Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Dairy Journal 15 (2005) 911–920
INTERNATIONAL DAIRY JOURNAL
www.elsevier.com/locate/idairyj

Phenotypic and PCR-based characterization of the microflora in Präst cheese during ripening

H.M. Østlie[a,*], L. Eliassen[b], A. Florvaag[c], S. Skeie[a]

[a]*Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, N-1432 s, Norway*
[b]*Østfold Technical School, Papeibakken 5, 1739 Sarpsborg, Norway*
[c]*Arcus AS, P.O. Box 6764, Rodeløkka, 0503 Oslo, Norway*

Received 15 April 2004; accepted 10 August 2004

Abstract

Microbiological sampling of Präst cheese from three cheese factories was done during ripening. The evolution of total bacterial counts, lactococci, lactobacilli, enterococci presumptive leuconostoc and pediococci was investigated after 30, 90, 180 and 270 days of ripening. Isolates (140) of non-starter lactic acid bacteria (NSLAB) from 12 Präst cheeses after 90, 180 and 270 days of ripening were examined. The isolates were tested by physiological and biochemical assays, species-specific PCR and 16S rDNA sequencing. The predominant NSLAB species was *Lactobacillus paracasei*. The development and evolution of the NSLAB microflora in Präst varied according to dairy plant and ripening time.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Non-starter lactic acid bacteria (NSLAB); Cheese; Ripening; Identification; *Lactobacillus*

1. Introduction

Swedish cheese production comprises several specific cheese varieties. One of the oldest and most popular cheeses in Sweden is Präst cheese. Präst is a semi-hard cheese variety with irregular eyes made using mesophilic DL-starter including species of the genus *Lactococcus* and *Leuconostoc mesenteroides* subsp. *cremoris*. Präst is made with rind and the ripening period is commonly 4–5 months long (Walstra, Noomen, & Geurts, 1993). The commercial manufacture of Präst is a controlled process that utilizes pasteurized milk in a dedicated plant under hygienic conditions. However, despite the application of many precautions during cheese making, a secondary non-starter bacterial population develops in the curd.

Non-starter lactic acid bacteria (NSLAB) is a collective term used to describe the adventitious lactic acid bacteria (LAB) flora capable of growth under the selective conditions of ripened cheese. The NSLAB flora is dominated by mesophilic lactobacilli, though pediococci and leuconostoc may also be found (Peterson & Marshall, 1990; Crow, Curry, & Hayes, 2001). The significance of NSLAB in cheese is still equivocal (Fox, McSweeney, & Lynch, 1998), but since NSLAB dominates the microflora of long-ripened cheese for most of its ripening period they certainly may have the potential to affect and contribute to cheese maturation.

Phenotypic, biochemical and physiological tests have normally been used to identify the microbial flora in cheese taxonomically. These tests are often time-consuming and not fully reliable. The development of PCR-based molecular techniques for the identification of bacterial species offers new perspectives in microbial taxonomic studies (Mannu, Riu, Comunian, Fozzi, & Scintu, 2002; Berthier & Ehrlich, 1998; Ward & Timmins, 1999; Drake, Small, Spence, & Swanson, 1996). The advantages of genotyping include the stability of genomic DNA, its composition being independent of cultural conditions or preparation

*Corresponding author. Tel.: +47 64 948 562; fax: +47 64 943 789.
E-mail address: hilde.ostlie@umb.no (H.M. Østlie).

0958-6946/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.idairyj.2004.08.020

methods, and amenability to automation and statistical data analysis. However, very often the most accurate results are obtained when both phenotypic and genotypic attributes are used to make the correct appellation.

Several studies have focused on the NSLAB flora in Cheddar cheese. However, reports about the occurrence of NSLAB in semi-hard cheese varieties other than Cheddar are rather limited and needs to be elucidated. In this study convential and PCR-based methods were used to identify the predominant NSLAB species during the overall ripening process in Präst cheese made at different dairies.

The objective of the study was to evaluate the diversity of NSLAB in Präst cheeses according to different dairies and different ripening time. The cheese milk consisted of pasteurized or both pasteurized and microfiltered milk.

2. Materials and methods

2.1. Cheese samples

Präst cheese from three Swedish dairies (X, Y, Z) situated in different geographic parts of Sweden was analysed. The cheeses were produced each month, from February to September, using mesophilic DL-starter (*Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris*). Five cheeses from the same vat at each dairy were selected for the study. The cheeses were ripened for 30 days at 17–19 °C at dairy X and at 19–20 °C at dairy Y and for 22 days at 18 °C at dairy Z before sending it to the Department of Chemistry, Biotechnology and Food Science (Ås, Norway) for analyses and further ripening at 11 °C for 90 days. Further storage of the cheeses was made at 4 °C. One of four cheeses was cut in two for analysis after 30, 90, 180 and 270 days of ripening, respectively. The fifth cheese (control cheese) was sampled aseptically from the centre of the cheese after 30, 90, 180 and 270 days of ripening. The sampling point was filled with wax after sampling.

2.2. Microbiological sampling

Cheese samples were aseptically collected according to the IDF-standard 50C (IDF, 1995) after 30, 90, 180 and 270 days of ripening using a cork borer (1–1.5 cm in diameter) and 11 g were homogenized with 99 mL of 2% (w/v) sodium citrate solution (pH 7.5) in an omnimixer (OMNI, International, Waterbury, C.T. USA) for 2 min. Serial dilutions were made in Ringer's solution and plated on specific media for viable counts. Total bacterial counts were determined on plate count agar (PCA, Oxoid Ltd, Basingstoke, Hampshire, England), lactococci were enumerated on M17 agar (Oxoid), presumptive leuconostoc and pediococci were enumerated on De Man Rogosa Sharpe agar (MRS; Difco Laboratories, Detroit MI, USA) supplemented with 100 μg mL^{-1} vancomycin (Abott Scandinavia AB, Kista, Sweden) and presumptive pediococci were enumerated on MRS agar (Difco) supplemented with 50 μg mL^{-1} vancomycin (Abott Scandinavia AB) and 5% NaCl (w/v), all after incubation at 30 °C for 4 days. Lactobacilli were enumerated on *Lactobacillus* selective agar (LBS agar, Becton Dickinson Microbiology Systems, Sparks, MD, USA) incubated anaerobically in anaerobic jars at 30 °C for 4 days (BBL GasPakPlus System, Becton Dickinson Microbiology Systems) and enterococci were enumerated on Slanetz and Bartley agar (Oxoid) incubated aerobic at 44 °C for 2 days. After the incubation period, the plates with 10–300 colony forming units (cfu) were selected for enumeration. The number of colonies developed in each medium was expressed as cfu g^{-1}.

2.3. Collection strains

Defined type strains of different species were obtained from DSMZ (Deutche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany): *Lactobacillus casei* DSM 20011; *Lb. paracasei* subsp. *paracasei* DSM 5622; *Lb. plantarum* DSM 20174; *Lb. curvatus* DSM 20019; *Leu. mesenteroides* subsp. *mesenteroides* DSM 20343; *Leu. mesenteroides* subsp. *cremoris* DSM 20346; *Leu. mesenteroides* subsp. *dextranicum* DSM 20484. *Lb. rhamnosus* GG (ATCC 53103) was kindly supplied by Valio Ltd, Helsinki, Finland.

2.4. NSLAB isolation

Cheese produced in April was selected for NSLAB isolation. From LBS agar, MRS agar containing vancomycin and MRS agar containing vancomycin and NaCl, five single colonies from each agar were randomly picked from each cheese after 90, 180 and 270 days of ripening. In total, 140 colonies were isolated from 12 cheeses made at 3 dairies, i.e. 4 cheeses and 46 colonies per dairy. All colonies were subcultured to purity at least twice on MRS-agar. The pure cultures were frozen and stored at −80 °C in MRS medium containing 15% (v/v) glycerol. Working cultures were prepared from the frozen cultures by two consecutive transfers in MRS broth and incubation overnight at 30 °C.

2.4.1. Phenotypic characterization

Morphological studies were carried out on isolates grown on MRS agar incubated at 30 °C for 4 days. Growth at 5 °C, 10 °C, 40 °C and 45 °C were studied in MRS broth for 14 days and growth at different salt

Download English Version:

https://daneshyari.com/en/article/8979055

Download Persian Version:

https://daneshyari.com/article/8979055

Daneshyari.com