ELSEVIER

PII: S0308-8146(97)00005-8

Food Chemistry, Vol. 59, No. 4, pp. 547–554, 1997

0308-8146/97 $17.00+0.00

Bacterial starter cultures for meat fermentation

Marta Hugas & Josep Ma. Monfort

Institute of Food Research and Technology, Meat Technology Center, 17121- Monells, Spain

(Received 15 March 1996; revised version received 18 October 1996; accepted 18 October 1996)

Lactic acid bacteria play an essential role in the production of fermented meat products, *Lactobacillus* being the main species used in the European type of fermented products. In order to ensure sensory quality and good colour formation, lactic acid bacteria are not sufficient and the contribution of *Staphylococcus carnosus* is needed. Some strains of meat lactobacilli exhibit important properties from a technological point of view, such as the production of antimicrobials. The application of bacteriocinogenic lactic strains as starter cultures in fermented products could provide an additional tool for preventing the outgrowth of food pathogens in sausages as well as enhancing the competitiveness of the starter organisms in favour of the fortuitous flora. However, further research is needed before the application in meat products of these bacteriocins can be put into practice. The spectrum of application of lactic acid bacteria is wide; *lactobacilli* are good candidates as probiotic strains, thanks to their GRAS status and their ability to adhere to epithelian cells. In the near future, *lactobacilli* probiotic cultures will be included in new foods; fermented meat products are likely to be one of these foods. © 1997 Elsevier Science Ltd

THE ROLE OF LACTIC ACID BACTERIA IN MEAT FERMENTATION

Fermented sausage is defined as comminuted meat and fat, mixed with salt, nitrate and/or nitrite, sugar and spices (black pepper at least), which is stuffed into casings, and subjected to a fermentation time and then to a drying process. The final product preserves well and has an increased shelf-life as a consequence of the inhibition of pathogenic and spoilage bacteria.

In spontaneous meat fermentation, the lactic acid bacteria derived from the raw materials or the environment are responsible for both lactic acid production resulting from carbohydrate utilization, and also a low pH value (5.9–4.6). As a consequence of this, the muscle protein coagulates, resulting in the sliceability, firmness and cohesiveness found in the final product. Ripening is also favoured when pH values decrease and approach the isoelectric point of proteins. The development of curing colour occurs also in acidic conditions when nitric oxide is produced from nitrite and can then react with myoglobin. Finally, the inhibition of pathogenic and spoilage bacteria is a consequence of the accumulation of lactic acid as well as acetic acid, formic acid, ethanol, ammonium, fatty acids, hydrogen peroxide, acetaldehyde, antibiotics and bacteriocins.

Today the modern meat industry has to ensure high quality, reduce variability and enhance organoleptic characteristics in sausage production, which is not feasible using spontaneous fermentation methods. Therefore starter cultures have been developed during the last 40 years reducing fermentation times, ensuring low residual nitrate and nitrite contents in the end product and standardizing the organoleptic characteristics.

With regard to the importance of bacteria, it may be noted that most of the commercially available starter cultures are mixtures of a strain of lactic acid bacteria and a strain of the *staphylococci* and/or *micrococci* genus. Starter cultures for sausages produced with nitrite as a curing agent normally contain only lactic acid bacteria and staphylococci, whereas starters for sausages produced with nitrate as a curing agent include lactic acid bacteria, staphylococci and micrococci which possess the nitrate reductase activity.

Today's starter cultures for raw sausages can be divided into two categories (Buckenhüskes, 1994). First generation starter preparations contain lactic acid bacteria originating from plant material (*L. plantarum*, *P. pentosaceus*). The second generation starter cultures contain lactic acid bacteria originating from meat and thus are specially adapted to the ecology of meat fermentation.

The identification of 254 strains of lactobacilli isolated from Spanish spontaneously fermented dry sausages from 15 different producers showed that *L. sake* comprised the largest number of isolates (55%) followed by *L. curvatus* (26%), *L. bavaricus* (11%) and *L. plantarum* (8%) (Hugas *et al.*, 1993). So, *L. sake* and *L. curvatus* are the lactic acid bacteria most used in second generation starter cultures, and the results show that they

control the entire fermentation and ripening processes, inhibiting the growth of spontaneous lactic acid bacteria. This fact is very important because, when using first generation starters, the fermentation process is initiated as desired, but spontaneous lactic acid bacteria are able to grow and may have an undesirable influence on the sensory properties of the final product. Most lactobacilli are capable of forming hydrogen peroxide, and this peroxide leads to the discolouration of the nitroso heme pigment. Second generation starter cultures contain the catalase activity and by using them together with the catalase-positive cocci this type of problem can be avoided.

MAIN TRAITS OF SECOND GENERATION STARTER CULTURES

Flavour production

The aromatic and sensory qualities of dry sausages are achieved by the contribution of lactic acid bacteria together with micrococci and yeasts as starter organisms. The non-lactic starter organisms contribute to the organoleptic qualities of fermented products by means of several activities: nitrate reductase, nitrite reductase, catalase and lipase.

Several authors (Cantoni *et al.*, 1972; Wolf & Hammes, 1988; Wolf *et al.*, 1990) have shown that lactic acid bacteria involved in meat fermentation can also display the above-mentioned activities (Table 1). The catalase and nitrite reductase activities are either heme-dependent or independent since meat contains sufficient heme and therefore there is no limitation for heme-depending activities in fermenting sausages.

After the studies of Hammes *et al.* (1990) it was shown that a multiple strain starter culture with several nitrate-reducing lactobacilli achieved the reduction of nitrate and nitrite. However, the nitrate reduction was slow and, as a result, produced flavour and colour defects in the final product.

Members of the genus *Staphylococcus* are partly responsible for lipolysis together with tissue lipases. They contribute to the release of free fatty acids during sausages whereas *Micrococcus varians* would not participate in the lipolysis according to Talon *et al.* (1992); in 1971 Reuter showed that strains of *L. sake* and *L. curvatus* exhibit lipolytic activity *in vitro*. Up to now, the importance of lipolysis by the lactobacilli has not yet been looked into in fermenting sausages.

The contribution of bacteria to the mechanisms of proteolysis in sausages has not been studied in detail. Results, up to now, are consistent with a collaborative and consecutive role of muscle cathepsin D and bacterial enzymes in proteolysis, the former preparing peptide substrates for the latter (Verplaetse *et al.*, 1992). Proteolytic activity can be found *in vivo* in lactobacilli strains. Therefore, it would appear that after further studies it will be possible to determine whether a single lactobacilli starter culture produces sausages of a sensorial and technologically appropriate standard, avoiding the need to use micrococci starter cultures in combination with lactobacilli strains.

Genetic manipulation

The improvement or optimization of the starter cultures presently in use will be achieved in the future by means of genetic engineering. This technique can be used for introducing the genes coding for useful properties, which are present in other micro-organisms, into competitive starter strains.

The genetics of meat-borne lactobacilli have been increasingly investigated. The most suitable method for introducing DNA into lactobacilli is electroporation; this method has been adapted for strains of *L. sake* and *L. curvatus* by Gaier *et al.* (1990) and for *L. sake*, *L. curvatus*, *L. bavaricus* and *L. plantarum* by Aymerich *et al.* (1993) using different DNA vectors.

Different lactobacilli genes have been cloned: B-galactosidase gene (Obst *et al.*, 1992); catalase (Knauf *et al.*, 1992), bacteriocin genes (Tichaczek *et al.*, 1993, 1994; Axelsson *et al.*, 1993), etc. The cloned genes may serve as marker genes in vectors or for improving the metabolic qualities of the strains.

Genes from generally recognized as safe (GRAS) organisms other than lactic acid bacteria can also be cloned, for example the cloning of the lysostaphin gene of *S. staphylolyticus* into meat starter lactobacilli (Gaier *et al.*, 1992). With the introduction of the lysostaphin gene, the growth of *S. aureus* can be prevented and the hygienic status of meat products increased.

Table 1. Rare properties of meat-borne Lactobacilli

LAB	Catalase		Nitrate-reductase	Nitrite-reductase	
	Heme dependent	Heme independent		Heme dependent	Heme independent
L. plantarum	+	(+)	(+)	(+)	(+)
L. pentosus	+	—	+	+	—
L. farciminis	—	—	—	—	(+)
L. sake	+	—	—	—	(+)
L. curvatus	—	—	—	—	—
P. pentosaceus	—	+	—	(+)	—
P. acidilactici	+	—	—	—	—

+ = most strains; (+) = some strains. After Hammes *et al.* (1990).

Bacteriocins

The potential of meat-borne lactobacilli to produce antagonistic compounds has been investigated (Garriga *et al.*, 1993) and detected in strains of *L. curvatus*, *L. sake*, *L. bavaricus* and *L. plantarum* (Table 2).

These strains produce bacteriocins which are antimicrobial compounds of a peptidic nature, active against different indicator bacteria. Among these sensitive bacteria there are food-poisoning micro-organisms as well as lactic acid bacteria.

The application of the bacteriocinogenic lactic strains as starter cultures in fermented products could provide an additional tool for preventing the outgrowth of food pathogens in sausages as well as enhancing the competitiveness of the starter organisms towards the fortuitous flora.

For the application of new bacteriocin-producing isolates, their suitability as starter organisms has to be demonstrated with respect to their performance in the respective process and the product quality. Alternatively, well-stablished starter strains can be supplied with bacteriocin production capacity by transfer of the relevant genes which may be found in transmissible plasmids (Ahn & Stiles, 1992).

Several authors (Nielsen *et al.*, 1990; Winkowski *et al.*, 1993; Berry *et al.*, 1990, 1991; Foegeding *et al.*, 1992; Schillinger *et al.*, 1991; Campanini *et al.*, 1993; Degnan *et al.*, 1992) have conducted studies in order to assay the potential of bacteriocin-producing strains in meat systems. Most studies about the *in situ* behaviour of bacteriocins against food pathogens like *Listeria monocytogenes* have been conducted using bacteriocinogenic strains of *Pediococcus acidilactici*, which is the main starter culture used in the manufacture of American-style fermented meat products.

In fresh meat, Nielsen *et al.* (1990) observed a reduction of 2.2 log cfu g^{-1} of *L. monocytogenes* in the lots inoculated with a bacteriocinogenic strain of *P. acidilactici* compared to the control lots. In minced meat, several authors (Skytta *et al.*, 1991; Schillinger *et al.*, 1991; Berry *et al.*, 1991) have reported that the level of *L. monocytogenes* remained constant for 14 days by inoculating a bacteriocinogenic while in the control lot the pathogen grew exponentially. In American-style fermented sasuages (Berry *et al.*, 1990) a 1.3 log reduction of *L. monocytogenes* was observed after the inoculation of *P. acidilactici* JD1-23 compared to the uninoculated control lot.

There have been several reports about the use of bacteriocinogenic lactobacilli in European-style fermented sausages. In 1991, Schillinger and co-workers were the first to apply a bacteriocinogenic *L. sake* in a slightly fermented product (fresh Mettwurst) in order to evaluate the inhibition of *L. monocytogenes*. Although the bacteriocin produced by *L. sake* Lb706 was less effective in food than *in vitro* systems, the authors stated that it can be used as a bioprotective culture to prevent the outgrowth of *L. monocytogenes* in certain types of food.

Vogel *et al.* (1993) investigated the suitability of the bacteriocinogenic *L. curvatus* LTH1174 in comparative studies employing the bacteriocin producer, its non-producing derivatives and a highly competitive commercial starter. The results indicated that *L. curvatus* LTH1174 is well-adapted to the meat environment. The bacteriocin production enhances its competitiveness since the original strain is more competitive than the cured derivative and the commercial starter culture, resulting in more than 97 and 80% dominance after inoculation at cell counts of 10^5 and 10^3 cfu g^{-1} respectively.

In the adaptation of the producer to the habitat, the inhibitory spectrum of the bacteriocin is crucial and has to be taken into account as well as stability and production of the bacteriocin under process conditions; the bacteriocin cannot affect the growth of the other components of the starter preparations.

During the evaluation of the antimicrobial activity of *L. plantarum* MSC and its cured derivative *L. plantarum* MSC1 in Italian salami, Campanini *et al.* (1993) did not find significative differences in the evolution of *L. monocytogenes* in either strain. Again, the inactivation of *Listeria* was lower in meat products than in laboratory media where high levels of contamination are used.

Recently, Hugas *et al.* (1995) reported the inhibition of *Listeria* in artificially contaminated dry fermented sausages by *L. sake* CTC494 producing a bacteriocin named sakacin K. In the trials reported (Fig. 1), the counts of *Listeria* decreased from 10^3 cfu g^{-1} during the initial stages to 2.66 MPN g^{-1} at the end of the process, with a 1.25 log of difference compared to the non-bacteriocinogenic starter culture. These results

Table 2. Antagonistic strains isolated from fermented meat products

	% of strains with positive inhibition in agar spot test			
Indicator strain	*L. sake*	*L. curvatus*	*L. bavaricus*	*L. plantarum*
L. plantarum ATCC8014	9	6	0	9
L. plantarum DSM20174	5	0	0	100
L. curvatus MCD02739	12	6	15	0
L. curvatus CTC strain	14	14	11	9
L. sake DSM20017	4	0	0	4
No. of strains assayed	139	66	27	22

After Garriga *et al.* (1993).

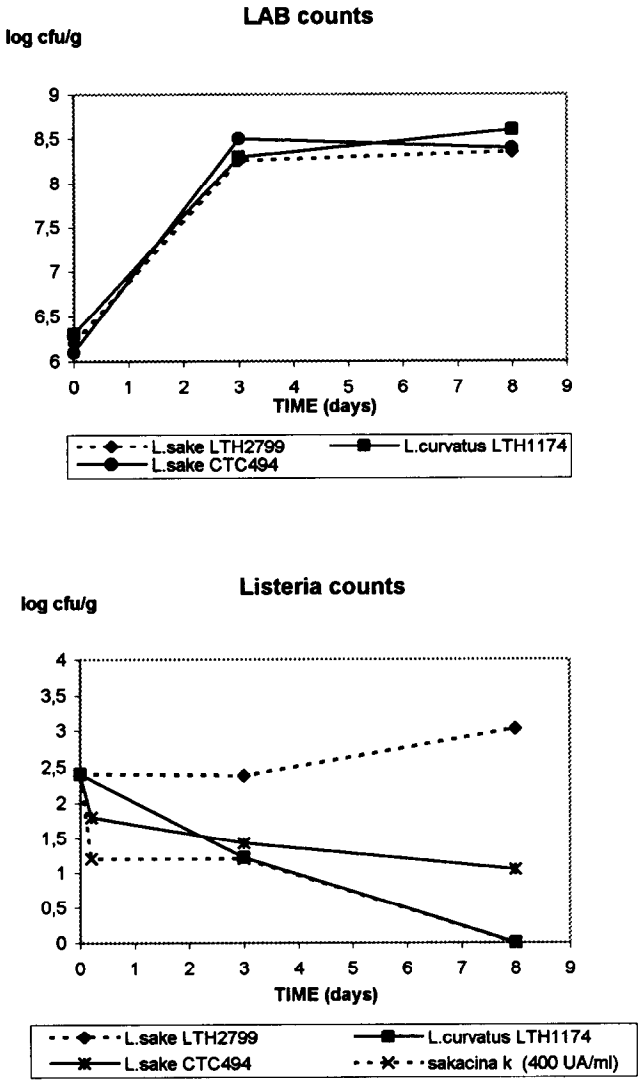

Fig. 1. Growth of lactic acid bacteria and *L. innocua* in fermented sausages. Values are the average of three experiments. *Listeria* counts below 10^3 cfu g^{-1} are given in log MPN g^{-1}.

demonstrate the effectiveness of sakacin K in controlling the outgrowth of *Listeria* in dry fermented sausages.

Up to now, several bacteriocins from *Lactobacillus* strains have been characterized, purified and the peptide sequenced (Table 2). Several strains are producing the same bacteriocin: sakacin A (Axelsson *et al.*, 1993) produced by *L. sake* Lb706 is the same compound as curvacin A (Tichaczek *et al.*, 1992) from *L. curvatus* LTH1174 and sakacin K (Remiger *et al.*, 1996) from *L.sake* CTC494; sakacin P from *L. sake* LTH673 (Tichaczek *et al.*, 1992) is like bavaricin A from *L. bavaricus* MI401 (Larsen *et al.*, 1993). Thus, even different strains from different, though closely related, species can produce the same bacteriocin.

As stated above, the production of a certain bacteriocin in laboratory media does not imply its effectiveness in a food system. When evaluating a bacteriocin-producing culture for sausage fermentation, it has to be considered that fermented sausages are a complex system with a number of factors influencing microbial growth; temperature, pH and the curing aids, nitrate and nitrite.

In order to assess the influence of formula and fermentation technology on the performance of bacteriocin-producing starter cultures, Hugas *et al.* (1996) assayed two different technologies, nitrate-nitrite cure and nitrate cure, as well as two ripening conditions. The experiments were performed with five different bacteriocinogenic cultures; two of them had already been assayed in suppressing the growth of *Listeria* in different meat products (Schillinger *et al.*, 1991; Hugas *et al.*, 1995).

In the nitrate and nitrite treatment, *Listeria* growth in all the sausages with bacteriocinogenic strains was lower than the values of the control standard starter strain. The best strains for inhibiting the growth of *L. ivanovii* LTH3097 were *L. curvatus* LTH1174 and *L. sake* CTC494. In these sausages the listerial count was reduced from 1×10^4 cfu g^{-1} to 2–4 MPN g^{-1} within eight days (Figs 2 and 3).

Listerial reduction continued until the end of the process. In the treatment with a nitrate cure, the sausages containing either *L. curvatus* LTH1174 or *L. sake* Lb706 acheived one log reduction of *Listeria* counts (from 1.6×10^4 cfu g^{-1} to 1.3×10^3 cfu g^{-1}) during the period of 30 days. No differences in the *Listeria* counts were observed between the standard starter strain and the rest of the bacteriocinogenic cultures.

It seems that nitrite might enhance the bacteriocin effect permitting a further reduction of *Listeria* counts. The reduction of pH to 5.5 and below has to be considered when discussing anti-listerial factors in sausage fermentation. In order to demonstrate that the production of bacteriocin enhances the effectiveness of starter cultures in inhibiting the survival of *Listeria*, the effect of the pH has to be excluded. In the above-mentioned experiment, the nitrate-nitrite treatment, the standard starter culture, *L. curvatus* LTH1174 and *L. sake* CTC494 decreased the pH to the same extent, allowing for the comparison of the anti-listerial effect of the strains.

Therefore, the bacteriocinogenic starter cultures have proved to be effective in inhibiting *Listeria* survival in fermented sausages, though the most appropriate cultures have to be selected according to the specific formulation and technology of fermentation.

BIOPRESERVATION AS A NEW REAL POSSIBILITY

Meat production has changed during the last decades. These changes were necessary and arose from the increased knowledge in nutrition and food hygiene as well as quality assurance. Distribution has also changed

from the small store to the hypermarket and the frequency of shopping has been reduced, thus the consumer seeks more stable and safer products with a longer shelf-life and which do not contain preservatives.

In this context bacteriocins produced by lactic acid bacteria associated with meat and meat fermentations such as *Pediococcus*, *Leuconostoc*, *Carnobacterium* and *Lactobacillus* spp. are likely to have much greater potential as meat preservatives (Stiles & Hastings, 1991; Shahidi, 1991; Yousef *et al.*, 1991).

L. monocytogenes, together with other food pathogens, are becoming emergent pathogens that can be isolated from foods of different origins, including meats and meat products. In meat processing plants, it may be present in the slicing rooms and may eventually contaminate pasteurized products during slicing and packaging.

Recently, some biopreservation techniques have been applied to meat products and these have involved the introduction of a competitive microflora of lactic acid bacteria as protective cultures to chill-stored ready-to-eat meat products, including bacteriocin-producing lactic acid bacteria and purified anti-listerial bacteriocins.

Yousef *et al.* (1991) investigated the growth of *L. monocytogenes* in packed wiener sausage, which is a fully-cooked cured meat product susceptible to contamination by *L. monocytogenes* before packaging. After the application of *Pediococcus acidilactici* or the purified pediocin, these authors provided evidence of the elimination of Gram-positive pathogenic bacteria in cooked meats during extended refrigerated storage.

In fresh meat there have been different reports. The antimicrobial activity of the pediocin produced by *P. acidilactici* was tested to determine whether the bacteriocin would reduce the populations of *L. monocytogenes* attached to a carcass, and remain stable enough to inhibit further contamination during processing (Nielsen *et al.*, 1990). The results of the study revealed that these bacteriocins, in association with red meat, have an inhibitory effect on *L. monocytogenes*. The effectiveness

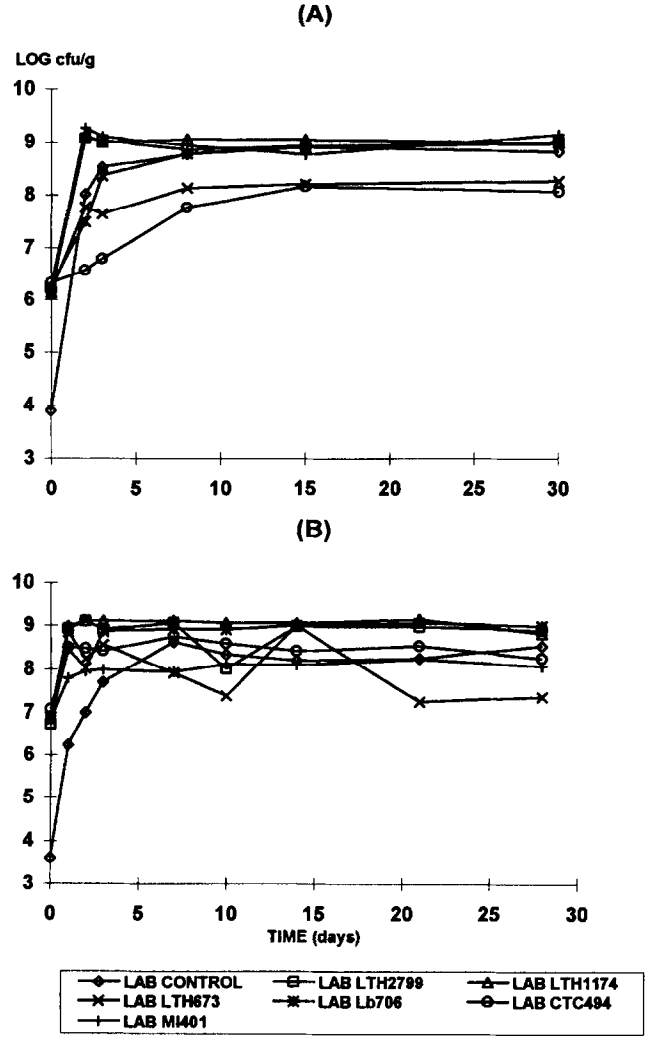

Fig. 2. Growth of lactic acid bacteria in fermented sausages inoculated with different Lactobacilli strains and with a non-inoculated control. (A) nitrate-nitrite cure, (B) nitrate cure.

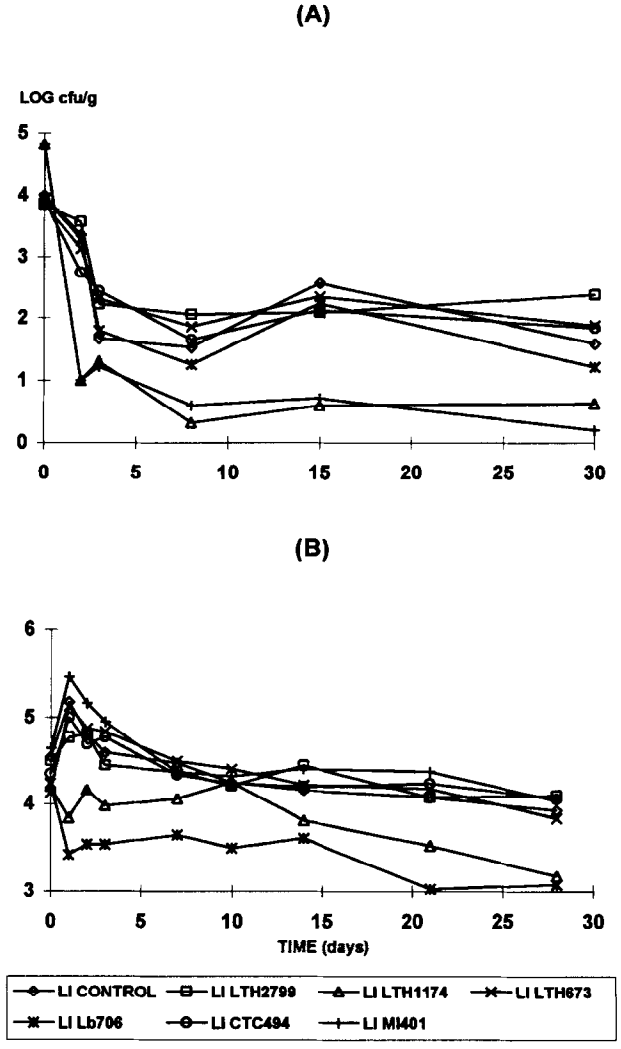

Fig. 3. Growth of *Listeria ivanovii* LTH3097 in fermented sausages inoculated with different Lactobacilli strains and with a non-inoculated control. (A) nitrate-nitrite cure, (B) nitrate cure.

of the bacteriocin appears to be dependent upon concentrations of both bacteriocin and bacteria.

In fresh chicken breast, the bacteriocinogenic strain *L. sake* CTC494, producing sakacin K, was inoculated by spraying the surface of chicken breasts previously contaminated with *Listeria*. The breasts were packed either in vacuum or in micro-aerophilic packages (Fig. 4). The vacuum-packed products displayed an absence of *Listeria* after 13 days of storage after an initial contamination of 230 cfu cm^{-2}. The *Listeria* counts in the control lot (vacuum-packed but with no bioprotective culture added) reached 440 cfu cm^{-2} during the same period of storage.

In modelised sliced vacuum-packed cooked ham, the kinetics of growth of inoculated *L. innocua* were evaluated using different bioprotective cultures *L. sake* LTH2799 (Bac^-), *L. curvatus* LTH1174 (Bac^+) and *L. sake* CTC494 (Bac^+). All the lactobacilli counts grew from 10^6 cfu g^{-1} to 10^8 cfu g^{-1} in eight days. *Listeria* counts in the samples with the non-bacteriocinogenic culture grew from 2.5 log cfu g^{-1} to 3 log cfu g^{-1}, while in the samples with bacteriocinogenic cultures, a decrease in *Listeria* numbers was observed. *L. curvatus* LTH1174 was the most effective strain in inactivating *L. innocua*. (< 3 MPN g). The same results were attained when purified sakacin K was added to the samples.

According to the above-mentioned results, an additional hurdle for preventing the growth of spoilage and pathogenic bacteria in a great variety of food products may be provided by using traditional methods of preservation in combination with bacteriocinogenic cultures of lactic acid bacteria or their bacteriocins alone.

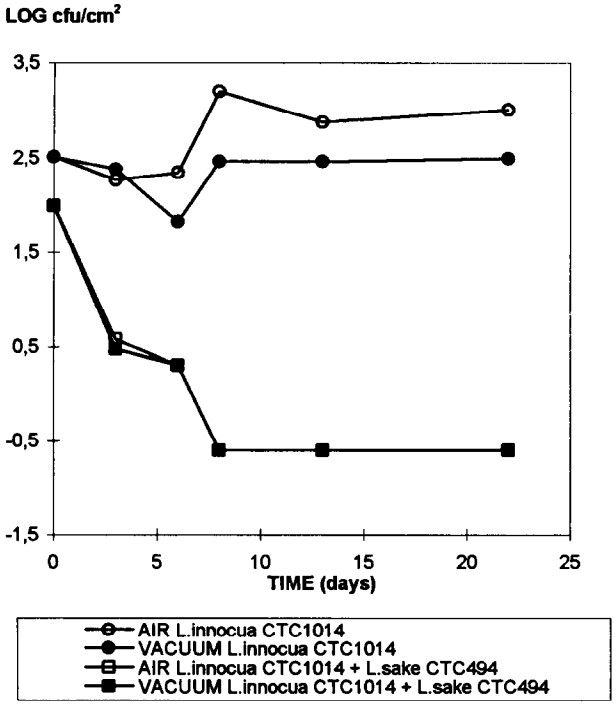

Fig. 4. Inhibition of *L. innocua* in fresh chicken breasts vacuum and air packed stored at 7°C.

Before bacteriocins can be applied in foods, they should be approved by government regulations for their application in meat products. Up to now, nisin is the only bacteriocin approved as a food additive. Unfortunately nisin is apparently not the most suitable bacteriocin for meat preservation despite its effectiveness in dairy products. Bacteriocins produced by meat-related bacteria seem to have better prospects for application. Further research is needed before application in meat products of these bacteriocins can become a reality.

COULD THE PROBIOTIC APPROACH BE ONE OF THE NEXT STEPS IN STARTER CULTURE DESIGN?

Lactic acid bacteria and specifically Lactobacilli are good candidates as probiotic strains because they are normal components of the gut microflora. The use of lactic acid bacteria in foods has a long history and Lactobacilli have a GRAS status and some of them have antagonistic properties towards pathogenic bacteria either by antimicrobial substance production or competitive exclusion.

Only recently have the scientific bases of probiotic studies been firmly established and clinical studies of some strains published. With the progression of clinical research, assessment and improvement of the stability of probiotic bacteria is being carried out, and we are now approaching the general acceptance of probiotics as functional ingredients, mainly in dairy products.

It is generally accepted that a probiotic strain has to be acid, lysozyme and bile resistant and able to colonize the human intestinal tract (at least temporarily) by means of some mechanisms for adhering or binding to the intestinal cells. Obviously, a probiotic will be a GRAS micro-organism and preferably of human origin.

However, there are a set of complementary conditions needed for a probiotic culture: (a) once administrated it must be capable of activating and growing quickly and remaining in the gut for an acceptable time (Juven *et al.*, 1991; Havenaar & Huis in't Veld, 1992); (b) it should be resistant to antibiotics normally present in foods, but never by plasmid coding for antibiotic resistance, and sensitive to those used in the therapy of acid lactic bacteria infections (normally penicillines or aminoglycosids) (Fonden, 1989); (c) no pathogenic, toxic, allergic, mutagenic or carcinogenic reactions must be caused by the strains or their fermentation products or cell compounds after bacterial death.

O'Sullivan *et al.* (1992) demonstrated that lactic acid bacteria are the micro-organisms in the highest quantity and regularity along the gastrointestinal tract, the main species being *L. acidophilus*, *L. casei*, *L. fermentum*, *L. salivarius*, *L. cellobiosus*, *L. brevis*, *L. reuteri*, *Ent. faecium*, *Ent. faecalis*, *Bifd. bifidum*, *Bifd. thermophilum*,

Bifb. pseudolongum and *P. pentosaceus*, though other species of lactic acid bacteria could be present.

The most interesting point is that the ability to remain in the intestine is strain-dependent, and is probably a function of the aggregation and adhesion capabilities. Naito *et al.* (1995) have demonstrated in piglets the differences in colonization and intestinal development of 20 biovars of *L. reuteri* and *L. acidophilus* from intestinal origin and the amount of time that they are in the gut. Consequently, the adhesion of lactic acid bacteria to intestinal epithelial cells is also a complex question. The Lactobacilli isolated from the jejunum, ileum and caecum showed a differential ability to adhere to the ileal epithelial cells, indicating that strains are specific to certain intestinal locations (Jin *et al.*, 1996). Therefore, the understanding of the adherence and aggregation mechanisms and their genetic coding and regulation in lactic acid bacteria will be one of the most interesting approaches in the probiotic field.

Because the probiotic properties of desirable bacteria are largely dependent on their ability to remain viable and to colonize the surface of human intestinal cells, sufficient numbers of viable bacteria must be present at the time of consumption, and in this sense it has been suggested that a viable cell number of 10^7–10^9 cells per day is necessary in order for any beneficial effects to develop in humans (Salminen *et al.*, 1993). Therefore, the daily intake of probiotic cultures in the diet requires the development of a wide range of fermented foods with these properties. Today, most probiotic strains are used in dairy products but, according to Lee and Salminen (1995), new probiotic functional foods will include infant formulae, baby foods, fermented fruit juices, fermented soy products, cereal-based products and, in our opinion, fermented meat products. Why not?

REFERENCES

Ahn, C. & Stiles, M. E. (1992). Mobilization and expression of bacteriocin plasmids from *Carnobacterium piscicola* isolated from meat. *J. Appl. Bacteriol.*, **73**, 217–228.

Axelsson, L., Holck, A., Birkeland, S. E., Aukrust, T. & Blom, H. (1993). Cloning and nucleotide sequence of a gene from *Lb. sake* Lb706 necessary for sakacin A production and immunity. *Appl. Environ. Microbiol.*, **59**, 2868–2875.

Aymerich, M. T., Hugas, M., Garriga, M., Vogel, R. F. & Monfort, J. M. (1993). Electrotransformation of meat lactobacilli. Effect of several parameters on their efficiency of transformation. *J. Appl. Bacteriol.*, **75**, 320–325.

Berry, E. D., Liewen, M. B., Mandigo, R. W. & Hutkins, R. W. (1990). Inhibition of *Listeria monocytogenes* by bacteriocin-producing *Pediococcus* during the manufacture of fermented semi dry sausage. *J. Food Protect.*, **53**, 194–197.

Berry, E. D., Hutkins, R. W. & Mandigo, R. W. (1991). The use of bacteriocin-producing *Pediococcus acidilactici* to control postprocessing *Listeria monocytogenes* contamination of frankfurters. *J. Food Prot.*, **54**, 681–686.

Buckenhüskes, H. J. (1994). Bacterial starter cultures for fermented sausage. *Meat Focus Inter.*, **12**, 497–500.

Campanini, M., Pedrazzoni, I., Barbuti, S. & Baldini, P. (1993). Behaviour of *Listeria monocytogenes* during the maturation of naturally and artificially contaminated salami: effect of lactic-acid bacteria starter cultures. *Int. J. Food Microbiol.*, **20**, 169–175.

Cantoni, A. C., Bianchi, M. A., Renon, P., Beretta, G., D'Aubert, S., Bertolani, G. & Giolitti, G. (1972). Trasformazioni bilogiche in prodotti alimentari stagionati. *Riv. di Biologia* **LXV**, 335–369.

Degnan, A. J., Yousef, A. E. & Luchansky, J. B. (1992). Use of *Pediococcus acidilactici* to control *Listeria monocytogenes* in temperature-abused vacuum-packaged wieners. *J. Food Prot.*, **55**, 98–103.

Foegeding, P. M., Thomas, A. B., Pilkington, D. H. & Klaenhammer, T. R. (1992). Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. *Appl. Environ. Microbiol.*, **58**, 884–890.

Fonden, R. (1989). *Lactobacillus acidophilus*. Les laits fermentés. *Actualité de la recherche* 35–40.

Gaier, W., Vogel, R. F. & Hammes, W. P. (1990). Genetic transformation of intact cells of *Lactobacilluscurvatus* Lc2 and *L. sake* Ls2 by electroporation. *Lett. in Appl. Microbiol.*, **11**, 81–83.

Gaier, W., Vogel, R. F. & Hammes, W. P. (1992). Cloning and expression of the lysostaphin gene in *Bacillus subtilisi* and *Lactobacillus casei*. *Lett. in Appl. Microbiol.*, **14**, 72–76.

Garriga, M., Hugas, M., Aymerich, T. & Monfort, J. M. (1993). Bacteriocinogenic activity of lactobacilli from fermented sausages. *J. Appl. Bacteriol.*, **75**, 142–148.

Hammes, W. P., Bantleon, A. & Min, S. (1990). Lactic acid bacteria in meat fermentation. *FEMS Microbiol. Rev.*, **87**, 165–174.

Havenaar, R. & Huis in't Veld, J. (1992). Probiotics: a general view. *The Lactic Acid Bacteria vol. 1*: The Lactic acid bacteria in health and disease. Ed. *Elsevier Appl. Sci.*, 151–170

Hugas, M., Garriga, M., Aymerich, T. & Monfort, J. M. (1993). Biochemical characterization of lactobacilli isolated from dry sausages. *Int. J. Food Microbiol.*, **18**, 107–113.

Hugas, M., Garriga, M., Aymerich, T. & Monfort, J. M. (1995). Inhibition of *Listeria* in dry fermented sausages by the bacteriocinogenic *Lactobacillus sake* CTC494. *J. Appl. Bacteriol.*, **79**, 322–330.

Hugas, M., Neumeyer, B., Pagés, F., Garriga, F. & Hammes, W. P. (1996). Die antimikrobielle Wirkung von Bakteriozinbildenden Kulturen in Fleischwaren: II) Vergleich des Effektes unterschiedlicher bakteriozinbildender Laktobazillen auf Listerien in Rohwurst. *Fleischwirtsch.*, **76**(6), 649–652.

Jin, L. Z., Ho, Y., Ali, M., Abdullah, N., Ong, K. & Jalaludin, S. (1996). *Letters in App. Microbiol.*, **22**, 229–232.

Juven, B. J., Meinersmann, R. & Stern, N. (1991). Antagonistic effects of lactobacilli and pediococci to control intestinal colonization by human enteropathogens in live poultry. *J. Appl. Bacteriol.*, **70**, 95–103.

Knauf, H. J., Vogel, R. F. & Hammes, W. P. (1992). Cloning, sequence and phenotypic expression of KatA, which encodes the catalase of *Lactobacillus sake* LTH677. *Appl. Environ. Microbiol.*, **58**, 832–839.

Larsen, G. A., Vogensen, F. K. & Josephsen, J. (1993). Antimicrobial activity of lactic acid bacteria isolated from sour doughs: purification and characterization of bavaricin A a bacteriocin produced by *Lactobacillus bavaricus* MI401. *J. Appl. Bacteriol.*, **75**, 113–122.

Lee, Y. & Salminen, S. (1995). The coming of age of probiotics. *Trends in Food Sci. and Tech.*, **6**, 241–245.

Naito, S., Hayashidani, H., Kameko, K., Ogawa, M. & Benno, Y. (1995). Development of intestinal lactobacilli in normal piglets. *J. Appl. Bacteriol.*, **79**, 230–236.

Nielsen, J. W., Dickson, J. S. & Crouse, J. D. (1990). Use of a bacteriocin produced by *Pediococcus acidilactici* to inhibit

Listeria monocytogenes associated with fresh meat. *Appl. Environ. Microbiol.*, **56**, 2142–2145.

Obst, M., Meding, E. R., Vogel, R. F. & Hammes, W. P. (1992). Two genes encoding the B-galactosidase of *Lactobacillus sake*. *Microbiol.*, **141**, 3059–3066.

O'Sullivan, M. G., Thornton, G., O'Sullivan, G. C. & Collins, J. K. (1992). Probiotic bacteria; myth or reality?. *Trends in Food Sci. and Tech.*, **3**, 309–314.

Remiger, A., Ehrmann, M. & Vogel, R. F. (1996). Identification of bacteriocin-encoding genes in lactobacilli by polymerase chain reaction (PCR). *System. Appl. Microbiol.*, **19**, 28–34.

Reuter, G. (1971). Laktobazillen und eng verwandte Mikroorganismen in Fleisch und Fleischwaren. 5. Mitt.: Proteolytische Enzymaktivitäten. *Fleischwirtsch.*, **51**, 1901–1906.

Salminen, S., Deighton, M. & Gorbach, S. (1993). In *Lactic acid bacteria* (eds S. Salminen & von Wright) 199–225. Marcel Dekker.

Schillinger, U., Kaya, M. & Lücke, F. K. (1991). Behaviour of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*.. *J. Appl. Bacteriol.*, **70**, 473–478.

Shahidi, F. (1991). Developing alternative meat-curing systems. *Trends Food Sci. Technol.* September, 291–322.

Skytta, E., Hereijgers, W. & Mattila-Sandholm, T. (1991). Broad spectrum antibacterial activity of *Pediococcus damnosus* and *Pediococcus pentosaceus* in minded meat. *Food Microbiol.*, **8**, 231–237.

Stiles, M. E. & Hastings, J. W. (1991). Bacteriocin production by lactic acid bacteria: potential for use in meat preservation. *Food Sci. Technol.*, **2**, 235–263.

Talon, R., Montel, M. C. & Cantonnet, M. (1992) Lipolytic *activity* of *Micrococacceae*. Proceed. 38th ICoMST: 843–846 Clermont-Ferrand. France.

Tichaczek, P., Nissen-Meyer, J., Nes, I. F., Vogel, R. F. & Hammes, W. P. (1992). Characterization of the bacteriocin curvacin A from *Lactobacillus curvatus* LTH1174 and sakacin P from *L. sake* LTH673. *System. Appl. Microbiol.*, **15**, 460–468.

Tichaczek, P. S., Vogel, R. F. & Hammes, W. P. (1993). Cloning and sequencing of *cur A* encoding curvacin A, the bacteriocin produced by *Lactobacillus curvatus* LTH1174. *Arch. Microbiol.*, **160**, 279–283.

Tichaczek, P. S., Vogel, R. F. & Hammes, W. P. (1994). Cloning and sequencing of *sak P* encoding sakacin P, the bacteriocin produced by *Lactobacillus sake* LTH673. *Microbiol.*, **140**, 361–367.

Verplaetse, A., Demeyer, D., Gerard, S., & Buys, E. (1992) Endogenous and bacterial proteolysis in dry sausages fermentation. Proceed. 38th ICoMST: 851–854 Clermont-Ferrand. France.

Vogel, R. F., Pohle, S., Tichaczek, P. & Hammes, W. P. (1993). The competitive advantage of *Lactobacillus curvatus* LTH1174 in sausage fermentations is caused by formation of curvacin A. *System. Appl. Microbiol.*, **16**(3), 457–462.

Winkowski, K., Crandall, A. D. & Montville, T. J. (1993). Inhibition of *Listeria monocytogenes* by *Lactobacillus bavaricus* MN in beef systems at refrigeration temperatures. *Appl. Environ. Microbiol.*, **59**, 2552–2557.

Wolf, G. & Hammes, W. P. (1988). Effect of hematin on the activities of nitrite reductase and catalase in lactobacilli. *Arch. Microbiol.*, **149**, 220–224.

Wolf, G., Strahl, A., Meisel, J. & Hammes, W. P. (1990). Heme-dependent catalase activity of lactobacilli. *Int. J. Microbiol.*, **12**(2/3), 133–140.

Yousef, A. E., Luchansky, J. B., Degnan, A. J. & Doyle, M. P. (1991). Behavior of *Listeria monocytogenes* in Wiener exudates in the presence of *Pediococcus acidilactici* or Pediocin AcH during storage at 4 or 25°C. *Appl. Environ. Microbiol.*, **57**, 1461–1467.