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Review

Application of proteomics to the characterisation of milk and dairy products

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Abstract

The use of proteomic tools allows a global and dynamic view of proteins that are expressed by bacteria. As an increasing number of bacterial genomes is currently available for homology searches, it is now possible to use such techniques to screen proteins expressed by microorganisms used in various fermented foods. Proteomic tools are also useful to investigate protein heterogeneity in protein-rich foods. In this paper the use of proteomic tools for the characterisation of milk proteins and in the study of protein expression of lactic acid bacteria used for manufacture of dairy products is reviewed; particular attention is given applied to advances in proteomic techniques available. The particular case of proteomics applied to cheese as an example of a complex food matrix [a mixture of animal (milk) and microbial proteins] is discussed, focusing on a novel strategy that allows the study of the enzymatic machinery found in situ in cheese.

Keywords: Proteomics; Milk; Cheese; Stress; Proteolytic enzymes; Glycolytic enzymes

Contents

1. Introduction

The term proteome refers to the proteins expressed by a genome at a particular point in time. The genome provides only static information, while the proteome provides an overall view of the cell machinery, which

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can be studied under various conditions and could provide information regarding dynamic processes. The elucidation of the expressed part of the genome is required to link genomic data to biological functions.

Proteomics, mainly based on high-resolution two-dimensional electrophoresis (2DE) (Patterson & Aebersold, 1995) coupled with mass spectrometry (MS), is a powerful tool for analysing several hundreds of proteins simultaneously in complex mixtures (Mann, Hendrickson, & Pandey, 2001). High performance liquid chromatography (HPLC) is another technique of choice for proteomic studies, especially for protein identification through peptide analysis, due to its ability to separate and identify lower molecular mass molecules. Multi-dimensional HPLC and tandem MS coupled on-line are systems that are capable of separating and identifying low-abundance and membrane proteins which escape 2DE analysis (Smith, 2002).

Among the broad applications of proteomics to samples with origins as varied as microbial, vegetable and animal sources, many applications to a variety of nutritionally relevant proteins have also been described. Proteomic tools have permitted the characterisation of food components, the study of their functional, nutritional and biological relevance, the study of protein conformation and of protein interactions, as well as food quality assessment (Carbonaro, 2004; Kvasnicka, 2003; Léonil, Gagnaire, Mollé, Pezennec, & Bouhallab, 2000). A number of proteomic techniques have been applied to the study of milk and milk products, allowing the separation of major proteins, including caseins (α_{s1}-, α_{s2}-, β- and κ-casein) and whey proteins (β-lactoglobulin, α-lactalbumin, bovine serum albumin).

Milk proteins are characterised by a great heterogeneity and the presence of several molecular forms. Important challenges facing the use of proteomics concern the elucidation of different genetic variants, changes in the degree of phosphorylation or glycosylation and the localisation of post-translational modifications of milk proteins. Milk also contains an important number of low abundance proteins, such as lactoferrin, immunoglobulins, glycoproteins, hormones and endogenous enzymes (Fox & Kelly, 2003), which may also be studied by the use of techniques with such high resolution.

Another important field of application of proteomic tools, based on 2DE, concerns profiling proteins from different micro-organisms (Washburn & Yates, 2000). Lactic acid bacteria (LAB) are used as starter cultures in the production a great variety of fermented food products in the agro-food industry. Among them, *Lactococcus* (*L.*), *Lactobacillus* (*Lb.*) and *Streptococcus* (*St.*) are the most frequently used genera for the production of milk products, where their main role involves rapid production of lactic acid from lactose, consequently resulting in acidification of the product, which inhibits pathogen growth, and avoidance of possible spoilage. One of the main aspects concerning LAB that may be achieved by the use of proteomic techniques is the study of their ability to adapt to different environmental stresses (Champomier-Vergès, Maguin, Mistou, Anglade, & Chich, 2002; Miyoshi et al., 2003).

This article aims to review the use of proteomic tools in the study of milk proteins and proteins expressed by LAB used in dairy technology. Attention will be paid to the progress made due to the advances in the application of 2DE and HPLC techniques to biological samples, especially due to the coupling of these techniques with mass spectrometry.

2. Milk proteins

2.1. Characterisation, genetic variants and post-translational modifications

During the 1980s several 2DE procedures were used as high-resolution methods for the separation of milk proteins (Felgenhauer & Hagedorn, 1980; Addeo, Mauriello, & di Luccia, 1988) and the first 2DE maps of various milk products were established (Trieu-Cuot & Gripon, 1981a; Cheng & Miller, 1988; Marshall & Williams, 1988).

Two-dimensional patterns of milk proteins of several mammals were obtained by combining isoelectric focusing (IEF) (pH 3–10) in the first dimension and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) or urea-PAGE in the second dimension, which allowed comparison of the major milk proteins in the different species. Human, murine and porcine milk proteins were revealed to be completely different from those of ruminant species (Kim & Jiménez-Flores, 1994; Goldfarb, 1999). The study of the evolution of milk protein profiles during different lactation periods using this technique revealed that casein proportions were reduced throughout the dry period and a number of peptides generated due to casein breakdown were identified (Aslam, Jiménez-Flores, Kim, & Hurley, 1994). Using the same pH gradient, some of the genetic variants of caseins and whey proteins and their phosphorylated forms were separated and identified (Holt & Zeece, 1988; Zeece, Holt, Wehling, Liewen, & Bush, 1989).

The advent of immobilised pH gradients (IPG) (Görg et al., 1983) allowed the creation of both wide and narrow pH gradients that enabled higher resolution leading to the visualisation of previously undetected proteins, improvement in the separation of isoforms and facilitating quantification. As an example of a recent application in the use of narrow IPGs to the analysis of bovine milk proteins, the creation of a linear pH 4–7