THE GROWING CHALLENGE FROM ALKALITROPHIC PSYCHROTROPHS IN MEAT PROCESSING.

Richard A. Holley, Dept Food Science, University of Manitoba

There is little question that the often meat borne bacterial pathogen *Listeria monocytogenes* is on the minds of folks in the cooked cured meat business today and with good reason. Although ubiquitous, often present in food, and carried by ≤ 10% of healthy persons in their upper respiratory tract, clinical cases are rare and physician experience is limited. This is because it causes only 0.03 cases of foodborne illness per 100,000 persons (U.S.). However it is highly lethal, killing one of every 6 persons infected. Similar figures for *E.coli* 0157:H7 and non-typhoid *Salmonella* are 0.6 and 13.4 per 100,000 with mortality rates of 1/1200 and 1/2600, respectively. Serious illness with chronic sequellae (eg. memory loss) or death are not confined to the very young, elderly or persons with underlying disease. More recent information indicates that 36% of cases do not have any known underlying or predisposing characteristics. Much remains to be done before we can adequately understand and appropriately address the presence of this organism in food. Ongoing work that examines virulence gene expression will compliment work which has yielded valuable information on how *Listeria* successfully invades tissue and establishes foci of infection in animals.

Back at the meat plant it is important to recognize the following factors: that conditions there (low temperature, humidity, unclean equipment for part of the day, use of alkaline cleaners); in cooked product (salt, nitrite, low numbers of competitors, low residual oxygen); during distribution (≤ 60 day shelf-life), and display (temperature often ≥4°C), favour survival and growth of *Listeria monocytogenes.* There is a lesson to be learned from the observation that while hot dogs or frankfurters have occasionally been responsible for causing outbreaks of listeriosis, bologna has not. Bologna is essentially made with the same ingredients as hot dogs, except for spices and is cooked similarly but packaged differently (often sliced). In addition, hot dogs are cooked again before serving while bologna is usually eaten without further heating. The lesson is that there is nothing magic about bologna to prevent it from becoming the next vehicle of a *Listeria* outbreak. Vigilance during handling at packaging and equipment sanitation are key. Our recent experience with a discolouration problem in bologna caused in large measure by organisms sensitive to the normal thermal process used in bologna manufacture illustrate the point and the level of product vulnerability.

There are two main types of discolouration problems in cooked cured meat products and both are triggered by hydrogen peroxide. There are "green cores" caused by organisms that are resistant to the thermal processing

conditions used (generally *Weissella viridescens*) and those that cause greenish rings to form at the outer edges of the product. These latter defects are caused by organisms that are not thermally resistant and generally contaminate cooked product at packaging (like *Listeria*). Earlier work (Grant *et al.* 1988) concluded that the acidiphilic *Leuconostoc*, *Lactobacillus* and *Pediococcus* species were responsible for the latter type of spoilage, and this was based largely on the use of meat-containing agar model systems. However, our recent work using these same media plus inoculated pack studies suggest it is highly likely that alkalitrophic *Aerococcus* and *Carnobacterium* species are more commonly responsible for the green discolouration defect in refrigerated vacuum packed cured meat products. It is notable that the defect is only produced after vacuum packages are opened and exposed to air. This exposure allows bacterial production of hydrogen peroxide which reacts with myoglobin in the meat to yield hydroperoxy-metmyoglobin and other compounds in reactions that are not well characterized to give products that are greenish in colour. It has been generally believed that any catalase negative organism capable of growth and H_2O_2 production in or on cured meats can produce the defect. We suspect that this is not always true, the amount of H_2O_2 produced and its ability (stability) to react with myoglobin being more important determinants of whether discolouration will occur. Levels of available carbohydrate can repress synthesis of some of the oxidase enzymes in bacterial cells responsible for H_2O_2 production.

In recent work with commercial vacuum packed sliced bologna (Peirson *et al.* 2002a), we were able to re-affirm that studies conducted almost 50 years ago and largely ignored in the recent literature, were valid in their identification of *Aerococcus viridans* as an agent causing this type of discolouration. However, we were unable to confirm that the catalase negative lactobacilli, leuconostocs, enterococci or pediococci caused similar changes. In contrast, we found, identified and named a new species of *Carnobacterium* (*Cb. viridans*) that was particularly adept at causing the discolouration problem in these products (Holley *et al.* 2002).

We also found (Peirson *et al.* 2002a) that a small volume of 3% (v/v) catalase prevented and a 10% (w/v) solution of sodium erythorbate delayed formation of green discolouration when spread on slices of inoculated bologna at package opening (after they had been held for a month in intact packages at 10°C). No hydrogen sulphide production was detected in these studies. Four strains of *A. viridans* and one of *Cb.viridans* isolated from spoiled commercial bologna as well as *W. viridescens* ATCC 12706 consistently produced green discolouration when irradiation-sterilized sliced bologna was inoculated, stored for a month at 9-10°C, opened and held 3 d at 4°C. However, to complicate matters, four other ATCC species of *Carnobacte-*

rium (*Cb. piscicola, Cb. divergens, Cb. gallinarum* and *Cb.mobile* as well as an ATCC culture of *A.viridans* (11563) did not discolour inoculated bologna. We cannot explain this observation at this point except to suggest that the different organisms generated varied amounts of H_2O_2 under the storage conditions used. There was poor agreement between the ability of organisms to generate colour reactions on model laboratory media containing meat or a test chromogen precursor and their ability to cause bologna discolouration.

All of the *A.viridans* and *Cb.viridans* meat isolates were salt tolerant and survived more than a month in saturated brine (26.4% NaCl) held at 4°C and are thus well adapted to survival in the meat processing plant. *Cb. viridans* did not grow in 4% NaCl but *A. viridans* was able to grow in 6.5% NaCl. It is particularly notable that aerococci species are easily transmitted in food plant environments through aerosols generated by human activity. Neither organism (*Cb.viridans* or *A.viridans*) was able to grow on acetate-containing Rogosa SL agar (*W.viridescens* could) but all three were able to grow at pH 9.1 or above. Interestingly, they share this latter characteristic in common with *Listeria, Yersinia, Vibrio* and *Campylobacter* and one must wonder whether our extensive use of alkaline cleaners and sanitizers in food plants provides a selective advantage for these alkalitrophic spoilage and pathogenic organisms in these environments. This is particularly important since all of the above organisms except for *Campylobacter* can grow at 4°C.

In a follow up study on spoilage, we formulated commercial bologna batter with either or both 3% sodium lactate or 0.3% sodium diacetate, cooked and inoculated the product with *A.viridans, Cb.viridans* or *W. viridescens*. Slices were vacuum packed and stored at 10°C for 10 weeks. In untreated bologna, *Cb.viridans* caused loss of red meat colour within one week, but meats inoculated with the other organisms did not show colour defects until 35d of storage. *A. viridans* (Fig.1) and *Cb. viridans* (not shown) were similarly inhibited by the acetate and lactate salts and discolouration was prevented by all lactate treatments. Lactate was more effective than diacetate against both organisms and both salts together were more effective against these organisms. Growth and discolouration by *W. viridescens* were not prevented by the treatments (Peirson *et al.* 2002b). Nonetheless, these salts have been proven effective against *L. monocytogenes* in cured meats (Mbandi & Shelef, 2002), and treatments such as these to prevent growth of *L.monocytogenes* may also address to some extent periodic discolouration problems. We found that diacetate alone and in combination with lactate tended to reduce the redness of the bologna. Lactate alone had a lesser but measurable negative effect on colour.

Finally we examined the thermal resistance of *Cb.viridans, A.viridans* and *W. viridescens* cultures along with a variety of other organisms including a strain of *L.monocytogenes* and *Enterococcus faecalis* to benchmark the thermal challenge method used. Thermal resistance for most organisms was lower in broth than in meat batter. At 60°C in broth *E.faecalis* was unaffected, but *W.viridescens* did not survive and the $D_{60°C}$ value for *L.monocytogenes* was 2.5 min. We found the $D_{65°C}$ value for *E.faecalis* was 1.07 min in MRS broth while the $D_{60°C}$ values for all the other organisms in meat batter (except *W. viridescens*) were ≤ 1.3 min. The $D_{60°C}$ value of 14.7 min for *W.viridescens* in meat reflected its higher resistance to thermal challenge (Peirson *et al.* 2002b).

Clearly then, discolouration when caused by *A. viridans* and *Cb.viridans* occurs as post-cook contamination and can be prevented by blocking their access to products at packaging or by inhibiting their growth with sodium lactate and sodium diacetate. If *W.viridescens* is present in uncooked meat batter in high numbers its growth and the discolouration it can cause will not be prevented by the acetate and lactate salts or by cooking to an internal temperature of 69°C. Good equipment sanitation, monitoring and validation of GMP's are important key elements that will reduce the occurrence of post-cook contaminants that can shorten product shelf-life and affect safety thresholds. Based on available information, epidemiological experience and known characteristics of frequently occurring psychrotrophic spoilage and pathogenic bacteria, it is likely that alkali tolerance is presently an important factor contributing to their survival in food plants. This tolerance may play a more significant role in the future through enhanced selection of these bacteria which can shorten shelf-life and change foodborne illness statistics.

Acknowledgements:
This work was supported in part by Maple Leaf Consumer Foods and the Agri-Food Research and Development Initiative, Manitoba.

References:

Grant, G.F., A.R. M^{c}Curdy and A.D. Osborne (1988). Bacterial greening in cured meats: a review. Can Inst. Food Sci. Technol. J. 21:50-56.

Holley, R.A., T.Y Guan, M. Peirson and C. Yost (2002). *Carnobacterium viridans* sp.nov., an alkaliphilic, facultative anaerobe isolated from refrigerated, vacuum-packed bologna sausage. Int. J. Sys. Evol. Microbiol. 52:1881-1885.

Mbandi, E. and L.A. Shelef (2002). Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. Int. J. Food Microbiol. 76:191-198.

Peirson, M.S., T.Y Guan and R.A. Holley (2002a). Aerococci and carnobacteria cause discolouration in cooked cured bologna. Food Microbiol. (Accepted Sept).

Peirson, M.S., T.Y Guan and R.A. Holley (2002b). Thermal resistance and lactate and diacetate sensitivities of bacteria causing bologna discolouration. Int. J. Food Microbiol. (Submitted July).