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Note

Retention of *Listeria* and *Salmonella* cells contaminating skim milk by tangential membrane microfiltration ("Bactocatch" process)

MN Madec, S Mejean, JL Maubois *

INRA, Laboratoire de Recherches de Technologie Laitière,
65, rue de St-Brieuc, 35042 Rennes Cedex, France

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Summary — Retention by tangential membrane ultrafiltration of non-virulent *Listeria* and *Salmonella* cells whose morphological and physiological characteristics were close to pathogenic strains was studied. The bacteria were added to raw milk, pasteurized milk or previously microfiltered milk. Decimal reductions observed at 35°C were close to 1.9 units for *Listeria* and 2.5 units for *Salmonella*. The reduction was not influenced by contamination level (between 10^2 and 10^6 CFU/ml). An increase of microfiltration temperature resulted in a significant increase of *Salmonella* retention (only 0.05% of the bacteria added was found in the micro-filtered milk) but had no effect on *Listeria* retention. The possible obtention of cheeses made from raw milk with a satisfactory level of hygiene is discussed.

milk / microfiltration / *Salmonella* / *Listeria*

Résumé — Rétention de *Listeria* et *Salmonella* par le procédé de microfiltration tangentielle «Bactocatch». *La rétention de souches avirulentes, mais de caractéristiques morphologiques identiques à celles des souches pathogènes de* Listeria monocytogenes *et de* Salmonella typhimurium *par la membrane de microfiltration mise en œuvre dans le procédé «Bactocatch» a été étudiée. Ces bactéries étaient ajoutée à du lait cru, à du lait pasteurisé et à du lait préalablement microfiltré. Les réductions décimales observées étaient voisines de 1,9 unités pour* Listeria *et de 2,5 pour* Salmonella *à 35 °C. Elles n'étaient pas influencées par le niveau d'ensemencement, étudié entre 10^2 et 10^6 UFC/ml. L'élévation de la température de microfiltration à 50 °C n'avait pas d'influence significative sur la rétention des* Listeria *mais accroissait significativement la rétention des salmonelles (seulement 0,05% des populations ajoutées était alors dénombré dans le lait microfiltré). La possibilité de garantir des fromages de lait cru de qualité hygiénique satisfaisante grâce à l'emploi du procédé Bactocatch est discutée.*

***lait* / *microfiltration* / Salmonella / Listeria**

* Correspondence and reprints

INTRODUCTION

Despite an extended variation reported according to country, Griffiths (1989) estimated that *Listeria monocytogenes* occurs in less than 5% of collected milks. The level of *Listeria* in raw milk is estimated to be 10 CFU/ml (Beckers *et al,* 1987). Thus, bulked silo milk probably contains 0.5 CFU/ml of *Listeria*. But *Listeria monocytogenes* is able to grow at low temperatures (Donnelly and Briggs, 1986) and taking into account a lag phase of 24–48 h and a generation time of 20 h, it can be assumed that even after 96 h of low temperature storage, untreated milk would approach levels of 5 to 10 CFU/ml (Griffiths, 1989). Information on the concentration of *Salmonella* cells in raw milk supplies seems to be lacking in the literature (Marth, 1969) but it is likely that contamination of raw milk by *Salmonella* is similar to that of *Listeria* (Johnson *et al,* 1990).

HTST pasteurization has been shown to reduce *L monocytogenes* populations by 3.7 log units (Bunning *et al,* 1988) and *Salmonella* counts by more than 5 log units (d'Aoust, 1989). Consequently, dairy products made from pasteurized milk, if not recontaminated post-process, are hygienically safe. Cheesemaking characteristics of raw milk and organoleptic qualities of ripened cheeses are altered by heat treatment. Consequently, most French and European cheeses defined by law as "Fromages d'appellation d'origine contrôlée" are made from raw milk which with its natural flora gives a unique flavour to the resulting products. Such cheeses must evidently be totally safe for consumption.

From the results recently generated by our laboratory (Trouvé *et al,* 1991) on the bacterial epuration of raw skim milk through the use of membrane microfiltration (MF) technology ("Bactocatch" process), we have examined specific retention by the 1.4-µm pore diameter MF membrane of *Listeria* and *Salmonella* cells added to various skim milks. Our purpose was to quantify this specific retention as a function of MF temperature and levels of added cells.

MATERIAL AND METHODS

Bacterial strains

Listeria innocua No 80.11 was a gift of Institut Pasteur (Paris). This strain was chosen because it is of the same size and has the same optimal growth temperature (37 °C) as *Listeria monocytogenes* without pathogenicity, and is consequently easy to handle. Growth was conducted at 30 °C for 48 h in tryptic soy broth (TSB) (Biokar OBK 028). Enumeration (24 h at 37 °C) in TSB and in artificially contaminated milks samples was carried out on modified Oxford medium (AES 151 992) supplemented with selective components mixture (cefotetan, cycloheximide, colistine and fosfomycine) (AES 184 122) when the natural milk flora was high compared to the level of *Listeria*.

Salmonella abortus ovis used in this study was a gift from Dr Pardon (INRA, Tours). It was a non-virulent strain of similar size (3–4 µm; 0.6 µm) to the pathogenic counterpart. Growth was also conducted on TSB (Biokar No OBK 028) at 30 °C for 48 h. Enumeration was performed on tryptic soy agar (TSA) (Difco 0369-01) supplemented with an antibiotics mixture (Pardon, personal communication) after 48 h at 37 °C.

Milks utilized and levels of artificial contamination

Three types of milk were inoculated with 3 levels (10^6 CFU/ml; 10^4 CFU/ml; 10^2 CFU/ml of both *Listeria* and *Salmonella* cultures in the feed vat of the microfiltration equipment. The milk examined in this study consisted of: raw bulk skim

milk with less than 0.05% fat and a total mesophilic flora of around 10^5 CFU/ml; HTST pasteurized (72 °C; 15 s) bulk skim milk with less than 0.05% fat and a total mesophilic flora of around 10^4 CFU/ml and HTST pasteurized (72 °C; 15 s) bulk skim milk, "Bactocatch" treated as described by Maubois (1991) with a total mesophilic flora of around 10^2 CFU/ml.

Microfiltration experiments

Bacterial retentions were studied with MFS-1 Alfa-Laval equipment, containing a 1 P 19 Membralox (SCT Tarbes, France) 1.4-μm pore size cartridge (0.85 m length; internal diameter of the channels: 4 mm; area 0.2 m^2). Hydraulic system was obtained with 3 centrifugal pumps: a feeding pump giving an inlet flow of 147 $l.h^{-1}$ and maintaining inlet MF pressure at 3.2–3.5 bars; a retentate recirculation pump allowing a MF membrane recirculation speed of 5.8 $m.s^{-1}$; a microfiltrate recirculation pump giving a uniform transmembrane pressure of 0.55 bar and a recirculating flow of 500 $l.h^{-1}$.

Extraction fluxes were maintained at 700 l. $h^{-1}.m^{-2}$ for the microfiltrate and 35 $l.h^{-1}.m^{-2}$ for the retentate, *ie* a volumic concentration of 20. Microfiltrate and retentate were continuously recycled; 30 l of milk were used for each experiment.

Two temperatures (35° and 50 °C) were investigated. Samples were aseptically taken out through septums inserted in feed and recirculation loops pipes, every 3 min after a period of 10 min following addition of the bacterial cultures to the feed vat.

RESULTS AND DISCUSSION

Effects of milk pretreatments on Listeria *cells retention by MF*

Table I shows decimal reduction observed at 30 °C on raw skim milk, "premicrofiltrated" pasteurized skim milk and pasteurized skim milk artificially contaminated with 10^6 CFU/ml *Listeria* cells. Each result is the average of 16 enumerations (4 successive enumerations and 4 experiments).

The MF membrane used in "Bactocatch" equipment retained 99.5% of *Listeria innocua* cells added in raw skim milk achieving a decimal reduction of 2.28 ± 0.17 log units. No significant difference was observed with pasteurized skim milk: the decimal reduction was 2.14 ± 0.16 log units. Such results agree well with the previous observations of Trouvé *et al* (1991). *Listeria innocua* has a cellular volume similar to that of *Pseudomonas fluorescens* for which Trouvé *et al* (1991) found a decimal reduction of 2.12 log units.

Retention of *Listeria* cells was much lower when "premicrofiltrated" milk was investigated. Only 95% of added cells were retained by the 1.4-μm pore size membrane. No evident reason appears to explain this result. Milk from "Bactocatch" equipment does not contain any somatic cells (Maubois, 1991), and there might be some interaction between leucocytes and *Listeria* as shown by Doyle *et al* (1987) favouring MF *Listeria* retention in raw and

Table I. Decimal reductions (DR) observed with the different milks inoculated with 10^6 *Listeria* cells/ml.
Réductions décimales observées sur les différents laits inoculés avec 10^6 cellules/ml de Listeria.

Types of milk	*DR* *
Raw skim milk	2.28 ± 0.17
HTST pasteurized skim milk	2.14 ± 0.16
Premicrofiltrated HTST pasteurized skim milk	1.30 ± 0.16

* Average of 16 trials (log inlet milk–log outlet microfiltrate).

pasteurized skim milk. Another hypothesis is that premicrofiltration of milk leads to a general reduction of milk components involved in the fouling of the 1.4-μm membrane and consequently, membrane transfer of *Listeria* cells during the subsequent MF would be easier because of reduced fouling. More work is required to understand this phenomenon.

Effect of contamination level and temperature on Listeria *and* Salmonella *cells retention by MF*

Table II summarizes the different results obtained with 3 levels of contamination and 2 temperatures with HTST pasteurized skim milk. This type of milk was chosen because enumeration of *Listeria* cells added at the lowest level (10^2 CFU/ml) was easier than in raw skim milk.

Decimal reduction of both *Listeria* and *Salmonella* cells is similar, regardless of the level of artificial contamination. Such results agree with those of Trouvé *et al* (1991) and Jaubert *et al* (1991) who also found that MF membrane acts as a depth filter and not as a screen filter.

MF membrane with 1.4-μm pore size showed a better retention of *Salmonella* cells than *Listeria* cells. This may be related to the larger cellular volume of *Salmonella*. At 35 °C, 99.7% of *Salmonella* cells are retained in the MF retentate compared with 98.6% for *Listeria* cells. Heating milk to 50 °C increases decimal reduction of *Salmonella* by MF treatment because of heat sensitivity of this bacterial genus (D value at 51.8 °C is 1.267 s for *S typhimurium* according to Bradshaw *et al,* 1987). Consequently, holding contaminated milk at 50 °C for 20 min in association with a MF treatment removes 99.95% of *Salmonella* flora initially present in milk. Temperature has a much less significant effect on *Listeria*, which is known to be more heat-resistant than *Salmonella*. *Listeria* D value at 63.3 °C is 33.3 s (Bunning *et al,* 1986) compared with 6.7 s for *Salmonella* (Bradshaw *et al,* 1987). On the other hand, heat treatment applied to *Listeria* contaminated

Table II. Decimal reductions (DR) observed at different levels of contamination with *Listeria* and *Salmonella* at 35 °C and 50 °C.
Réductions décimales observées à différents niveaux de contamination en Listeria *et* Salmonella *à 35 et 50 °C.*

		DR	
		Listeria innocua	Salmonella abortus ovis
Level of contamination 35 °C	10^2 CFU/ml	1.81 ± 0.18	2.46 ± 0.10
	10^4 CFU/ml	1.83 ± 0.39	2.37 ± 0.13
	10^6 CFU/ml	1.87 ± 0.31	2.46 ± 0.16
MF temperature	35 °C	1.85 ± 0.38	2.52 ± 0.22
	50 °C	1.99 ± 0.21	3.30 ± 0.19
10^6 CFU/ml added			

milk in the MF equipment (50 °C; 20 min) is in the neighborhood of the conditions (56 °C; 50 min) recently described by Busch and Donnelly (1992) as injuring *Listeria* cells and minimizing enumeration on TSB medium. Nevertheless, if such an injury did occur with the *Listeria* strain used it was the same for retentate and microfiltrate populations, both liquids being recycled in our trials, and consequently could not affect the determined decimal reduction.

CONCLUSION

Use of the "Bactocatch" process for treating skim milk at 50 °C reduces 99.0 and 99.95% *Listeria* and *Salmonella* populations respectively. Such a bacterial reduction is not as effective as pasteurization, but it could be relatively satisfactory when cheesemaking with raw milk is envisaged. "Bactocatch" treatment of raw milk containing 0.5–10 CFU/ml of potentially pathogenic *Listeria* and *Salmonella* species (Griffiths, 1989), according to the process described by Maubois (1991) with an UHT sterilization of cream to be added for fat standardization and a MF treatment of skim milk will allow a count between 2–40 CFU/1 000 ml in cheese milk. Envisaging that 90% of these contaminating bacteria will be kept in the 100 g of cheese resulting from the original 1 000 ml of cheesemilk leads to 0.2–4 *Listeria* or *Salmonella* cells per 10 g of cheese. Such a hypothetical calculation gives a result which could be in the upper part of the range slightly higher than the French regulation (no *Listeria* in 25 g of cheese (Dehove, 1991) but taking into consideration a likely constant increase in hygiene at milk production and processing, it can be claimed that use of microfiltration will considerably increase the safety of raw milk cheeses.

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