JOURNAL OF PROTEOMICS XX (2013) XXX–XXX

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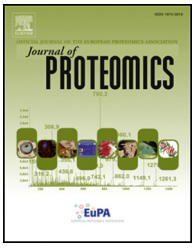

Fermentation and proteome profiles of *Lactobacillus plantarum* strains during growth under food-like conditions

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ARTICLE INFO

Article history:
Received 14 May 2013
Accepted 1 November 2013

Keywords:
Lactobacillus plantarum
Adaptation
Food-like conditions

ABSTRACT

This study aimed at investigating the proteomic adaptation of *Lactobacillus plantarum* strains. Cultivation of *L. plantarum* strains under food-like conditions (wheat flour hydrolyzed, whey milk, tomato juice) affected some metabolic traits (e.g., consumption of carbohydrates and synthesis of organic acids) compared to de Man, Rogosa and Sharpe (MRS) broth. The analysis of the fermentation profile showed that the highest number of carbon sources metabolized by *L. plantarum* strains was found using cells cultivated in media containing low concentration of glucose or no glucose at all. The proteomic maps of the strains were comparatively determined after growth on MRS broth and under food-like conditions. The amount of proteins depended on strain and, especially, on culture conditions. Proteins showing decreased or increased amounts under food-like conditions were identified using MALDI-TOF-MS/MS or LC-nano-ESI-MS/MS. Changes of the proteome concerned proteins are involved in carbohydrate transport and metabolism, energy metabolism, Sec-dependent secretion system, stress response, nucleotide metabolism, regulation of nitrogen metabolism, and protein biosynthesis. A catabolic repression by glucose on carbohydrate transport and metabolism was also found. The characterization of the proteomes in response to changing environmental conditions could be useful to get *L. plantarum* strains adapted for specific applications.

Biological significance

Microbial cell performance during food biotechnological processes has become one of the greatest concerns all over the world. *L. plantarum* is a lactic acid bacterium with a large industrial application for fermented foods or functional foods (e.g., probiotics). The present study compared the metabolic and proteomic profiling of *L. plantarum* strains during growth under food-like conditions and under optimal laboratory conditions (MRS broth). This study provides specific mechanisms of proteomic adaptation involved in the microbial performances (carbohydrates utilization, energy metabolism, stress resistance etc.) affecting the main biotechnological tracts of *L. plantarum* strains. The finding of this study provides evidences that may be exploited to get strains adapted for specific applications in food biotechnology.

Abbreviations: ABC, ATP binding cassette; CCR, carbon catabolite repression; CFU, colony forming units; CGH, comparative genome hybridization; EF, Electro Focusing; KEGG, Kyoto Encyclopedia of Genes and Genomes; LCQ, liquid chromatography quadrupole; MDLC, multidimensional liquid chromatography; MRS, de Man, Rogosa and Sharpe; PTS, phosphotransfer system; TJ, tomato juice; UDP, uridine diphosphate; WFH, wheat flour hydrolyzed; WM, whey milk, % VOL, relative volume.

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1874-3919/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.jprot.2013.11.003

1. Introduction

Lactobacillus plantarum, proposed as *Streptobacterium plantarum* by Orla-Jensen in 1919, is a facultative hetero-fermentative lactic acid bacterium, which is largely distributed in nature [1,2]. This bacterium is part of the adventitious or starter microbiota of starchy and cereal foods, and dairy, meat, fruit, vegetable and fish products [2–5]. Selected probiotic *L. plantarum* strains are also used to develop functional foods and live oral vaccines [6–8]. The large industrial application of *L. plantarum* supports the ambition to better understand the proteome and, consequently, the metabolism features to optimize the survival during industrial and downstream processing [9]. Genomes of five strains of *L. plantarum* were completely or partially sequenced (WCFS1, GenBank AL935263, [10]; JDM1, GenBank CP001617; ST-III, GenBank CP002222; ATCC14917, GenBank ACGZ00000000; NC8, GenBank AGRI00000000.1, [11]). The comparative analysis provided detailed insight into core genes, variable or accessory genes and gene cassettes, genome synteny, transposable elements, and functional adaptation to growth on various substrates [12]. Despite the successful examples of genotype–phenotype matching strategies, the above approach intrinsically relies on diversity among strains at the level of genome-content [9]. The importance of differential regulation of the phenotype features, which relies on the expression of conserved genes, has to be assessed [13]. The genome diversity of several lactic acid bacteria strains was also assessed by DNA-microarray-based comparative genome hybridization (CGH) approaches, providing one-directional gene absence/presence patterns [2,14,15]. Such datasets, in combination with random forest (RF)-based correlation analysis linking multiple-genome or with CGH data, led to the identification of the genetic determinants, which are responsible for some strain-specific phenotypes [16]. There is an accumulating evidence that plant fermentation, storage conditions and, especially, chemical composition of plant matrices markedly affect the functional features of *L. plantarum* strains [9,17,18]. Based on the *L. plantarum* genome, a metabolic model was created to describe the growth and synthesis of metabolites, when this bacterium grows on minimal media containing glucose. Despite recent progress in unraveling details of *L. plantarum* genome, the mechanisms of proteomic adaptation remain largely unclear [9]. Proteomics plays a pivotal role to link genome and transcriptome to potential biological functions. To date, only a few studies were carried out on the proteomic adaptation of *L. plantarum* [19]. Almost all the previous proteomic studies on *L. plantarum* used de Man, Rogosa and Sharpe (MRS) broth, the standard rich medium for laboratory cultivations [20–25]. The cultivation of *L. plantarum* on MRS broth as well as on other laboratory media has to be regarded as much different from the conditions encountered by *L. plantarum* during food fermentations [5,18]. To the best of our knowledge, only one proteomic study was carried out on *L. plantarum* cells cultivated on cucumber juice and liquid pig feed [19]. Recently, the adaptive growth and survival strategies of *L. plantarum* during plant fermentation and storage were studied using a panel of various metabolomic approaches and elaborated by multidimensional statistical analyses. Different metabolic traits emerged depending on the strain, and on fermentation and storage processes, which resulted in alternative energy routes such as malolactic fermentation, catabolism of branched chain free amino acids, and synthesis of volatile compounds or volatile free fatty acids [18].

This study aimed at investigating the proteomic adaptation of *L. plantarum* strains, which were previously isolated from various fermented foods and intestinal tract, and were in part characterized for the phenotypic behavior under environmental conditions, which mimicked those characterizing vegetables and fruits [18]. Strains were cultivated under laboratory conditions (MRS broth) or under environmental conditions (wheat flour hydrolyzed, whey milk and tomato juice), which mimicked various natural food ecosystems. Proteins were identified by MALDI-TOF-MS/MS and multidimensional liquid chromatography (MDLC) coupled to nano-ESI-MS/MS to get a global picture of the proteome adaptation of *L. plantarum* strains.

2. Materials and methods

2.1. Bacterial strains and culture conditions

L. plantarum strains were previously identified by 16S rRNA gene sequence analysis. Strains were isolated from sourdough (strain DB200) [26], cheese (CC3M8) [27], tomato juice (POM1) [28] and human intestinal tract (LP40, Sacco S.r.L., Como, Italy) [29]. *L. plantarum* DB200 was previously selected for some phenotypic traits during sourdough fermentation [30]. *L. plantarum* CC3M8 and POM1 were previously characterized for adaptive metabolome responses under culture conditions, which mimicked those of various food ecosystems [18]. *L. plantarum* LP40 was previously selected for heat stress resistance during manufacture of Fior di Latte cheese, which incorporated probiotic lactobacilli [29].

Strains were cultivated on MRS broth (Oxoid Ltd., Basingstoke, UK) (pH ca. 6.20), wheat flour hydrolyzed (WFH), whey milk (WM) (Sigma-Aldrich, St. Louis, MO, USA) (5% w/v) [18] or tomato juice (TJ) [28] at 30 °C for 24 h. WFH was obtained by preliminary incubation of a suspension of wheat flour (20% w/v, in tap water) at 30 °C for 18 h, under stirring conditions (200 rpm). After incubation, the suspension was filtered through a Whatman apparatus (Polycarp 75 SPF, Whatman International, Maidstone, UK) and supplemented with yeast extract (0.3% w/v), glucose, sucrose and maltose (0.25% w/v each). WFH was sterilized by filtration on 0.22 μm membrane filters (Millipore Corporation, Bedford, MA, USA) and stored at 4 °C before use. The pH of the WFH was ca. 5.6. Based on previous results [18], WM was supplied with yeast extract (0.3% w/v) before sterilization to increase the growth of *L. plantarum*. The final pH of WM was ca. 6.0. TJ was obtained by centrifugation of tomato homogenate at 12,500 rpm for 20 min at 4 °C, sterilization at 121 °C for 10 min and further centrifugation at 12,500 rpm for 20 min at 4 °C. TJ was sterilized by filtration on 0.22 μm membrane filters (Millipore Corporation) and stored at 4 °C before use. The final pH of TJ was ca. 4.4.

Please cite this article as: Siragusa S, et al, Fermentation and proteome profiles of *Lactobacillus plantarum* strains during growth under food-like conditions, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.11.003