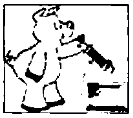

UNDER THE MICROSCOPE: THE LACTIC ACID BACTERIA FRIEND OR FOE

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BIOGRAPHIC DETAILS

Frances Nattress has a Ph.D in Molecular Genetics from McGill University (1994). She subsequently conducted research in McGill University's Department of Food Science and Agricultural Chemistry (1995-1996) and taught undergraduate microbiology laboratories in the Natural Resource Sciences Department. She joined Agriculture and Agri-Food Canada's Lacombe Research Centre in 1996 with a mandate to establish a research program in meat microbiology.

In 4 years at Lacombe Research Centre, Frances has initiated a research program addressing four important areas of meat microbiology: 1) physical and chemical intervention strategies to slow or prevent meat spoilage and improve meat safety; 2) the effect of ante-mortem animal husbandry on the gastro-intestinal carriage of selected pathogenic and non-pathogenic bacteria using cultural and molecular methods; 3) enumeration of pathogenic bacteria using cultural and molecular methods; and 4) molecular characterization of selected meat-borne bacteria that grow during anoxic storage, including identification and gene expression studies.

THE LACTIC ACID BACTERIA FRIEND OR FOE

The lactic acid bacteria comprise a large group of Gram positive bacteria and could well be the most interesting group of bacteria. They can grow almost anywhere in nature, however, they can be quite difficult to maintain in the laboratory. They are resistant to conditions that easily control most other types of bacteria. For example they are acid tolerant, in fact they often prefer a somewhat reduced pH. They are more heat resistant than Gram negative bacteria such as *E. coli* but they do not form spores so that they are not as heat resistant as spore formers such as *Bacillus* spp. or *Clostridium* spp. They can grow in the absence of oxygen and many can grow in the presence of carbon dioxide.

Of importance to the meat industry is that vacuum packaging and packaging in the presence of low or high levels of carbon dioxide does not inhibit lactic acid bacteria. Those conditions are usually quite inhibitory to many other organisms, particularly if the packaging is accompanied by storage at refrigeration temperature. These conditions are in fact, the perfect environment for the growth of lactic acid bacteria. Temperatures of below 0°C will slow the growth of lactic acid bacteria but many will grow quite vigorously at 2°C and 4°C, typical meat storage temperatures. Lactic acid bacteria are great competitors. Many produce antimicrobial substances or cause changes in the growth environment so that other organisms are inhibited, not only those that are closely related as is usually the case with bacteria but also unrelated organisms.

Many of our food and beverage products as well as animal feeds result from fermentation of raw materials by lactic acid bacteria and the development of well-liked flavour profiles. The fine flavours of wines are enhanced by malo-lactic fermentation by lactic acid bacteria. Cheeses, yoghurt and kefir are produced by lactic acid fermentation of milk and lactic acid bacteria are included in the starter cultures for these processes. Metabolic activity of the natural flora of cabbage results in sauerkraut and many gourmet meats are produced by inoculation with lactic acid bacteria and the resulting fermentation.

Why are lactic acid bacteria important to the producer of fresh meats?

In traditional meat processing where the carcass is hung for a period of time to allow natural aging and the meat is then cut and consumed within a few days, or frozen, the lactic acid bacteria are of minimal importance. However, as the industry attempts to prolong storage life to access foreign markets and to allow for national distribution of fresh meats, vacuum packaging of subprimal cuts has become the accepted practice for packaging for distribution and storage. Eighty percent or more of fresh meat is packaged in vacuum at the processor and is then shipped under refrigeration to its ultimate destination. A typical microbiological profile of fresh beef during six weeks of storage in vacuum at refrigeration temperature is shown in Figure 1.

Meat stored in air will spoil in a few days due to the putrid odour produced by growth of the aerobic spoilage bacteria, pseudomonads, whereas vacuum packaged meat develops a "sour" spoilage odour sometime after the lactic acid bacteria reach their maximum population density. Throughout vacuum packaged storage the lactic acid bacteria are the dominant microflora. By 21 days of storage at refrigeration temperatures their numbers are approaching their maximum and the quality of the meat is being influenced by them. A sensory evaluation of vacuum packaged meat including off odour intensity and odour acceptability characteristics (Figure 2) shows that only after five weeks of storage did the odour acceptability become moderately unacceptable. There was only a slight off odour during the whole six week storage time, despite relatively high numbers of lactic acid bacteria.

Most meat processors have, however, encountered complaints from retailers regarding their vacuum packaged product. Common customer complaints could include bubbling in the package or a greening of the meat accompanied by an objectionable odour when the package is opened and the meat is exposed to oxygen. If a microbiological analysis of this product were done the microbial profile would likely resemble that of unspoiled meat of the same age and it would be dominated by the lactic acid bacteria. This is because some of the lactic acid bacteria wear "black hats" (Les Jeremiah, personal communication) and if meat becomes contaminated by these types of organisms premature spoilage can result. Most of the time the lactic acid bacteria do not cause premature spoilage and it is only after their numbers reach their maximum that the acid or sour odour becomes strong enough to be objectionable. Flavour changes may precede the development of objectionable odours but these changes would only be noticed once the meat has been prepared by the consumer.

When the processor attempts to find the source of these organisms it becomes very difficult because, in most cases, the numbers of lactic acid bacteria on fresh meat are so low that they cannot be recovered using traditional microbiological techniques. Also differentiation of strains using cultural characteristics is very time-consuming and results can be ambiguous. Hence a more reliable identification can be attained by analysing the genetic makeup of the bacteria and an evaluation of the sources of contamination using differentiation based on genotypic differences between strains could be useful (Lawrence and Gilmour, 1995, Bjorkroth and Korkeala, 1997, Yost and Nattress, 2000). An example of the proportions of the different stains of lactic acid bacteria isolated from beef during six weeks of storage at refrigeration temperature is shown in Figure 3. The population was differentiated using a multiplex polymerase chain reaction-based analysis of colonies isolated from de Man Rogosa and Sharpe agar (Yost and Nattress, in press). A further analysis can be used to differentiate the large group of *Leuconostoc* spp. to further pinpoint the potential spoilage organisms (Figure 4).

The challenge for the meat industry is to keep product as free of lactic acid bacteria as is possible. Unfortunately some of the intervention strategies such as pasteurization that have been put in place by the meat industry to reduce numbers of pathogens such as *E. coli* O157:H7 may not necessarily reduce numbers of lactic acid bacteria. The interventions are used before carcass breaking and that process could be a source of contamination with lactic acid bacteria, particularly psychrotrophic types. Good manufacturing practices, a HACCP system and great attention to sanitation procedures will provide the best protection against contamination with lactic acid bacteria that is currently available. Locating the source of contamination with a particular strain is possible but it is technically demanding. Such an analysis could be done in the event of a persistent problem. Research is continuing on controlling the numbers and types of lactic acid bacteria that can grow during storage. Approaches include innovative packaging techniques such as packaging in 100% carbon dioxide and the inclusion of natural antimicrobials such as bacteriocins either by coating the meat or by incorporating the antimicrobial into the packaging material.

References:

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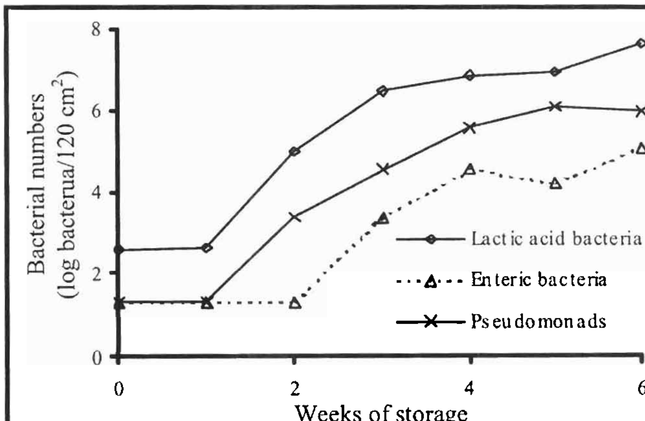

Figure 1. Bacterial growth on beef steaks during six weeks of storage at 2°C in vacuum packages.

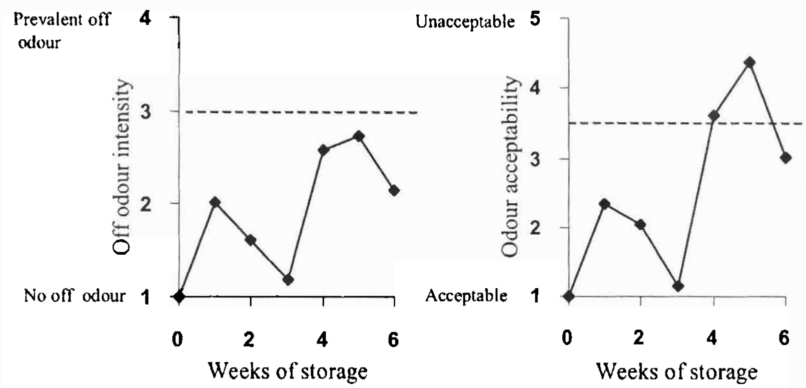

Figure 2. Off odour intensity and odour acceptability of beef steaks stored in vacuum packages for six weeks at refrigeration temperature.

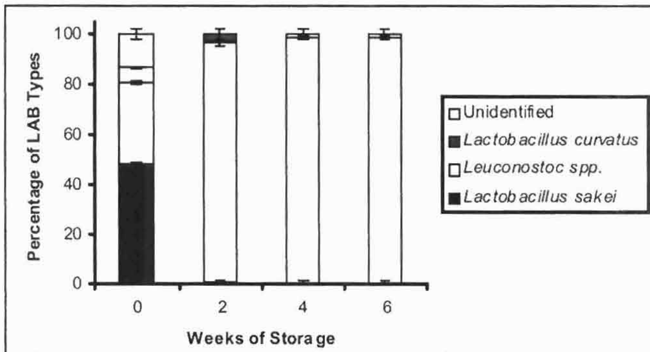

Figure 3. Development of the community of lactic acid bacteria growing on beef steaks during six weeks storage at refrigeration temperature.

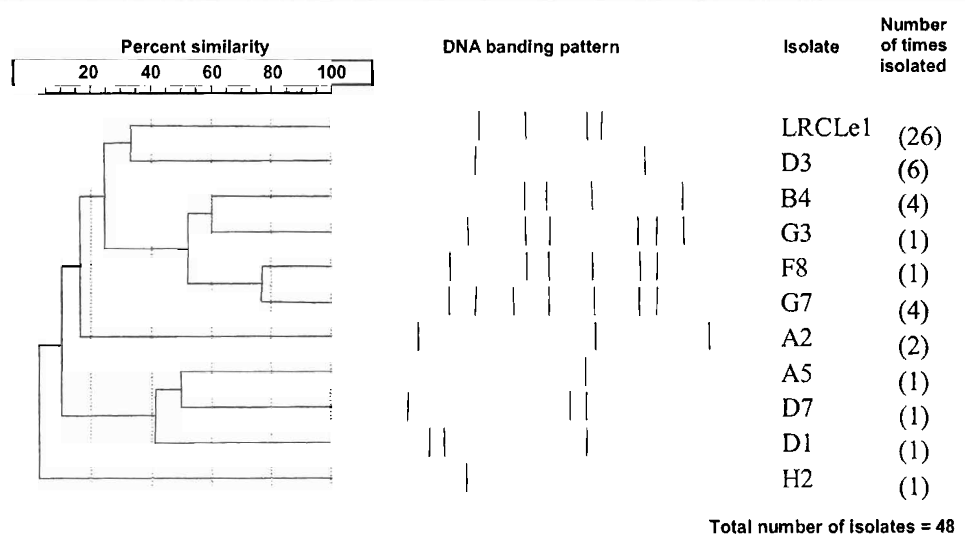

Figure 4. Dendogram showing the relatedness of the *Leuconostoc* spp. as determined by a randomly amplified polymorphic DNA analysis. For the purpose of this analysis, strains that are 80% similar are considered to be the same.