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Review

Preserving non-fermented refrigerated foods with microbial cultures—a review

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The use of microbial cultures offers a natural temperature-responsive preservation method. The inhibition at refrigeration temperatures is associated with production of low molecular weight compounds including lactic acid, hydrogen peroxide and bacteriocins. Bacteriocin-producing cultures are not effective against Gram-negative bacteria and special strategies are needed. The role of intrinsic and extrinsic factors in the antibiosis is reviewed with the examples in a range of refrigerated foods. The suggested protocol for practical applications includes the selection of cultures, screening in microbiological media, trials in food and the strategies to enhance the inhibitory effect.

Freshness has been identified as the most important trend in food preservation of the next decade (Sloan, 2001). Refrigeration is a preservative-free method, which traditionally has been applied to extend the shelf-life of such perishable foods as raw meat, dairy and small goods. Psychrotrophic *Listeria monocytogenes* and *Pseudomonas* species are examples of the FPSOs (food poisoning or spoilage organisms) of concern (Grau, 1996). The new generation of minimally processed ready-to-eat convenience foods such as REPFEDs (refrigerated processed foods of extended durability) including sous vide (cooked-in-a-bag) and MAP (modified atmosphere packed) foods are gaining popularity. This is the most dynamic sector of the market. It is predicted that in Europe the sale of chilled prepared foods will reach 15 billion Euros by the year 2003 (Sheard, 1999). The small scale of production and the large variety of products coupled with the high speed of new product introduction often does not allow for proper validation of preservation methods. Mild processing coupled with a changed storage atmosphere through vacuum packaging or MAP can give selective advantage to pathogens, such as *Clostridium botulinum* for example (Notermans, Dufrenne, & Lund, 1990), which otherwise would not be significant. A single case of food poisoning can damage these novel technologies. Temperature is often the only controlling factor and additional preservation techniques are needed (Gould, 1999).

Use of chemical preservatives is not compatible with the 'fresh' image of these foods. The latest trend is a synergetic combination of subtle preservation factors including biopreservation. Biopreservation is the extension of storage life and enhancing of safety of foods using the natural or controlled microflora and/or their antimicrobial products (Stiles, 1996).

Protective cultures

Similar to probiotic and starter cultures, PCs (protective cultures) are food-grade bacteria (most likely lactic acid bacteria or LAB), which may or may not be strains naturally present in the food type. They are selected for their ability to grow in a product and inhibit a FPSO rather than to deliver a desired texture/favour profile as in fermentation, or health benefits of probiotics. Under normal storage conditions PCs should not effect product sensorial qualities (Leroi, Arbey, Joffraud, & Chevalier, 1996).

The unique advantages of using a PC are: additive-free preservation; natural image; and temperature—responsive inhibition—it can 'switch on' only when temperature abuse takes place. Furthermore, the constant production of bacteriocins by viable cultures can overcome the problem of their decomposition and binding to food particles when used as an additive (Scott & Taylor, 1981). Although, gradual production of bacteriocins can also simulate a stepwise exposure to

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increasing concentrations and, thus, generate resistance (Mazzotta & Montville, 1997).

It is possible that indigenous bacteriocin-producing LAB (Garver & Muriana, 1993) already contributes to the safety of many refrigerated foods including ready-to-eat foods (Table 1). Theoretically, such foods can be re-formulated or processing parameters re-designed in order to encourage the growth of these species. However, as with fermentation, the addition of PCs directly into the product offers a controlled process in terms of desired safety outcomes and in the prevention of uncontrollable spoilage.

Safety

Potential health risks associated with LAB are the rare cases of bacteraemia (Donohue, 1998) associated with vancomycin therapy (Stiles, 1996). Strong evidence of non-pathogenicity, such as being non-haemolytic and antibiotic sensitive (Embarek, Jeppesen, & Huss, 1994), should be given for the cultures isolated from foods but not routinely used in food fermentation/production. BLIS (bacteriocin-like inhibitory substance to which the producer has specific immunity) produced by *Bacillus* species (Lyver, Smith, Austin, & Blanchfield, 1998) rather than the live bacteria can be considered for potential applications. The horizontal transfer of plastids may dramatically alter the phenotypes of *Bacillus* species in terms of their pathogenicity (Helgason *et al.*, 2000). Unlike LAB, anti-botulinal *Clostridium perfringens* (Smith, 1975) and anti-listerial *Enterococcus* (Embarek *et al.*, 1994) are not GRAS-level (Generally Recognised as Safe, US).

The consumption of LAB induces specific secretory immunity and enhances the gut's inflammatory immune response (Perdigon, Vintini, Alvarez, Medina, & Medici, 1999) and can be viewed as a part of the 'improving safety through nutrition' trend. Furthermore, PCs with probiotic qualities offer additional marketing opportunities.

Table 1. Populations of LAB including bacteriocin-producing strains (bac^+ lab)[a] from retail ready-to-eat foods

Food sample	Total LAB (cfu/g)	Bac^+ LAB (cfu/g)
Raw meat		
Ground beef	1.3×10^5	1.8×10^3
Ground chicken	1.2×10^4	4.0×10^1
Ready to eat meat (cold cuts)		
Bologna[b] No. 1	2.4×10^3	9.4×10^1
Bologna[c] No. 2	1.3×10^5	5.4×10^4
Ham[c] No. 1	2.8×10^3	2.0×10^1
Ham[c] No. 2	4.1×10^4	1.0×10^2
Roast Beef[b] No. 1	3.1×10^7	2.4×10^5
Roast Beef[c] No. 2	8.4×10^2	2.0×10^1
Chicken[c]	2.3×10^5	2.0×10^3

[a] On MRS agar.
[b] Commercially sliced and vacuum-packaged.
[c] Sliced at the deli counter.
Adopted from Garver and Muriana (1993).

Nature of inhibition

LAB can be isolated from a wide variety of habitats associated with plants and animals including foods such as dairy products, meat and vegetables. Nutritional requirements of LAB are complex and require a rich media for growth (Holt, Krieg, Sneath, Staley, & Williams, 1994). LAB ferment and spoil, or ferment uncontrollably, milk, meat, cereals, bakery products, fruits and vegetables. Being a strong competitor in its natural habitat, LAB evolved the ability to produce a variety of inhibitory substances including lactic acid and other low molecular weight metabolites (hydrogen peroxide, alcohols, carbon dioxide, diacetyl, benzoic acid, etc.). Bacteriocin-producing LAB are identified as producing relatively low molecular weight proteins that are normally bacteriocidal to other Gram-positive bacteria (Ray & Daeschel, 1994).

In designing a preservation system the understanding of the nature of inhibition and factors effecting it is important. Different natures of inhibition can be observed even within the same species: at 5 °C one of two strains of *Carnobacterium piscicola* produced a proteinaceous anti-listerial compound; and another inhibited *L. monocytogenes* 057 by possible nutrient depletion and/or occupying vital ecological niches (Nillson, Graham, & Huss, 1999). It is also possible that several mechanisms can take place at the same time.

Reduction of the pH to 4.6 delayed the production of toxin by *C. botulinum* in pea soup by co-incubation with non-bacteriocin producing *Lactobacillus plantarum* ATCC 8014 at 5, 15 and 25 °C (Skinner, Solomon, & Fingerhut, 1999). *L. alimentarius, L. delbrueckii* subsp. *bulgaricus* and *Streptococcus xylosus*, on the other hand, did not reduce the pH of ham and inhibited *Pseudomonas* by hydrogen peroxide production (Gilliland & Speck, 1975; Kotzekidou & Bloukas, 1996). D-Lactate was utilised to produce hydrogen peroxide in the presence of lactate oxidase (Villegas & Gilliland, 1998).

In the majority of cases, the inhibition is connected with bacteriocin production. Bacteriocins are effective at low concentrations (10 mg/kg of nisin, for example) and do not affect the product's sensorial quality. Bacteriocins form poration complexes in the membrane of Gram-positive bacteria through a multi-step binding and insertion process (Montville & Chen, 1998). This causes the leakage of essential cellular materials such as amino acids, minerals and intracellular adenosine triphosphate from the cell and in severe cases—lysis (Ramseier, 1960). Bacteriocin-producing PCs are suitable for refrigerated products with reduced oxygen atmosphere. The microflora of such products is dominated by Gram-positive bacteria—LAB, bacilli and

clostridia including pathogens such as *C. botulinum* (Betts & Gaze, 1995) and *B. cereus* (Carlin, Guinebretiere, Choma, Schmott, & Nguyen-The, 1999).

The examples of bacteriocin-mediated antigenesis at refrigerated temperatures are as follows: *L. curvatus* and *Lactococcus lactis* inhibited *Bacillus cereus, Staphylococcus aureus* and *C. perfringens* (Garver & Muriana, 1993); *Pediococcus pentosaceus* 43200 prevented toxigenesis by *C. botulinum* (Crandall, Winkowski, & Montville, 1994); *L. monocytogenes* was inhibited by *L. bavaricus* (Winkowski, Crandall, & Montville, 1993) and other LAB isolated from retail cuts of meat (Lewus & Montville, 1991). Non-LAB can produce BLIS at low temperatures. The BLIS of *Bacillus* spp. was effective against *C. botulinum*, *L. monocytogenes, S. faecalis, S. aureus* and *B. cereus* in raw and cooked surimi-shrimp (Lyver *et al*., 1998).

The limitations in applying bacteriocinogenic cultures are: their spectrum is narrow; possible development of resistance (Embarek *et al*., 1994); and inability to inhibit Gram-negative bacteria (Helander, von Wright, & Mattila-Sandholm, 1997). Aerobic psychrotrophic Gram-negative pathogens *Yersinia enterocolitica, Aeromonas hygrophilia*, *Salmonella* and *Escherichia coli*, as well as spoiling *Pseudomonas* are significant FPSOs for refrigerated foods. Control of Gram-negative bacteria by a bacteriocinogenic PC is questionable. The outer membrane of Gram-negative bacteria, which contain lipopolysaccharides and no phospholipids, acts as a permeability barrier against macromolecules and hydrophobic solutes such as bacteriocins. Gram-negative bacteria can be even stabilised by bacteriocins against the action of acids (Helander & Mattila-Sandholm, 2000).

Inhibition of Gram-negative bacteria can be enhanced by the addition of chelating agents. They make the membrane permeable to bacteriocins, although they are often toxic. Alternatively, synergistic combinations with lactoperoxidase, pimaricin and hydrolytic enzymes can be applied (Helander *et al*., 1997). A PC can be selected which inhibits by the production of lactic acid and/or other hydrophilic substances. Benzoic acid, 5-methyl-2,4-imidazolidinedione, tetrahydro-4-hydroxy-4-methyl-2H-pyran-2-one and 3-(2-methylpropyl)-2,5-piperazinedione produced by *L. plantarum* inhibited the growth of Gram-negative *Pantoea agglomerans* at 10 ppm in syneresis with lactic acid (Niku-Poavola, Laitila, Mattila-Sandholm, & Haikara, 1999). A PC can be manipulated through selection or genetic engineering (Kim, 1993) to enhance the production of these compounds.

Factors affecting PC performance

Unlike preservation with chemicals, the simple addition of a PC into the product does not guarantee a successful outcome—the microbial antigenesis is a dynamic process. The interaction will depend on the rate of growth, both of the PC and the FPSO, the rate of production of inhibiting substances and toxins (in the case of an exo-toxinogenic pathogen). This, in turn, is determined by the intrinsic characteristics of the food type and extrinsic factors such as processing parameters, the quantity and identity of the PCs used.

Temperature effect

Growth rate

The PC should be able to survive in products under refrigeration and be able to grow and exert an antagonistic effect at the desired temperatures. Failure to delay the toxin formation by *C. botulinum* in sous vide beef with gravy was attributed to the possible inability of *P. pentosaceus* to grow at 4 and 10°C (Crandall *et al*., 1994). *P. acidilactici*, on the other hand, grew and delayed the formation of botulism toxin in chicken salad at 10–35°C (Hutton, Chehak, & Hanlin, 1991). *L. plantarum* did not grow at 4°C, but survived well for 21 days in meat (Tanaka, Traisman, Lee, Cassens, & Foster, 1980). The temperature increase from 4 to 8°C stimulated the growth of the LAB isolates from ready-to-eat vegetables (Vescovo, Torriana, Orsi, Macchiarolo, & Scolari, 1996).

The growth rate of a PC should be higher to that of a FPSO (Breidt & Fleming, 1997). *Enterococcus faecium* (1.3×10^4 cfu/ml) failed to inhibit *L. monocytogenes* at 3 or 5°C due to a higher growth rate of the latter (Smith, 1975). If the inoculated product is intended for cooking, the heat resistance of the PC should be taken into consideration. *L. alimentarius* was destroyed in ham during heating to a core temperature of 72°C, thus leaving the product unprotected (Kotzekidou & Bloukas, 1996). None of the selected LAB survived sous-vide cooking (Bolton, 1998).

Bacteriocin-producing ability

Bacteriocins production rate and their activity depends on the incubation temperature. Detection of nisin produced by *Lc. lactis* coincided with the reduction in populations of non-proteolytic *C. botulinum* in co-incubation trials at 10°C (Fig. 1). However, the rate of nisin production was not fast enough to prevent botulinum toxin formation (Rybka-Rodgers, Kailasapathy, Cox, Peizis, & Arumugaswamy, 2001). Nisin lost its effectiveness with an increase in temperature from 15 to 35°C (Rogers & Montville, 1994).

FPSO sensitivity

The temperature range at which a FPSO is sensitive to inhibition should be taken into account. Psychrotrophic strains of LAB isolated from commercial fresh salads inhibited different pathogens at different temperatures: *Aeromonas hydrophiea* and *L. monocytogenes* at 8°C but not at 37°C and S*taphylococcus aureus* and coliforms

conversely (Vescovo *et al*., 1996). None of the selected LAB could prevent toxogenesis by *C. botulinum* at temperatures above 25°C in a model gravy system (Crandall & Montville, 1993). Generally, lowering the temperature increases the sensitivity of pathogens, such as *L. monocytogenes* (Smith, 1975; Winkowski *et al*., 1993) and *C. botulinum* (Rogers & Montville, 1994; Rybka-Rodgers *et al*., 2001).

PC and FPSO identity

A PC can be effective against one or several FPSOs (Table 2). The inhibitory substance production rate by a PC and the sensitivity of a FPSO to inhibition depends on the identity of the interactive cultures. *Salmonella* was less sensitive than *Pseudomonas* to the selected LAB (Raccach & Baker, 1979). Eighteen strains of *C. botulinum* had limited, moderate and extreme spore sensitivity to nisin (Okereke & Montville, 1991a).

The hydrogen peroxide-producing ability of selective cultures was ranked in the following order: *L. lactis* > *L. delbrueckii* subsp. *bulgaricus* > *P. cerevisiae* (Gilliland & Speck, 1975). *L. alimentarius* was a better inhibitor for micrococci and staphylococci than *S. xylosus* in cooked vacuum-packed ham (Kotzekidou & Bloukas, 1996). *L. casei* and *P. pentosaceus* 43200 provided the best protection amongst the selected LAB strains against *C. botulinum* (Montville, Rogers, & Okerede, 1992; Rogers & Montville, 1994). The most resistant FPSO and the most effective PC strain should be chosen for challenge studies.

Inoculum effect

The size of the inoculum impacts not only on the speed of the development of preservation factors, but on the sensory quality of the product and the cost-effectiveness of the method. Generally, high inoculums (10^6–10^9 cfu/g) are needed (Table 2). The PC inoculation levels depended on the growth medium, the initial load of the FSPO and the identity of the PC used (Okereke & Montville, 1991b; Rogers & Montville, 1994). Low inoculums of *E. faecium* (1.3×10^4 cfu/ml) did not inhibit

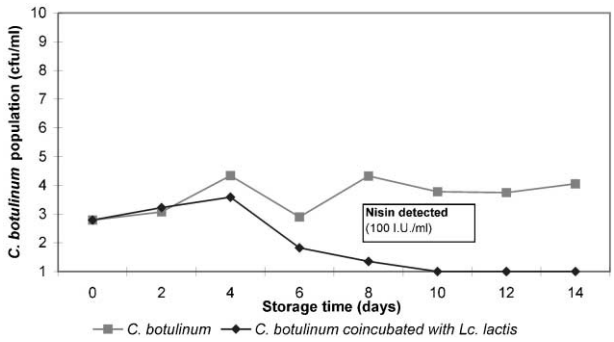

Fig. 1. Growth of non-proteolytic *C. botulinum* 17B at 10°C in TPGY broth with and without nisin-producing *Lc. lactis* 146 inoculated at 10^6 cfu/ml. Adopted from Rybka-Rodgers *et al.* (2001).

10^2 cfu/ml of *L. monocytogenes* and high populations (1.6×10^7 cfu/ml) were needed (Smith, 1975). Successful inhibition of psychrophiles in milk at refrigerated temperatures required at least 10^8 cfu/ml of LAB (Gilliland & Speck, 1975). Excessively high populations may restrict the growth of the culture, which is necessary for bacteriocin production (Yang & Ray, 1994).

Food effect

Food composition and structure have a significant effect on the dynamic and final outcomes of an interaction. Naturally present ingredients can favour or inhibit the interactive cultures. Food composition can be manipulated to achieve the desired effect.

Salts, acids, spices, added preservatives and bacteriocins have a synergetic effect with PCs. The combination of LAB and 4% NaCl at 15°C, for example, completely inhibited *C. botulinum* spore growth (Rogers & Montville, 1994). Food can also contain substances promoting bacteriocin production such as glucose, yeast extract and biotin (Yang & Ray, 1994). Indigenous microflora can produce its own inhibitory effect on the pathogen of interest. For example, LAB in raw and *Bacillus* species in cooked surimi nuggets prevented toxigenesis by *C. botulinum* type E (Lyvez *et al*., 1998).

The low pH of a product can favour bacteriocin production and increase their activity (Yang & Ray, 1994). The decrease in pH from 6 to 4 increased the concentration of pediocin AcH produced by *P. acidilactici* H from negligible to 3.2×10^4 AU/ml (Biswas, Purbita, Johnson, & Ray, 1991). The high pH of pasteurized eggs, on the other hand, rendered the environment unfavourable for the inhibition of background microflora by LAB (Raccach & Baker, 1979). The reduction in pH through production of lactic acid can be stimulated by the addition of 0.5% sucrose (Tanaka *et al*., 1980), 0.5% glucose (Ernsaw, Mitchell, & Banks, 1989; Winkowski *et al*., 1993) or 1% dextrose (Hutton *et al*., 1991). However, the overdosing of simple sugars can cause undesirable over-acidification (Ernshaw *et al*., 1989).

The food structure has an effect on the dynamics of microbial growth and inhibitory substances diffusion rate. Growth rates are usually slower in structured foods than in liquid media (Robins & Wilson, 1994). To the author's knowledge there have been no studies comparing the inhibitory effect in structured versus homogenized/liquid foods. The differences of *Listeria* species sensitivity to inhibition in broth and agar medium (Coventry *et al*., 1997) can be indicative.

The even distribution of PCs and their metabolites throughout a product is critical to prevent niches where the product is left unprotected. Chicken a la king inoculated with *C. botulinum* and a mixture of *Lc. lactis* and *L. delbrueckii* subsp. *bulgaricus* became toxic as a result of inadequate mixing (Saleh & Ordal, 1955).

Table 2. Application of PC to non-fermented refrigerated foods

PC	Temperature (°C)	Inoculum (cfu/g)	FSPO	Food	Reference
Raw meat, milk and eggs					
L. delbrueckii subsp. *bulgaricus* *P. cerevisiae*	5	5×10^8	*Pseudomonas* spp.	Ground beef	Gilland and Speck (1975)
P. cerevisiae	3	10^9	*Pseudomonas* spp.	Raw poultry	Raccach, Baker, Regenstein, and Mulnox (1979)
Lc. Lactis	7	5×10^7	*E. coli* O157:H7	Raw chicken meat	Brashears, Reilly, and Gilliland (1998)
L. delbrueckii subsp. *bulgaricus*	5, 7	10^8	*P. fragi* Psychrotrophs	Milk Fresh crab meat	Gilliland and Speck (1975)
P. cerevisiae *L. plantarum*	3	10^9	*Pseudomonas* spp. *S. typhimurium*	Pasteurised liquid whole egg	Raccach and Baker (1979)
Ready-to-eat meals					
E. faecium	3	10^4, 10^7	*L. monocytogenes* *C. botulinum* *C. perfringens* *B. thermosphacta*	Sous vide fish	Smith (1975)
Lc. lactis *L. delbrueckii* subsp. *bulgaricus*	30	10^6	*C. botulinum*	Chicken a la king	Saleh and Ordal (1955)
L. plantarum ATCC 8014	5, 15, 25	10^6	*C. botulinum* type A, B and E	Refrigerated can pea soup	Skinner *et al.* (1999)
L. bavaricus	4, 10	10^3,10^5	*L. monocytogenes*	Sous-vide beef	Winkowski *et al.* (1993)
L. plantarum	4	4×10^6	*C. botulinum*	Cured meat	Tanaka, Meske, Doyle, Traisman, Thayer, and Johnson (1985)
P. acidilactici	7				
L. alimentarius *S. xylosus*	4	10^{10}	Micrococci Staphylococci *Brochothix thermosphacta* Aerobic bacteria *Pseudomonas*	Cooked ham	Kotzekidou and Bloukas (1996)
L. casei *L. plantarum* *Pediococcus* spp.	4, 8	10^8	*L. monocytogenes* *S. typhimurium* *S. aureus* *Aeromonas hydrophila*	Ready-to-eat vegetables	Vescovo *et al.* (1996)
P. acidilactici	10–35	10^6	*C. botulinum*	Chicken salad	Hutton *et al.* (1991)
L. delbrueckii subsp. *Bulgaricus*	6	4×10^9	Background microflora	Tuna and potato salad	Raccach *et al.* (1979)
Seafood					
Leuconostoc spp *L. plantarum*	5	10^6,10^7	*L. monocytogenes* *Yersinia enterocolitica*	Shrimp extracts	Jeppesen ad Huss (1993)
Carnobacterium piscicola *L. plantarum*	4	10^8	H_2S-producing bacteria Yeasts and molds	Smoked salmon	Leroi *et al.* (1996)
Lc. lactis subsp. *lactis* ATCC 11454	10	3×10^6	*L. monocytogenes*	Cold-smoked salmon	Wessels and Huss (1996)
Carnobacterium piscicola *L. plantarum*	5	5×10^6	*L. monocytogenes*	Cold-smoked salmon	Nillson *et al.* (1999)
Others					
L. plantarum *L. mesenteroides* *P. pentosaceus*	15	10^8	*Salmonella* *L. monocytogenes*	Model food slurries	Ernsaw *et al.* (1993)
Lc. lactis *P. pentosaceus*	10	10^6 10^7	*C. botulinum* 17B	TPGY broth	Rybka-Rodgers *et al.* (1989)
L. plantarum	15	10^8–10^9	*S. typhimurium* *Staphylococcus aureus*	Buttered brain heard Infusion Broth	Raccach *et al.* (1979)

Gravy added to cooked beef cubes served as a matrix to promote the diffusion of bacteriocin in order to reach targeted pathogens (Winkowski *et al.*, 1993). Some LAB consume oxygen in the storage atmosphere (Ishibashi & Shimamura, 1993), which can extend the product shelf-life (Kotzekidou & Bloukas, 1996) and favour the growth of *C. botulinum* (Gaze, 1992). Thus, the final stages of challenge studies should be performed in a real food system reflecting the product composition, structure and background microflora.

Applications

Examples

The effect of PCs was demonstrated in raw meat, eggs, ready-to-eat meals and salads, seafood and other foods as well as microbiological media (Table 2). LAB can be used to improve the safety of minimally processed fruits and vegetables (Breidt & Fleming, 1997). The 'Wisconsin process'—a combination of LAB and sucrose to prevent botulinum toxigenesis in reduced nitrite bacon (Tanaka *et al.*, 1985)—was one of the early applications. Inoculation of salads or dressings with *L. acidophilus*, *L. plantarum, L. delbrueckii* and *P. acidilactici* (Hutton *et al.*, 1991; Nederland, 1998) are more recent examples. In Australia, *L. plantarum, L. rhamnoseus* and *P. freudenreichii* targeting *L. monocytogenes* and clostridia are marketed for use in cheeses, salads and ready-to-eat meals. To the author's knowledge, there have been no commercial applications in non-fermented foods. Despite the fact that these cultures have been used in food fermentation, a new standard would be required.

Protocol for application of PC

Practical applications would provide more data, which would allow quantitative assessment of the significance of the factors involved. The suggested approach in preserving non-fermented refrigerated foods with PCs is described in Fig. 2.

PCs, as any other preservation technique, should not be used to mask a microbiologically poor quality product. The fact that LAB often represents the majority of the indigenous microflora does not mean that it will have the desirable attributes. They can spoil the product (Kotzekidou & Bloukas, 1996) or may not inhibit the targeted FPSO (Winkouski *et al.*, 1993). The presence of the species with desirable qualities in a product can not be guaranteed. Considering the importance of food safety risks and complexity of interactions between PCs and FSPOs, the reliance on the species naturally occurring in a product is not desirable. At the same time the indigenous species can be used as potential candidates for PCs during initial screening. Such cultures are likely to be accepted by consumers as being a natural part of the product and their ability to survive the processing is another important advantage.

Thus, the search for potential PCs and targeted FPSOs can start with studying the product's microflora as well as consulting literature sources. It is desirable that the state of microbiological media used for the initial screening of PCs and the state of food is the same—solid or liquid. The physiological condition of a PC, frozen or freeze-dried, should be taken into account. Achieving sufficient concentrations of inhibitory substances in non-fermented foods without changing their sensory quality can present a challenge (Breidt & Fleming, 1997).

The strategies for enhancing inhibition are based on the knowledge of the factors effecting the interaction. The synergetic effects can be achieved through a mixture of PCs producing different bacteriocins (Mulet-Powell, Lacoste-Armynot, Vinas, & Buicherg, 1998) or through

Table 3. Factors effecting PCs performance in non-fermented refrigerated foods

Factor	Possible impacts
Increase in PC inoculation level	• Reduces the time for production of sufficient quantity of inhibitory substance • Excessively high inoculums can prevent culture growth and bacteriocin production
Increase in incubation temperature	• Increases the growth rate of PC and FPSO • Can reduce bacteriocin-producing capacity and bacteriocin activity • Can reduce FPSO sensitivity
Choice of PC strain	• FPSO target range • Rate of inhibiting substance production • Minimum growth temperature • Possible alteration of redox potential • Ability to spoil the product • Survival during processing • Possible health benefits/hazards
Type of food	• Growth and inhibitory substances diffusion rate • Indigenous microflora can produce its own inhibitory effect • Antimicrobial substances can have a synergetic effect with the antibiosis • Growth and bacteriocin-production promoting substances can enhance the antagonistic effect

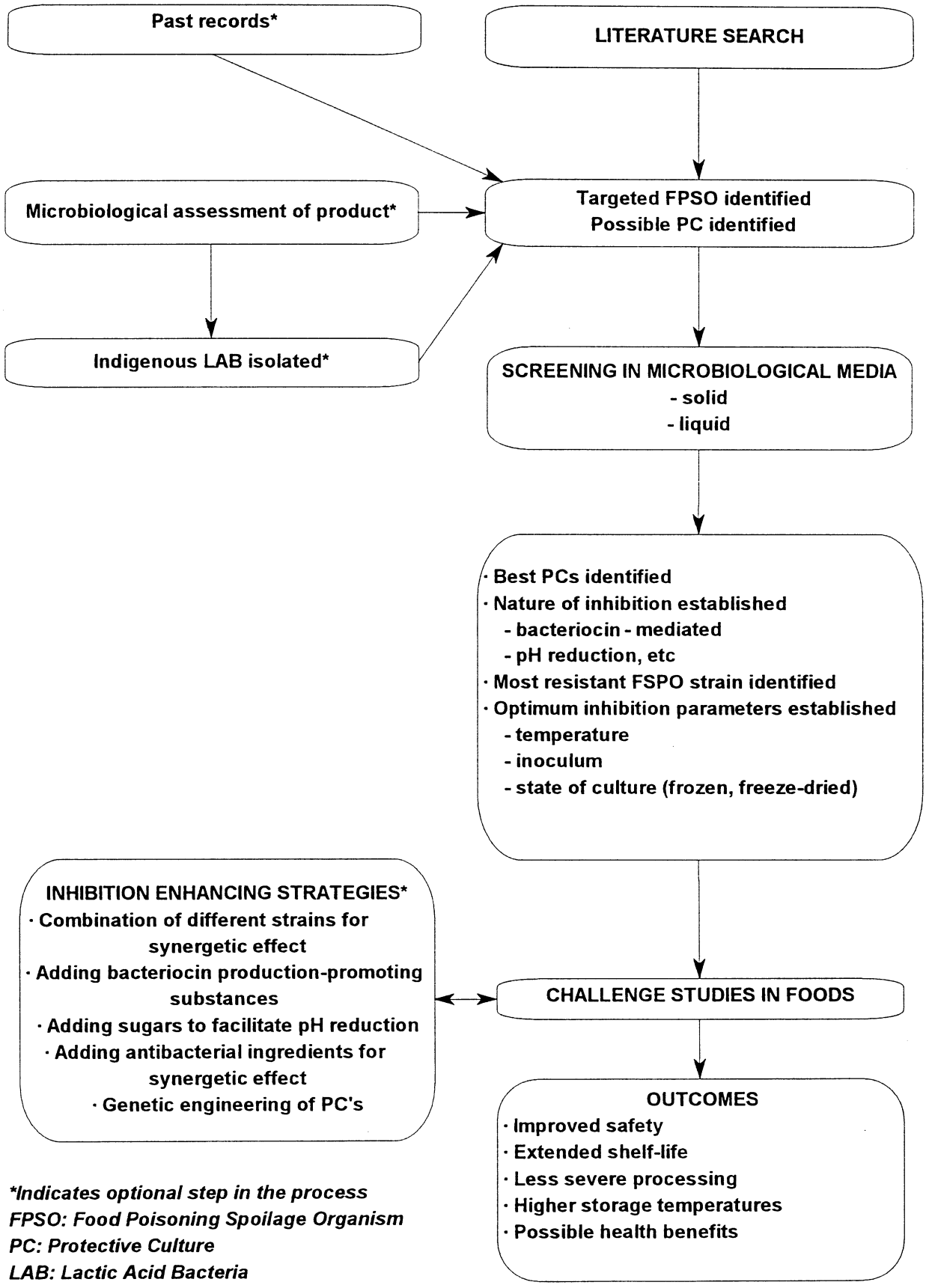

Fig. 2. Protocol for the application of protective cultures.

genetic manipulation by combining the genes responsible for production of several bacteriocins in one PC (Horn *et al*., 1999).

The possible outcomes of PCs application include the improvement of the safety of the product, decrease in the severity of processing and extension of shelf-life. Savings can be made in the reduction of energy costs and wastage. PCs can be selected to grow during normal storage conditions or only when temperature abuse takes place.

Conclusion

A number of factors are important for the outcome of antigenesis (Table 3). Some of them have conflicting effects and some are interrelated: both bacteriocin production and its activity depend on the pH of the medium; temperature impacts on the PC and FSPO growth rate, inhibitory substance production and diffusion rate as well as the sensitivity of the FPSO to inhibition.

As in fermentation, the development of preservation effect is gradual, but unlike fermentation, it should not effect the product's sensorial properties. Commercial applications are limited owing to the complexity of the inhibitory effect, ineffectiveness of bacteriocins against Gram-negative bacteria, heat sensitivity of cultures and the uncertainty of their legal status. In the future, PCs would be used possibly in synergetic relationships with other subtle hurdles as a part of sophisticated temperature-sensitive preservation systems, which would offer 'natural' hurdles with a potential to save processing costs.

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