on media such as Plate Count agar and APT agar is probably the most suitable non-selective medium for the isolation of lactic acid bacteria from meats (Baird and Patterson, 1980). MRS agar is widely used and whilst it permits growth of most strains which grow on vacuum-packaged beef, some fail to grow and others grow poorly. Unfortunately another problem sometimes arises. Packaged meats often carry a high population of *Brochothrix thermosphacta* and some strains are not only able to grow on MRS agar but are catalase-negative on it (A. F. Egan, unpublished data). In order to obtain a count of presumptive lactic acid bacteria in this situation, it is necessary to patch colonies from the MRS agar onto a medium such as APT agar and again test for catalase. Further, some strains of gram-negative bacteria may also form colonies on MRS agar. To date there appears to be no selective medium available which is entirely satisfactory for the recovery and enumeration of lactic acid bacteria growing on meats.

Cured meats

Lactic acid bacteria are a major component of the microbial flora of most types of cured meats (Kitchell and Shaw, 1975). They are able to grow in the presence of salt and nitrite and the addition of fermentable carbohydrates results in a favourable nutrient medium. The flora of raw cured hams and bacon is usually a mixture of micrococci, coagulase-negative staphylococci, lactobacilli and some gram-negative rods. Micrococci may dominate on high salt products but vacuum-packaging in gas-impermeable materials favours the growth of lactic acid bacteria.

Vacuum-packaged sliced meat products fall into two main classes. Raw cured meats such as bacon and (raw) ham commonly have a high salt content and as a result may be stable at room temperature. Lactobacilli may dominate on

Table 1. Relative significance of selected bacteria in the spoilage of vacuum-packaged sliced corned beef at 5 °C

Organism	Minimum time in days required [1] for		
	Aroma defect	Flavour defect	Reduced acceptability
Brochothrix thermosphacta	0	0	0
Heterofermentative lactobacilli	24	11	16
Homofermentative lactobacilli	>22	22	22

[1] Time from when the bacterial population reached 10^8/g to the onset of significant spoilage.

vacuum-packaged bacon and eventually cause spoilage by souring (Kitchell and Shaw, 1975).

Vacuum-packaged sliced cooked meats usually have a lower salt content and a pH of 6.0–6.5. Such products, which include cooked ham, corned beef and emulsion-type sausages are more susceptible to spoilage and require refrigeration for an adequate shelf-life. Lactic acid bacteria cause spoilage of these products, the microbiology of which is discussed by Mol et al. (1971).

Spoilage of cured meats by lactic acid bacteria is commonly due to the development of a sour "off" flavour, but how significant is this type of spoilage compared to that produced by other bacteria? Recently spoilage of vacuum-packaged sliced corned beef and ham by selected strains of lactic acid bacteria was compared with that produced by *B. thermosphacta*. Table 1 gives the minimum times required to produce spoilage subsequent to the bacterial population reaching 10^8/g in each case (Egan et al., 1980). *B. thermosphacta* caused spoilage much more rapidly than did the lactobacilli.

As well as souring, other organoleptic and visual defects may be caused by the growth of lactic bacteria on cured meats. Production of slime (dextran) may occur if sucrose is present. This is often caused by leuconostocs but unclassified streptobacteria and *Lactobacillus viridescens* may also be responsible (Sharpe, 1962). Greening of processed meats may be caused by lactic acid bacteria (Sharpe, 1962). Some strains, particularly *Lb. viridescens*, are able to produce hydrogen peroxide and, if catalase in the meat has been destroyed by heating or by the presence of nitrite, this may accumulate. Reaction between hydrogen peroxide and meat pigments results in the formation of choleglobin which is green.

Fermented sausages

Fermented or dry sausages are raw cured meat products which may be stored without refrigeration. They owe their stability largely to a combination of re-

duced water activity and low pH, which prevent undesirable bacterial growth. Early investigations established that lactic acid bacteria are usually the predominant component of the microbial flora of fermented sausages (discussed by Nurmi, 1966), and the fermentation of carbohydrates by them is responsible for the decrease in pH of these products (with a typical ultimate pH of 4.8–5.2). There have been numerous studies of the microbiology of fermented sausages and a detailed discussion is not possible here. Genigeorgis (1976) lists many of the major references.

Traditional methods of manufacture rely upon a natural fermentation. During the early stages of the process, there is a change from a catalase-positive gram-negative aerobic flora to one dominated by lactic acid bacteria. These organisms, favoured by a decrease in the oxidation-reduction potential and by their resistance to the inhibitory effects of curing salts, grow rapidly and may reach a count of about 10^8/g after only a few days. Micrococci also grow and may number up to 10^6/g, but gram-negative bacteria decline rapidly in numbers (Coretti, 1971).

The safety of fermented sausages depends upon fermentation, and failure of this process may have drastic consequences resulting in growth of organisms such as *Salmonella* spp. Whilst lactic acid bacteria inhibit the growth of pathogens primarily by producing acid and lowering pH, they also produce antibacterial compounds, such as hydrogen peroxide and antibiotics (Genigeorgis, 1976).

Traditional fermentations depend upon bacteria present on the raw materials and acquired from the environment in which processing is occurring. Hence large variations in the nature and size of the inoculum occur. Further, modern techniques of chilling and sanitation mean that the average natural inoculum nowadays is likely to contain fewer bacteria than in the past. These factors led to the development of starter cultures which consist of suitable strains of lactic acid bacteria. Correctly used, starter cultures not only ensure a microbiologically safe product, but also impart desirable flavour characteristics and aid in texture development (Nurmi, 1966; Coretti, 1977; Bacus and Brown, 1981).

Whilst lactic acid bacteria are essential to the production of safe fermented sausages, they may also cause colour and flavour defects. As discussed previously certain strains may cause greening. Further, if the numbers of lactic acid bacteria rise above the normal level, excessive acidity may result and colour, flavour and aroma may all be adversely affected. The defects of fermented sausages have been extensively studied and are described by Coretti (1971).

VACUUM-PACKAGED RAW CHILLED MEAT

When fresh meat is packaged in plastic films of low gas permeability, respiration by the muscle tissue consumes oxygen and produces carbon dioxide. This results in a gas atmosphere containing 20–40 % carbon dioxide and considerably

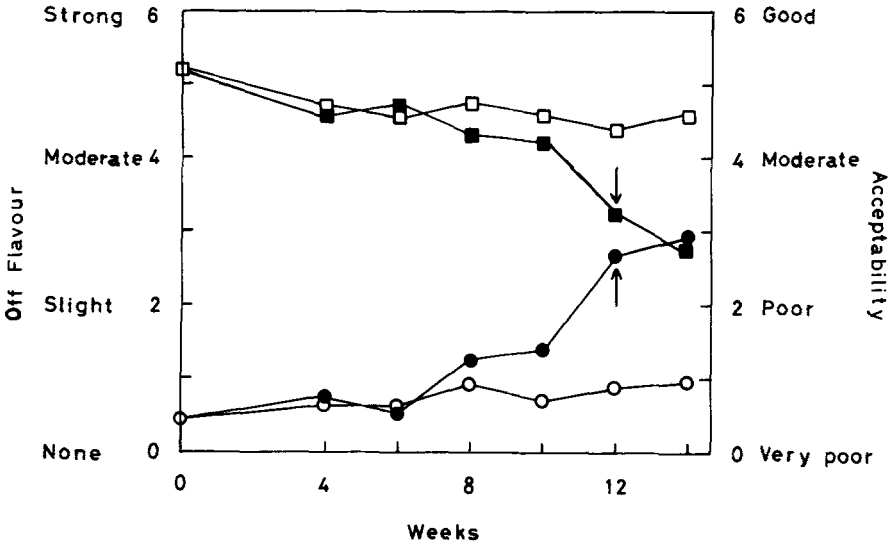

Fig. 1. Taste panel assessments of vacuum-packaged fresh beef of pH 5.6–5.8 stored at 0–1 °C. Samples packaged in bags with an oxygen permeability of 25 ml/m^2·24h·atm were compared to frozen control samples.

● "off" flavour and ■ acceptability of packaged meat; ○, □ corresponding attributes of frozen control samples. Arrows indicate the times at which samples first became statistically significantly different from the controls.

less than 1% oxygen (Dainty et al., 1979). Such conditions inhibit the growth of pseudomonads and the bacterial flora which develops at chill temperatures consists of lactic acid bacteria, *B. thermosphacta* and psychrotrophic strains of Enterobacteriaceae (Gill and Newton, 1978).

The microbiology of vacuum-packaged beef has been studied for some years and there are a number of apparently conflicting observations in the literature. Basically, a combination of meat pH and packaging film permeability controls the composition of the microbial flora which develops on this product (Campbell et al., 1979).

Provided pH is normal (5.4–5.9) and the permeability of the packaging film is sufficiently low (oxygen permeability less than about 100 ml/m^2·24h·atm at 25 °C), then lactic acid bacteria grow on the lean surface to the virtual exclusion of all other types. If meat pH is higher than about 5.9 or the packaging film used has a higher permeability, there is increased growth of gram-negative organisms (Dainty et al., 1979) and *B. thermosphacta* (Egan and Grau, 1981).

Assuming lactic acid bacteria dominate the flora of vacuum-packaged beef, what is the storage life of this product? Using a trained analytical taste panel to evaluate the spoilage of vacuum-packaged beef, results as shown in Fig. 1 were obtained. Beef of pH 5.6–5.8 was vacuum-packaged in bags made of plastic film with an oxygen permeability of c. 25 ml/m^2·24h·atm (measured at 25 °C

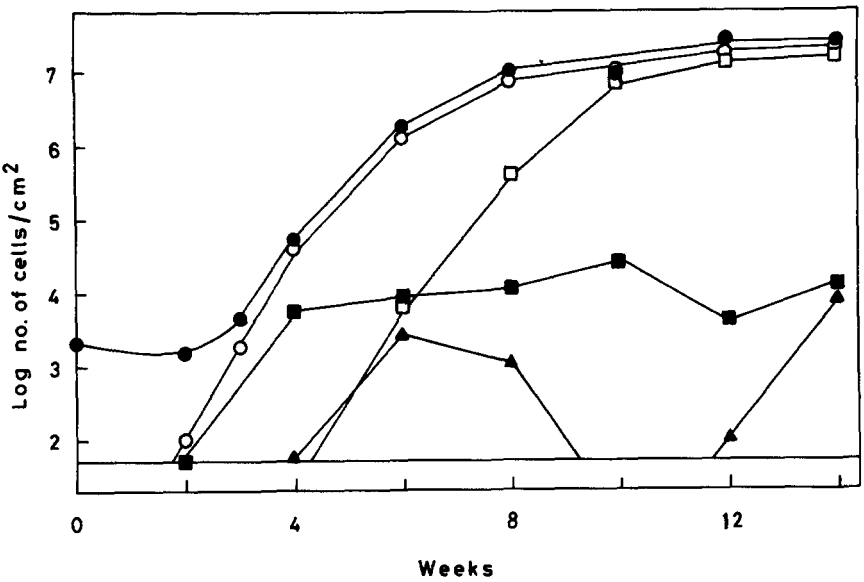

Fig. 2. Growth of bacteria on vacuum-packaged beef of pH 5.6–5.8 stored at 0–1 °C. Incubation temperature 25 °C.
● total viable count; ○ count on MRS agar, and □ on Rogosa SL agar; ▲ *B. thermosphacta*; ■, gram-negative bacteria.

and 98% relative humidity) and stored at 0–1 °C. The packaged samples gradually developed an "off" flavour which first became apparent after 10 weeks storage. Flavour became significantly different from that of frozen control samples after 12 weeks storage (P being < 0.05, and after 14 weeks < 0.01). Acceptability gradually declined and became significantly lower than that of the frozen control samples after 12 weeks storage ($P < 0.05$). No significant "off" aroma developed and there was no visual spoilage.

The development of the microbial flora during this experiment is shown in Fig. 2. This pattern of growth is typical of that which occurs under the conditions specified. Of an initial population of about 10^3 per cm^2, less than 10% are usually psychrotrophs and so fail to grow at 0–1 °C. Of the psychrotrophs only a small proportion are lactic acid bacteria. On Australian beef, the average count of these organisms is probably less than 10 per cm^2, however this figure may be climate-dependent.

When the bacteria are counted upon incubation of plates at 25 °C, there is an apparent lag period of about two weeks, followed by growth of psychrotrophs which reach a maximum population usually in the range of 3×10^7 to 10^8 per cm^2. After 6 weeks the count is usually about 10^7 per cm^2.

Commonly, more than 99% of the organisms present are catalase-negative and the number able to grow on MRS agar is usually, but not always, equal

to that on the non-selective agar. In contrast, the number of organisms able to grow on Rogosa SL agar may be considerably lower than that on MRS agar during the early stages of storage but this changes as storage progresses (Fig. 2). However on some occasions the count on Rogosa SL agar may approximate that on MRS agar early in the storage period suggesting that in such cases the initial population of lactic acid bacteria was aciduric in nature.

Experiments of the type discussed above demonstrate a shelf-life of up to 12 weeks for vacuum-packaged beef of normal pH stored at 0–1 °C in bags made of plastic film of low permeability. Spoilage is due mainly to the flavour defect described by tasters as sour, acid, cheesy and less frequently as bitter and liver-like. It is important to note that spoilage occurs some weeks after the population of bacteria reaches its equilibrium level, and thus a total count alone is of little value in predicting shelf-life.

Recently, it was shown that vacuum-packaged fresh beef spoils slowly, even in the absence of a contaminating bacterial flora, and that the rate of spoilage is increased by the presence of pure cultures of lactic acid bacteria (Egan and Shay, 1982). Not unexpectedly, the rate of spoilage produced by such pure cultures appears to be strain-dependent (A. F. Egan, unpublished data).

Very little information has been published concerning the growth and significance of lactic acid bacteria on the fat surface of vacuum-packaged beef stored at 0 °C. Preliminary studies in our laboratory have shown that these organisms are the dominant component of the microbial flora. Their numbers rarely exceed 10^7 per cm^2 and their significance in spoilage is not yet understood.

As compared to beef, there have been fewer studies on the microbiology of vacuum-packaged lamb and pork. These contain a greater proportion of fat and have a higher incidence of meat of high pH than does beef. Although lactic acid bacteria are present as a signifcant component of the flora, gram-negative organisms and *B. thermosphacta* are commonly present in much higher numbers than found on vacuum-packaged beef (Shaw et al., 1980). This situation resembles that found with beef of high pH which spoils more rapidly than that of normal pH (Dainty et al., 1979). Packaging under high concentrations of carbon dioxide suppresses these organisms and results in a flora composed largely of lactic acid bacteria (Enfors et al., 1979; Christopher et al., 1980).

Thus the existence of a flora dominated by lactic acid bacteria is considered desirable and can normally be expected to signify a maximum shelf-life for vacuum-packaged fresh meats. Unfortunately, this is not always the case. Recently, we described a *Lactobacillus* spp. which produces hydrogen sulphide during growth on vacuum-packaged beef of normal pH and this results in spoilage (Shay and Egan, 1981). Now several other strains with this property have been isolated and two of these have been associated with mild greening of vacuum-packaged meat although this problem occurred towards the end of the normal commercial storage life. Greening is a problem which commonly occurs if meat of high pH is vacuum-packaged. It is caused by the growth of hydrogen sulphide

producing gram-negative bacteria and under such circumstances shelf-life is greatly reduced (Taylor and Shaw, 1977).

Properties of lactic acid bacteria isolated from meats

Compared with the strains of dairy origin, there have been relatively few studies on the properties of lactic acid bacteria of meats. The most extensive studies are those of Reuter (1975), who examined in considerable detail lactobacilli isolated from German meat products. Cavett (1963) produced a scheme for the identification of strains isolated from bacon and this and other early studies are discussed by Kitchell and Shaw (1975).

Organisms commonly found on meat products include streptobacteria (often *Lb. plantarum*), betabacteria (*Lb. brevis* and *Lb. viridescens*), leuconostocs and pediococci. Many atypical strains have been isolated and these have proved difficult to identify, presumably because the methods are based upon the properties of organisms isolated from quite different environments.

Atypical streptobacteria have been shown to be a major group found on vacuum-packaged sliced cooked meats (Mol et al., 1971), on bacon (Cavett, 1963), on a variety of meat products (Reuter, 1975) and more recently on vacuum-packaged beef (Hitchener et al., 1982). Kitchell and Shaw (1975) pointed out that the relationships between the various groups of organisms designated as atypical streptobacteria are not clear, since different criteria were used in each study. Unfortunately, this comment is still largely valid.

Patterson and Baird (1977) reported that 45% of isolates from vacuum-packaged beef clustered with *Leuconostoc* spp. and only 10% with *Lactobacillus* spp. but no further details are provided. In contrast, atypical streptobacteria and betabacteria were the dominant groups in a recent study of Australian vacuum-packaged beef (Hitchener et al., 1982).

Recent studies by Kandler and co-workers (O. Kandler, personal communication) have begun to clarify the relationships between organisms isolated in some of the studies mentioned above. They have shown that *Lb. sake* and *Lb. curvatus* are very common on German meat products. Further, there appears to be a close relationship between these organisms and some of the atypical lactobacilli of Reuter (1975) and the atypical streptobacteria of Hitchener et al. (1982). The atypical betabacteria isolated by the latter group of workers are very unusual in that they produce only L(+)-lactic acid and possess L-lactate dehydrogenases which are activated by Mn^{2+} and fructose-1:6-diphosphate.

Hitchener et al. (1982) noted considerable variation in the nature of the organisms in different packs of beef. Further studies have shown that there may be relatively few types present and some packs appear to carry a "pure-culture" (A. F. Egan, unpublished data). This is perhaps not surprising when one considers the extremely low initial count.

Substrate utilization may be of major significance in controlling the growth and composition of mixed populations of bacteria growing on meats and little information on this subject is yet available. Another neglected area is that of the lipolytic and proteolytic properties of lactic acid bacteria found on meats.

Origins of lactic acid bacteria found on meats

The origins of lactic acid bacteria found in meats has been discussed by Sharpe (1962) and Kitchell and Shaw (1975). Organisms such as *Lb. viridescens* appear to be widely distributed in meat-processing plants and atypical streptobacteria have been found on the skins of freshly killed pigs.

It is generally accepted that the psychrotrophic bacteria found on hides and carcass meats originate from soil, water and vegetation. Atypical streptobacteria have been isolated from straw (Kitchell and Shaw, 1975) and from silage (Keddie, 1959). More studies into the relationship between strains found on meats and those from soil and vegetation would be useful.

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