Proteomic analysis of log to stationary growth phase
*Lactobacillus plantarum* cells and a 2-DE database

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*Lactobacillus plantarum* is part of the natural microbiota of many food fermentations as well as the human gastro-intestinal tract. The cytosolic fraction of the proteome of *L. plantarum* WCFS1, whose genome has been sequenced, was studied. 2-DE was used to investigate the proteins from the cytosolic fraction isolated from mid- and late-log, early- and late-stationary phase cells to generate reference maps of different growth conditions offering more knowledge of the metabolic behavior of this bacterium. From this fraction, a total of 200 protein spots were identified by MALDI-MS and a proteome production map was constructed to facilitate further studies such as detection of suitable biomarkers for specific growth conditions. More than half (57%) of the identified proteins were predicted to be involved in metabolic pathways of the bacterium. The protein profile changed during the growth of the bacteria such that 29% of the identified proteins involved in anabolic pathways were at least twofold up-regulated throughout the mid- and late-exponential and early-stationary phases. In the late-stationary phase, six proteins involved in stress or with a potential role for survival during starvation were up-regulated significantly.

Keywords:
2-DE / Differential production / Growth phases / *Lactobacillus plantarum*

1 Introduction

One of the few *Lactobacillus* species that is also a natural inhabitant of the human gastro-intestinal tract (GIT) is *Lactobacillus plantarum*. *L. plantarum* is a versatile, Gram-positive, fermentative bacterium that can be found in a range of habitats, including dairy, meat, and many plant fermentations. Thus, the *L. plantarum* strains are able to adapt to different environmental conditions [1–3]. Furthermore, *L. plantarum* has the advantage that it can grow to high cell densities which is desirable for industrial applications. *L. plantarum* strain WCFS1 is a single-colony isolate from the human pharyngeal strain NCIMB8826 [4] and has been demonstrated to survive the GIT of men [5] and mice [6]. A variety of *L. plantarum* has been described to have beneficial effects on the host and have been marketed as a probiotic [2]. For several years, much research has been done on the mechanisms of adaptation and survival of *L. plantarum* under various environmental conditions [6, 7]. The complete genome of *L. plantarum* WCFS1 has been sequenced [8]. Analysis of the sequence predicted 3052 proteins from which 209 proteins are located extracellularly. The genome sequence allowed the design of specific DNA microarrays for use in transcript profiling of this species [9, 10]. Recently, the global gene expression of *L. plantarum* has been investigated to obtain a greater insight into the inhibitory effects of lactic acid on growth of this organism, the major end product of fermentation [11]. Although transcriptomics provides...
detailed information about global gene expression under a certain condition, the short half life of the mRNA can be a limiting factor for certain studies. Proteins have the advantage of greater stability. In particular the high turnover rate of mRNA allows only the use of biopsies for analysis of bacterial behavior in the intestinal tract, but since proteins are more stable in time, the far more accessible fecal samples can be used. Furthermore, post-transcriptional regulations at the protein level may be investigated.

In this study, a proteome reference map of cytosolic proteins from *L. plantarum* WCFS1 was generated, for the purpose of aiding physiological investigations involved in environmental adaptation to conditions such as food-processing, stress, or passage of the intestinal tract. Furthermore, proteome maps of the cytosolic fraction of different growth conditions of *L. plantarum*, were made to give a global picture of the relative abundance of proteins during growth phases in the selective medium for lactobacilli. The relative abundance measured in this study takes into account protein production as well as other processes such as protein turnover rate and protein modification and translocation [12]. The proteome maps were used to indicate the dynamics of the proteome of *L. plantarum* WCFS1 during growth.

2 Materials and methods

2.1 Microorganism and growth condition

The microorganism used for these experiments was *L. plantarum* WCFS1, a single-colony isolate from *L. plantarum* NCIMB8826, and was obtained from NIZO Food Research in Ede, The Netherlands. The complete genome sequence of *L. plantarum* NCIMB8826, and was obtained from NIZO Food Research in Ede, The Netherlands. The complete genome sequence of this organism is available [8]; (GenBank, AL935263).

*L. plantarum* WCFS1 was cultured in 10 mL MRS [13] medium (Difco, Surrey, UK) at 37°C. For generation of proteome maps, precultured cells in stationary phase were used to inoculate a fresh culture, which was incubated under the same growth conditions. Samples were taken at OD$_{600}$ of 0.5, 1.6, 4.7, and 8.0. These OD$_{600}$’s corresponded to mid-log, late-log, early-stationary, and late-stationary phase growth, and to $1.2 \times 10^4$, $5.0 \times 10^4$, $1.4 \times 10^5$, and $3.7 \times 10^5$ CFU/mL, respectively. The samples were aliquoted and used for protein extraction from different cellular fractions as described below.

2.2 Preparation of cytosolic protein fraction

*L. plantarum* WCFS1 cells were harvested and washed with PBS and double distilled water (ddH$_2$O), respectively, and centrifuged at 500 $\times$ g for 10 min at 4°C. The supernatant was discarded and the cells were resuspended in buffer containing 8 M urea (BioRad, Hercules, CA, USA), 2% w/v CHAPS (Sigma, St Louis, MO, USA) 65 mM DTT (Sigma), 0.5% v/v IPG buffer (pH 3–10 NL; Amersham Bioscience, Uppsala, Sweden). The bacteria were lysed mechanically by beating with zirconium beads (diameter 0.8 mm) using a minibeat beater (BioSpec Products, Bartlesville, OK, USA) for 5 $\times$ 1 min with 1 min intervals on ice. The cell debris was removed by centrifugation and the supernatant was collected and centrifuged at 20,000 $\times$ g for 30 min at 4°C. The supernatant was collected and stored at $-80^\circ$C until use.

2.3 SDS-PAGE

Prior to gel electrophoresis, the protein concentration was determined using a Bradford-based DC-protein assay (BioRad), using BSA as the standard. SDS-PAGE was used to confirm protein quality of all protein extracts [14]. Briefly, 20 $\mu$g of protein was loaded on a 12.5% SDS-PAGE gel that was run constantly at a voltage of 110 V until the Bromophenol blue front had run off. Afterwards, the gels were stained with silver nitrate according to Shevchenko et al. [15]. Gel images were obtained by using a GS-800 calibrated densitometer (BioRad).

2.4 IEF and 2-DE

*L. plantarum* WCFS1 cytosolic proteins were first separated by IEF. Proteins (60 $\mu$g) were loaded on Immobiline Dry Strips (pH range 3–10, nonlinear, 24 cm long; Amersham). IEF was performed on an IPIFhor electrophoresis unit (Amersham), operating at a constant temperature of 20°C. The strips, containing the samples, were first actively rehydrated at 30 V for 12 h. The IEF program was as follows: 250 V for 1 h, 500 V for 1 h, 1000 V for 1 h, 1000–8000 V for 2 h, and 8000 V for 52,000 V. Prior to running the second dimension, the strips were equilibrated for 15 min in 50 mM Tris-HCl, pH 6.8, 6 M urea, 30% v/v glycerol, 2% w/v SDS, 1% DTT, and for 15 min in 50 mM Tris-HCl, pH 6.8, 6 M urea, 30% v/v glycerol, 2% w/v SDS, 2.5% iodoacetamide. The strips were placed on 12.5% SDS-PAGE gels and covered with 0.5% agarose containing a trace of Bromophenol blue. The gels were run at a constant voltage of 200 V in a Dodeca Cell system (BioRad) until the marker dye had reached the base of the gel. The gels were stained with silver nitrate as described above. The images were obtained with a GS-800 calibrated densitometer (BioRad).

2.5 Protein identification

Image analysis was performed using the PDQuest software (version 7.2) (BioRad). Four gels were produced for every growth condition and spots present on at least three gels per growth condition were used for comparison of the obtained spots. Proteins were considered differentially produced when spot intensities passed the threshold of at least a two-fold difference in up- or down-regulation in combination with a student’s $t$-test using a statistical 95% reliability score.

Protein bands and spots were excised manually from the gels and subjected to in-gel tryptic digestion according to Bouwman et al. [16]. The peptide mass fingerprints of the
3 Results and discussion

A proteome map of *L. plantarum* WCFS1 was constructed, which can be used as a reference map for studying proteome dynamics of *L. plantarum* under different conditions. The map has been used initially in this study to investigate the proteome of *L. plantarum* WCFS1 during growth.

3.1 Generation of a 2-DE reference map from cytosolic *L. plantarum* proteins

The cytosolic protein fraction of *L. plantarum* WCFS1 having reached the stationary phase was used to construct an initial 2-DE reference map of the proteome of *L. plantarum* grown in standard medium. Using a standard medium facilitates the comparative analysis of the proteome when different conditions are used.

A substantial part of the proteome of the bacterium could be visualized by using a pH range of the IPG strips from 3 to 10 (nonlinear), 2-DE followed by silver staining revealed approximately 500 spots (Fig. 1). A total of 190 well-separated and prominent spots were subjected to MS and finally 123 proteins were identified by PMF. The latter are indicated on the 2-DE protein reference map with an identification number (Fig. 1). The protein names and specific information on each of the proteins, whose function has been annotated in the database [8], are listed in Supplementary Table 1. The proteins have been classified into 14 groups based on Kyoto Encyclopedia of Genes and Genomes (KEGG) database [17]. Those proteins that could not be classified clearly were grouped together as miscellaneous. The majority of the proteins were involved in metabolic pathways (39.6%), especially carbohydrate and energy metabolism. A further group of 14 proteins coded by potential ORFs in the *L. plantarum* genome but without assigned function were also identified. Thus, on this initial 2-DE reference map, 25% of the proteins from 2-DE gel spots were identified and belonged to the *L. plantarum* WCFS1 genome, and by extrapolation this corresponds to 3.3% coverage of the genome. This percentage is in good accordance with previously published bacterial proteome maps that varied between 2 and 12% [18–20].

3.2 Proteome dynamics during *L. plantarum* growth

The proteomic approach was used to follow the changes of the *L. plantarum* proteome during growth and into stationary phase, offering a dynamic view of different processes during the growth of the bacterium. Protein extracts were prepared from cells in the mid- and late-log, and early- and late-stationary phases of growth. *L. plantarum* WCFS1 showed a diminished growth between the late-log and early-stationary phase (Fig. 2). It was previously reported that *L. plantarum* WCFS1 exhibits two consecutive growth phases in MRS medium, and the second phase with a lower doubling time occurs approximately between OD600 3.0 and the stationary phase [21]. Consequently, the late-log and early-stationary phases were also investigated on the protein level to gain more insight in this phenomenon. Representative 2-DE gels of the four different growth phases are presented in Fig. 3A–D. These maps show a moderate change in the proteome between the log and stationary phases. Detailed analysis of the images revealed the highest numbers of detected spots in the late-log and the early-stationary phases, 600 ± 73.8 and 597 ± 68.3, respectively. The gels of the early-log and late-stationary phases showed 532 ± 58.4 and 510 ± 30.4, respectively. Further analysis showed that 154 protein spots were at least twofold up- or down-regulated during growth.

A substantial number of proteins (approximately 400) present on the 2-DE gels did not show a significant change in relative abundance during growth. Based on the results of the initial proteome reference map, these proteins were mainly associated with translation, transcription, and carbohydrate metabolism, and presumably do not change in time as a constant level of activity is needed for cell survival at all times.

A total of 154 protein spots showed a differential regulation pattern (at least twofold up- or down-regulation) during the different growth conditions. Firstly, 41 of these differentially regulated proteins spots were identified using the initial proteome reference map, based on their pI and molecular weight (MW). Another 96 regulated unidentified protein spots was subjected to MALDI-TOF MS for identification. An additional set of 70 proteins in total could be identified which was a good efficiency for a silver-stained gel [22]. The (predicted) functions of all the identified proteins that showed difference in relative abundance during the various growth phases are listed in Supplementary Table 2. Based on their relative abundance patterns these proteins could be clustered into 12 different groups (Supplementary Table 2). Representative proteins of each group are illustrated in Fig. 4.

3.2.1 Soluble proteins produced from the mid-log to early-stationary phases

Proteins that differed in their relative abundance during growth could be assigned to groups that showed the same regulation pattern. Proteins belonging to groups 1, 2, 3, and
Figure 1. A 2-DE proteome initial reference map of L. plantarum WCFS1 grown in MRS medium at 37°C at cell density of OD600 8.0 with a pH interval between 3 and 10 (nonlinear). The identified proteins are indicated by numbers and the names are listed in Supplementary Table 1.

4 (Supplementary Table 2) showed a higher relative abundance during the log phase (and in some instances in the early-stationary phase), but showed a strong decrease in abundance during the late-stationary phase. The majority of these proteins were involved in (cellular) energy metabolism, translation of RNA to protein, as well as protein, lipid, and nucleotide biosynthesis. Proteins involved in energy generation, such as glucokinase and phosphoglycerate mutase, were significantly up-regulated (≥twofold) during the log phase. The putative transcriptional regulator lp_3416, also up-regulated, was located upstream of the gene coding for phosphoenolpyruvate carboxy kinase and was therefore also predicted to be involved in energy metabolism. As expected, the majority of the proteins that showed differences in relative abundance during the log phases were involved in the generation of sufficient energy for growth.

Throughout growth, the relative abundance of the antimicrobial plantaricin biosynthesis protein PInX’s (group 2; Supplementary Table 2) was high but decreased in the late-stationary phase. The production of plantaricins is controlled by a three-component regulatory system consisting of a membrane-associated histidine protein kinase, response
Figure 2. Growth curve of *L. plantarum* WCFS1 in MRS medium at 37°C. Protein samples were taken at the four different time points indicated by the arrows: OD<sub>600</sub> 0.5, 1.6, 4.7, and 8.0.

Figure 3. Silver-stained 2-DE gels of *L. plantarum* WCFS1 grown in MRS medium at 37°C at four different points during growth. Protein samples were taken at OD<sub>600</sub> 0.5 (A), 1.6 (B), 4.7 (C), and 8.0 (D). A sample area of spots having a difference in relative abundance has been enclosed in the box and is enlarged. The encircled spots were identified as cystathionine β-lyase and aspartate semialdehyde-dehydrogenase, respectively, with MALDI-TOF MS.
Figure 4. A representative of each group (1–10) with its specific differential twofold increased or decreased abundance pattern of protein spots during growth of *L. plantarum* WCSF1.

regulator, and a peptide pheromone with bacteriocin-like properties [23]. Maldonado et al. [24] demonstrated in *L. plantarum* strain NC8, an autoinducible mechanism, such that the pheromone or plantaricin controls its own regulation. The organizational structure of the gene cluster coding for plantaricins and the regulatory system of *L. plantarum* WCFS1 showed high similarity with the strains mentioned above. Our data suggested indeed that reduced abundance in the stationary phase led to decreased induction of the plantaricin.

A large number of the proteins of groups 3 and 4 (Supplementary Table 2) that showed a higher relative abundance during the log-phase and the early-stationary phase included amino acid metabolism and protein biosynthesis genes. Some proteins involved in cell division (lp_3199; lp_2193) and DNA replication and repair (lp_1976) were also induced during these growth phases. Notably, several enzymes showing a high relative abundance in the late-log phase were involved in lipid metabolism. ORFs lp_1680 and lp_1681 (group 4) code for enzymes belonging to the fatty acid biosynthesis pathway that is the main route for the formation of membrane phospholipid acyl chains in bacteria [25]. The ORF lp_3174, predicted to encode the enzyme cyclopropane-fatty-acyl-phospholipid (CFA) synthase showed a similar regulation pattern: greater abundance during mid- and late-log phase. This enzyme catalyzes the methylation of the unsaturated moieties of phospholipids which reside in the hydrophobic interior of the phospholipid bilayer. The physiological effect of this phenomenon is not completely understood. In *Escherichia coli*, this enzyme was shown to increase in concentration during the log to stationary phase transition, and was regulated by a double promoter and an unstable CFA synthase enzyme [26, 27]. This enzyme was also induced during acid stress in *L. lactis* and it was proposed that its activity was reflecting a general stress response [28].

Proteins of groups 5 and 6 (Supplementary Table 2) showed the highest relative abundance during the late-log or early-stationary phases. Among these, were proteins predicted to be involved in protein degradation (lp_1321) and folding (lp_2231/ppiB). In addition, proteins involved in cell division and DNA repair featured strong induction during the early-stationary phase. In contrast to the mid- and late-log phases (groups 1, 2, and 4) described above, proteins involved in energy metabolic pathways showed reduced relative abundance during the early-stationary phase.

In conclusion, the most important reaction occurring during the log phase was generating sufficient energy, while in the late-log phase the energy metabolic pathways decreased and the biosynthesis of proteins and cell division became more important. Thus, the metabolism of the cells had shifted from energy generation pathways in the log phases to synthesis of macromolecules in the stationary phase. Furthermore, reinforcement of the cell membrane appeared to occur during the late-log to early-stationary phase based on induction of enzymes involved in fatty acid biosynthesis, needed for the formation of phospholipids.

3.2.2 Proteome changes during the early- and late-stationary phase

The proteins described to be abundant or highly abundant in the mid- and late-log phases above were at very low levels in the stationary phase, indicating that growth arrest had occurred. Proteins of groups 8 and 9 (Supplementary Table 2) essentially increased in relative abundance throughout the log phase and showed highest abundance in the early- and late-stationary phases. Proteins in group 10 (Supplementary Table 2) were highly abundant in the late-stationary phase alone. These groups included a number of proteins, transporters, and enzymes predicted to be involved in the uptake (mannose/glucose phosphotransferase system (PTS) EIIAB; lp_0575) and conversion of the carbohydrates mannose (lp_2384), mannitol, and the amino sugar glucosamine.
glucosamine-6-phosphate isomerase) into fructose-6-phosphate. Thus, all these sugars might be used as a carbon source for cellular physiology. In the log phase, fructose-6-phosphate was formed by the action of the enzyme glucokinase (group 1) that converted glucose from the MRS growth medium into the glucose-6-phosphate intermediate of the glycolytic pathway; this enzyme was repressed in the late-stationary phase. The utilization of mannose, mannitol, and glucosamine in the early and late-stationary phases suggested that _L. plantarum_ was switching from the use of glucose as a carbon source to alternative sources and pathways, which could be explained by the decreasing glucose content of the medium at the later growth or stationary phases.

Protein levels for substrate specific proteins (EIAB; _lp_0575) belonging to the mannose/glucose PTS complex increased substantially in the stationary phase compared to the log phase (group 9 and 10; Supplementary Table 2). Interestingly, two different isoforms of the latter presumably due to PTM were identified at two different locations on the gels (spots no. 7503 and 7507; Figs. 3A–D). The exact modification is unclear though it is plausible that phosphorylation of the initial molecule has occurred. The addition of a phosphate group is likely, because the enzyme IIAB components belonging to the PTS phosphorylate the sugar substrate during transport into the cell [29].

PTS transport systems are energetically efficient as the phosphorylated substrate can directly enter glycolysis saving ATP in contrast to ABC transporters which require an extra ATP. The former is advantageous under limiting energy conditions such as in the stationary phase of growth [30]. In accordance, a putative amino acid ABC transporter (_lp_2823) was significantly reduced in abundance during the late-stationary phase (group 3; spot no. 7402).

A response regulator (_rrp11; _lp_3191) showed a high relative abundance only in the stationary phase. The _rrp11_ gene is located together with a histidine kinase gene (_hpk11_), upstream of the _dacA1_ gene that encodes a penicillin-binding protein (PBP) with DD-carboxypeptidase activity. It is likely that _rrp11_ and _hpk11_ are members of a two-component regulatory signal transduction system involved in the regulation of the downstream _dacA1_ gene [10]. This enzyme has been demonstrated to modulate the peptidoglycan cross-linking of the cell wall in _E. coli_ [31, 32], and the DD-carboxypeptidases were shown to be involved in the maintenance of cell shape [33]. The regulation of PBP-proteins in lactobacilli is unclear [34]. In this study, the induction of this putative system suggests that structural cell wall changes may be occurring during late-stationary phase in order to reinforce the cell wall for protective purposes.

Galactokinase and UDP-glucose 4-epimerase were increased in relative abundance during the late-log phase and stationary phase (group 9). Both enzymes belong to the Leloir pathway [35]; galactokinase is involved in the formation of galactose to glucose-1P, and UDP-glucose 4-epimerase is responsible for the reversible conversion of UDP-glucose to UDP-galactose. Both UDP-sugars are precursors for the synthesis of exopolysaccharides (EPS) and other cell-surface associated sugars [36–38]. However, _L. plantarum_ WCFS1 lacks the eps genes for the regulation, polymerization, and exporting of the EPS [39]. Interestingly, a disruption of _Streptococcus thermophilus_ _php26_ gene coding for a PBP-protein blocked the EPS production of this lactic acid bacteria (LAB) [40]. Stingele and Mollet [40] suggested that peptidoglycan and EPS synthesis are interconnected pathways; the conversion of glucose-1P to dTDP-rhamnose can be performed by the enzymes coded by the genes _rfbABCD_. It was shown that the lactobacilli species possess rhamnose structures in cell wall polysaccharides [40, 41]. Thus, the increased abundance of both the putative regulator of the _dacA1_ gene described above and the _gal_ genes in the stationary phase might be linked to each other and play a role in strengthening the cell wall.

The pyruvate oxidase enzyme (_poxB_ gene; _lp_0852; group 10) was mainly induced in the late-stationary phase. This enzyme is responsible for the conversion of pyruvate into acetyl phosphate which in turn is metabolized to acetate and excreted into the medium. It was shown by Northern blotting that in _L. plantarum_ _Lp80_ the _poxB_ gene was down regulated in the presence of glucose, and during the early stage of the stationary phase _poxB_ expression was maximal [42]. In our study, during the exponential phase, the abundance of pyruvate oxidase is decreased and is increased in the stationary phase. The latter can be explained by the coupling between acetate and ATP production [42]; production of acetate is accompanied with increased ATP production which appears to be required during the stationary phase.

A number of proteins implicated in a stress response were induced in the stationary phase, including the stress-induced DNA binding protein (_lp_3128), heat shock protein (GrpE; _lp_2028), catalase, and endopeptidase ClpP at two different spots ( _lp_0786; spot 2304 and 4408). The latter protein localized at spot number 2304 is probably a monomer (observed MW 20 kDa) and the other spot might be a trimer of the ClpAP complex; alternatively, it may represent ClpP in complex with another molecule, generating the observed MW of 33 kDa [43, 44].

Both the diminishing nutrients and high concentration of lactic acid in the medium would cause stress especially in the late-stationary phase. The stress-induced DNA binding protein has a conserved DPS (DNA protecting protein under Starved conditions) domain. DPS is a protein that was initially found in _E. coli_ to crystallize in starved cells to protect DNA against oxidative stress [45]. Crossprotection between the responses to starvation and oxidative stress has been described for various bacteria [46, 47]. As a result of this crossprotection against starvation, also catalase was found to be increased during the stationary phase, which would facilitate the tolerance of _L. plantarum_ WCFS1 to possible oxidative stress and reducing lipid oxidation [48]. In support of the latter, up-regulation of catalase in _L. plantarum_ WCFS1 in response to lactic acid stress was demonstrated by a transcriptomic approach [11].
In conclusion, during the early and late-stationary phases, the use of glucose as the main carbon source and energy generation via glycolysis was replaced by alternative pathways for carbohydrate metabolism such as switching from glycolysis to the Leloir pathway for yielding energy. In the late-stationary phase, many stress proteins were strongly induced due to the unfavorable conditions such as high acid and diminished nutrients. Enzymes involved in the synthesis of cell wall structures were mainly in greater abundance during the late-stationary phase, presumably to strengthen the cell wall and to maintain the bacterial morphology.

4 Concluding remarks

A database of intracellular soluble proteins of *L. plantarum* WCFS1 has been established using a proteomic approach. A reference proteome map was generated using data from the stationary phase of *L. plantarum*. The dynamic proteome map was established with data from growth phases namely, mid-log, late-log, early-, and late-stationary phases of growth, including time points representing the two consecutive growth phases of this strain in MRS. Overall, this provided a detailed insight into metabolic pathways and their regulation in time during growth of *L. plantarum* WCFS1 under controlled conditions.

The most important reactions occurring during the log phase were metabolic pathways for generating sufficient energy. Interestingly, in the late-log phase and early-stationary phase, the period of reduced growth rate on MRS, more protein spots were present on the 2-DE gels compared to the mid-log and late-stationary phases. Thus, the highest rate of biosynthesis of proteins was during the late-log and early-stationary phase and this may play a role in the overall lower growth rate during this period. In the late-log phase, the biosynthesis of proteins and cell division pathways, i.e. synthesis of macromolecules, increased. Furthermore, strengthening of the cell membrane appeared to occur during the late-log to early-stationary phase, based on greater abundance of enzymes involved in fatty acid biosynthesis, needed for the formation of phospholipids. During the early- and late-stationary phases, the lower levels of glucose in the medium provoked alternative pathways for carbohydrate metabolism as the main carbon source for energy generation. Proteins predicted to be involved in cell wall structures such as UDP-sugars and PBp-protein were increased in relative abundance at the beginning of the early-stationary phase at the cost of the glucose-1 phosphate pool, which would result in less availability of glucose-1 phosphate for glycolysis. Overall, the energy required for induction of alternative pathways, and alterations of the cell wall, possibly in preparation for the stationary phase, may cause reduced growth rate around late-log to early-stationary phases. It was previously reported that diminished growth prior to the stationary phase coincided with alteration of the permeability of the cell envelope [21].

In the late-stationary phase, many stress proteins were strongly induced due to the harsher conditions such as high acid and reduced nutrient levels. Enzymes involved in the synthesis of cell wall structures showed mainly a higher relative abundance during the late-stationary phase, presumably to strengthen the cell wall and to maintain bacterial morphology at low pH and high lactate conditions.

The 2-DE reference map and the dynamics of the proteome of *L. plantarum* can facilitate further studies and information for industrial applications about the activity and metabolic processes of the cells under various conditions, such as those in food preparations and passage of the intestinal tract.

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5 References


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