Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Dairy Journal 16 (2006) 119–130
INTERNATIONAL DAIRY JOURNAL
www.elsevier.com/locate/idairyj

Assessing the proteolytic and lipolytic activities of single strains of mesophilic lactobacilli as adjunct cultures using a Caciotta cheese model system

Raffaella Di Cagno[a], Maurizio Quinto[b], Aldo Corsetti[c], Fabio Minervini[a], Marco Gobbetti[a,*]

[a]*Dipartimento di Protezione delle Piante e Microbiologia Applicata, Facolta di Agraria, University of Bari, Via Amendola 165/a, 70126 Bari, Italy*
[b]*DiSACD, University of Foggia, 71100 Foggia, Italy*
[c]*Department of Food Science, University of Perugia, 06126 Perugia, Italy*

Received 1 September 2004; accepted 31 January 2005

Abstract

Seventeen strains of mesophilic lactic acid bacteria, isolated from cheese (non-starter lactic acid bacteria, NSLAB) or sourdough, were used individually as adjunct cultures in a Caciotta cheese model system. Adjunct cultures were monitored by randomly amplified polymorphic DNA analysis and their cell counts mainly varied from ca. 9.0 to 8.0 $\log$ cfu g^{-1} throughout 36 days of ripening. Adjunct cultures influenced differently cheese proteolysis. Both NSLAB and sourdough strains caused an extensive secondary proteolysis; however, some NSLAB strains produced the highest concentration of free amino acids. Principal component analysis (PCA) differentiated cheeses manufactured with NSLAB strains *Lactobacillus parabuckneri* B_9F_{ST}, *Lb. paracasei* $B_{61}F_5$, *Lb. curvatus* 2768 and *Lb. rhamnosus* ATCC 7469 based on the accumulation of Lys, Glu, Phe, Hist, Asp and Met. Assessment of cheese lipolysis showed that: (i) highest concentrations of free fatty acids (FFA) were found with NSLAB strains *Lb. rhamnosus* ATCC 7469 and *Lb. casei* subsp. *pseudoplantarum* 2742 (ca. 10 500 mg kg^{-1}); (ii) PCA differentiated cheeses manufactured with NSLAB strains *Lb. rhamnosus* ATCC 7469 and *Lb. casei* subsp. *pseudoplantarum* 2742 based on the accumulation of palmitic (C16:0) and linoleic (C18:2) acids, and those with *Lb. curvatus* 2768 and *Lb. parabuckneri* B_9F_{ST} based on the high concentration of short chain FFA; (iii) the cheese made with sourdough strain *Lb. sanfranciscensis* CB1 had the highest levels of unsaturated FFA.

Keywords: Cheeses; Adjunct cultures; NSLAB; Proteolysis; Lipolysis

1. Introduction

Non-starter lactic acid bacteria (NSLAB) is a group of micro-organisms that dominate the microflora of Italian (e.g., Pecorino varieties) (Di Cagno et al., 2003), Spanish (e.g., Idiazabal and Roncal) (Arizcun, Barcina, & Torre, 1997), New Zealand (e.g., Cheddar) (Crow, Curry, & Hayes, 2001) and Irish (e.g., Cheddar) (Fitzsimons, Cogan, Condon, & Beresford, 1999) cheese varieties during ripening. Although pediococci and micrococci are also included in the NSLAB, mesophilic lactobacilli have been found to dominate (Crow et al., 2001). NSLAB are usually present because of post-pasteurization contamination, but may also constitute part of the raw milk microflora and survive pasteurization (Turner, Lawrence & Levriere, 1986; De Angelis et al., 2004). NSLAB usually grow from $\sim<2.0$ $\log_{10}$ cfu g^{-1} to ~ 9.0 $\log_{10}$ cfu g^{-1} at the end of cheese ripening (McSweeney, Fox, Lucey, Jordan, & Cogan, 1993).

Several studies have outlined the role of NSLAB in cheese making. Cheddar cheese manufactured using

*Corresponding author. Tel.: +39 080 5442949; fax: +39 080 5442911.
E-mail address: gobbetti@agr.uniba.it (M. Gobbetti).

0958-6946/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.idairyj.2005.01.012

aseptic cheese vats and without NSLAB is considered to lack full mature flavour (Fox, McSweeney, & Lynch, 1998). Studies on the inclusion of some strains of NSLAB with the starter lactococci (Fox et al., 1998; McSweeney et al., 1994), and the use of raw milk (Lane & Fox, 1996), or of blends of raw and pasteurised milk (Rehman et al., 2000) in Cheddar cheese manufacture have indicated that NSLAB contribute positively to the release of free amino acids and fatty acids during ripening. Moreover, the flavour and texture of other semi-hard cheeses were improved, or ripening was accelerated, when NSLAB was used as an adjunct to the starter culture (Gomez, Gaya, Nunez, & Medina, 1996; Corsetti, Gobbetti, Smacchi, De Angelis, & Rossi, 1998).

Nevertheless, the role of NSLAB in cheese ripening is strain-dependent and the selection of adjunct cultures from NSLAB strains is the most time-consuming but productive way to improve the cheese flavour and accelerate ripening. Moreover, the selection of mesophilic lactobacilli, which are isolated from different food ecosystems (e.g., sourdough), may also supply potential candidates for adjunct cultures in cheese making (Fox et al., 1998).

Assessment of the performance of a large number of enzymes, starter cultures or adjunct cultures in cheese is expensive. Thus, a model system in which their performance can be assessed rapidly is desirable, provided that standard cheese conditions can be replicated closely. Rehman, McSweeney, and Fox (1998) developed a model cheese system, with a gross composition, texture and consistency very similar to that of Cheddar cheese; the time and cost of manufacture and ripening with model system are greatly reduced. This model was successful for assessing the proteolytic activities of single strains of *Lactococcus* during ripening of Cheddar cheese (Rehman, Pripp, McSweeney, & Fox 1999).

This work was aimed at using the above cheese model system to permit the selection of adjunct cultures from a large number of mesophilic lactobacilli of different origin. Seventeen strains of mesophilic lactic acid bacteria, isolated from different cheese varieties or sourdoughs, were used individually as adjunct cultures to assess the proteolytic and lipolytic activities during ripening of model Caciotta-type cheeses.

2. Materials and methods

2.1. Lactic acid bacteria and culture conditions

Lactobacillus plantarum DC400, *Lb. pontis* 30, *Lb. hilgardii* 52B, *Lb. farciminis* 2I, *Lb. alimentarius* 15 M, *Lb. fructivorans* DA106, *Lb. fermentum* 6E, *Lb. brevis* 18B, *Lb. sanfranciscensis* CB1 and *Weissella confusa* (formerly *Lb. confusus*) 14A, were previously isolated from sourdoughs and belong to the Culture Collection of the Dipartimento di Protezione delle Piante e Microbiologia Applicata, University of Bari. These strains were cultivated in modified MRS broth (Oxoid, Basingstoke, Hampshire, England) containing fresh yeast extract (5%, v/v) and 28 mM maltose, and which had a final pH of 5.6.

Lb. casei subsp. *pseudoplantarum* 2742, *Lb. casei* subsp. *casei* 2752, *Lb. plantarum* 2739, *Lb. curvatus* 2768, *Lb. paracasei* $B_{61}F_5$ and *Lb. parabuckneri* B_9F_{ST}, previously isolated from cheeses and belonging to the same culture collection or to the culture collection of the Institute of Sciences of Food Production, CNR Bari, and *Lb. rhamnosus* ATCC 7469 were cultivated in MRS broth (Oxoid).

All the lactic acid bacteria were incubated at 30 °C for 24 h, with the exception of *Lb. hilgardii* 52B and *Lb. fermentum* 6E which were cultivated at 37 °C.

2.2. Protocol for the manufacture of miniature model cheeses

The raw cows' milk used in this study had the following characteristics: lactose 4.9%, protein 3.2%, fat 3.6% and pH 6.6.

Streptococcus thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus* were used as starter culture. The freeze-dried preparation (Sacco Srl., Como, Italy) was propagated in sterile milk at 45 °C until a cell count of ca. 9.5 $\log_{10}$ cfu g^{-1} was reached. After cultivation in MRS or modified MRS, lactic acid bacteria strains used as adjunct cultures were harvested by centrifugation at 9000 *g* for 15 min at 4 °C. The resultant pellet was washed with 50 mM Tris-HCl buffer, pH 7.5, containing 0.1 M $CaCl_2$ and re-suspended in sterile milk at a concentration of ca. 10.0 $\log_{10}$ cfu g^{-1}.

The method of Rehman et al. (1998), with some modifications was used for the manufacture of miniature model cheeses. For each model cheese, raw cows' milk was pasteurised at 72 °C for 30 s, transferred to 6 wide-mouth plastic centrifuge bottles (200 mL), cooled at 45 °C and inoculated with starter culture (5%, v/v). The milk was then incubated at 45 °C until the pH dropped to 6.4, cooled to 37 °C, and then inoculated (5%, v/v) with individual adjunct culture strains. After 30 min, liquid calf rennet (Estratto Concentrato di Caglio Liquido, Caglificio Clerici, Como, Italy) was added at a level of 0.1 mL L^{-1} (as recommended by the manufacturer) and the milk was held for 30–40 min until a firm coagulum formed. The coagulum in the bottles was cut manually using wire cutters spaced 1 cm apart in a frame. The curd particles in the whey were stirred slowly for 20 min using glass rods. Curds and whey, in the centrifuge bottles, were centrifuged at room temperature for ca. 60 min at 1700 *g* in order to achieve a moisture