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Microbial background flora in small-scale cheese production facilities does not inhibit growth and surface attachment of *Listeria monocytogenes*

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ABSTRACT

The background microbiota of 5 Norwegian small-scale cheese production sites was examined and the effect of the isolated strains on the growth and survival of *Listeria monocytogenes* was investigated. Samples were taken from the air, food contact surfaces (storage surfaces, cheese molds, and brine) and noncontact surfaces (floor, drains, and doors) and all isolates were identified by sequencing and morphology (mold). A total of 1,314 isolates were identified and found to belong to 55 bacterial genera, 1 species of yeast, and 6 species of mold. *Lactococcus* spp. (all of which were *Lactococcus lactis*), *Staphylococcus* spp., *Microbacterium* spp., and *Psychrobacter* sp. were isolated from all 5 sites and *Rhodococcus* spp. and *Chryseobacterium* spp. from 4 sites. Thirty-two genera were only found in 1 out of 5 facilities each. Great variations were observed in the microbial background flora both between the 5 producers, and also within the various production sites. The greatest diversity of bacteria was found in drains and on rubber seals of doors. The flora on cheese storage shelves and in salt brines was less varied. A total of 62 bacterial isolates and 1 yeast isolate were tested for antilisterial activity in an overlay assay and a spot-on-lawn assay, but none showed significant inhibitory effects. *Listeria monocytogenes* was also co-cultured on ceramic tiles with bacteria dominating in the cheese production plants: *Lactococcus lactis*, *Pseudomonas putida*, *Staphylococcus equorum*, *Rhodococcus* spp., or *Psychrobacter* spp. None of the tested isolates altered the survival of *L. monocytogenes* on ceramic tiles. The conclusion of the study was that no common background flora exists in cheese production environments. None of the tested isolates inhibited the growth of *L. monocytogenes*. Hence, this study does not support the hypothesis that the natural background flora in cheese production environments inhibits the growth or survival of *L. monocytogenes*.

Key words: flora, cheese, small scale, *Listeria monocytogenes*

INTRODUCTION

To produce safe food for consumers, food producers have a strong focus on hygiene during production. Cleaning and disinfection are the most common means of obtaining safe production environments, but even though daily cleaning and disinfection will significantly reduce the bacterial load in the production environment, surfaces are rarely sterile (Langsrud et al., 2012). At the same time, although most food products (including meat, fish, fruits, and vegetables) are produced or processed in clean environments, food types exist that are produced in the presence of a rich microbial background flora. In the production of cheese, disinfection is often avoided or performed rarely in the storage facilities to preserve a bacterial background flora, as it is considered to be important for the quality of the product (Retureau et al., 2010).

In recent years, cheese and other milk products have been the source for several outbreaks of listeriosis (Carrique-Mas et al., 2003; Gaulin et al., 2003; Makino et al., 2005; Bille et al., 2006; Swaminathan and Gerner-Smidt, 2007; Vít et al., 2007; Fretz et al., 2010; Johnsen et al., 2010; Gaulin et al., 2012). *Listeria monocytogenes*, the causative agent of listeriosis, is known to survive and grow well under conditions that inhibit a range of other bacteria, including low temperatures and high salt concentrations (Larson et al., 1999; Lado and Yousef, 2007). It does, however, not grow well at low pH (Lado and Yousef, 2007) and the presence of lactic acid bacteria, both naturally occurring and added during production, would hence inhibit further growth of *Listeria* in the cheese during the ripening period. However, as surface cultures (molds or bacteria) are commonly added during ripening of the cheese, a rise in pH may occur and conditions may allow for growth of *Listeria* to high numbers, especially close to the cheese surface (Bell and Kyriakides, 2005).

As *Listeria* is killed by pasteurization (Lado and Yousef, 2007), the main cause for the presence of vi-

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able *Listeria* in pasteurized milk and cheese from pasteurized milk is recontamination from the processing environment. It has been shown that *L. monocytogenes* is able to survive and persist in food production environments (Romanova et al., 2002; Di Bonaventura et al., 2008) and due to this, may pose a threat to food safety. Unpasteurized milk may contain *Listeria* even if no source for recontamination from the cheese production environment exists (Bemrah et al., 1998) but it has been shown that the bacterial background flora in raw milk may inhibit the growth of *L. monocytogenes* (Schvartzman et al., 2011). It is hence of interest to investigate whether the naturally occurring background flora in the cheese production environment may also have a protective effect against *Listeria*.

To establish whether the microbial background flora in the cheese production environment may have any protective effect against *L. monocytogenes*, it is necessary to know the composition of this flora. While many studies have focused on the bacterial flora in raw milk or cheese (Retureau et al., 2010; Fricker et al., 2011), few have studied the background flora in the whole cheese production environment, despite the fact that the flora on materials in direct contact with cheese has been shown to vary at different locations (Licitra et al., 2007; Mariani et al., 2007). Several studies have shown that various bacterial species can influence survival, growth, and adherence of *L. monocytogenes* to surfaces (Leriche et al., 1999; Leriche and Carpentier, 2000; Carpentier and Chassaing, 2004; Guðbjörnsdóttir et al., 2005), but no direct connection has been made between the common background flora in cheese-producing environments and the growth and survival of *L. monocytogenes* in production environments. This study shows in detail the composition of the microbial background flora in 5 Norwegian small-scale cheese production sites and how the isolated strains influence the growth of *L. monocytogenes* in a model system and in co-culture biofilms.

MATERIALS AND METHODS

Sampling and Microbial Culture Conditions

Five small-scale cheese-producing farms (ranging from 1 to 7 employees and production volumes from 2 to 10 t per year) in Norway were visited in the period between February and May 2010. All farms had their own milk production and used one set of production equipment to produce several types of cheese, including at least 1 type of soft cheese (Camembert or Brie type). Ripening rooms contained more than 1 type of cheese at all producers, but sampling was conducted in areas adjacent to soft cheeses. Four farms used pasteurized milk in their production, whereas 1 farm (producer 4) used unpasteurized milk. All farms produced cheeses that were salted in brines and inoculated with surface molds. At each farm, 8 sites were sampled (Supplemental Table S1, available online at http://dx.doi.org/10.3168/jds.2012-6395) using sampling swabs in Letheen broth (3M Norge AS, Skjetten, Norway) as well as at least 1 out of 2 *Listeria*-selective all-in-one swabs (InSite Listeria Test; Labolytic, Trondheim, or the Path-Chek Hygiene Listeria; Interfarm AS, Asker, Norway). In addition, plate count agar (**PCA**) plates (Oxoid Ltd., Basingstoke, UK) were placed without lids for 10 min in the production rooms and the ripening rooms for air samples. Brines (1 mL) were sampled directly by pipetting. For each selected location, 2 areas of 5 × 5 cm within 10 cm distance were swabbed thoroughly and the swabs were kept in a cooling bag until they reached the laboratory within 24 h after sampling. Appropriate dilutions of the Letheen broth and the brine were plated on PCA and the plates were incubated aerobically at 20°C for 5 d. The *Listeria*-selective swabs were kept following the manufacturer's instructions and examined for color changes after 1 and 2 d as indicated in the manuals.

Determination of the Microbial Flora: Bacteria

From each sample, 24 colonies were picked randomly from PCA plates and subjected to partial sequencing of the 16S ribosomal DNA (**rDNA**) gene to identify the isolated strains. All colonies were recultured on PCA for 3 d at 20°C to rule out contaminations. From these plates, 1 to 15 colonies (15 for small colonies to ensure enough cell material) were picked and mixed with Tryptone soy broth (TSB; 45 μL; Oxoid Ltd.), guanidine thiocyanate (GTC; 4 mol/L, 135 μL; Merck KGaA, Darmstadt, Germany) and Mag Prep Silica Particles (10 μL; Merck KGaA) in wells of a 96-well Greiner U plate (Greiner Bio-One GmbH, Frickenhausen, Germany). The DNA was isolated on a Biomek 2000 Workstation (Beckman Coulter Inc., Fullerton, CA) using magnetic silica particles and sarkosyl (Skånseng et al., 2006). Polymerase chain reaction of the 16S rDNA was conducted using DyNAzyme II Hot Start Polymerase (Finnzymes, Espoo, Finland) with primers MangalaF and MangalaR (Nadkarni et al., 2002) according to the producer's instructions. The PCR products were cleaned using ExoSAP-IT (USB Europe GmbH, Staufen, Germany). Sequencing PCR was carried out using the Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, CA) according to the manufacturer's instructions, with primer MangalaF. Samples were prepared for sequencing by using the Applied Biosystems BigDye XTerminator