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Lactic acid bacteria and proteomics: current knowledge and perspectives

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Abstract

Lactic acid bacteria (LAB) are widely used in the agro-food industry. Some of the LAB also participate in the natural flora in humans and animals. We review here proteomic studies concerning LAB. Two methods of research can be distinguished. In the first one, a systematic mapping of proteins is attempted, which will be useful for taxonomy and to function assignment of proteins. The second one focuses particularly on proteins whose synthesis is induced by various environmental situations or stresses. However, both approaches are complementary and will give new insights for the use of bacteria in industry, in human health and in the struggle against bacterial pathogens. Interest in LAB is growing, showing thus an increasing concern of their rational use and one can foresee in the near future an increasing use of proteomics as well as genomics.

Keywords: Proteomics; Lactic acid bacteria

1. Introduction

The lactic acid bacteria (LAB) emerged around 3 billion years ago, probably before the photosynthetic cyanobacteria. Their expansion has really began with the apparition of milk producing mammals, over 65 million years ago. However, the first registered usage comes from the discovery of small vases punched by small holes, near the Neufchatel Lake, over 3000 years BC. Since these days, humans are able to control milk curdling. Rapidly, these bacteria were used and contributed to produce various fermented food products like cheese, bread, fish, meat, etc. [1].

Nowadays, well defined, controlled starter cultures of lactic acid bacteria are of great importance in the agro-food industry. Strains have long been maintained in food products and have adapted to survival in that environment. With the establishment of the food industry, the use of selected and defined cultures became practice. Strains have been chosen for their survival, resistance against bacteriophages, bacteriocin production, impact on the taste of products and rapid acidification which was, in ancient times, probably the most important role of lactic acid since it inhibits pathogen growth, thus avoiding food spoilage.

With the expansion of their use in industry, it was

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necessary to acquire information for rational typing and selection of bacteria. During the 1980s with the use of molecular genetic techniques, the genus *Streptococcus* was separated into the three genera *Enterococcus*, *Lactococcus* and *Streptococcus*. Now, the lactic acid bacteria associated with food include species of the genera *Carnobacterium*, *Enterococcus*, *Leuconoctoc*, *Oenococcus*, *Pediococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* and the most used in the milk industry, *Lactobacillus*, *Lactococcus* and *Streptococcus* [2]. At first, classification was based on chromosome GC content. A more accurate classification was subsequently obtained on the basis of the analysis of ADNr 16S [1]. However, these classifications are of use for taxonomy but of little help for the routine use of LAB in industry. Practically, the use of a given strain or a combination of strains to obtain suited results largely remains an empirical process based on the know-how of each manufacturer and its strain collection. Lactic acid bacteria, in particular the genera *Streptococcus* and *Lactobacillus*, are implicated in health concerns, either as beneficial micro-organisms for health, probiotics (for example, see Ref. [3]) or in contrast as pathogens [4] but to our knowledge, no proteomic study has been performed on these particular bacteria.

Proteomics is a tool initially developed on the basis of two-dimensional electrophoresis (2-DE) [5]. Several recent improvements, like immobilised pH gradients, new accurate and simple mass spectrometers, large amounts of information available in databases, renewed the interest toward this technique which is able to separate several hundreds of proteins and to reveal their relative abundance. While the genome sequence is a static view of an organism, proteomics and transcriptomics introduce a quantitative and dynamic vision of the RNA and protein variations throughout cell life, respectively. However, it has been shown that the expression level of an mRNA did not preclude of the amount of a protein it encodes [6] and proteomics has some limitations [7]. However, when developed in conjunction, they seem, currently, the methods of choice to investigate cell life. In the world of LAB, only a few genomes were sequenced and their recent release did not yet allow the development of DNA chips. Therefore, most of the studies we review here concern proteomic analysis using 2-DE.

In the case of micro-organisms, two main approaches using proteomics can be envisaged [8,9]. The first one is to establish a systematic cartography of a bacteria in a given state. Growth conditions should be perfectly monitored to ensure reproducibility of the revealed patterns. All the proteins are then identified by powerful mass spectrometry measurements and research of databases. These studies, are per se, important because they give information on the relative distribution and abundance of proteins and thus, patterns of several strains can be compared. Function of proteins that were not assigned by genome annotation can be investigated. Combined with already known information obtained during an industrial process, one can envisage a prediction of the technological properties of a new strain of potential industrial interest. Finally, the genera classification can be modulated by introducing data on protein synthesis. It should be noted however that this approach is fastidious by defining what are ''reference and reproducible conditions''. The second one is a differential approach. Basically, it consists of comparing protein patterns of a given strain, submitted to different environmental conditions. This approach allows one to study the effects of various stress, drugs, culture conditions, etc.; differentially expressed proteins are then identified. This second approach is probably the most straightforward and most used approach today in the world for various types of cells or biological fluids.

In this review, we will describe both aspects listed above. The most advanced proteomic studies were performed on the genera *Enterococci*, *Lactobacilli* and *Lactococci*. We will detail the different approaches chosen, depending on a great part on the interest of industry and the problems encountered during the fabrication processes or the starter conservation. Perspectives offered by proteomics in the various fields of lactic acid bacteria study will be foresighted, especially for biomedical applications where no proteomic study has been reported, to our knowledge.

2. Two-dimensional (2D) reference map of lactic acid bacteria

The proteome was defined as ''the total protein complement of a genome'' [10]. Thus, one of the

goals of proteomics was to reveal and identify the maximum of proteins on a single 2D gel. However, it rapidly became clear that this goal was unrealistic. For example, *Mycoplasma genitalium* is an organism with the smaller known genome (580 kbases), which has the potential to express only 480 genes. In a very complete study [11], it was possible to detect up to 427 of the expected proteins, using 2-DE with four overlapping pH windows. Only 158 of these proteins were identified using mass spectrometry data and at this date, it can be considered as the most complete proteome analysed.

The situation is far more complex for organisms with a higher number of proteins. Because a 2D gel reveals the proteins expressed in a given condition but is restricted by the well known limitations of the technique, it is unlikely that any 2-DE analysis will fulfil the non-restrictive definition of the proteome.

However, the proteomic studies being essentially based on a comparative approach, the building of the most complete and reproducible reference gels is a prerequisite. Nevertheless, it must be emphasised that, for a given organism, a global view of the protein synthesis capacity will necessitate several distinct experiments, and many reference gels.

In the recent past, the development of a reference map consisted of an accurate indexation of spots, together with identification of proteins by N-terminal sequencing. Such a study was performed for *Streptococcus thermophilus* grown in M17-lactose medium [12]. We describe here the procedure followed because it is particularly interesting and can be used as an example for other studies. Three independent analyses were performed: (1) the silver stained gels revealed an average of 250 spots distributed between pH 4 to 7. (2) Whole cell soluble proteins were fractionated in parallel using ammonium sulfate. The protein fraction corresponding to the precipitation by 100% saturation was applied on a phosphocellulose column. (3) Fractions corresponding to major peaks of absorbance were analysed by one-dimensional electrophoresis (1-DE). A part of these fractions containing almost pure proteins were submitted to 2-DE and localised on the proteomic map, becoming internal markers for all future 2-DE. Another part was submitted to 1-DE, electroblotted and proteins were submitted to Edman degradation for identification.

Thus, the combination of chromatographic and electrophoretic techniques allowed one to identify proteins and to obtain the internal markers necessary to check the reproducibility of 2-DE and to allow comparison with profiles of other strains.

The ''marker'' proteins were identified and among 12 proteins, four were enzymes involved in carbon metabolism and four were stress proteins. A comparison of proteomic maps of two strains of *Streptococcus thermophilus*, following a metabolic labelling was undertaken. Several proteins were found to be common to both strains and one was specific for one strain. This work will benefit future studies, like the comparison of the differentially expressed proteins of *S. thermophilus* grown in MRS versus milk, which are currently under progress (C. Guimont, personal communication). The reference gel will allow one to visualise and compare the synthesis of proteins involved in the adaptation to a far less favourable medium.

More recently, a reference gel of *Lactococcus lactis* NCDO763 grown in a chemically defined medium was published by two of us (P.A. and J.-F.C.); 450 silver stained spots were detected on 2-DE after focalisation on a pH 4–7 gradient [13]. Fifteen proteins were identified by peptide mass fingerprinting followed by querying the general protein databases. Two peptidases which play an essential role in the nitrogen nutrition of this demanding bacteria were identified by this method. The recent publication of the genome sequence of *L. lactis* IL1403 [14] offers the possibility to systematically identify proteins resolved on 2D gels. Thanks to the availability of annotated genome of this bacteria, we have analysed the repartition of the proteins it encodes (Fig. 1). It can be seen that proteins from *L. lactis* are clearly distributed in two groups, an acidic which is seen by 2-DE and a basic one which is under-represented on pH 3–10 gradients (data not shown). It is also noticeable that high and low MM proteins are not seen (comparison between Fig. 1A and Fig. 2A). We are facing here the well-known limitations of 2-DE and a combination of improved electrophoretic [15] and chromatographic techniques will be necessary to detect all 2310 proteins predicted by the genome sequence.

For the purpose of this review, 2-DE gels of four lactic acid bacteria strains grown in the same rich synthetic MRS medium were performed (Fig. 2). The gels show some similarities: a dozen of spots are

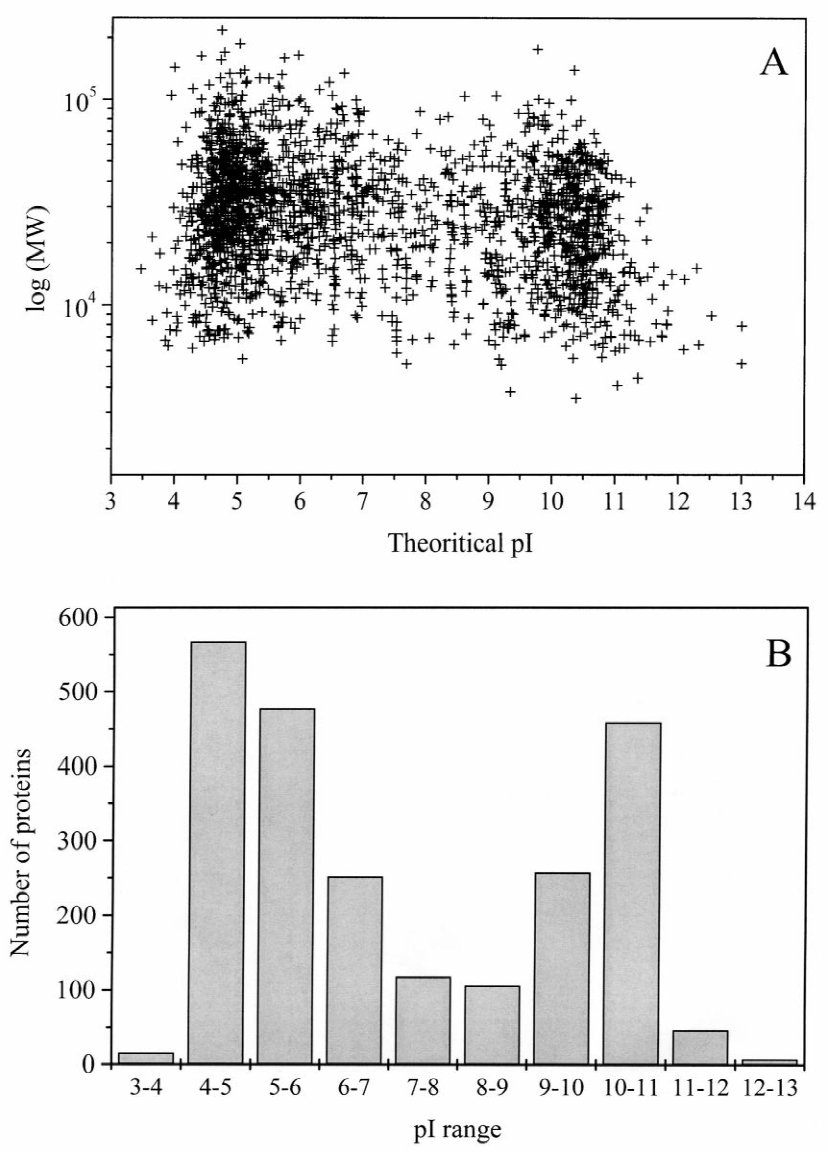

Fig. 1. Two representations of the theoretical proteome of *Lactococcus lactis* IL1403 computed from the data in Ref. [14]. (A) Graphical view of theoretical proteome by plotting MM vs. p*I*. The relative abundance of proteins is not taken into account. (B) Number of proteins in each p*I* range: 1297 proteins are located between pH 4 and 7, compared to the 450 viewed by experimental 2D gels (Fig. 2A) and 942 proteins are located between pH 7–11, a zone generally under represented in 2D gels.

probably glycolytic enzymes and represent almost 20% of the total protein detected on this acidic proteomic map. It is also striking that the number of proteins expressed in the rich broth medium is similar between the four LAB species having a similar small genome size (1.8–2.3 Mbases). An overcrowded area between pH 4.5 and 5.5 is also noticeable. This observation which is not particular to LAB, will impose the use of narrow pH ranges to resolve this region. The systematic identification of

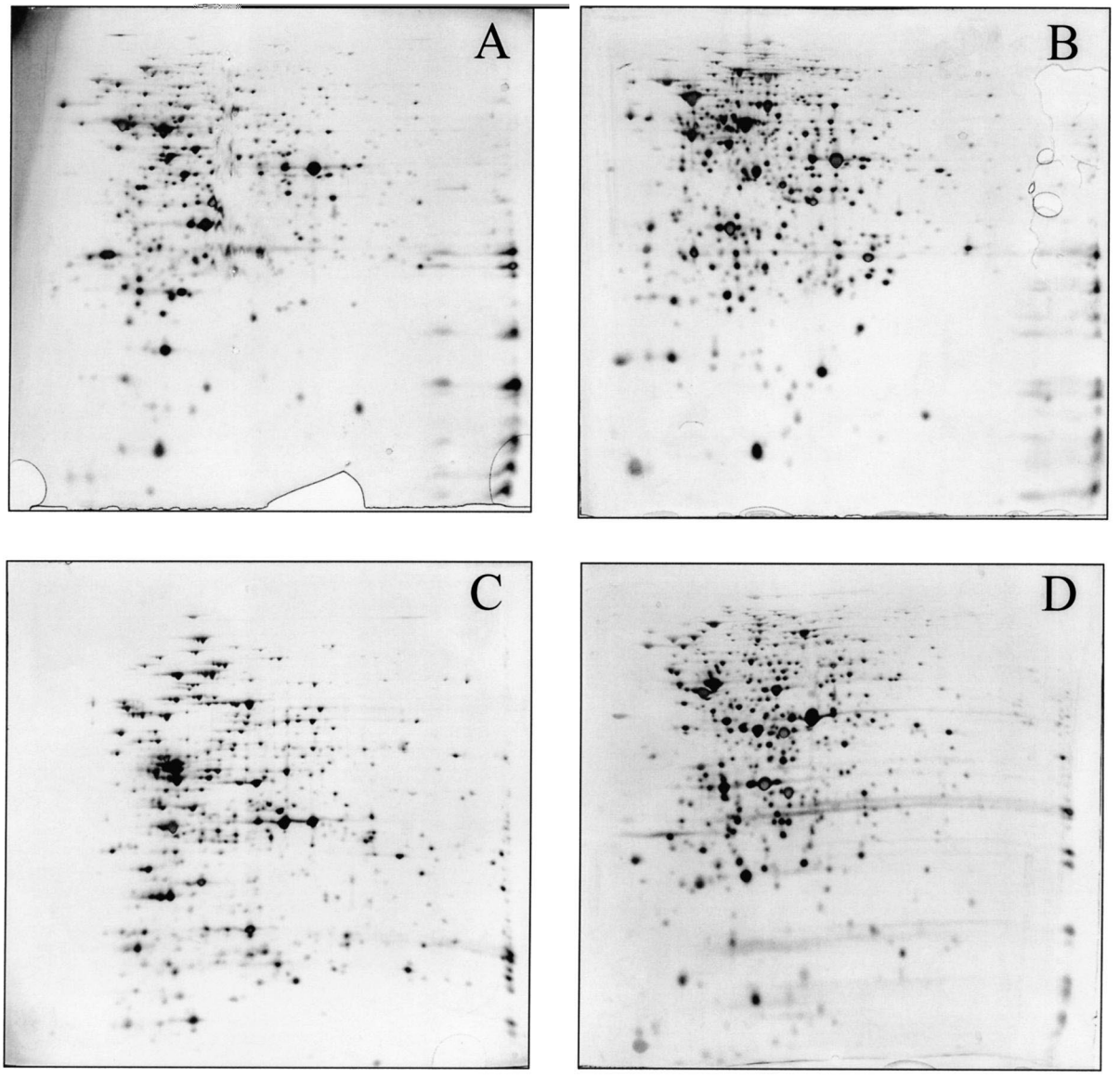

Fig. 2. 2-DE silver stained protein patterns of four lactic acid bacteria: *Lactococcus lactis* MG1363 (A), *Streptococcus thermophilus* (B), *Lactobacillus delbrueckii* subsp. *bulgaricus* ATCC11842 (C), *Lactobacillus sakei* (D). Cells were grown in MRS medium and harvested in late exponential phase. A 50-μg amount of a soluble protein extract was resolved on pH 4–7 gradient for the first dimension, and separated by 12.5% SDS–PAGE in the second dimension. These gels are shown only for pattern comparison; no systematic protein identification was performed. Some proteins were previously identified in Refs. [13,38].

the proteins should reveal additional common points between these fermentative bacteria. Additionally, the differences should provide some clues to the understanding of the metabolic adaptation to milk or meat medium. Because the genome sequencing programs for the four LAB are in their final phase it is likely that such a task will be feasible in the coming months.

A question remains open: will the reference maps obtained on one particular strain of a genera be relevant for the study or functional characterisation of the other strains? This point is particularly crucial in the case of these economically important bacteria where the phenotypic diversity, that is likely to be reflected at the proteome level, is well known and used by the industrials. The same kind of observation can be made concerning bacteria affecting health. The development of proteomic databases should keep in mind variability and diversity and this will represent a major scientific challenge.

We have described above the construction of proteomic maps and their use as ''protein catalogues''. We will focus now on applications of proteomics to LAB. The most intensive use of proteomics probably concerns stresses, due to particular uses of these bacteria in human activities.

3. Analysis of stress responses in LAB by a proteomic approach

Proteins whose synthesis is affected in response to environmental stresses can be clustered in few groups:

—General stress proteins: the most studied in all kind of stress and probably all kind of bacteria. They are generally induced non-specifically by several stresses and are involved in DNA or protein repair (chaperons DnaK GroEL, GroES, or proteases such as Clp proteases).

—Specific stress proteins for a given stress which we will focus on.

—Proteins of general metabolism which can be affected by some specific stresses (for example, the proteins of the glycolytic pathway).

In the past, most proteomic studies in LAB were mainly descriptive in relation to number of induced or repressed proteins by stresses or adaptation but identification of these proteins was often lacking. Quantitative data were obtained in different ways (^{35}S labelling or protein silver staining, etc.) but led to difficulties when undertaking a fine comparative analysis and in obtaining solid conclusions.

Increasing knowledge on genome sequences in LAB led to a better identification of proteins together with genetic analysis of encoding genes, transcript analysis and mutant construction in some cases. Only a few species are concerned by these studies: *Lactococcus lactis*, some *Lactobacillus* strains (*L. sakei, L. bulgaricus*), *Enterococcus faecalis*, *Oenococcus oeni*, *Streptococcus mutans*, *S. thermophilus*, *S. oralis*.

Like all bacteria, LAB can be affected by various stresses but due to their use in industrial processes or as probiotics passing the gastrointestinal tract, some of them are particularly studied in order to understand and to improve their responses. Thus, variations in temperature, presence of NaCl, oxidative shock, acidic environment, nutrient limitation are situations to which LAB are routinely submitted and where the proteomic approach can be of invaluable help. The results presented below are summarised in Table 1.

3.1. Heat shock

The lactic acid bacteria genera includes mesophilic (*L. lactis*, *L. casei*, etc.) as well as thermophilic (*L. delbrueckii*, *L. helveticus*, etc.) bacteria which may encounter during industrial processes restrictive heat conditions.

It is now well established that the heat shock (HS) response phenomenon is universal among prokaryotes and eukaryotes. HS destabilises non covalent interaction and leads to protein denaturation. The major aspect of the HS response is the induction of a set of chaperons and proteases which both act at the refolding or elimination of damaged proteins. The HS response has been studied in numerous bacteria and well characterised in *E. coli* and *B. subtilis*, the Gram negative and Gram positive bacterial models. Therefore, the investigation of the LAB heat shock responses could constitute a basis for comparative studies.

It was established in the first place, that several LAB had heat inducible response, 2-DE analysis then revealed a number of heat inducible proteins (heat shock proteins, Hsps) and immunodetection allowed the identification of a few of the well conserved chaperons (Table 1). More insights on the *L. lactis* HS response were obtained from studies focused on *dnaK* [16], *clpB, clpC, clpE, clpP* [17,18] and *ctsR* [19] mutants.

The combined use of genetic, mRNA and 2-DE

Table 1
Summary of the results on stress induced proteins obtained by proteomics

analysis allowed one to establish the regulatory role of two Hsps in *L. lactis*. The study of a *dnaK* mutant showed that at 30 °C (optimal growth temperature) several Hsps (HrcA, GroES, GroEL, DnaK, Hsp84, Hsp85 and Hsp100) were over synthesised in the mutant compared to the wild type strain. Thi observation strongly suggested that DnaK is normally involved in the repression of *hsp* genes at 30 °C. It also exemplified that transcriptomic and proteomic approaches are complementary since these techniques allowed to monitor the expression or synthesis of different elements of the HS response.

In *L. lactis*, the role of the CtsR repressor was also established using a combined approach. Comparisons of 30 °C *ctsR* or wild type cultures using 2-DE analysis revealed only two Hsps (ClpP and ClpE) which were accumulated to higher level in the *ctsR* mutant whereas, mRNA analysis showed increased expression of all the *clp* genes (*clpP*, *clpE*, *clpB*, *clpC*) in the mutant. Although ClpC seemed to be expressed in too low amounts to be visualised on 2-DE, the discrepancy between mRNA and protein monitoring was not explained for the *clpB* gene products. In that example, the mRNA analysis led to more information than 2-DE analysis and allowed to include the four *clp* genes in the CtsR controlled regulon. However, the biological effect of the CtsR inactivation may be better reflected by 2-DE analysis which revealed the disappearance of ClpB instead of its accumulation. It is also noteworthy that previous proteomic study showed that the ClpB protein had two isoforms [17] which would not have been detected without 2-DE analysis.

3.2. Cold shock

Lactic acid bacteria play an important role in the food industry because of their widespread application as starter cultures in many fermentation processes. Low temperature adaptation is highly relevant from a practical point of view since many LAB fermentations are initiated by the addition of frozen starters cultures that should benefit from a high freeze survival capacity. Moreover, LAB are also exposed to cold stress during low temperature fermentation and storage of fermented products prior consumption. Cold shock alters (i) the usual crystalline nature of the membrane and changes it to a gel phase state, (ii) the DNA supercoiling and (iii) the stability of at least few mRNAs which encode proteins involved in the cold shock response [20]. It was reported that the cytoplasmic membrane, the nucleic acids and the ribosomes are implicated in sensing the temperature in bacteria.

It was shown that cold pretreatment can improve *L. lactis* [21,22], *S. thermophilus* [23] and *Enterococcus faecalis* [24] survival to freezing or freeze–thaw cycles.

Using a proteomic approach, the rapid induction of specific sets of protein upon cold shock was observed in a variety of bacteria. Among LAB, cold shock responses of *L. lactis*, *S. thermophilus* and *L. plantarum* have been studied at the genetic level. The 2-DE approach was used with *L. lactis* and *S. thermophilus* and revealed, respectively, 22 and 24 of so-called cold induced proteins (CIPs). Among the *L. lactis* CIP, Wouters and co-workers [25,26] identified proteins involved in translation process, sugar metabolism, chromosome structuring and signal transduction. The low molecular mass (about 7 kDa) CIP proteins are often distinguished from the others since they putatively belong to the cold shock protein (Csp) family. Some of these Csps appeared to have a major role in the cold shock survival and regulation of CIPs in other bacteria than LAB [20]. The number of Csps appeared to vary upon LAB strains since one and six Csp proteins were revealed in *S. thermophilus* PB18 [27] or CNRZ302 [23], respectively. Similarly for the *L. lactis* species, the complete genome sequence of IL1403, a *L. lactis* subsp. *lactis* strain revealed only two *csp* genes [14] although genetic and 2-DE analysis revealed seven Csps in *L. lactis* subsp. *cremoris* strain MG1363 [28]. It is noteworthy that for the *L. lactis* subsp. *cremoris* strain MG1363, the use of degenerated-polymerase chain reaction amplification allowed the isolation of only five *csp* genes [29]. The two other proteins which were named CspF [30] and CspG [28] were only visualised via 2-DE analysis. This data suggests strongly that MG1363 has two different families of Csp encoding genes.

It is obvious from these data that 2-DE is an efficient method to determine and characterise the protein modifications correlated with an increased stress resistance. However, the absence of genome sequence remains a limiting factor for the identifica-

tion of the CIPs in several LAB and therefore hampers the understanding of that physiological phenomenon.

3.3. Acid shock

The major characteristic of LAB is to ferment sugars in lactic acid. This LAB ability is the basis of their industrial use for food fermentation. The ability of LAB to withstand acidic environment may also favour the survival of probiotic strains in the digestive tract, during the transit into the stomach. However, it is also the basis of the involvement of some LAB species in the development of dental caries. As a consequence the acid stress responses of the neutrophilic LAB have been studied in order to improve (i) the use of LAB in industrial applications and (ii) the understanding of the initiation and caries progression.

For neutrophilic bacteria, the decrease of internal pH resulting in a lower external pH has multiple effects on the cellular metabolism. Acidification leads to dissipation of the proton motive force which constitutes an energy source for the bacteria. It also alters the enzymatic activities, may denature proteins and damages nucleic acids [31]. Furthermore, acidification by lactic acid results in intracellular accumulation of lactate which has negative effect on the bacterial physiology.

2-DE studies of the acid adaptive responses of several LAB revealed the induction of a large number of proteins. The acid response induced 33 proteins for *L. lactis* [32], 64 in *S. mutans* [33], 29 in *S. oralis* [34] and 9 or 10 proteins with *S. thermophilus* during exponential growth or stationary phase, respectively [35]. Moderate acidity resulted in the accumulation of 32 proteins with *E. faecalis* [36], 15 proteins in *Lactobacillus sanfranciscensis* [37], more than 30 proteins in *Lactobacillus bulgaricus* [38] and with *L. acidophilus*, nine acid response proteins were expressed in stationary phase. Except in the most recent study concerning *S. oralis*, only a limited number of proteins were identified due to the lack of genome sequence data. Therefore, despite several 2-DE studies, major contributions to the understanding of LAB adaptation mechanisms so far result of genetic or biochemical studies.

For all the LAB studied, 2-DE confirmed the induction of at least some of the subunits of the F_oF_1 ATPase (also called H^+-ATPase) which expells protons from the bacteria at low pH. The 2-DE studies also revealed that some heat shock proteins (mostly chaperons) are induced during acid adaptation, however, the identity of the induced chaperon(s) varied from a species to another. GroES and GroEL were induced in *L. lactis*, GroEL and DnaK were accumulated in *E. faecalis* and in *L. bulgaricus*, all three (GroES, GroEL and DnaK) were over synthesised. In contrast, in *L. sanfranciscensis*, GroES, DnaK and DnaJ amounts remained unaltered by acidity whereas GrpE increases. As mentioned above, acid induced proteins of *S. oralis* were analysed by peptide mass fingerprint and tentatively identified using the *S. pneumoniae* genome information. The results established that acid adaptation had a major impact on the glycolytic pathway and might redirect the carbon flux. It also confirmed that the synthesis of proteins from a number of functional categories were modulated at low pH. However, the acid-specific stress response proteins remained to be determined since some of the induced proteins may be nonspecific.

3.4. Salt stress

Micro-organisms generally response to salt stress by accumulating osmoprotective substances such as proline, glycine betaïne or glutamate. Following an hyperosmotic shock, potassium is first accumulated and then exchanged by osmoprotectants. Potassium transport systems and proteins involved in osmoprotectant accumulation systems have their synthesis induced in case of salt stress.

The use of 2-DE to analyse the response to salt stress in LAB led to major insight in NaCl shock regulation. It has been shown that response to salt stress overlaps with heat stress in *L. lactis* [39]. The general stress proteins DnaK, GroEL, GroES have been identified in 2D gels. Nevertheless, no specific NaCl-induced protein has been detected in *L. lactis*. In fact, it was shown in other bacteria that transporters which are induced by the presence of NaCl are integral membrane proteins which are not easily solubilised or detected on 2D gels.

In *Enterococcus faecalis,* 13 proteins are induced by salt stress (0.5 *M* NaCl). Eleven of these proteins

are no longer induced when cells are grown in the presence of glycine betaïne [40]. This data confirms the physiological role of osmoprotectants. It has also been shown that salt stress induces cross tolerance to lethal treatments [41] with bile salt, SDS, ethanol or hydrogen peroxide.

3.5. Bile salts, SDS stress

The effects of these detergents were studied in *E. faecalis* [36,42]. The synthesis of 45 proteins was induced after treatment by bile salts whereas the SDS treatment induced 34 proteins. Twelve of these proteins were common to both responses. Nevertheless, these stresses seemed to lead to different responses in *E. faecalis* with the involvement of different proteins. In fact, synthesis of DnaK and GroEL is strongly induced by bile salts while it is not affected by SDS treatment.

3.6. Starvation

In their natural environment bacteria are often submitted to growth-restricting conditions. Their response to such unfavourable condition generally leads to the expression of a set of genes under the control of alternative sigma factors [43]. Nevertheless, the exact counterpart of these factors were not found in the genome of the LAB species which have been sequenced. In fact, it should be mentioned that when cells enter the stationary phase, several factors affect their development: carbon source starvation, pH (this is specially important in the case of LAB), oxygen availability. They are thus submitted to several stresses at the same time and it becomes particularly difficult to determine the nature of the inducing stimulus for each protein.

However, the proteomic approach was thus successfully undertaken to obtain information on the response to starvation.

For several LAB, 2-DE established that starvation induced the synthesis of specific proteins. In *L. lactis* two different studies have dealt with glucose starvation. They report, respectively, the induction of 14 and 21 proteins [44,45].

In *S. thermophilus*, 10 proteins are over-synthesised in the stationary phase whereas nine are repressed [35]. In *Lactobacillus acidophilus*, 16 proteins are induced in stationary phase. It was shown that seven of these proteins are induced by the stationary phase itself whilst the induction of the nine other rather resulted from low pH at the end of the culture [46]. In *Streptococcus mutans*, 58 proteins are induced by starvation, 11 of them being specifically induced by the starvation itself; 20 proteins have their synthesis reduced at the onset of starvation [33].

Forty two proteins are induced by glucose starvation in *E. faecalis*. Surprisingly, some are common with the response to a cadmium chloride treatment [47]. Glucose starvation also induces multi resistance in *E. faecalis* against heat treatment, hydrogen peroxide, acid or ethanol but not against UV irradiation [48].

One of these glucose-starvation-induced proteins was identified as a carbamate kinase. This protein belongs to the *arc* operon involved in arginine degradation by the arginine deiminase pathway. This metabolic pathway leads to ATP production and is known to be regulated by catabolite repression [49]. It is assumed that this pathway is an alternative route for providing energy under glucose starvation conditions.

Another glucose starvation induced protein was also induced by several stresses. It was identified as a general stress protein (named Gls24). Due to the availability of the complete genome sequence of *E. faecalis*, the corresponding gene and its surrounding region were analysed. Gls24 belongs to an operon containing six ORFs. The *gls24* gene is followed by an ORF (named *orf4*) showing strong similarity with *gls24*. It seems thus that this gene has been duplicated. Nevertheless, it is probable that these two paralogues in the same operon are devoted to different physiological roles. Indeed, a mutant in *gls24* is severely affected in growth, cell shape and is less resistant to bile salts but has an unmodified resistance to other stresses such as ethanol, hydrogen peroxide. A mutant in *orf4* does not display these behavioural modifications [50].

Thanks to 2-DE it has also been shown that response to oligotrophic environment overlaps with that of glucose starvation. In fact, 42 and 51 proteins are induced, respectively, by glucose or growth in oligotrophic environment. Sixteen are common to both responses.

3.7. General stress proteins

Comparison of 2-DE analysis performed after treatments by several stresses revealed proteins specifically induced by a combination of stresses. Thus, comparison of stress proteomes of *E. faecalis* leads to the identification of six of these proteins which are called ''general stress proteins'' (Gsps, [51]). Two were identified as the chaperones DnaK and GroEL [52]. Another of these general stress protein (Gsp65) was recently identified thanks to N-terminal sequencing and subsequent cloning of the corresponding gene. This protein has homologies with hydroperoxide resistance (Ohr) proteins. Transcriptional analysis confirmed that the *gsp65* encoding gene was induced in response to hydrogen peroxide, heat shock, acidic pH, ethanol, sodium chloride, bile salts and *tert*.-butyl hydroxyperoxide [53]. However, a *gsp65* mutant showed increased sensitivity to *tert*.-butyl hydroperoxide and ethanol but the other treatments did not lead to behavioural difference compared to the wild type strain.

These examples, taken in the field of LAB proteomics, show that identification of proteins by 2-DE, followed by the study of genes expression, is the prerequisite for the understanding of the bacterial stress responses.

4. Proteomics and proteins of unknown function: a case study

Proteomics is considered to be powerful enough to help to find a function for proteins annotated in a fully sequenced genome. The classical approach is to delete the gene encoding the protein of interest and to observe phenotypic variation between wild type and mutant strain. Proteomics gives a new dimension to this kind of study by allowing one to observe and quantify the amount of synthesis of a great number of cell proteins. We describe here the main results of such a study [54].

In the presence of a rapidly metabolisable sugar in their environment, bacteria generally switch their metabolism to repression of genes involved in other carbon source utilisation. This central regulation mechanism is known as catabolite repression. In Gram positive bacteria, this mechanism involves the catabolite control protein (CcpA) and the Hpr protein of the phosphotransferase sugar transport system (PTS). The phosphorylated form of Hpr (ser-P) and CcpA bind together on *cre* sequences (catabolite responsive element) which are located upstream the promotors of the target genes. This leads to transcriptional repression of these genes and also to transcriptional activation in some cases. This central regulatory role of CcpA has been investigated recently in *E. faecalis* by a proteomic approach in order to have a global view of positively or negatively regulated targets.

In a *ccpA* mutant, 16 proteins have their synthesis induced and six are repressed. Thirteen of the induced proteins are also glucose starvation proteins (see above). Among the induced proteins, four have been identified after Edman degradation. They belong to the four-gene operon. This operon contains an ORF sharing identity with glycerol dehydrogenase, two ORFs sharing identity with dihydroxyacetone kinase and an ORF of unknown function. The last induced protein has a similarity with the β chain of L-serine dehydratase and belongs to a four-gene operon also encoding the α chain of this enzyme and a seryl tRNA synthetase. The induction of these proteins was also confirmed by Northern analysis.

This study also revealed the effect of CcpA on phosphorylation state of Hpr. In fact in the *ccpA* mutant, the formation of the unphosphorylated form of Hpr is reduced by half whilst the Hpr (ser-P) is twofold induced. Both forms of this protein can be detected and quantified on 2D gels.

From this survey of the literature, it can be seen that proteomics by itself gives new and interesting results concerning the regulation of protein synthesis during stresses. However, the last described study seems particularly interesting because it demonstrates not only that a combination of proteomics with genetic analysis can give new insights on metabolism regulation mechanisms but it also illustrates specially that the function of unknown proteins can be determined.

5. Conclusion

As described above, a few proteomic maps have been now completed for lactic acid bacteria. The

interest is rather turned toward the study of specific functions like metabolism and reaction to various stresses or adaptation to media, situations encountered by these bacteria during industrial processes. However, an increasing number of genomes are fully sequenced and interest in this approach will develop in the near future, in conjunction with studies on transcriptomics [55]. With the development of high throughput analysis techniques [56] one can foresee not only more proteomic studies but fully integrated approaches including genomics, transcriptomics, proteomics and bioinformatics. New emerging techniques sometimes referred to as interactome could also be envisaged in order to establish which interactions occur in bacteria between proteins. Such an interesting approach was recently made for *Helicobacter pylori* [57] using the yeast double hybrid technique.

Lactic acid bacteria are also used in human health where it is believed that their ingestion can protect against various virus infections by stimulating immune system [58]. In the same field of interest, it has been shown that they can act in colon cancer prevention [59]. A well documented review has been recently published on this subject [60]. However, the mechanism of protection is not fully understood and the study of the different benefit strains in the presence of eukaryotic tissues or pathogenic bacteria by proteomics could give new insights on the mechanism of protection.

Lactic acid bacteria are also present in normal or in pathological conditions in mucosal surfaces of gastrointestinal or genitourinary tracts [4]. There have been increasing reports of lactobacilli causing serious infections [61]. *Lactobacilli* present in dental plaque can acidify the oral cavity and pose an acidogenic challenge to teeth [62,63]. Thus, proteomics can become an invaluable tool to investigate epidemiology and taxonomy of microbial pathogens, the identification of their mechanisms of action and their drug resistance [64].

In conclusion, lactic acid bacteria are present in various aspects of human activities, in particular in industry and health. However, proteomic studies on these bacteria are still at their beginning and one can hope in a near future great developments on the knowledge of these bacteria and their rational use.

6. Nomenclature

1-DE	One-dimensional electrophoresis
2-DE	Two-dimensional electrophoresis
ADNr	Ribosomal ADN
LAB	Lactic acid bacteria
MM	Molecular mass
mRNA	Messenger RNA
MRS	deMan Rogosa Sharpe medium
p*I*	Isoelectric point
SDS–PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

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