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Full Length Research Paper

Microflora of biofilm on Algerian dairy processing lines: An approach to improve microbial quality of pasteurized milk

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Bacterial contamination of pasteurized milk may originate from different sources: raw milk, process equipment surfaces and packaging materials. It is hypothesized that post-pasteurization contamination along the milk processing lines is responsible of reducing shelf life of Algerian pasteurized milk. This assumption was investigated through assessment of both the microflora of biofilms in milk pipeline systems at five dairy plants of Northwestern Algeria and the effectiveness of a quaternary ammonium based compound used for the disinfection of the plant equipment. Samples were collected before and after cleaning-in-place (CIP) systems from different segments of pasteurization lines with sterile cotton swabs. Quantitative assessment showed little reduction of the total bacteria count after CIP. On the average bacterial numbers were 5.6×10^3, 1.2×10^4, 5.1×10^4, 2.5×10^5 and 9.7×10^7 cfu/cm^2, respectively, in the different units. Phenotypic identification of isolates revealed predominance of Gram-positive bacilli belonging to *Bacillus* and notably the *Bacillus cereus* group, at maximal levels of 72 and 21% respectively. The other Gram-positive microflora included *Staphylococcus* (30%) and *Micrococcus* (10%). In contrast, the incidence of the Gram-negative bacteria was relatively low. Two genera, identified as *Pseudomonas* (9%) and *Enterobacter* (6%), were found only in two dairies. Three dairies were Gram-negative bacteria-free. That should be the result of common contamination sources or highly environmental selective pressure. Further studies have to address these hypotheses. Treatment of experimental *Bacillus cereus sensu lato* strains biofilms with a 50, 100 and 150 ppm of quaternary ammonium disinfectant, showed a significant resistance of biofilms to this product even after long exposure time (15 min). This study emphasized the importance of aerobic spore-forming bacteria in dairy-processing equipment as they are able to built recalcitrant biofilms on the inside equipment surfaces with subsequent resistance to conventional CIP system and potential transfer to pasteurized milk. Therefore, in order to reduce the contamination levels of spore-forming bacteria and improve the quality and shelf life of the product, these dairies have, besides improvement in the hygienic status of the plant equipments, also to monitor either the pasteurization process or the contamination from raw material (that is, milk powder).

Key words: Milk contamination, pasteurization, biofilm, cleaning-in-place (CIP).

INTRODUCTION

In the dairy industry, equipment surfaces are recognized to be a major source of contamination of processed milk with both spoilage and pathogenic microflora. Adhered bacteria can detach and contaminate the product as it passes the surfaces (Bagge-Ravn et al., 2003; Kusumaningrum et al., 2003; Brooks and Flint, 2008). In this case cross-contamination is a crucial economic and sanitary problem. Indeed, Biofilms are known to threaten

the quality and safety of dairy products and to significantly reduce their shelf-life (Austin and Bergeron, 1995; Chmielewski and Frank, 2003; Salustiano et al., 2009). Due to their resistance to heat treatments and to antimicrobial agents, biofilms developed on dairy processing lines are also difficult to remove even with acceptable cleaning and disinfecting procedures (Bore and Langsrud, 2005; Bremer et al., 2006; Brooks and Flint, 2008). In addition, bacterial re-contamination of food processing lines surfaces has been reported to occur again during cleaning-in- place procedures, due to the re-adhesion phenomenon (le Gentil et al., 2010). Several reviews in this field (Chmielewski and Frank, 2003; Shi and Zhu, 2009; Simoes et al., 2010; Vlkova et al., 2008) highlighted the significant emergence of resistant bacteria to conventional antimicrobial treatment and emphasize the need to develop new biofilm control strategies.

A recurrent problem in the dairy industry is the microbial quality of pasteurized milk. This product is exposed to middle heat treatments that do not ensure complete destruction of both spoilage and pathogenic bacteria. Despite improvement in the dairy technology, contamination of pasteurized milk especially with aerobic spore-forming bacteria remains a specific biological barrier that limits shelf life and quality of the product (Huck et al., 2007; Novak et al., 2005; Ranieri et al., 2009). Numerous studies were conducted throughout the world to solve this problem in order to extend pasteurized milk shelf life. However, the limiting factor varies from a country to another depending on the process conditions. Different potential contamination sources of pasteurized milk are reported: raw milk (Bartoszewicz et al., 2008; Lin et al., 1998; Ranieri and Boor, 2009), equipment surfaces (Salutiano et al., 2009; Sharma and Anand, 2002; Svensson et al., 2004) and packaging materials (Petrus et al., 2010; Simon and Hanson, 2001; Zygoura et al., 2004). Temperatures used for the pasteurization processes are also reported to affect processed milk shelf life (Aires et al., 2009; Hanson et al., 2005; Ranieri et al., 2009) as well as the somatic cell count of raw milk (Barbano et al., 2006).

Nevertheless, among these limiting factors, pasteurization process appears to be a key step with regard to spore-forming bacteria because the role of temperature on spore activation. Data from some reports indicated that temperature affects the microbial population of pasteurized milk in terms of the amount and type of microorganisms present following pasteurization, with higher bacterial number in milk processed at higher temperatures (Hanson et al., 2005; Ranieri et al., 2009). The role of temperature on spore activation should be then stressed. In addition, when persisting on the dairy-processing equipment, the selective thermoresistant aerobic spore-forming microflora may develop biofilms that are difficult to remove and may compromise the quality and safety of the final product.

In Algeria, reconstituted pasteurized milk that is a widely consumed beverage is subject to high pasteurization at 85°C for 5 to 10 min. The level of bacterial contamination remains too high in the processed product, and consumers must boil milk again before any consumption.

Once post-pasteurization recontamination of processed milk is hypothesized, an approach to improve the quality of pasteurized milk and avoid this double heat treatment is to minimize contamination from biofilms on processing lines. However, in Algerian dairy manufacturing plants very little is known about the persistent microflora colonizing dairy equipment surfaces, and the development of biofilms. Therefore, until now, strategies for biofilm control rely mainly on the effectiveness of cleaning and disinfection procedures. Consequently, knowledge of the biofilm ecology is necessary to elaborate efficient cleaning and disinfection procedures that would target dominant species and successfully eliminate biofilms from the process equipment surfaces.

In the present study, identification, characterization of the dominant bacterial component in pasteurized milk lines and assessment of the effectiveness of a commonly used sanitizer on biofilm removal were the essential objectives.

MATERIALS AND METHODS

Collection of samples origin

Samples were collected from five (05) dairy plants located in the West of Algeria. All dairies produce pasteurized milk from medium heat skim milk powder imported from several countries. One of them uses also raw cow milk. The 5 plants adopt the same cleaning procedure: water pre-rinsing followed by, a caustic wash (2% NaOH at 70°C/5 min), and rinse with water. An acid wash (1% HNO_3 at 70°C/5 min). A final rinse with water completes the cleaning process. For the sanitization process, the dairies use chemical disinfection, especially with ammonium based products and occasionally with chlorine, or peracetic acid based products. A final rinse with water completes the process.

The samples were collected from different segments along the pasteurized milk production line, in the closed system. This includes essentially pre- and post-pasteurization sections of milk pipelines. The samples were taken either from cleaned and disinfected surfaces (after CIP) or from surfaces at the end of production (just before CIP), with sterile swabs (wooden applicator, cotton tipped, Batch M 20, Italy).

Dominant bacteria in pasteurized milk lines

After sampling, the swabs were transferred to 10 ml physiological

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water (0.85% NaCl) with 0.1% peptone (Merck, Germany) and tenfold dilutions were performed and spread on Luria agar plates (LA): 10 g l^{-1} tryptone, 5 g l^{-1} yeast extract and 5 g l^{-1} NaCl (Microbiology Fermtech Merck Germany). The agar plates were incubated at 30°C for 72 h. The number of colony-forming unit was counted and the colony morphology was noted. A representative number of colonies were isolated randomly from the agar plates and pure cultured on Luria-agar. They were then frozen-stored for further identification and characterization.

Identification and characterization of selected isolates

The identification of isolates was based on colony morphology, Gram's reaction and biochemical tests following standard procedures.

A screening for the *Bacillus cereus* group species was performed among the isolated aerobic spore forming bacteria, according to the AFNOR (1996) standard recommendations. Five colonies with the typical mannitol negative and lecithinase positive characteristic of *B. cereus* were then selected from MYEP plates. Identification of isolates to the *B. cereus* group was confirmed by growth on blood agar (Fluka Biochemica Spain) and microscopical observation of endospores.

Selected isolates were also characterized on their important characteristics with respect to biofilm formation and their physiological significance in the dairy industry (Pirttijarvi and al., 2000; Sharma and Anand, 2002). These include growth at 7 and 10°C for 10 days, at 37 and 55°C for 24 h and hydrolysis of food components: starch, casein, tested with skim milk and lipid, tested with tween 80 (Fluka UK).

Effect of a quaternary ammonium disinfectant on experimental biofilms

Preparation of biofilm

To investigate the effectiveness of the quaternary ammonium compound, 24 h Biofilms were formed on stainless steel chips by microorganism carrier-surface method of Maris (1992). Stainless steel chips (AISI 304 L, 2 x 2 cm) were treated according to the protocol proposed by Peng et al. (2002). Two *B. cereus* strains were used for this purpose: *B. cereus* ATCC11778 USA, (Bc R) and *B. cereus* (Bc 6) isolated from pasteurized milk processing line in one of the dairies studied.

Disinfection treatment

The disinfection test was performed according to the procedure described by Peng et al. (2002). 0.1 ml of planktonic cells or chips containing biofilms were exposed in 20 ml of the sanitizer Divosan® (Divosan® QC Johnson Diversey F), 50, 100 and 150 ppm, one chip per test tube, at room temperature. After time exposure (5, 10 and 15 min), the planktonic cells or chips were removed and immediately mixed with neutralizing buffer solution (Difco). Viable cells were then enumerated in plate count agar.

RESULTS AND DISCUSSION

Count of bacterial contaminants

The mean values before CIP system application were high and ranged from 3. 5 × 10^5 to 2.5 × 10^9 cfu/cm^2 (Table 1). Such high levels of bacteria suggested a wide contamination of milk lines that could be traced mainly to skim milk powder. After CIP we found also high numbers (1.0 × 10^7 and 5.7 × 10^7 ufc/cm^2) in two dairies, respectively D3 and D5. This indicates that important microflora still colonizes the surfaces. Logarithmic reduction of the total count obtained after CIP system is thus very low, 0,039, 2,202, 2,398, 0,236 and 1,231 respectively in D1, to D5 respectively. The maximal value (2.398) was reached in D3 plant. In this plant a subsequent residual number of bacteria (1.0 × 10^7 cfu/cm^2) was observed. These results show clearly that the cleaning and disinfection procedures were insufficient in all dairies and failed to adequately remove adhered bacteria from the process equipment.

The role played by initial contamination of milk on the microbial quality of the processed product is highlighted, and until now many studies focused on investigation of the microbial ecology of raw milk and its effects on the quality and shelf life of the heat treated milk (Huck et al., 2007; Martin et al., 2011; Ranieri and Boor, 2010). In the case of Algerian pasteurized milk the raw material is the medium-high heat milk powder imported from different countries. It is contaminated with spore-forming bacteria (unpublished data) and should be a possible secondary contamination source of recombinated pasteurized milk, while equipment surfaces should be a possible primary contamination source.

Indeed results show that bacterial contamination of the process equipment occurred at high levels. It may result in the development of thick and recalcitrant biofilms whose removal with conventional cleaning and disinfection procedures is more difficult. In another hand, density up to 10^8 cfu/cm^3 has been reported to result on biofilms structures consisting of several layers (Gibson et al., 1999). These thick biofilms can then reduce the efficiency of heat transfer when they occur at location such as plate heat exchanger leading to lower the pasteurization treatment. To solve this problem, processors increase heat treatments. Such practice affects negatively nutritional and sensorial quality of processed milk without any improvement in the microbial quality. It has been showed that increasing heat treatments does not necessary lead to lower bacteria number in the final product. Inversely affect microbial numbers during storage of pasteurized milk (Hanson et al., 2005; Ranieri et al., 2009). The role of heat on selecting spore forming bacteria is well-known.

New strategies that permit the right management of these heat treatment processes are then required. Recently, non-thermal preservations methods of pasteurized milk such as pulsed electric fields and microfiltration (Sepulveda et al., 2009; Walking-Ribeiro et al., 2011) were investigated and should be an interesting alternative.

Table 1. Bacterial contamination of pasteurized milk processing lines in Five West algerian dairy plants before and after cleaning and disinfection.

Dairies		Mesophilic aerobic flora (cfu/cm^2)		
		Before CIP	After CIP	Decimal reductions
Dairy 1	min	2.5×10^4	2.7×10^3	0.967
	Mean	3.5×10^5	3.2×10^5	0.039
	Ma×	2.4×10^6	2.1×10^4	0.058
Dairy 2	min	3.5×10^5	3.2×10^4	0.039
	Mean	3.5×10^6	2.2×10^4	2.202
	Ma×	9.7×10^8	6.7×10^7	1.161
Dairy 3	min	1.5×10^6	2.3×10^4	1.814
	Mean	2.5×10^9	1×10^7	2.398
	Ma×	4.5×10^9	1.1×10^7	2.612
Dairy 4	min	3.4×10^5	2.2×10^5	0.189
	Mean	4.3×10^5	2.5×10^4	0.236
	Ma×	3.5×10^6	2.1×10^4	1.222
Dairy 5	min	1.5×10^6	2.5×10^4	1.778
	Mean	9.7×10^8	5.7×10^7	1.231
	Ma×	3.5×10^9	2.1×10^7	1.113

Dominant microflora of pasteurized milk processing lines

One hundred and eight-six isolates were selected from different stainless steel segments of the five dairies. The distribution pattern of the isolates (Table 2 and Figure 1) reveals a large dominance of Gram-positive strains with emerging aerobic spore forming rods, belonging to the genus *Bacillus*. High numbers of bacilli were found at the five dairies. They were isolated before and after pasteurization segments of the processing lines. Levels ranged from 51 to 72%. These results are in agreement with those obtained by Sharma and Anand (2002) who respectively found 59 and 64% bacilli in two dairy plants investigated.

Bacilli are recognized to dominate on processes involving heat treatment. That may activate the spores and kill the competing non-sporeforming microflora. Consequently, these organisms are predominant contaminants of heat-treated milk and are incriminated in the deterioration and keeping quality of the product. Recently, the majority of aerobic spore-forming bacteria in pasteurized milk was, indeed, assigned to *Bacillus* and *Paenibacillus* genera (Ranieri and Boor, 2009) or *Bacillus* and among other representative of the genus, type strains of species belonging to the *Bacillus cereus* group (Coorevits et al., 2008; Zhou et al., 2008). The occurrence of potentially toxic members of the latter group in both raw and heat-treated milk was also reported (Bartoszewicz et al., 2008).

In the present study, members of the potentially pathogenic *Bacillus cereus* group were also found in all the investigated plants as well, levels varied between 10 and 21%. Data from literature showed the wide spread of *B. cereus* in the dairy environment. Several potential sites of contamination by these bacteria are identified along the entire milk production line. Indeed, the bacterium was isolated from milk silo tanks (Moussa et al., 2004; Svensson et al., 2004), pasteurizers (Svensson et al., 2000; Te Giffel et al., 1997), and the filling machine (Eneroth et al., 2001). Now, it is well known that post-pasteurization sections are reservoirs of *B. cereus* (Salustiano et al., 2009). Besides heat resistance, members of the *B. cereus* group are described to adhere easily to surfaces and to be excellent biofilm formers (Faille et al., 2001; Peng et al., 2002). Therefore, Wijman et al. (2007) observed that thick biofilms of *B. cereus* developed in industrial piping systems that are partly filled during operation or where residual liquid has remained after a production cycle. Shaheen et al. (2010) have even found that dairy silo tank isolates possessing hot-alkali resistant spores were capable of germinating and forming biofilm in whole milk, not previously reported for *B. cereus* at that time.

The other Gram-positive bacteria included *Staphylococcus* and *Micrococcus* genera, which are, with

Table 2. Distribution pattern of isolates based on primary identification

Type of organism	% total isolates				
	Dairy 1	Dairy 2	Dairy 3	Dairy 4	Dairy 5
Gram + rods	71	60	51	60	72
Gram - rods	00	00	15	00	5
Gram + cocci	29	35	28	30	23
Yeast	00	00	3	3	00
Moulds	00	00	00	7	00

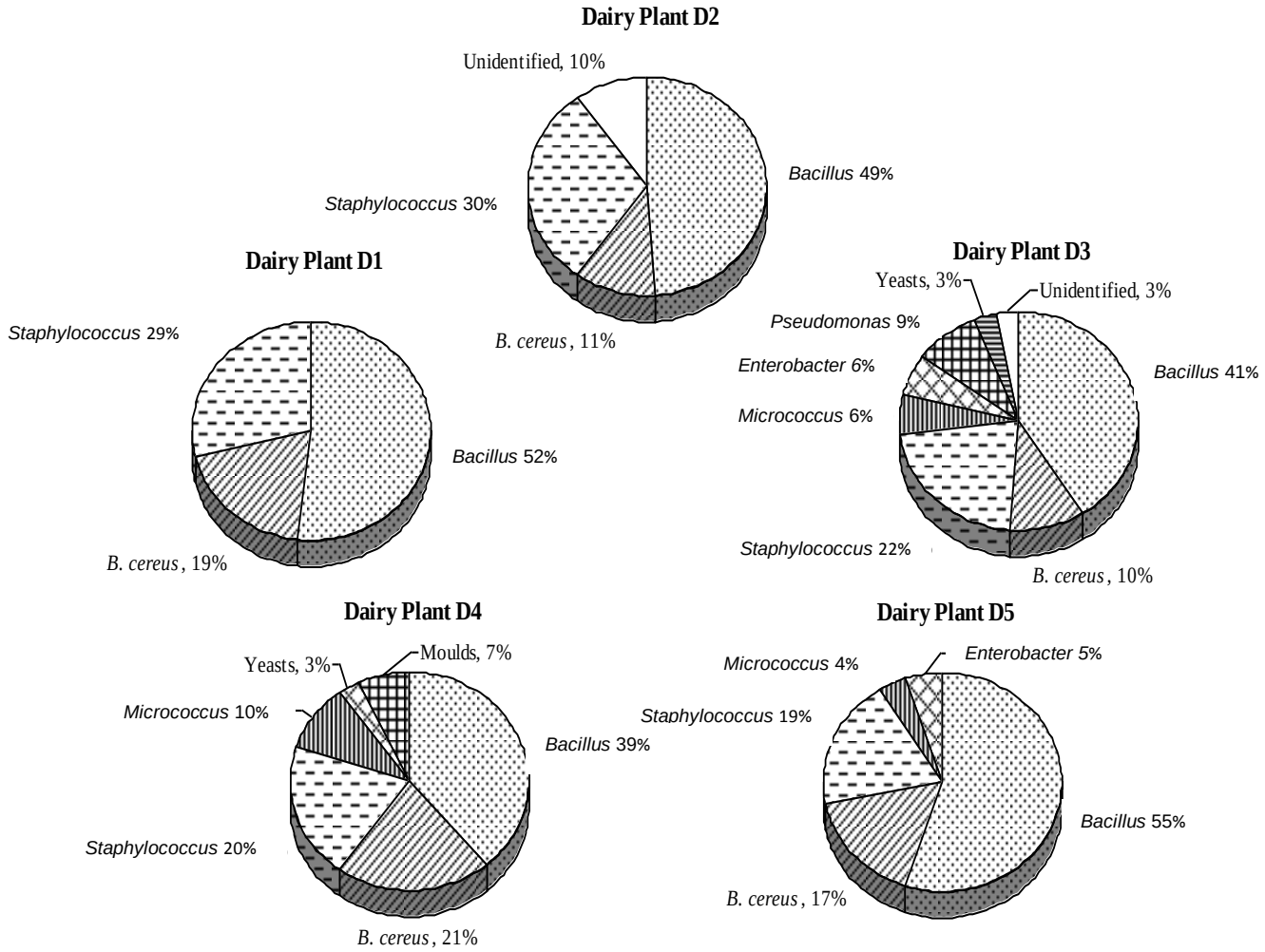

Figure 1. Distribution pattern of isolates in five Algerian dairy plants.

Lactobacillus, *Listeria* and *Streptococcus*, the most commonly encountered bacteria in dairy environments (Sharma and Anand, 2002). *Staphylococcus* is well represented and account for 29, 30, 22, 20, and 19% in the five dairy plants respectively. It is the second genus after *Bacillus*. Both biofilm-positive *Staphylococcus epidermidis* (Schlegelova et al., 2008) and *Enterococcus* especially *E. faecalis* and *E. faecium* (Necidova et al., 2009), were also isolated from dairy plants. Data from some reports indicated that a more various microflora compose biofilms formed on the surfaces of the processing equipment depending on the various food industries. *Pseudomonas*, *Staphylococcus* and yeasts were found to be the dominant groups of microorganisms

in a caviar-processing plant (Bagge-Ravn et al., 2003). Moreover, as outlined by these authors every industry possesses its own in-house-flora reflecting the product produced. Food processing plants producing the same food product will also have different biofilms, as processes will never be 100% alike.

Our work showed that in the dairy plants investigated Gram-positive bacteria are well represented and constitute the in-house flora of the dairies. However the absence of lactic acid bacteria that is, *Lactobacillus*, from the pasteurized milk processing line should be noted. This may be due to the nature of the raw material that is processed, since high heat milk powder is used instead of cow's raw milk in most instances.

Similarly, the Gram-negative bacteria were found in two dairies only: D5 and D3, at levels of 5 and 15% respectively. They were identified as *Pseudomonas* and *Enterobacter* which are also wide spread in dairy facilities. In addition to these genera, Sharma and Anand (2002) described the presence of *Shigella* spp. and *E. coli*. In this study, three dairies were entirely Gram-negative bacteria free which firstly argues for a plant-specific microflora. Otherwise, these results support also the fact that predominance of Gram-positive bacteria may happen because a higher proportion of Gram-negative cells were not capable of surviving pasteurization (Carpentier et al., 1998). Moreover, It has also been reported that Gram-positive bacteria such as *Streptococcus thermophilus* and *Bacillus* spp organized in biofilms are more difficult to remove than Gram-negative bacteria by conventional cleaning and disinfection procedures in the dairy industry (Bremer et al., 2006). Single species of bacteria often dominate in biofilm (Flint et al., 1997). In contrast Bagge-Ravn et al., (2003) found that the Gram-positive flora was significantly reduced by cleaning and disinfection and Gram-negative bacteria such as *Pseudomonas, Acinetobacter* and Neisseriaceae were the remaining microlora on the processing equipment of the fish plants investigated. According to these authors most of the microorganisms isolated are typical members of the normal fish microflora. These results strengthened the idea of the in-house-flora that will change from product to product and from processing unit to processing unit (Bagge-Ravn et al., 2003). However, the remaining bacteria may also be a reflection of cleaning and disinfection regimes adopted by these plants. According to Svensson et al. (2004) the in-house-flora also means that the cleaning system of the process equipment may not be satisfactory.

At last it is possible to note that general microflora of biofilm on pasteurized milk production lines in these dairy plants consists mainly of bacteria; yeast and moulds were found at low levels (from 3 to 7%), only in two plants (D3 and D4). Therefore the composition of this in-house-microflora little varied between the different plants. The dissemination of Gram-positive cocci and spore-forming bacteria may suggest highly selective technological processes, notably inadequate pasteurization, and sanitizing treatments. Indeed bacteria are known to survive otherwise lethal stress treatments when they are inappropriate, and become more tolerant to them. Once in the five dairies it has been demonstrated that heavy contamination remains overall milk processing line, subsequent and recalcitrant biofilms may notably develop and resist the cleaning system. Biofilms may also affect adversely the efficiency of the pasteurization treatment. However, initial contamination of raw material (that is, milk powder) could not be neglected as well.

Distribution of selected isolates according to their properties related to food hygiene

Characterization of selected isolates indicated that most of the strains selected produced different enzymes (proteases, lipases and amylases), which is of concern in food hygiene because of their hydrolytic activities on food components. Several *Bacillus* species are known to be strongly proteolytic and producing lecithinase activity regarding the *B. cereus* group. It is well known that in pasteurized milk, these enzymes will cause protein and fat degradation during storage and produce off-flavors. Moreover such bacteria are considered as good biofilms producers (Carpentier et al., 1998).

Strains were also able to grow at a large range of temperature from 7 to 55°C (Table 3). The frequency of occurrence of the psychrotrophic microflora was relatively low in D3 (6%) and D4 (7%) and null in D5. While in the same plant about 32% of the isolates grew at 55°C, and 21% in D4. Whereas, none of the selected isolates obtained from D3 were able to grow at this temperature. Given that raw material of these dairy plants is milk powder, it is not surprising that isolates were mostly mesophilic or moderately thermotolerant and not psychrotrophic bacteria. In the case of *B. cereus*, Te Giffel (1997) showed the absence of psychrotrophic strains in milk powder and attributed that to the process used to make powder. Growth at 55°C should then be due to thermophilic *bacilli* belonging to the genus *Bacillus* as well as other genera that are also frequent contaminants in the dairy industry. We can cite *Geobacillus* and *Anosybacillus* which are recognized as commonly occurring during the production of milk powder (Rueckert et al., 2005).

Inactivation of *B. cereus* biofilms by sanitizer

B. cereus was found among the microflora of pasteurized milk production line, in the various units studied. This bacterium is described as an excellent biofilm former due to the pronounced ability to its spores to adhere to

Table 3. Distribution pattern of strains based on the growth temperatures.

Dairy plant	Psychrotrophic	Mesophilic (%)	Thermophilic
D3	7%	93	00
D4	6%	62	32%
D5	00	79	21%

Table 4. Inactivation of *B. cereus* biofilm by a quaternary ammonium sanitizer.

Strain	Bacterial live organisation	Disinfectant concentration (ppm)	Population reduction[(a)] after treatment with disenfectant		
			5 min	10 min	15 min
Bc R	planktonic cells	50	3.16	3.69	3.84
	Biofilms		1.27	1.29	1.39
Bc 6	planktonic cells		3.37	3.50	3.80
	Biofilms		1.10	1.16	1.24
Bc R	planktonic cells	100	3.98	4.13	4.77
	Biofilms		1.36	1.42	1.56
Bc 6	planktonic cells		3.84	3.98	4.80
	Biofilms		1.12	1.19	1.40
Bc R	planktonic cells	150	5.61	5.64	5.92
	Biofilms		2.02	2.19	2.46
Bc 6	planktonic cells		4.23	5.02	5.35
	Biofilms		1.47	1.55	1.69

[a]obtained by subtracting final population (log cfu/mL or cm^2) after treatment from original population (log cfu/mL or cm^2).

stainless steel surfaces (Peng et al., 2002), and it is also known for its resistance to chemical disinfectants (Faille et al., 2001; Peng et al., 2002).

For disinfection with the quaternary ammonium Divosan®, supplier recommended minimal concentration of 50 ppm and a contact time of 5 min. The present study has shown that *B. cereus* biofilms were resistant to disinfection in these conditions, maximal decimal reduction of biofilm cells did not exceed 1.4 log cfu/ cm^2 (Table 4). Increasing the concentration of sanitizer to 100 and 150 ppm and exposure time to 10 and 15 min, did not result on any significant effect on biofilms of both Bc. 6 and Bc. R strains, compared to planktonic cells. It seems clear that quaternary ammonium based disinfectants have better effe**c**t on planktonic cells than on biofilms as outlined in the litterature (Peng et al., 2002, Shi and Zhu, 2009).

Resistance of biofilm to antimicrobial is well documented. Several hypotheses are formulated to explain this phenomenon. In the food industry, dissemination of resistance has been attributed to inefficient biofilm control by conventional cleaning and disinfection regimens. Bacteria submitted to sublethal concentrations of sanitizer agents have been demonstrated to exhibit highly

adaptative responses (Simoes et al., 2009). Another reason for this is species association that occurs within biofilm. Protection of species one another are then assumed to increase biofilm resistance to chemical and mechanical treatment (Simoes et al., 2009; Vlkova et al., 2008). Nevertheless, according to Kim et al. (2008) the major reason for antimicrobial tolerance of biofilms is the presence of dormant cells. Indeed, physiological heterogeneity in biofilms has been reported (Stewart and Franklin, 2008). Bacteria that are in a wide range of physiological states result on variability of phenotypes with different patterns of resistance. Thus, within biofilms, spores which are inactive cells, may exhibit a double resistance in addition to their natural resistance to aggressive environments. Data from several reports showed that *B. cereus* spores are more difficult to remove from stainless steel surfaces than vegetative cells using CIP procedures (Faille et al., 2001; Peng et al., 2002).

The success of sanitizer effect also depends on the efficiency of the cleaning regimes which must lead to the removal of cells and organic debris as well as the elimination of viable cells (Parkar et al., 2004). Less than 0.85 cfu/cm^2 *B. cereus* adhesion was found by Salutiano et al. (2009) after treatment of *B. cereus* biofilms with sodium hypochlorite following an adequate cleaning regime. Guinebretière et al. (2003) also described in a zucchini purée processing line efficient cleaning procedures used for equipment surfaces which prevent the installation of *B. cereus*. These included three successive steps of washing with hot water and at the end of each processing day, surfaces were cleaned with disinfectant solutions containing, among other sanitizers, specifically one *B. cereus* sporocide. Sporocide products are then required in disinfectant formulations destined to sporeformer bacteria biofilms.

Another approach to kill spores inside biofilms is to activate them prior to their submission to any sanitizer agent in order to make easy their elimination as geminating cells. Different strategies are adopted for this purpose. These include spore sensitivity (Shaheen et al., 2010) and use of spore germination inducers (Hornstra et al., 2007), treatments before any sanitation process application. According to the latters, up to 80% of the germinated *B. cereus* spores could be removed from the surface tested with germination inducers, as germinating spores lose their resistance capacities instantaneously. This could be then a valuable strategy to improve the control of spore-forming bacteria biofilms.

Conclusion

Gram-positive bacteria mainly *Bacillus* spp. and members of the *B. cereus* group were shown to be dominant bacteria of the processing equipment in the dairy plants analyzed. As a consequence, potentially extensive and recalcitrant biofilms may develop on these equipments and contribute to notably reduce the efficiency of the pasteurization and sanitation treatments as well as to potentially re-contaminate processed milk. Therefore this typical microflora which is partly a reflection of the raw material used and partly a reflection of highly selective technological processes requires a specific cleaning regime to both target the dominant species and suit the conditions of the plants. This means that sanitizer procedures must allow effectively reaching spores inside the biofilm. To achieve this goal disinfectant products have to be chosen for their sporocidal effect as well as for their activity against biofilms.

On the perspective of this study is it the molecular characterization of the selected isolates using a PCR-RAPD based method that should verify whether equipment surfaces are really the major source of pasteurized milk contamination?

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