GROWTH OF *BROCHOTHRIX THERMOSPHACTA* AND *LACTOBACILLUS SAKEI* STRAINS IN REFRIGERATED VACUUM-PACKAGED MEATS

Graciela M. Vignolo, Aída P. de R. Holgado and Guillermo Oliver

Centro de Referencia para Lactobacilos - CERELA - CONICET. Chacabuco 145, (4000) San M. de Tucumán, Argentina.

Background

The characteristic microbial population that develop in meat products result from the effect of the prevailing environmental conditions on the growth of the microorganisms initially present in the raw materials or introduced later by cross-contamination or processing. The environmental factors involved, which are mainly related with storage temperature as well as composition and relative humidity of the gaseous atmosphere surrounding the meat, are often manipulated to extend the shelf-life of meat products (1). CO_2, produced during the growth of aerobic microflora, constitutes an important naturally occurring selective agent. Storage of chilled meat in gas-impermeable packs restricts the growth of *Pseudomonas* so that *Lactobacillus* and *Brochothrix thermosphacta* become the major components. Under conditions which restrict the growth of *Pseudomonas*, competition between facultative anaerobic bacteria determines the nature of the final flora. *Lactobacillus* can grow more rapidly on meat under anaerobic conditions than either *B. thermosphacta* or *Enterobacter* and, if initially present in sufficient numbers, it tends to dominate the flora in vaccum-packaged meats (2). In addition to an advantage in growth rate over competitors, lactobacilli are able to inhibit the growth of others species through the competition for nutrients and the production of antibacterial substance like bacteriocins (3).

Objectives

This study was conducted to evaluate the effect of different conditions on the growth rate of *B. thermosphacta* and *L. sakei* at 2°C with the aim to inhibit the former using *L. sakei* during the storage of refrigerated vacuum-packaged meat.

Methods

Lactobacillus sakei ATCC 15521, 160x1 and G3 and *Brochothrix thermosphacta* ATCC 11509, 160x8 and 8727 isolated from meat were used and cultured in LBS (4) and TVG5P (5) respectively.

Semimembranosus muscle was excised from commercial beef carcases. After trimming the flamed meat layer the underlying muscle was cut into pieces of 3 cm diameter, placed in Petri dishes and sterilized by U.V light (60-wat germicidal bulbs; 50-cm distance from tissue, 30 min). Top surfaces of meat discs were inoculated using Whatman paper N°1 soaked up in the cell suspensions (prepared to yield a final concentration of 10^3 cells/cm^2) of *L. sakei* and *B. thermosphacta*.

To simulate a "ripening process" in the meat, 100 µl of peptone (10%), glucose (20%) and peptone+glucose were spread on the surface of meat. In an additional experiment meat was also subjected to a maturation during 24 h at 25°C before to be inoculated. Petri dishes containing the inoculated meat discs were packed in laminated film Cryovac B-series bags (cryovac, GRACE Packaging, Argentina) with a diffusion coefficient of 1 cm^3/m^2/24h/atm to O_2 at 25°C and 75% RH. The vacuum bags were evacuated using a TURBOVAC 320/1, sealed and heat-shrunk. Finally, meat samples were incubated at 2°C for 18 days. Microbiological analysis were carried out at time intervals using the following media: for total viable counts, TVG5P (incubated at 25° C for 48 h); *B. thermosphacta* on Streptomycin Thallous Acetate Actidione Agar, STAA (11) (25°C for 3 d) and *L. sake* on LBS agar (25°C for 4 d).

Results and discussions

The growth rates of the strains of *L. sakei* ATCC 15521, G3 and 160x1 and *B. thermosphacta* ATCC 11509, 8727 and 160x8 were determined and are shown in Fig.1 and 2. *Lactobacillus* strains grew from an initial inoculum of *c.a* $8 \times 10^2 - 5 \times 10^3$ cells/cm^2 to $5.3 \times 10^4 - 3.6 \times 10^6$ cells/cm^2. *L. sake* ATCC 15521 was observed to be the strain that reached the maximal cell number per cm^2 after 18 days (3.6×10^6 cells/cm^2) (Fig.1). When *Brochothrix thermosphacta* was inoculated, all strains grew more rapidly under the study conditions and by day 18 had produced a final population of between 2.4×10^6 and 5×10^8 cells/cm^2. The maximal cell densities attained by *Lactobacillus* strains were about two log cycles lower than *Brochothrix* at the end of the incubation period and the length of lag phase was observed to be 7 days in contrast with only 4 days for *B. thermosphacta*. These results are in agreenment with Roth and Clark (6), who observed that *Brochothrix thermosphacta* tolerates high concentrations of CO_2 and grows on vacuum-packaged fresh beef. Eventhough, Newton and Gill [19] stated that *Lactobacillus* grew more rapidly than other species between 2° and 15° and the advantage in growth rate of *Lactobacillus* increased with decreasing temperature.

With the aim of reducing the lag phase duration of *Lactobacillus*, the addition of glucose 20% (w/v), peptone 10% (w/v) and a mixture of glucose + peptone on the meat slices was assayed at 2°C (Fig. 3). In this conditions *L. sake* ATCC 15521 had the best growth rate when a peptone solution was spread on the meat showing an increase of the viable counts of more than one log cycle at day 7 when compared to the control, leading to a population of 2.6×10^4 cells/cm^2 in contrast with 8.2×10^3 and 1.5×10^3 cells/cm^2 found when glucose +peptone and glucose, respectively, were added to the meat. This evidence would indicate that neither nitrogen sources, amino acids or the low molecular weight peptides required for +bacterial growth would be available in the meat at a high enough concentrations. Under the assumptions that in anaerobic conditions the only fermentable substrates available in meat for lactobacilli are glucose and arginine (7) and that even glucose is utilized by the two species, it is the only substrate for *B. thermosphacta*, the increase in the growth rate of *L. sake* cannot be explained in the above way due to the fact that *Lactobacillus* shows the lowest affinity for glucose. But, it attacks arginine, which is not utilized by the competing species. These facts probably allow *Lactobacillus* to attain a density at which the metabolism of the competing species is

inhibited. When the growth of *B. thermosphacta* was studied under the above conditions, the addition of peptone and glucose did not affect the cell density obtained at 7 days (3.4×10^4 cells/cm^2) (data not shown).

Based on these assumptions, a "ripening process" was performed on the meat by ATCC 15521, *B. thermosphacta* ATCC 11509 and a mixed culture of both organisms are shown in Fig 4. Both organisms grew at a higher rate when meat was pre-incubated at 25°C (Fig. 4a and 4b). When they were inoculated in a mixed culture *L. sake* outgrew *B. thermosphacta* after 7 days of incubation at 2°C, reaching a cell densitiy of 1×10^8 cells cm^{-2} at 18 days while *B. thermosphacta* only attained 5×10^4 cells/cm^2 (Fig 4c) in the same period of time. These results would indicate that the pre-incubation of meat at 25°C for 24 h allowed the release of peptides and amino acids from meat proteins that would make possible the growth of *Lactobacillus* over *Brochothrix* in vacuum-packaged meats stored at 2°C. No doubt, the effect of temperature was a determining factor in the protein breakdown, this process being mediated by a number of different endogenous proteolytic enzymes like lysosomal proteinases, aminopeptidases system and calcium-dependent proteinases, (8).

Conclusions

Although, the nature of the inhibition of *B. thermosphacta* cannot be explained solely in terms of competition of nutrients, from a technological point of view, the simple fact of mantaining the meat at room temperature during 24 h before vacuum-packed, would be a great advantage for lactobacilli to start growth and restrict the development of *B. thermosphacta* on meat stored anaerobically at chill temperatures.

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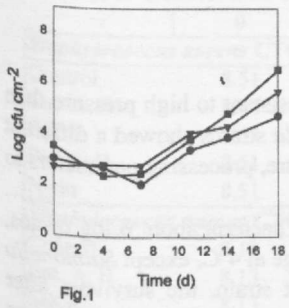


Fig.1

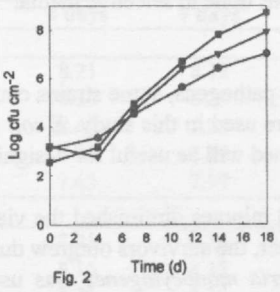


Fig. 2

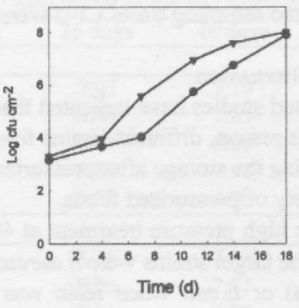
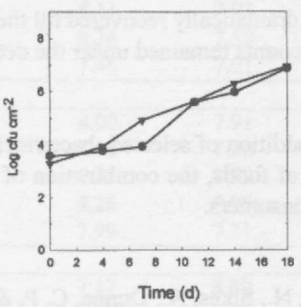


Fig. 1: Growth on vacuum-packaged meat at 2°C of *L. sakei* strains G3 (▼), 160x1 (•) and ATCC 15521 (■).

Fig. 2: *B. thermosphacta* strains 8727 (▼), 160x8 (•) and ATCC 11509 (■).

Fig. 3: Effect of glucose (▼), peptone (■) and glucose+peptone (♦) on the growth of *L. sakei* ATCC 15521 in vacuum-packaged meat incubated at 2°C. Control (•).

Fig. 4: Effect of incubation at 25°C during 24 h of the meat (ripening process) on the growth of (a) *L. sakei* ATCC 15521; (b) *B. thermosphacta* ATCC 11509 and (c) *L. sakei*+*B. thermosphacta*. Control (•), ripening process (▼).



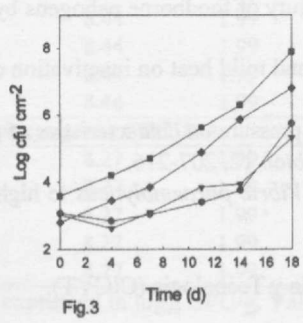


Fig.3

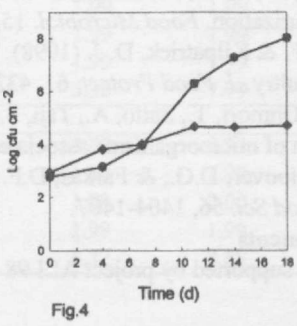


Fig.4