Analytica Chimica Acta 788 (2013) 193–199

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Capillary isoelectric focusing of probiotic bacteria from cow's milk in tapered fused silica capillary with off-line matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification

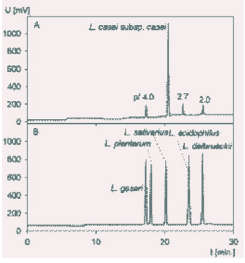
Marie Horká[a,*], Pavel Karásek[a], Jiří Šalplachta[a], Filip Růžička[b,1], Marie Vykydalová[a], Anna Kubesová[a], Vladimír Dráb[c,2], Michal Roth[a], Karel Šlais[a]

[a] Institute of Analytical Chemistry of the ASCR, v. v. i., Veveří 97, 602 00 Brno, Czech Republic
[b] Department of Microbiology, Faculty of Medicine, Masaryk University Brno, Czech Republic
[c] MILCOM a.s., Dairy Research Institute, Tábor, Czech Republic

HIGHLIGHTS

- We found method for rapid separation of *Lactobacillus* in tapered FS capillary.
- CIEF separation capacity and efficiency was increased.
- CIEF was coupled with off-line MALDI-TOF MS identification.
- Method was tested on spiked milk.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 March 2013
Received in revised form 6 May 2013
Accepted 18 May 2013
Available online 10 June 2013

Keywords:
Capillary isoelectric focusing
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
Tapered fused silica capillary
Lactic acid bacteria
Milk

ABSTRACT

In this study, combination of capillary isoelectric focusing (CIEF) in tapered fused silica (FS) capillary with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is presented as an efficient approach for unambiguous identification of probiotic bacteria in real sample. For this purpose, bacteria within genus *Lactobacillus* were selected as model bioanalytes and cow's milk was selected as a biological sample. CIEF analysis of both the cultivated bacteria and the bacteria in the milk was optimized and isoelectric points characterizing the examined bacteria were subsequently determined independently of the bacterial sample origin. The use of tapered FS capillary significantly enhanced the separation capacity and efficiency of the CIEF analyses performed. In addition, the cell number injected into the tapered FS capillary was quantified and an excellent linearity of the calibration curves was achieved which enabled quantitative analysis of the bacteria by CIEF with UV detection. The minimum detectable number of bacterial cells was 2×10^6 mL^{-1}. Finally, cow's milk spiked with the selected bacterium was analyzed by CIEF in tapered FS capillary, the focused and detected bacterial cells were collected from the capillary, deposited onto the cultivation medium, and identified using MALDI-TOF MS afterward. Our results have revealed that the proposed procedure can be advantageously used for unambiguous identification of probiotic bacteria in a real sample.

* Corresponding author. Tel.: +420 5 32290221; fax: +420 5 41212113.
E-mail addresses: horka@iach.cz (M. Horká), filip.ruzicka@fnusa.cz (F. Růžička), sbirka@vum-tabor.cz (V. Dráb).
[1] Tel.: +420 5 43183117; fax: +420 5 43183089.
[2] Tel.: +420 381 252 980; fax: +420 381 252 980.

0003-2670/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2013.05.059

1. Introduction

Detection and identification of trace amounts of similar components in biological samples require appropriate selection of separation techniques and procedures. Reliability and reproducibility of the results are important prerequisites. Suitable analytical techniques should meet the basic requirements regarding speed, sensitivity, selectivity, and costs [1]. Capillary isoelectric focusing (CIEF) is an example of analytical technique suitable for trace analysis of amphoteric components in complex biological matrices [2–11]. In CIEF, the analytes are focused and simultaneously concentrated into narrow zones whose positions are characterized by the local pH equal to their respective isoelectric points (p*I*) [7]. However, apart from electroosmotic flow, high concentrations of salts can decrease the separation efficiency of bioanalytes, e.g., analysis of proteins or microorganisms in physiological fluids [2–5]. On the other hand, the presence of chloride ion considerably improves the limit of detection [3,6].

Our previous studies on zone focusing in electrophoretic migration [12–14] have shown that the separation capacity in CIEF can be improved if a tapered fused silica (FS) capillary is used instead of a cylindrical one. Modulation of cross-section area of the separation capillary by inserting cylindrical fibers at different distances into the capillary was used in capillary zone electrophoresis (CZE) [15]. Application of continuously tapered capillaries results in a higher number of separable compounds and in improved resolution of sample components as predicted previously [12–14]. Recently, it was shown that tapered FS capillaries can reproducibly be prepared by etching with supercritical water [16] and the improved separation efficiency of CIEF in tapered capillaries was achieved as described in our latest study [17].

In the present contribution, tapered FS capillary was used in CIEF separation of probiotic bacteria. Lactic acid bacteria (LAB) [18–20] within genus *Lactobacillus* were chosen as model bioanalytes. The physicochemical properties of bacterial cells within the genus *Lactobacillus*, determined by the chemical moieties on the cell surface [8,21–23], are strongly species- or strain-dependent, which can be expediently employed in the analyses of the bacteria. These properties determine in a large extent the behavior of the cells and their non-specific interactions with the environment [24].

A large variety of methods applicable to food samples are used for identification of LAB [25]. Capillary electrophoretic techniques (CE) have some advantages [25] compared to other techniques, e.g., polymerase chain reaction [26]. Separation of intact cells by CE enables fast and efficient identification of microorganisms in various sample types, including foodstuff. Hjertén et al. were the first who used *Lactobacillus casei* [27] in CE. Thereafter, various LAB were analyzed in different sample matrices (e.g., yoghurt) using CZE or microchip electrophoresis [28–30]. In addition, CZE or CIEF were also used for the analysis of *Enterococcus faecalis* in our previous studies [31–33]. CE can also be used for the analysis of milk proteins as a suitable alternative to gel electrophoresis [34–37]. In recent years, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been reported a powerful, rapid, cost-effective, and reliable analytical tool for the classification and identification of various bacterial and fungal species [38–42]. In this technique, the identification is based on species-specific fingerprint, i.e., the set of mass/charge (m/z) values, obtained by MALDI analysis of either intact cells or cell lysates. Recently, MALDI-TOF MS was successfully used to identify LAB isolated from various dairy and meat products [43,44].

The aims of this study were to assess capability of CIEF in tapered capillary to detect probiotic bacteria in a milk sample and to investigate feasibility of combination of CIEF with MALDI-TOF MS to unambiguously identify the bacteria in real sample.

2. Experimental

2.1. Chemicals

The high resolution ampholyte, pH 2.0–4.0, ampholyte pH 3.0–4.5, 2-morpholino-ethanesulphonic acid monohydrate, 3-morpholino-propanesulphonic acid, and *N*-[tris-(hydroxymethyl)-methyl]-3-amino-2-hydroxy-propanesulphonic acid were obtained from Fluka Chemie (Buchs, Switzerland). The solution of synthetic carrier ampholytes (Biolyte, pH 3–10) was obtained from Bio-Rad Laboratories (Hercules, CA, USA). *N*-(2-acetamido)-2-aminoethanesulphonic acid and 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulphonic acid were obtained from Merck (Darmstadt, Germany). L-Aspartic acid was obtained from LOBA Chemie (Wien, Austria). Other spacers (tartaric, malic, formic (FA), succinic, acetic, pivalic, glutamic and nicotinic acids), non-ionogenic detergent Brij 35, ethanol (EtOH), acetonitrile (ACN), and trifluoroacetic acid (TFA) were purchased from Sigma (St. Louis, MO, USA). Polyethylene glycol, M_r 10,000 (PEG 10,000), bovine serum albumin (BSA, M_r 67,000, p*I* 4.9 [45]), β-lactoglobulin (M_r 35,000, p*I* 5.1 [45]), and L-cysteine hydrochloride were obtained from Sigma–Aldrich (Milwaukee, WI, USA). 3,5-Dimethoxy-4-hydroxycinnamic acid (SA) and calibration mixture ProMix2 were purchased from LaserBio Labs (Sophia-Antipolis Cedex, France). The specifications of the spacers used [46,47], all simple ampholytic electrolytes, are described in Ref. [31]. The low-molecular-mass p*I* markers, p*I* = 2.0, 2.7 [48], 4.0 [49], and 4-morpholinyl acetic acid [47] were synthesized at the Institute of Analytical Chemistry of the ASCR. All chemicals were of electrophoresis or analytical grade.

2.2. Fabrication of the tapered fused silica capillary

The tapered fused silica (FS) capillary has been prepared by etching with supercritical water in an apparatus designed and built in our laboratory [13]. Undeactivated, polyimide-coated capillary (100 μm I.D., 360 μm O.D.) was delivered by Agilent Technologies, Waldbronn, Germany (Part No. 160-2634-10). Water treatment prior to use included double distillation, purification with reverse osmosis system (Ultra Clear UV, Barsbüttel, Germany) and stripping with helium (Linde, Brno, purity 4.5) to remove oxygen and CO_2. The capillary with 250 mm long separation space tapered from the inlet I.D. 170 μm to the 100 μm I.D. at the detection point (total length 450 mm) were prepared by dynamic flow-mode method with water flow rate 0.272 g min^{-1}, temperature 400 °C, pressure 300 bar, and treatment duration 40 min. To minimize the pressure drop along the treated capillary, a FS restrictor (50 μm I.D., 130 cm) was located at the system outlet downstream of the cooling unit (Grant LTD6G, Grant Instruments, United Kingdom). The diameter changes were checked with optical microscope (Olympus BX-51, Prague, Czech Republic) equipped for operation in both reflected and transmitted light and featuring an image processing software (Deep Focus ver. 3.1.). The average relative standard deviation (n = 5) of internal diameters in capillary-to-capillary test did not exceed 5%.

2.3. Microbial sample and cow's milk

The strains included in this study: *Lactobacillus gasseri* CCDM 340, *Lactobacillus plantarum* CCM 7039, *Lactobacillus salivarius* CCDM 216, *Lactobacillus acidophilus* CCDM 149, and *Lactobacillus delbrueckii* CCDM 707 were obtained from the Culture Collection of Dairy Microorganisms (CCDM, Laktoflora, Tábor, Czech Republic). The strains of *L. casei* subsp. *casei* CCM 7089, *Lactobacillus rhamnosus*^{T} CCM 1825were obtained from the Czech Collection of Microorganisms (CCM, Brno, Czech Republic).