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Application of Two-Dimensional Gel Electrophoresis to Microbial Systems

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1. Introduction

Proteome analysis represents large-scale analysis of the proteins in an organism, simultaneously (Pandey and Mann, 2000), thus facilitating with the elucidation of gene function (Bro and Nielsen, 2004).

The term proteomics was coined by Marc R. Wilkins in 1994, whilst he was developing the concept as PhD student in Macquarie University (Wilkins *et al.*, 1996a). Proteomics was mainly derived from the field of two-dimensional gel electrophoresis (2-DE). With the arrival of the concept of proteomics or proteome analysis, the method of 2-DE has gained greater significance. Although alternative methods of protein separation for proteomics have been developed, 2-DE has remained the core technology of choice for protein separation.

Two-dimensional gel electrophoresis is derived from 1-D SDS-PAGE, and expands the number of proteins resolved on an electrophoresis gel by separating the proteins based on their native charge and molecular mass. Smithies and Poulik (1956) separated serum proteins using a 2-D combination of paper and starch gel electrophoresis for the first time. The basic method of 2-DE was introduced over 30 years ago (O'Farrell, 1975; Klose, 1975) and represents a technology crucial to the field of proteomics. The coupling of isoelectric focusing (IEF) for the first dimension separation with SDS-PAGE in the second dimension resulted in a 2-D method in which proteins were being distributed across the two-dimensional gel profile (Dunn & Görg, 2001). The Proteomic approach was created by combining 2-DE technology with mass spectrometry and bioinformatics. Any improvements, both in mass spectrometers to allow higher imaging quality and more sensitivity and resolution as well as in software for more efficient data mining provides more and detailed information of the proteome. This state-of-the-art technology involves separation, identification and quantification of proteins. It offers many advantages which include the identification of target proteins amongst a pool of gene products in cell and tissue extracts. Having high resolving power, the method provides a greater level of the purified protein for subsequent characterization using mass spectrometry; and the proteins that are separated on the gel are not destroyed and can thus be used for further analysis (Westermeier, 2005).

Proteomics has influenced microbiological research to a large extent, ranging from environmental to medical aspects of microbiology. The genome sequence of several microorganisms has been analyzed with computational tools and now most research studies are in the postgenomic era. In 1997, Blattner and colleagues predicted a proteome, extracted from the genome sequence of *Escherichia coli* MG1655 which consisted of 4405 proteins (Fig. 1). In fact the bacterium, *E. coli*, has served as a model system for the development of 2-DE (O'Farrell, 1975; Cash, 2009).
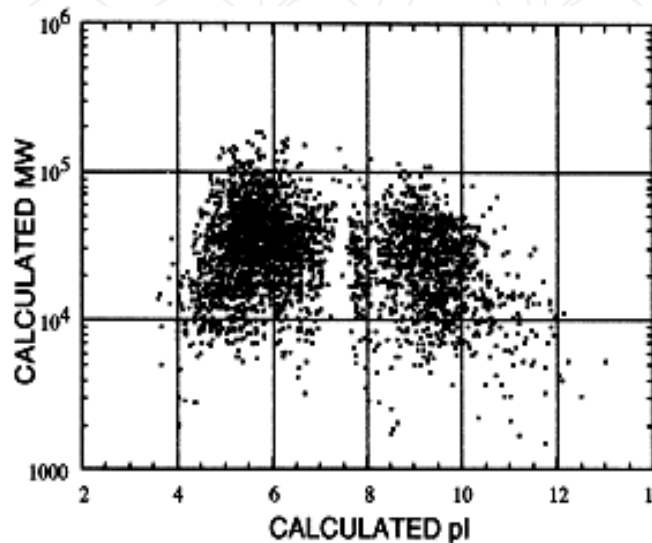

Fig. 1. A predicted *E. coli* proteome. The predicted isoelectric point (pI) versus the predicted molecular mass (M_r) of predicted open reading frames (ORFs) for *E. coli* is depicted. Each dot represents a protein. This plot shows the range of pI and M_r values for the proteins that may be detected by 2-DE gels (VanBogelen *et al.*, 1999).

At that time, proteomes of some other microorganisms such as *Mycoplasma genitalium, M. pneumoniae, Methanocaldococcus jannaschii* and *Synechocystis* sp. were also predicted from their DNA sequences (VanBogelen *et al.*, 1997; Link *et al.*, 1997a; Link *et al.*, 1997b; Wasinger *et al.*, 1997). Protein expression profile of a single sample under different circumstances is determined by using 2-DE gels. It may be compared to that of a control condition to examine the changes in protein expression of microbial cells in response to a foreign stimulus resulting from chemical, physical or biological changes. A set of proteins that responds specifically to a particular status of the cell termed as a proteomic signature, relates to a certain metabolic conditions such as the redox state, protonmotive force, etc. This can be used to diagnose the cellular states of microbial organisms (VanBogelen *et al.,* 1999). In addition, 2-DE was extensively employed to differentiate and classify the microbial isolates. Discrimination of bacterial isolates is important in the study of the molecular taxonomy and epidemiology of bacterial pathogens (Cash, 2009). 1-D SDS PAGE and 2-DE were used as successful rapid typing methods generating reliable and reproducible data. Furthermore, the proteome studies have a remarkable contribution to detection of antigens which may be used for diagnostics and vaccine candidate prediction (Jungblut and Hecker, 2007).

This chapter outlines major topics associated with the application of 2-DE to microbial systems, which include: characterization of proteins, characterizing protein modifications, protein-protein interactions, metabolic engineering, characterization of mutant proteins,

microbial systematics and epidemiology, and evaluation of proteins involved in the toxic response.

The chapter will also include future perspectives regarding the use of 2-DE.

2. Charactarization of proteins

Proteomics is regarded as a powerful approach as far as biochemical research is concerned, because it directly studies the key functional components of biochemical systems, namely proteins (Freed *et al.*, 2007). Approximately 70% of a microbial cell is composed of proteins (Frantz & Maccallum, 1980) and can aid in discriminating and distinguishing between different species and different bacterial strains (Fagerquist *et al.*, 2005).

Although alternative protein separation technologies have been developed for proteome analysis, 2-DE polyacrylamide gel electrophoresis is regarded as a powerful technique, because it can be used to separate and resolve complex protein mixtures into thousands of individual components (Görg *et al.*, 2004). It should be noted that spots are often composed of several proteins. However, longer gels and narrower pH ranges can increase the chances of getting only one protein per spot. Depending on the gel size, 2-D PAGE is capable of resolving more than 5000 proteins simultaneously (ca. 2000 proteins routinely) and can detect less than 1 ng of protein per spot.

Sample preparation is regarded as a very important step which should be as simple as possible to increase reproducibility. In order to avoid artifactual spots in 2D gels, protein modifications during sample preparation must be minimized. Hence, proteolytic enzymes that may be present in the sample must be inactivated. Furthermore, samples that contain urea must not be heated so as to avoid charge heterogeneities caused by the carbamylation of the protein by isocyanate formed during the decomposition of urea (Dunn, 2000).

Proteomic mapping (the protein complements expressed by a genome, cell or tissue), is being used to verify and provide new protein targets, in order to explore mechanisms of action or toxicology of compounds, and to discover new disease biomarkers for clinical and diagnostic applications (Bunai & Yamane, 2005; Kamelia *et al.*, 2009). Proteomics is regularly used to analyze the reaction of organisms and cells to a changed environment, for example growth under different culture conditions and different food sources, such as temperature, nutrients, oxygen, osmotic stress and toxins (Molly *et al.*, 2011).

The differential proteomics approach has also been made use in many pharmacological studies (Vlahou & Fountoulakis, 2005). Such an approach has been very useful in comparing different strains of microorganisms (Kamelia *et al.*, 2009). In addition to the genetics, this technique delivers more levels of complexity at which homologies and differences between different microbial strains can be analyzed.

3. Posttranslational modification (PTM)

There are possible modifications to the expression of a protein that are not encoded by the sequence of its gene alone. Most proteins show some form of posttranslational modification (Jensen, 2004; Walsh, 2006). A series of protein modifications are involved in the signaling pathways, from membrane to nucleus, in response to external stimuli (Table 1).

PTM type	Average MH+	Modified amino acid residue	Postion	Remarks	Reported in Pub Med (case)
Acetylation	42.04	S	N-term	Reversible, protein stability,	11,069
		K	anywhere	regulation of protein function	
Phosphorylation	79.98	Y, S, T, H, D	anywhere	Reversible, regulation of protein activity, signaling	103,235
Cys oxidation			anywhere	Reversible, oxidative regulation	
disulfide bond	-2.0	C		of proteins	23,538
glutathionylation	305.31	C			63
sulfenic acid	16.00	C			228
sulfinic acid	32.00	C			642
Acylation				Reversible, cellular localization	
farnesylation	204.36	C	anywhere	to membrane	1,349
myristoylation	210.36	G	N-term		644
		K	anywhere		
palmitoylation	238.41	C (S, T, K)	anywhere		681
Glycosylation			anywhere	Reversible, cell-cell interaction	
O-linked	>800	S, T		and regulation of proteins	24,115
(O-Glc-NAc)	203.20,				
N-linked	>800	N			
Deamidation	0. 98	N, Q	anywhere	N to D, Q to E	711
Methylation			anywhere	Regulation of gene expression,	29,889
monomethylation	14.03	K		protein stability	
dimethylation	28.05	K			
trimethylation	42.08	K			
Nitration	45.0	Y		Oxidative damage	62
S-Nitrosylation	29.00	C			399
Ubiquitination		K	anywhere	Reversible/irreversible	1951
Sumoylation		K	[ILFV]K.D		104
Hydroxyproline	16.00	P		Proein stability	11,424
Pyroglutamic acid	-17	Q	N-term		710

Table 1. The list of protein modifications in the signaling pathway (Seo & Lee, 2004).

These modifications, such as deamidation or oxidation of old cellular proteins may represent aging of the protein (Hipkiss, 2006) or they can occur in an enzymatically regulated fashion after the proteins are translated (Zhang, 2009).

In fact the primary sequence which contains motifs that allow different PTMs (Gupta *et al.*, 2007) are actually found on a protein at any given time in a specific tissue, and cannot be predicted.

Posttranslational modification can bring about change in parameters such as molecular weight and the isoelectric point of proteins (Halligan, 2009). There are in fact more than 360 known chemical modifications of proteins (http://www.abrf. org), which include natural PTMs such as phosphorylation, glycosylation and acylation , as well as artifacts such as oxidation or deamination that might occur naturally inside cells but can also act as artifacts during protein preparation (Benson *et al.*, 2006). It should be noted that shifting of the protein spot (pI or M_r) is usually more likely to be a kind of protein modification.

Oxidation is one of the most common modifications that affect mainly methionine and cysteine residues, but also lysine, histdine, tryptophan and proline, which are oxidized to carbonyl derivatives. Furthermore, the carbonyl content of protein has been used to estimate oxidation of the protein during aging (Stadtman, 1992; Madian & Regnier, 2010). We could

end up missing all peptides containing either of the amino acid, because their masses will increase from 2 to 48 Da.

The pulse - chase experiment allows the estimation of the half life of protein degradation. Phosphorylation of proteins can be observed very conveniently *by in vivo* metabolic labelling using ^{32}p- or ^{33}p - ortho phosphate (Cohen, 2000). The sensitivity of metabolic labeling with ^{35}s amino acids is about three to five times higher than protein detection by the silver staining method (Gygi *et al.,* 2002).

Films or phosphorimagers can be used to detect radioactivity incorporated into the proteins. It is also possible to use two films, and covering the gel with aluminum foil can deliver the ^{35}S and ^{32}P signals differentially.

Posttranslational modification enhances the structural diversity and functionality of proteins directly by providing a larger repertoire of chemical properties than is possible using the 20 standard amino acids specific for the genetic code. Therefore, it is regarded as a dynamic phenomenon with a central role in many biological processes.

The applications of two dimensional gels are extensive, particularly in regard to detecting and quantifying modification in genome expression during development under different environmental and stress conditions (Anderson & Anderson, 1998; Wilkins *et al.,* 1996b). If the modified group can be removed by chemical or enzymatic treatment, then 2D gels can be used to identify the position of the modified proteins (Nyman, 2001).

Phosphorylation is the most commonly occurring form of posttranslational modification and the most significant form of regulatory modification in both prokaryotic and eukaryotic cells. The quantitative aspect of this process is important because the phosphoproteome is not only complex but also extremely dynamic (Reinders & Sickmann, 2005). Several reviews have reported a variety of techniques and methodologies for the analysis of phosphoproteins (Thingholm *et al.,* 2009; Paradela & Alber, 2008; Parker *et al.,* 2010).

The primary difference in the detection of the phosphorylated protein is the staining method. This method must be specific for phosphorylated proteins and must not interact with the nonphosphorylated species. Since many phosphorylation-regulated proteins are present in small quantities, the staining or labeling procedures must be sensitive. These criteria are met when using both ^{32}p labeling and immunostaining procedures.

The radioactive proteins are used during fractionation procedures, such as 2D-PAGE or HPLC, so as to identify the amino acid types that are modified; the phosphoproteins are completely hydrolyzed and the phosphoamino acid content determined. The specific sites of phosphorylation can be determined by proteolytic digestion of the radiolabeled protein, separation and detection of phosphorylated peptides (e.g. by two-dimensional peptide mapping), followed by Edman sequencing. To measure differences in relative abundances of phosphorylation,^{32}p-labeled proteomes can be separated by 2D–PAGE and the relative spot intensities are subsequently compared (Cohen, 2000).

One of the most common forms of posttranslational modification that occurs within the cell involves the attachment of carbohydrates to proteins. In fact 2D-GE is the method of choice for visualizing glycoproteins (Schäffer *et al.,* 2001).

Standard techniques for in-gel or on-membrane protein staining appear to work poorly with glycoproteins and special methods are required. The periodic acid-Schiff method, biotin-hydrazide, fluorescein, semicarbazide and the glycoprotein specific staining reagent (pro-Q Emearald, which is 50 times more sensitive than other methods) are used for detection and staining of glycoproteins (Steinberg *et al.*, 2001).

For identification of glycosylated proteins, identification of the sites of glycosylation, quantitation of the extent of glycosylation of each site, identification of the number of different glycoforms and structural characterization of the glycolytic side chain are necessary. Detection of glycoproteins can be achieved by 2DGE and polyvinylidene difluoride membranes (Harvey, 2001).

Glycosylation is the most common and complex PTM, (Sharon & Lis, 1997) and has many different biological roles (Varki, 1993; Varki *et al.*, 1999). These roles vary from those which are related primarily to general effects of the size and shape of the glycan, such as protein folding and assembly of protein complexes (Helenius & Aebi, 2001), to those which depend upon the specific configuration of the branched glycan structures, such as cell recognition, cell - cell interaction and immune responses (Rudd *et al.*, 2001).

The main methods that have been used for preparation of glycosylated samples can be divided into two main categories:

- sample preparation for detailed characterization of a relatively pure protein.
- sample preparation for analysis of low level, complex proteomic samples.

It is possible to prepare proteomic samples for glycosylation-specific analysis by combining 2 D-GE separation with glycoprotein-specific staining, (Hart *et al.*, 2003) but this is subject to the limitation of 2D-GE procedures including limited throughput and difficulties in membrane glycoproteins.The most established affinity techniques (lectin affinity chromatography) can be used to enrich samples for subsequent analysis (Kuster *et al.*, 2001).

4. Protein – Protein interactions

The majority of proteins of a cell usually function as part of much larger complexes that are often in the static or transitory form, and it is the latter form which takes part in signaling and metabolic pathways.

Proteins within the cells often interact with small molecules, nucleic acids and/or other proteins (Guidi *et al.*, 2010). In order to study interactions between proteins, methods have been devised that are based on the detection of binary interactions which involve interactions between pairs of proteins , and those that are based on the detection of complex interactions that consist of interactions between multiple proteins that form complexes (Phizicky *et al.*, 2003). Such interactions can be confirmed or rejected by the many available genetic and bioinformatics methods. In fact, by applying classical genetics that combines different mutations in small cells or organisms and observing the resulting phenotype, a shortcut to functionally significant interactions that have a recognizable effect on the overall phenotype can be provided (Giot *et al.*, 2003).

Another genetic approach involves a strategy that screens for enhancer mutations. In this situation the individual mutations in the genes that code for proteins X and Y do not

prevent interaction and are therefore considered as viable, however the presence of simultaneous mutations in both genes prevents the interaction and leads to what is called a lethal phenotype, thus confirming that these proteins are part of the same complex in the same biochemical or signaling pathway (Tong *et al.*, 2001).

In the bioinformatics approach, three methods have been developed for the confirmation of protein interaction directly from genomic data (Halligan *et al.*, 2004). One of the methods is referred to as the domain fusion or Rosetta stone method (Marcotte 2000). It is based on the principle that protein domains are structurally and functionally independent units that can operate either as discrete polypeptides or as part of the same polypeptide chain. Hence, multidomain proteins in one species may be represented by two or more interacting subunits.

Another of the three methods is the comparative genomic method, which is based on the fact that bacterial genes are usually arranged into operons and although their sequences are diverse, such genes are often functionally related. Thus, if two genes are located next to each other in a series of bacterial genomes, it may be that they are functionally related even if their products are found to interact (Marcotte, 2000). However, there is also evidence that genes whose functions are apparently unrelated may be organized into operons (Marcotte, 2000).

The third bioinformatics-based method is associated with phylogentic profiling, which makes use of the evolutionary conservation of genes with the same function. For example the presence of three or four conserved genes of unknown function in 20 aerobic bacteria and their absence in 20 anaerobic ones indicate that the products of such genes are highly likely to be involved in aerobic metabolism (Marcotte, 2000).

In addition to the above approaches, biochemical and physical methods are required to verify the genetic and bioinformatic methods. However biochemical and physical procedures are often faced with limitations with regard to the number of interactions thus making such approaches unsuitable for the purpose of analyzing protein interactions on a global scale.

In contrast to the above approaches, the gel-based proteomics approach, otherwise known as two-dimensional electrophoresis (2-DE), has been found to be especially useful in the study of protein-protein interactions, because it allows for an improved separation of proteins as well as the detection of specific interacting protein isoforms of a protein that arise from posttranslational modification (Mann & Jensen, 2003). In fact the detection of protein interactions by the proteomics methodology has been considered as one of the most challenging and rewarding approaches. The objective of single studies is often to identify all interacting partners of a single protein, but when several studies are taken together, they can be used to identify all interactions within a single signaling module (Bader *et al.*, 2003).

Interactions of a proteomic nature have been analyzed only in some exceptional studies (Ho *et al.*, 2002; Krogen *et al.*, 2006). The results obtained from such studies are often incomplete because of the temporal and unstable nature of protein-protein interactions, and the acquisition of different results from different methods and their complexities. Proteomic parameters can change, from seconds or minutes (e.g. signaling) to hours, days and ever longer time periods, such as in degenerative diseases.

It is important to analyze all these interactions on a proteomic scale. Several proteomic studies have in fact significantly enhanced our knowledge regarding the interacting partners and functions of single proteins, or whole protein complexes. Nevertheless, based on the methods used, it may be difficult to understand whether, for example a protein shows a weak but specific interaction or a strong but nonspecific interaction (Jensen, 2004).

Hence, comparing and combining data from different studies must be carried out with a great deal of care and precision, because of the application of different technologies during such studies.

5. Metabolic engineering

It is obvious that all proteins of a living cell are not expressed simultaneously. This makes it possible to compare the proteome maps under different conditions. The up-regulated and down-regulated proteins which are stimulated by withdrawal of nutrients, or any other physical and chemical changes, can be determined by 2-DE. In this way proteins of high significance are found. Proteomics has become an important tool in understanding gene function with regard to many metabolic engineering strategies. In this section, the potential use of proteomic analysis in metabolic engineering will be illustrated.

Metabolic engineering involves the optimization of genetic and regulatory processes within cells to increase production of certain substances of human interest by the cells. It involves the alteration of the cells genetic makeup, in order to obtain a specific phenotype (Vemuri *et al.*, 2005). This strategy often tries to minimize cellular energy associated with cell reproduction and proliferation and also attempts to reduce the production of cellular wastes.

One of the main features of metabolic engineering involves metabolic pathway manipulation, which has been classified by Cameron & Tong (1993) into five groups. These are: Improvement of the yield and productivity of products made by microorganisms; expanding the spectrum of substrates that can be metabolized by an organism; forming new and unique products; improving cellular properties, and degradation of xenobiotics.

The genetic and regulatory changes can have significant effects on the complex cellular machinery and hence the cells' ability to survive. In metabolic engineering strategies, a great deal of focus is currently being placed on regulatory networks in the cell to engineer an efficient metabolism besides the direct deletion of or overexpression of the genes that code for metabolic enzymes (Vemuri *et al.*, 2005). Because of the significance of regulatory control in metabolic processes several investigations are currently being carried out to understand regulation at various levels of the metabolic hierarchy (both local and global). The availability of biological data has helped with the identification of the individual components (genes, proteins, and metabolites) of a biological system, thus making it easier to unravel interactions between such components that culminate in what is known as the phenotype. Hence, in addition to an integrated understanding of physiology, but also for practical applications of using biological systems as cell factories, it is very important to reveal and identify such components. Recent "-omics (genome, trancriptome, interactome, proteome, metabolome, fluxome)" approaches have extended knowledge regarding regulation at the gene, protein, and metabolite levels, and thus have had a great influence on

the progress associated with metabolic engineering. A combination of global information derived from various levels of metabolic hierarchy is absolutely essential in comprehending and assessing the relationship between changes in gene expression and the resulting phenotype (Vemuri *et al.,* 2005).

One of the "omics" that has allowed a better comprehension of regulation is proteomics. The awareness of protein abundance helps with understanding the extent to which regulatory proteins and transcription binding factors take part in the subsequent change that occurs in the gene expression profile. The initiation of translation and the following regulation is mainly dependant on the ribosome-binding site. After the detection of a signal, the regulatory proteins bind to the promoters and recruit RNA polymerase enzymes to the transcription start site (Vemuri *et al.,* 2005). Two-dimensional (2D) gel electrophoresis has so far been the method of choice in analyzing proteomes, with a good turnover of information. However, one of the drawbacks of this method is that it mostly detects proteins expressed at high concentrations. In order to counteract this problem, staining methods have been developed to improve the accuracy and the sensitivity of protein detection and quantification. Nevertheless, several regulatory proteins are present in the cell at very low concentrations, hence, alongside 2D-gel electrophoresis, sensitive high-throughput methods for accurate protein detection and quantification is widely acknowledged (Vemuri *et al.,* 2005) and is currently being implemented (Bernhardt *et al.,* 2003).

A wide array of microbial systems has been and is currently being used for production of many products that are of biotechnological and metabolic significance. Here presented are a few examples.

5.1 Lactic acid bacteria

There have been only a few studies of the regulation of carbon flow in Lactic acid bacteria (LAB) with regard to the "omic" technologies. In fact little is known regarding the regulation of glycolytic enzymes in the emerging pathogen *Enterococcus faecalis*. In a study by Mehmeti and colleagues (2011) regulation of central carbon metabolism at the level of biosynthesis of the participating proteins was demonstrated in *E. faecalis* V583 . Such research is of vital interest because of the pathogenic aspects of *E. faecalis,* in addition to significance of lactic acid production for LAB, and the fact that LAB are widely used for production of lactic acid in fermented food. The data derived from such research will inevitably help with metabolic engineering strategies associated with product development in LAB. During homolactic fermentation in LAB, pyruvate is converted to lactate in addition to a number of minor metabolites, such as acetic acid, acetaldehyde, ethanol, acetoin, and acetate, but, under certain environmental conditions, metabolism switches to heterolactic fermentation (mixed-acid producing formate, acetate, acetoin, ethanol, and CO_2 as the final products (Mehmeti *et al.,* 2011). An example of such a situation was observed in *Lactococcus lactis*, where mixed-acid fermentation has been shown to occur at low growth rates under microaerobic conditions (Jensen *et al.,* 2001; Mehmeti *et al.,* 2011), under true carbon-limited conditions, and while growing at low pH on carbon sources other than glucose (Melchiorsen *et al.,* 2002; Mehmeti *et al.,* 2011)). Mixed-acid fermentation has also been observed in *E. faecalis* V583, but only after removal of the lactate dehydrogenase (LDH) activity (Jonsson, *et al.,* 2009)). This emerging pathogen has two *ldh* genes, but it is the *ldh-1* gene which is the main contributor to lactate production (Mehmeti *et al.,* 2011).

The "omics" approach that involves metabolic, transcriptomic and proteomic technologies will no doubt assist in elucidating the shift from homolactic to heterolactic fermentation in LAB. This has already been demonstrated in *L. lactis*, where enzyme levels have been found to be regulated in response to growth conditions, and correlations between metabolic and transcriptomic or proteomic data have been established (Dressaire *et al.*, 2009; Mehmeti *et al.*, 2011). In this latest research, Mehmeti and colleagues (2011) compared a constructed lactate dehydrogenase (LDH)-negative mutant of *E. faecalis* V583 *(ldh1.2)* with its wild type via metabolic, transcriptomic, and proteomic analyses. The mutant was found to grow at the same rate as the wild type but converted glucose to ethanol, formate, and acetoin. Microarray analysis was also performed showing that LDH deficiency had overwhelming transcriptional effects, where 43 genes in the mutant were found to be upregulated, and 45 were found to be downregulated. Most of the upregulated genes were identified as those involved in energy metabolism and transport. Subsequently, 45 differentially expressed proteins were identified using the technique of two-dimensional (2D) gel analysis in conjuction with MALDI-TOF/MS analysis. A comparison of transcriptomic and proteomic data suggested that for several proteins the level of expression is regulated beyond the level of transcription. Comparative proteomic and transcriptomic and metabolic analyses showed that the pyruvate catabolic genes, including the truncated *ldh* gene, showed highly increased transcription in the mutant., and that protein expression was regulated beyond the level of transcription, as suggested by Mehmeti and colleagues (2011), who also demonstrated that these genes, along with a number of other differentially expressed genes, are preceded by sequences with homology to binding sites for the global redox-sensing repressor, Rex, of *Staphylococcus aureus*. The results of this research demonstrates that the NADH/NAD ratio is involved in the transcriptional regulation of these genes, thus having a critical role in the regulatory network controlling energy metabolism in *E. faecalis* (Mehmeti *et al.*, 2011).

5.2 *Vitreoscilla stercoraria*

The obligate aerobic bacterium, *Vitreoscilla stercoraria, produces* the oxygen binding protein, *Vitreoscilla* hemoglobin (VHb), the expression of which under hypoxic conditions in various organisms, such as bacteria, yeasts, fungi, and plant cells, , improves growth, increases protein secretion and metabolic productivity and stress resistance, mediates ATP synthesis and detoxifies the adverse effects of nitric oxide. However, the mechanism of VHb action has still not been clearly understood. With such positive effects, VHb is currently being applied to various cell-based biotechnological processes including metabolic engineering, production of valuable metabolites, and fermentation (Isarankura-Na-Ayudhya *et al.,* 2008).

In the study by Isarankura-Na-Ayudhya and colleagues (2008), the effect of VHb production on the protein expression profile of *E. coli* was investigated using 2-DE and peptide mass fingerprinting. Following fusion of VHb with green fluorescent protein (GFP), GFPuv was selected as a reporter molecule to demonstrate *vgb* gene expression, which is under the control of *lac* promoter. In fact, the location of the fusion protein (VHbGFP) on the 2D gels can easily be distinguished from other high abundant proteins. The resulting protein spots were then identified by MALDI-TOF mass spectrometry.

Data showed that in addition to the loss of tryptophanase that is responsible for tryptophan, cysteine and serine catabolism, VHbGFPuv expression also down-regulated proteins involved in various metabolic pathways, such as glycerol kinase, isocitrate dehydrogenase,

aldehyde dehydrogenase, and D-glucose-D-galactose binding protein. The data obtained from this proteomic approach point to the critical roles that VHb plays at the level of cellular carbon and nitrogen consumptions. It may also be involved in the regulation of other metabolic pathway intermediates, via autoregulation of the catabolite repressor regulons (Isarankura-Na-Ayudhya *et al.*, 2008) .

5.3 *Amycolatopsis balhimycina*

The identification and unraveling of biochemical pathways and enzymes by the proteomics approach has helped greatly with targeting proteins of interest for eventual genetic manipulations, so as to optimize the reactions that culminate in the production of beneficial products in various microbial systems. A recent example of this technology has involved the use of the differential proteomic approach which has shown that the production of the antibiotic balhimycin in batch culture is associated with the up-regulation of enzymes involved in the biosynthesis of antibiotic precursors. These proteomic data point to the possible sustainable balhimycin production through availability of increased levels of precursors, such as tyrosine, that has also proved to be effective in balhimycin production (Gallo *et al.*, 2010).

Chemostat cultures are one of the techniques that help with understanding the relationship between the physiology of a microorganism and its metabolism. In a study by Gallo and colleagues (2010), chemostat cultures of *Amycolatopsis balhimycina* were used to reach steady-state conditions for the purpose of biomass accumulation under the same growth rate conditions, with or without balhimycin production. The minimal defined media consisted of low Pi concentrations and proficient glucose, or high Pi levels and limiting glucose concentrations. The biomass obtained from these cultivations were then analyzed by a comparative proteomic study in order to reveal the expression of genes that are involved in *A. balhimycina* primary and secondary metabolism which are associated with biomass production and antibiotic synthesis (Gallo *et al.*, 2010). The medium containing low Pi levels produced balhimycin. Accordingly, quantitative RT-PCR revealed up-regulation of the *bal* genes, responsible for balhimycin biosynthesis, and of phoP, phoR, pstS and phoD, which are involved in the Pi limitation stress response (Gallo *et al.*, 2010).

The analysis of the biomass by 2-D Differential Gel Electrophoresis (DIGE) and the protein identification carried out by mass spectrometry and computer-assisted 2-D reference-map (http://www.unipa.it/ampuglia/Abal-proteome-maps), showed a differential expression for proteins involved in many metabolic pathways such as the central carbon and phosphate metabolism. Furthermore, the DIGE technique demonstrated that in the presence of low Pi levels, proteins involved in the production of primary metabolic intermediates and cofactors required for balhimycin biosynthesis were up-regulated. The bioinformatic approach was subsequently used to show the presence of PHO box-like regulatory elements in the upstream regions of nine differentially expressed genes (Gallo *et al.*, 2010). The proteomic approach illustrated a relationship between primary metabolism and antibiotic production, which can be used in metabolic engineering strategies to increase antibiotic yield (Gallo e*t al.*, 2010).

5.4 *Escherichia coli*

According to Renzone and colleagues (2005), identification of regulators and regulatory networks is essential to control, predict or engineer bacterial behavior. In fact, the

identification of crucial stress-related genes in particular will uncover targets for a) specific manipulation to promote or limit cellular growth, b) development of useful tools to screen for tolerant or sensitive strains and c) evaluation of the culture fitness (an indication whether bacteria are fully adapted or stressed), which can help to optimize parameters for growth in culture (Renzone *et al.,* 2005).

In a study by Han *et al.* (2001), recombinant *E. coli* strains harboring heterologous polyhydroxyalkanoate (PHA) biosynthesic genes that were shown to accumulate unusually large amounts of PHA were analyzed. In this study, integrated cellular responses of metabolically engineered *E. coli* to the accumulation of poly (3-hydroxybutyrate) (PHB) in the early stationary phase were analyzed at the protein level by two-dimensional gel electrophoresis. In the presence of accumulated levels of PHB, 20 proteins showed altered expression levels, 13 of which were identified using the mass spectrometry technique. Three heat shock proteins, GroEL, GroES, and DnaK, were significantly up-regulated in PHB-accumulating cells, but, proteins involved in the protein biosynthetic pathways were adversely affected. The proteomics approach illustrated that the increased synthesis of two glycolytic enzymes and one enzyme belonging to the Entner-Doudoroff pathway was likely to have arisen from the cellular demand for large amounts of acetyl coenzyme A and NADPH that are required for PHB biosynthesis. Furthermore, in the presence of accumulated levels of PHB, the expression of the *yfiD* gene was highly induced; this gene codes for a 14.3-kDa protein that is usually produced at low pH levels. This analysis suggests that the accumulation of PHB in *E. coli* represents a situation of stress leading to a reduction in the cells' ability to synthesize proteins and induction of the expression of various protective proteins (Han *et al.*, 2001, 2011).

5.5 *Xanthophyllomyces dendrorhous*

The bacidiomycete, *Xanthophyllomyces dendrorhous,* is one of the best sources of the antioxidant carotenoid astaxanthin, and is used for the microbiological production of this antioxidant. Being of biotechnological significance, many investigations have recently been carried out, including metabolic engineering strategies to improve astaxanthin yield. The first proteomic analysis of the strain ATCC 24230 has been carried out recently by Matinez-Moya and colleagues (2011). The technique of 2-DE in conjunction with matrix-assisted-laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) were used to produce protein profiles before and during the induction of carotenogenesis . Approximately 600 protein spots were observed, 131 of which were found to be non-redundant proteins. In addition, 50 differentially expressed proteins that were classified as a result of distinct expression patterns were also identified. Proteomic analyses revealed that enzymes involved in acetyl-CoA synthesis were present at higher concentrations prior to the process of carotenogenesis (Martinez-Moya et al., 2011). However, redox- and stress-related proteins were up-regulated during the induction of carotenogenesis. Higher levels of the carotenoid biosynthetic enzymes, mevalonate kinase and phytoene/squalene synthase, were observed during induction and accumulation of carotenoids. Nonetheless, the usual antioxidant enzymes, such as catalase, glutathione peroxidase and the cytosolic superoxide dismutases, were not detected. The gel-based proteomic approach succeeded in identifying the potential carotenogenesis-related proteins, which also included those involved in carbohydrate and lipid biosynthetic pathways, and the several redox- and stress related

proteins. Martinez-Moya and colleagues (2011) suggest that these proteomic-derived data also indicate that *X. dendrorhous* accumulates astaxanthin under aerobic conditions, so as to scavenge the reactive oxygen species (ROS) generated during metabolism (Martinez-Moya *et al.,* 2011). Nevertheless, for the sake of both basic research and metabolic engineering, detailed transcriptomic, proteomic and metabolomic studies are required to produce an integrated understanding of the biochemical, physiological and biological processes of *X. dendrorhous*, (Martinez-Moya *et al.,* 2011).

6. Characterization of mutant proteins

The elucidation of many regulatory pathways in various microorganisms has involved the creation of mutations in genes that code for enzymes involved in the regulatory pathways and networks. In this regard the presence of mutant proteins has been investigated using several techniques. However, one technology which allows us to identify mutant proteins in the cell proteome and understand the effects of such mutants on other proteins, with insight into the interconnections between the regulatory networks in the proteome under different environmental conditions, is proteomic analysis involving the use of 2-DE. This section will summarize the applications of 2-DE in this regard.

The characterization of mutant proteins in response to elucidation of regulatory proteins and pathways has been greatly aided by the use of 2D-gel electrophoresis. The application of this technique to this area of research in microbial physiology has been extensive and has managed to unravel many regulatory components associated with bacterial metabolism (van Vliet *et al.,* 1998; Coppee *et al.,* 2001; Cheng *et al.,* 2009; Kint *et al.,* 2009; Egan *et al.,* 2002; Panmanee *et al.,* 2008; Cox *et al.,* 2009; Shin *et al.,* 2007; Friedman *et al.,* 2006). Some applications of this technique are described below.

6.1 *Bacillus subtilis*

Recent advances in the application of 2D-gel electrophoresis have been applied to the metabolic system of the Gram positive bacterium, *B. subtilis* (Bernhardt *et al.,* 2003; Hecker *et al.,* 2009).

The introduction of the highly sensitive technique of 2D-gel electrophoresis into bacterial physiology by Neidhardt and van Bogelen more than 20 years ago, otherwise known as "proteomics" in the mid-90s (Wilkins *et al.,* 1996b), opened a new era in this field of research (Bernhardt *et al.,* 2003; Hecker *et al.,* 2009).

The complete genome sequence and protein annotation of *B. subtilis* have been made available, which has provided advantages in many proteomic studies. (Kunst *et al.,* 1997; Seul et al., 2011). Extensive information can simply be derived from the genome sequence. However, it is the proteome that signifies the phenotype, representing the cell at the molecular level (Bernhardt *et al.,* 2003). Up to 10,000 proteins (Klose & Kobalz, 1995; Bernhardt *et al.,* 2011) can be separated on one single 2D-gel, with the majority of all proteins synthesized in bacterial cells being visualized simultaneously (including alkaline and extracellular cells). Identification of proteins by mass spectrometry (MALDI TOF MS, ESI MS, etc.) supported by N-terminal sequencing will no longer be problematic once the genome sequence of the organism is known (Bernhardt *et al.,* 2003).

According to Bernhardt *et al.*, 2003, physiological proteomics provides information at the protein level regarding the physiological state of a cell , however, the next step in proteomics involves analyzing the kinetics of the protein pattern under different environmental conditions that represent nature. The growth of *B. subtilis* in the upper layers of soil, involves short periods of growth followed by long non-growth periods brought about by stress and starvation. To survive such situations, *B. subtilis* has developed cellular adaptation strategies derived from complex genetic and regulatory networks (Bernhardt *et al.*, 2003). Such networks are comprised of many regulons, that have a unique adaptive function and are controlled by one global regulator. In order to comprehend bacterial physiology, the analysis of this network, its dissection into single regulons, and a definition of the adaptive function of all proteins within the regulons is essential (Msadek, 1999; Bernhardt *et al.*, 2003). Usually more than one regulon is induced by extracellular stimuli; this group when expressed in the protein form is referred to as the stimulon and contributes to stress adaptaion (VanBogelen & Neidhardt, 1990). In order to elucidate adaptaional networks, stimulons and their respective regulons must be defined and distinguished. This is where the role of identifying and characterizing mutant proteins for comparative purposes takes on a significant role. Proteins/ genes belonging to regulons can be identified if mutants in global regulators are available (Bernhardt *et al.*, 2003). The structure of presently known as well as still unknown regulons can be analyzed by comparing wild-type protein expression patterns to deregulated mutant strains carrying inactivated global regulatory genes as demonstrated by the σ^B-dependent general stress regulon of *B. subtilis* as a model (Hecker & Völker, 1998; Hecker *et al.*, 2009).

In order to understand global gene regulation, the study of single regulons derived from the genome is not appropriate because single regulons are part of a tight adaptational network. However, in order to understand and unravel global gene regulation, the use of a comprehensive computer-aided inspection and matching of various 2D gels loaded with radioactively labeled proteins becomes the method of choice (Antelmann *et al.*, 2000; Bernhardt *et al.*, 2003). Although DNA array techniques can be comprehensive and generate a huge quantity of data, but they are often very difficult to interpret. However, a fast gel-based proteomics method that can assist with this kind of analysis, involves the use of the dual channel imaging of 2D protein gels (Bernhardt *et al.*, 1999; Hecker *et al.*, 2009), which has made the search for proteins belonging to stimulons or regulons more practical. In this technique, proteins are assigned to stimulons or regulons simply by detecting red (newly induced) or green (repressed) proteins. In fact, the green proteins which comprise a substantial part of the protein pool in the non-growing cell, cannot be detected when using DNA arrays. According to Bernhardt and colleagues (2003), the dual channel imaging technique combines staining techniques to visualize accumulated proteins and autoradiography to uncover proteins that are synthesized at defined states. Two digitized images of 2D gels have to be generated and combined in using alternate additive color channels. One of them—the densitogram— showing proteins accumulated in the cell that had been visualized by (silver) staining techniques is pseudocolored green. The second image of an autoradiograph showing the proteins synthesized during a 5-min L-[^{35}S]-methionine pulse label is pseudocolored red. As a result, accumulated as well as newly synthesized vegetative proteins take on a yellow color. After imposition of a glucose-starvation stimulus, however, proteins newly synthesized in response to that stimulus are

red because they have not yet accumulated in the cell. Proteins, the synthesis of which, have been switched off by the stimulus, change their color from yellow to green (Bernhardt *et al.*, 2003). The major advantage of this technique is that it allows for the matching of protein patterns on a single gel as opposed to many different gels, which is one of the bottlenecks in data analysis (Bernhardt *et al.*, 1999; Hecker *et al.*, 2009)). Hence, dual channel imaging helps with providing a large quantity of information regarding the relative amount and rate of synthesis of each single protein (Bernhardt *et al.*, 2003).

Fig. 2. Dual channel image of autoradiograms (protein synthesis) of *B. subtilis* 168 (red) and the isogenic *sigB*-mutant strain ML6 (green), transformed with the Delta2D two-dimensional gel analysis software. Samples were taken from cultures in the transient growth phase. Already identified spots synthesized at a higher rate in the wild type are indicated by text labels, and nonidentified spots by white circles (taken from the article by Bernhardt *et al.*, 2003). For a list of protein spots that are induced in a σ^B-dependent manner refer to Bernhardt *et al.* (2003).

2D protein gel electrophoresis when used in conjunction with MALDI TOF mass spectrometry and dual channel imaging technique can provide a comprehensive outline of the physiology of a bacterial cell population entering the stationary growth phase, as has been demonstrated by Bernhardt and colleagues (2003) to evaluate and visualize the overall regulation of protein synthesis during glucose starvation. A sequential series of overlays obtained at specific points of the growth curve helps with visualization of the developmental processes at a proteomics scale. A substantial reprogramming of the protein synthesis pattern was found during glucose starvation, where 150 proteins were synthesized *de novo* and the synthesis of almost 400 proteins was terminated. These 150 proteins were identified as belonging to different general and specific regulation groups, which were

demonstrated by the analysis of mutants in genes encoding global regulators. Following glucose starvation, a series of proteins are induced that belong to two main regulatory groups: general stress/starvation responses induced by different stresses or starvation stimuli (σ^B-dependent general stress regulon, stringent response, sporulation), and glucose-starvation-specific responses (decline of glycolysis, utilization of alternative carbon sources, and gluconeogenesis). Hence, in addition to being able to identify stimulons and regulons by the dual channel approach, the fate of each single protein could also be followed by the three-color code system in this technique (Fig. 2) (Bernhardt *et al.*, 2003; Hecker *et al.*, 2009).

Mutant protein characterization using the gel-based proteomics approach was also recently used to understand the global regulation by the bacterial PyrR regulatory protein (Seul *et al.*, 2011). The enzymes involved in the denovo biosynthesis of uridine monophosphate (UMP) and cistrones encoding a uracil permease , together with the regulatory protein PyrR are encoded by the *B. subtilis* pyrimidine biosynthetic (*pyr*) operon. The PyrR is a bifunctional protein that possesses both *pyr* mRNA-binding regulatory function and uracil phosphoribosyltransferase activity (Seul *et al.*, 2011).

In a study by Seul and colleagues (2011), proteomic analysis of PyrR-associated protein expression in *B. subtilis* cells was carried out, where the cellular proteome of *B. subtilis* DB104 was compared with that of a *pyrR* knockout mutant (*PyrR Mut*) using a gel-based differential proteomic strategy. In order to have an insight into global regulation by the *pyrR* deletion, the cellular proteomes of both *B. subtilis* DB104 and *B. subtilis* DB104 ΔpyrR were compared following growth in minimal medium without pyrimidines. The proteins were analyzed by MALDI-TOF mass spectrometry and matched with database search. Variations in levels of expression of the cytosolic proteins in both strains were demonstrated by 2D-gel electrophoresis. Proteomic analysis revealed, approximately 1,300 spots, 172 of which showed quantitative variations; amongst the latter 42 high quantitatively variant proteins were identified on the silver stained 2D-gel with an isoelectric point (pI) between 4 and 10. These results showed that production of the pyrimidine biosynthetic enzymes (PyrAA, PyrAB, PyrB, PyrC, PyrD, and PyrF) were significantly increased in *B. subtilis* DB104 ΔpyrR (Seul *et al.*, 2011). Proteins associated with carbohydrate metabolism, elongation protein synthesis, metabolism of cofactors and vitamins, motility, tRNA synthetase, catalase, ATP–binding protein, and cell division protein FtsZ were also overproduced in the PyrR–deficient mutant. Analytical data suggests in addition to pyrimidine biosynthesis, the PyrR protein might be involved in a number of other metabolisms in the bacterial cell (Seul *et al.*, 2011).

In conclusion, this gel-based differential proteomics study enabled to elucidate an example of global regulation in the model bacterial strain *B. subtilis* affected by a gene deletion.

7. Microbial systematics and epidemiology

Classification and identification of microorganisms are traditionally carried out on the basis of cell morphology, cellular metabolism, cell structure and cell components such as fatty acids, carbohydrates and quinines. The uncertainty of conventional methods to identify the microbial strains has arisen from the lack of distinctive structures in most strains and the lateral gene transfer between some species. Modern microbial classification puts emphasis on molecular systematics, using genetic techniques like guanine-cytosine ratio determination, DNA hybridization, and sequencing of conserved genes such as the

ribosomal RNA gene. Molecular phylogenetics uses DNA sequences as information to build a relationship tree showing the possible evolution of various organisms. Application of molecular phylogenetics for organism's evolutionary relationship was pioneered in the 1970s by Charles G. Sibley. Recently new molecular technologies based on genomics and proteomics methods have been developed to identify and characterize bacteria. Although these new molecular technologies can be high throughput and rapid, they are counted as complement techniques for traditional methods (Emerson *et al.*, 2008).

Molecular techniques capable of typing bacteria on the basis of their proteomes are used for pathogenesis and epidemiological investigations (Cash, 2009). In 1990, Costas presented a new approach to bacterial typing by 1-D SDS PAGE. Costas and colleagues paid particular attention to analyze a whole cell protein using a high resolution SDS PAGE. Besides, they considered a detailed analysis of gel data by using high-resolution densitometers and software. Three groups of bacterial isolates belonging to *Acromobacter*, *Neisseriaceae* and *Providencia* were analyzed by this method, and subsequently the strategy of 1-D protein electrophoresis was widely used for typing of a number of bacterial isolates such as *Streptococcus porcinus*, *Bacillus thuringiensis*, *Helicobacter pylori*, etc (Duarte *et al.*, 2005; Konecka *et al* 2007; Costas *et al.*, 1990). The *Haemophilus influenzae* proteome was analyzed by 2-DE and extended to three other members of the *Haemophilus* genus in 1997. Data analysis was carried out by the Nonlinear Dynamics Phoretix 2D (version 3.1) analytical software (Cash *et al.*, 1997). The *E. coli* isolates collected from clinical specimens were also discriminated by 2-DE. This technique provides a high resolution large-scale screening for differentiation of bacterial isolates. Despite the advantages of 2-DE for taxonomic and epidemiological studies, 1-D SDS PAGE is mostly preferred because it is a simple and straightforward method (Cash, 2009).

Differential proteomics is very helpful for rapid detection of bacteria. Surface enhanced laser desorption ionization (SELDI)-time of flight which is a rapid MALDI MS based technology compatible with the ProteinChip Array has been developed. This is a valuable technique to determine the microbial phylogeny and discriminate different bacterial strains. The protein expression profiles of different strains of *Streptococcus pneumoniae* grown under different conditions were evaluated by SELDI analysis. This protocol was also successfully applied to a wide range of Gram positive and negative bacteria (Barzaghi *et al.*, 2004). This technology has a potential to be used as a complement to 2-DE gel electrophoresis for the purpose of microbial proteome analysis.

An MS-based proteomics approach for bacterial evolutionary studies has been recently presented. In this method, a bacterial proteome database was prepared from protein coding ORFs found in 170 fully sequenced bacterial genomes. By analysis of amino acid sequences of tryptic peptides obtained by LC-ESI MS/MS, phylogenetic profiles of these peptides were obtained. It has been shown that the application of this proteomic method is possible to classify the bacterial isolate at the strain level using sufficient amounts of sequence information derived from the MS/MS experiments (Dwornzanski *et al.*, 2006).

In a similar approach, differential proteomics can be used to compare single residues and oligopeptide compositions of the organisms' proteomes. The oligopeptides which are either universally over- or under-abundant, constituting overall properties of the proteomic landscape, as well as oligopeptides whose over- or under-abundance is phyla- or species-specific have been proposed. Principal component analysis (PCA) provides a new method to

study the landscape of compositional motifs among different species and deduce their phylogenetic relationships (Pe'er *et al.,* 2004).

The differential proteomics approach has been known as a powerful method for evaluating evolutionary relationships amongst prokaryotes or eukaryotes at different evolutionary units such as strains, species, genera and even kingdoms (Enard *et al.,* 2002, Roth *et al.,* 2009, Smithies & Poulik, 1956).

The proteome of different strains of bacteria can be analyzed to find the biomarkers related to various diseases. For this reason, a microbial proteomics database system was set up at the Max Planck Institute for Infection Biology based on 2DE/MS. Many studies have been undertaken to detect biomarkers for various conditions using differential proteomics. Distinguishing pathological from harmless bacteria and identification of a biomarker for a pathogen by LC MS and LC MS/MS analyses have been reported (Mini *et al.,* 2006, Mamone *et al.,* 2009). Certain related examples are presented as follows.

7.1 *Helicobacter pylori*

H. pylori eventually causes gastric/duodenal ulcers or even gastric cancer. *Helicobacter* infections are the reason for approximately one million people dying annually. Chemotherapy alone is not sufficient for *Helicobacter* eradication. Therefore, effective vaccines are considered as promising strategies to control this important pathogen. To develop a protective vaccine, a comprehensive list of all possible protein antigens has been provided by the *H. pylori* genome database (Alm *et al.,* 1999). Since the clinical isolates of *H. pylori* differ remarkably in their genome sequences, antigens for a subunit vaccine should be selected from a core set of 1281 genes (Salama *et al.,* 2000). It needs large-scale screening and clinical trials to find a protective *Helicobacter* antigen amongst all possible candidates (Ferrero & Labigne, 2001). To overcome this problem, global techniques such as DNA microarray and proteomics have been employed to identify the promising vaccine antigen subsets rapidly. The proteome of *H. pylori* has been analyzed by 2-DE and LC-MS (Bumann *et al.,* 2001, Govorun *et al.,* 2003). Immunoblotting of 2-DE gels using human sera has provided global information on the immunoproteome of *H. pylori* (Jungblut *et al.,* 2000). A comparative proteomic and immunoproteomic analysis has been carried out to identify the antigenic patterns of different *H. pylori* strains. The results have shown that immunoblotting is suitable as a diagnostic test (Mini *et al.,* 2006). Recently, 2-DE protein maps of *H. pylori* strain 10K, probed against single sera from *H. pylori*-positive patients and immunoreactive spots were identified by MALDI-TOF–MS (Lahner *et al.,* 2011). The *H. pylori* proteome, subproteomes including immunoproteomes, serotome and surface exposed proteins data are stored in a proteomics database (*http://www.mpiib-berlin.mpg.de/2D-PAGE/*) which is necessary for vaccine development. It has been shown that an antioxidant protein, alkylhydroperoxide reductase (AhpC), is an abundant and important antioxidant present in *H. pylori*. Oxidative stress-induced AhpC with chaperone activity *in vivo* was investigated by co-immunoprecipitation, 2-DE followed by nano-liquid chromatography coupled with tandem mass spectrometry (nanoLC-MS/MS). Consequently a significant correlation between the AhpC magnitude of inflammatory damage was detected by immunoblotting assays and endoscopic examinations. AphC was thus suggested as a biomarker for gastric patients (Huang *et al.,* 2011).

7.2 *Mycobacterium*

The *Mycobacterium tuberculosis* H37Rv genome encoding approximately 4000 proteins has been completely sequenced (Camus *et al.*, 2002). This suggests that *Mycobacterium* is an ideal model organism for proteomics. Proteome analysis of the virulent and attenuated mycobacterial strain has been carried out in order to identify those proteins having a significant role in its pathogenicity and persistence in the host (Schmidt *et al.*, 2004). Culture supernatant proteins of *M. tuberculosis* were analyzed by combination of high resolution 2-DE, MS-based techniques consisting of MALDI-MS peptide mass finger printing (PMF), ESI-MS/MS, MALDI-MS PMF and N-terminal sequencing by Edman degradation, and 137 different proteins were finally identified. A small set of protein-specific signature peptide masses was designated as the minimal protein identifier (MPI) upon database comparisons of MALDI spectra. The MPI approach takes into consideration the proposition of protein identity of two sample mass spectra. It is a powerful approach for both a reliable identification of low molecular mass proteins and protein fragments as well as tracking proteins in 2-DE gels. The MPI approach was successfully employed to identify the low molecular mass fragments of mycobacterial elongation factor EF-Tu (Tuf; Rv0685). The 14 kDa antigen (HspX; Rv2031c), the 10 kDa chaperon (GroEs; Rv3418c) and the conserved hypothetical protein Rv0569 of *M. tuberculosis* H37Rv were tracked in 2-DE gels by this approach (Mattow *et al.*, 2004).

The genome of *M. leprae*, an obligate intracellular pathogen causing the disease leprosy, has completely been sequenced mainly in order to identify those genes that are expressed during mycobacterial pathogenesis. Proteins from the cytosol and membrane subcellular fractions were separated by 2-DE and identified by mass spectrometry. The proteins identified in the membrane fraction were analyzed by ESI-MS/MS. They were mostly associated with protein synthesis, secretion and heat shock. Proteins present in the cytosol fraction were separated by 2-DE and 172 spots were analyzed by ESI-MS/MS. The complete list of proteins in both fractions has been described and those involved in virulence, adaptation, detoxification and intermediary metabolism have been identified (Marques *et al.*, 2004).

7.3 *Vibrio cholerae*

V. cholera, the causative agent of severe diarrheal disease, is a Gram-negative bacterium with two different physiological states, in the aquatic environment and in the human small intestine. The whole cell proteome of the *V. cholera* strain N16961 under anaerobic conditions, approximating the *in vivo* microenvironment, was separated by 2-DE and the protein spots compared with those in the aerobic environment. Under aerobic conditions, some proteins involved in substrate transport, amino acid metabolism and aerobic respiration were found to be abundant. The increased abundance of some proteins related to motility was observed when the bacterium was grown under anaerobic conditions, thus suggesting a meticulous correlation between *V. cholera* motility and pathogenesis (Kan *et al.*, 2004). This proteome analysis provides useful information for detection of the antigens by immunoproteomics for the purpose of vaccine development.

7.4 *Listeria monocytogenes*

The pathogen *L. monocytogenes* causes a severe food-borne infection leading to meningitis, encephalitis and spontaneous abortion in pregnant women. Surface proteins of pathogenic

bacteria mediate the main interactions between the bacterial cell and the host. The cell wall subproteome of *L. monocytogenes* was detected by 2-DE and then identified by N-terminal sequencing and MALDI mass fingerprinting after tryptic in-gel digestion and purification of the resulting peptides. Three proteins were found to have no orthologue in the nonpathogenic *L. innocua* and might be involved in virulence. Some cytoplasmic proteins such as enolase, glyceraldehyde -3-phosphate dehydrogenase, heat-shock factor DnaK and elongation factor TU were observed in the cell wall proteome unexpectedly. They have neither a secretion signal nor a known surface binding domain. Immunoelectron microscopy demonstrated that they are able to bind human plasminogen specifically. This may suggests that proteomic investigations are necessary to confirm the theoretical predictions of protein localization and function from genome sequence (Schaumburg *et al.,* 2004).

8. Evaluation of proteins involved in the toxic response

Proteomics can be employed to analyze the microbial or cell responses to an environmental change, such as different culture conditions. The regular stresses in nature are temperature, nutrients, oxygen and toxins. In differential proteomics, two or more sets of proteins from similar but distinct samples that are exposed to different conditions are compared. This is the main application of proteomics which can be used to identify biological markers (Lovrić, 2011). The Differential proteomics approach screens and analyzes proteins qualitatively and quantitatively in order to detect the differential proteins and identify them by mass spectrometric data.

Stimulon is a set of proteins whose amount or rate of synthesis changes in response to a single stimulus (Neidhardt *et al.,* 1990). Stimulons are directly identified using protein expression profiles as a quantitative list of proteins which are produced by cells under a given condition. A 2-DE gel run from a sample reveals most of a particular protein expression profile. The protein expression profile of a control condition can be compared with that seen in a test condition to identify the stimulon for that circumstance. Indeed, one of the prime objectives in proteomics is to define up- or down-regulated proteins when a cell is exposed to a certain stimulus. Regulon is a set of proteins whose synthesis is regulated by the same regulatory protein (VanBogelen *et al.,* 1999). The protein expression profiles of mutant strains can be compared to those of wild type strains in order to define proteins with the expression characteristics of a regulon member. Most stimulons consist of multiple regulons. For example, heat shock response of *E. coli* is controlled by at least two regulons of σ^S and σ^E (O'Connor *et al.,* 2000).

In medical microbiology, proteomics has a great role in defining the proteins synthesized by pathogenic bacteria following their exposure to eukaryotic cells. The synthesis of certain proteins has been found to be induced during cocultivation of bacteria with host cells. For example, the synthesis of the bacterial heat shock proteins is induced during *Brucella abortus* infection of bovine and murine macrophages. The changes in the patterns of *in vivo* gene expression of pathogens have been revealed using 2-DE combined with metabolic radiolabelling or immunoblotting (Cash, 2000). The expression pattern of proteins at higher levels in intracellular bacteria is generally similar to those induced in bacteria in response to stress conditions including extreme acidity, oxygen and high temperature. On the contrary, proteomic studies can be used to investigate the host response to microbial infections. It is possible to compare uninfected and infected whole protein patterns using 2-DE. This can be

used to find biological biomarkers. The identification of immunogenic proteins by using 2-DE, immunoblotting and polyclonal sera will significantly aid in vaccine development.
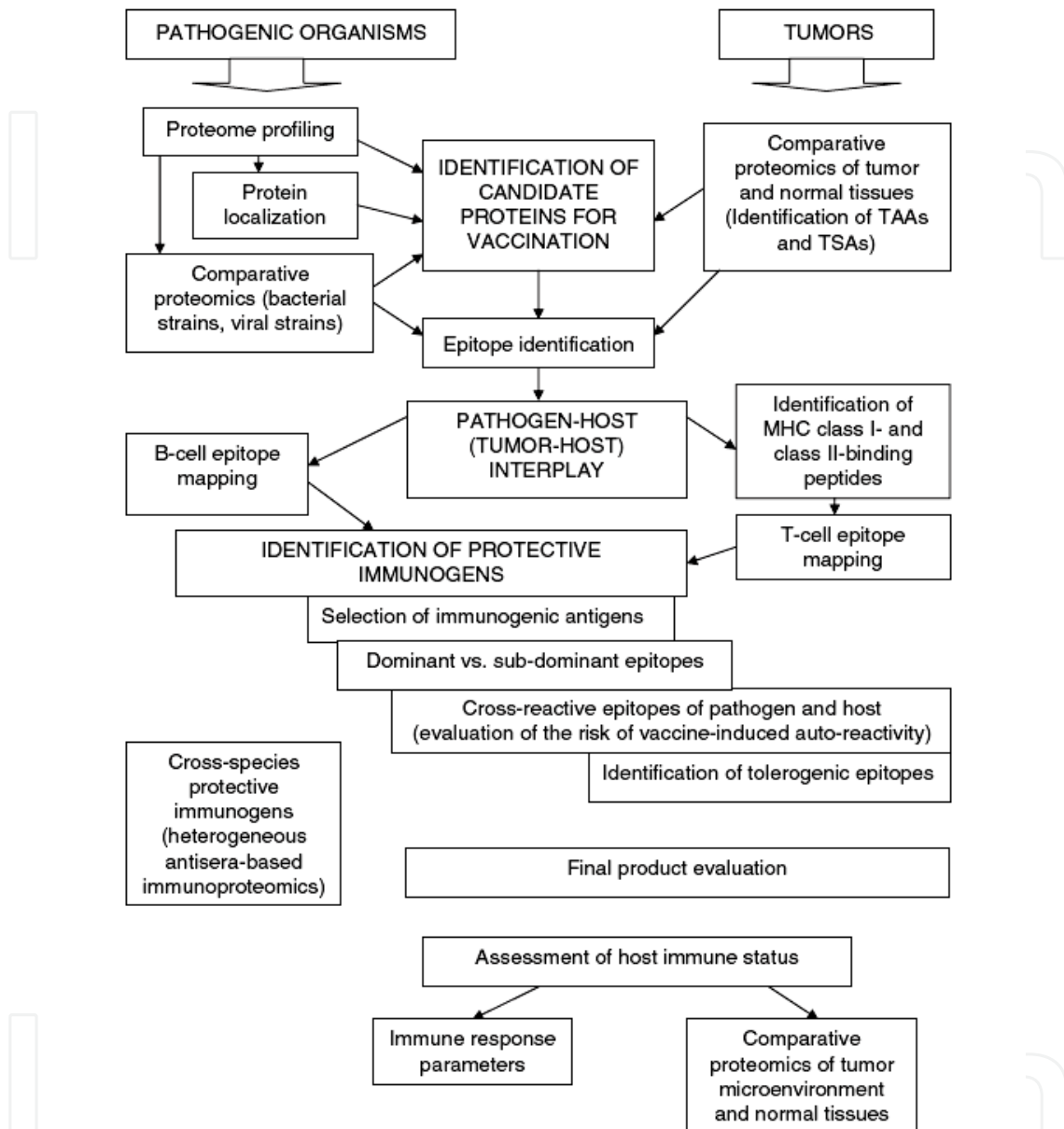

Fig. 3. Proteomic applications in vaccine development (From Adamczyk-Poplawska *et al.*, 2011).

One of the major problems for the control of infection is bacterial resistance to antibiotics. Bacteria recognize drugs and antibiotics as toxic materials and produce several proteins in response to these stresses. High resolution 2-DE has been employed to investigate the beta-lactam antibiotics-resistance in *Pseudomonas aeruginosa* and *S. pneumoniae* (Cash, 2000). Identification of those proteins involved in drug resistance will lead to improvements in future antimicrobials. In a comparative study, proteome of *H. influenza* in response to Ro-64-1874, a 2,4-diaminopyrimidine derivative like trimethoprim as dihydrofulate reductase inhibitor, and to standard antibiotics, such as trimethoprim and sulfamethoxazol were

analyzed. The expression profile resulting from exposure to Ro-64-1874 showed a good correlation with those in the database in response to standard antibiotics. Thus, the effectiveness of this new antimicrobial drug was confirmed (Grag & Keck, 1999). In this case, the identification of up- or down-regulated proteins is unimportant. Having a database of responses derived from known compounds is generally sufficient in the study of structure-function relationship. But more detailed analysis is required for compounds with novel modes of action.

Proteomic/immunoproteomic analyses of *H. pylori*, *Neisseria menangitidis*, *Streptococcus pyogenes*, *Bacillus anthracis* and *M. tuberculosis* have been carried out to find new potential vaccine candidates. A large data set has been provided by proteomic studies of different strains and a lot of potentially useful antigens have been introduced as vaccine candidates. However, the data derived from proteome analysis are not consistent. It may be due to the pan-genome, defined as a species gene pool, of some bacterial species being open (Adamczyk-Poplawska *et al.*, 2011). Recent vaccine developments using proteomic studies are summarized in Figure 3.

9. Concluding remarks

The relative simplicity of microbial cells has made them an attractive target for numerous extensive experiments directed toward understanding the physiology and function of the smallest part of life, in a quest for improved disease prevention and treatment and production of valuable metabolites used in various industries. Following decades of study on microbial systems that are considered as separate compartments, this is the new era of – omics to examine the holistic behavior of a microbial cell.

In this chapter, we focused on proteomics and its applications to microbial systems. Proteome (protein complement of a genome) should help to unravel biochemical and physiological mechanisms at the functional molecular level. We explained how the basic technology of 2-DE can be employed for the separation and characterization of proteins, identification of PTMs and detection of protein-protein interactions. This technique followed by powerful methods of data analysis has a remarkable role in manipulation of metabolic pathways, especially for the improvement of the yield and productivity of microbial products. Furthermore, microbial systematics and epidemiology can be studied by molecular technologies based on genomics and proteomics. The approach of differential proteomics, which compares the distinct proteomes of cells exposed to different conditions, such as normal versus treated ones, could be very helpful in the detection of any environmental change or stress. This review represents the extensive applications of 2-DE in the various areas of the basic science of biology, e.g., cell physiology and molecular biology as well as the applied science of biology, *viz.* medical and industrial microbiology.

10. Future perspectives

The fundamental goal of molecular biological research is to determine the function of genes, the role of proteins in metabolic pathways and networks and finally provide a detailed understanding of how these molecules interact and collaborate to work a biological system under different conditions. This is achieved by considering biological systems as a whole and not individual part, which is referred to as systems biology. The new biology is rapidly

growing through the concept of –omics. There are strict relations among the major –omics consisting of genomics, metabonomics and proteomics. The sub-groups of these major fields of –omics study the specific interactions/processes/molecules quantitatively and qualitatively. The entire data derived from the specific groups are necessary to achieve an understanding of the properties of a whole cell or system.

Obviously, proteomics provides more applicable data than genomics, although genomics is a pre-requisite for proteomics. The new –omics derived from proteomics are coming into being as peptidomics, glycomics, phosphoproteomics, interactomics etc. that try to supply more detailed information in the fields of function, regulation and interaction of peptides/proteins.

Two dimensional gel electrophoresis is still widely accepted as the powerful method capable of separating proteins from highly complex samples. However, sophisticated devices, authoritative techniques and dominant mathematical methods have been developed to analyze proteomics data. For example, the dual channel imaging technique for 2-DE analysis accompanied with MALDI-TOF mass spectrometry can provide comprehensive information of a proteome. Because of the remarkable capabilities of proteomics in the enhancement of our knowledge regarding the qualitative and qualitative properties of a whole cell, it has been employed as a beneficial tool in microbiological research. Those applications of proteomics in this field of study, as mentioned in this chapter, pave the way for improvement of new products by these mico-factories through deep information on microbial physiology, responses and interactions obtained from proteomic data. Therefore, there is no doubt that holistic information contributed by the major –omics is essentially required for the better understanding of a microbial cell.

11. References

Adamczyk-Poplawska, M., Markowicz, S., & Jagusztyn-Krynickac, E.K., (2011). Proteomics for development of vaccine. *Journal of proteomics*, doi:10.1016/j.jprot.2011.01.019

Alm, R. A., Ling, L.S., Moir, D.T., King, B.L., Brown, E.D., Doig, P.C., Smith, D.R., Noohan, B., Guild, B.C., DeJonge, B.L., Carmel, G., Tummino, P.J., Caruso, A., Uria-Nickelsen, M., Mills, D.M., Ives, C., Gibson, R., Merberg, D., Mills, S.D., Jiang, Q., Taylor, D.E., Vovis, G.F., & Trust, T.J., (1999). Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. *Nature*, Vol. 397, No. 6715, pp. 176-180.

Amycolatopsis balhimycina Proteomic Project. [http://www.unipa.it/ampuglia/Abal-proteome-maps].

Anderson, N.G., & Anderson, L., (1998). Proteome and proteomics: new technologies, new concepts, and new words. *Electrophoresis*, Vol. 19, No. 11, pp. 1853-1861.

Antelmann, H., Tjalsma, H., Voigt, B., Ohlmeier, S., Bron, S., van Dijl, J.M., and Hecker, M. 2001. A proteomic view on genome-based signal peptide predictions. *Genome Research*, Vol. 11, pp. 1484–1502.

Bader, G.D., Betel, D., & Hogue, C.W.V., (2003). BIND: the biomolecular interaction Network Database. *Nucleic Acids Research*, Vol. 31, No. 1, pp. 248–250.

Barzaghi, D., Isbister, J.D., Lauer, K.P., & Born, T.L., (2004) Use of surface enhanced LASER desorption/ionization- time of flight to explore bacterial proteome. *Proteomics*, Vol. 4, No. 9 , pp. 2624-2628.

Benson D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., & Wheeler, D.L., (2006). GenBank , *Nucleic Acids Research*, Vol. 34, Database Issue, pp. 16 – 20.

Bernhardt, J., Bu¨ttner, K., Scharf, C., and Hecker, M. 1999. Dual channel imaging of two-dimensional electropherograms in *Bacillus subtilis*. *Electrophoresis*, Vol. 20, pp. 2225–2240.

Bernhardt , J ., Weibezahn, J., Scharf, C., Hecker, M. (2003). *Bacillus subtilis* During Feast and Famine: Visualization of the Overall Regulation of Protein Synthesis During Glucose Starvation by Proteome Analysis. *Genome Research,* Vol. 13, pp. 224–237

Blattner, F.R., Plunkett III, G., Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F., Gregor, J., Davis, N.W., Kirkpatrick, H.A., Goeden, M.A., Rose, D.J., Mau, B., & Shao, Y., (1997). The complete genome sequence of *Escherichia coli* K-12. *Science*, Vol. 277, No. 5331, pp. 1453-1474.

Bro, C., & Nielsen, J., (2004). Impact of 'ome' analyses on inverse metabolic engineering. *Metabolic Engineering*, Vol. 6, No. 3, pp. 204-211.

Bumann, D., Meyer, T.F., & Jungblut, P.R., (2001). Proteome analysis of the common human pathogen *Helicobacter pylori*. *Proteomics*, Vol. 1, No. 4, pp. 473-479.

Bunai, K., & Yamane, K., (2005). Effectiveness and limitation of two-dimensional gel electrophoresis in bacterial membrane protein proteomics and perspectives. *Journal of Chromatography B*, Vol. 815, No. 1-2, pp. 227-236.

Büttner, K., Scharf, C., Bernhardt, J., Vo¨lker, U., & Hecker, M., 2001. A comprehensive two-dimensional map of cytosolic proteins of *Bacillus subtilis*. *Electrophoresis* 22: 2908–2935.

Cameron, D. C., Tong, I. T. 1993. Cellular and metabolic engineering. An overview. *Applied Biochemistry and Biotechnology* 38(1–2):105–140

Camus, J.C., Pryor, M.J, Medigue, C, & Cole, S.T., (2002). Re-annotation of the genome sequence of *Mycobacterium tuberculosis* H37Rv. *Microbiology*, Vol. 148, No. 10, pp. 2967-2973.

Cash, P., (2000). Proteomics in medical microbiology. *Electrophoresis,* Vol. 21, No. 6, pp. 1187-1201.

Cash, P., (2009). Proteomics in the study of the molecular taxonomy and epidemiology of bacterial pathogens. *Electrophoresis*, Vol. 30, No. S1, pp. S113-S141.

Cash, P., Argo, E., Langford, P.R., & Kroll J.S., (1997). Development of a *Haemophilus* two-dimensional protein database. *Electrophoresis*, Vol. 18, No. 8, pp. 1472-1482.

Cheng, Z., Wei, Y. Y. C., Sung, W. W. L., Glick, B. R., McConkey, B. J. (2009). Proteomic analysis of the response of the plant growth-promoting bacterium *Pseudomonas putida* UW4 to nickel stress.*Proteome Science,* Vol 7, pp. 18-26.

Cohen, P., (2000). The regulation of protein function by multisite phosphorylation, a 25 years update. *Trends in Biochemical Sciences*, Vol. 25, No. 12, pp. 596-601.

Coppee, J. Y., Auger, S., Turlin, E., Sekowska, A.,   Le Caer, J. P., Labas, V., Vagner, V., Danchin, A.,   Martin Verstraete, I. (2001). Sulfur-limitation-regulated proteins in *Bacillus subtilis*: a two-dimensional gel electrophoresis study. *Microbiology*, Vol. 147, pp. 1631-1640.

Costas, D.M., (1990). Numerical analysis of sodium dodecyl sulphatepolyacrylamide gel electrophoretic protein patterns for the classification, identification and typing of medically important bacteria. *Electrophoresis*, Vol. 11, No. 5, pp. 382-391.

Costas, M., Morgan, D.D., Owen, R.J., & Morgan, D.R., (1991). Differentiation of strains of Helicobacter pylori by numerical analysis of 1-D SDS-PAGE protein patterns: Evidence for posttreatment recrudescence. *Epidemiology and infection*, Vol. 107, No. 3, pp. 607-617.

Cox, K. H., Ruiz-Bustos, E., Courtney, H. S., Dale2, J. B., Pence5, M. A., Nizet, V., Aziz, R. K., Gerling, I., Price, S. M., Hasty, D. L. (2009). Inactivation of DltA Modulates Virulence Factor Expression in *Streptococcus pyogenes*. *PLoS One*, Vol.4, No. 4, pp. e5336 (1-10).

Dressaire, C ., Gitton, C ., Loubière, P ., Monnet, V ., Queinnec, I ., Cocaign-Bousquet, M. (2009). Transcriptome and Proteome Exploration to Model Translation Efficiency and Protein Stability in *Lactococcus lactis*. *PloS Computational Biology*, Vol. 5:e1000606.

Duarte, R.S., Barros, R.R., Facklam, R.R., & Teixeira, L.M., (2005). Phenotypic and genotypic characteristics of *Streptococcus porcinus* isolated from human sources. *Journal of Clinical Microbiology*, Vol. 43, No. 9, pp. 4592-4601.

Dunn, M.J. (2000). From genome to proteome: Advances in the practice and application of proteomics, Wiley-VCH.

Dunn, M.J., & Görg, A., (2001). *Two-dimensional polyacrylamide gel electrophoresis for proteome analysis*, in Proteomics, From Protein Sequence to function (Pennington, S.R., & Dunn, M.J., eds. BIOS Scientific Publisher, Oxford, pp: 43-63.

Dwornzanski, J.P., Deshpande, S.V., Chen, R., Jabbour, R.E., Snyder, A.P., Wick, C.H., & Li, L., (2006). Mass spectrometry-based proteomics combined with bioinformatic tools for bacterial classification. *Journal of Proteome Research*, Vol. 5, No. 1, pp. 76-87.

Egan, S., James, S., Kjelleberg, S. (2002).Identification and Characterization of a Putative Transcriptional Regulator Controlling the Expression of Fouling Inhibitors in *Pseudoalteromonas tunicate*. *Applied and Environmental Microbiology*, Vol. 68, No. 1, pp. 372-378

Emerson, D., Agulto, L., Liu, H., & Liu, L., (2008). Identifying and characterizing of bacteria in an era of genemoics and proteomics. *Bioscience*, Vol. 58, No. 10, pp. 925-936.

Enard, W., Khaitovich, P., Klose, J., Zöllner, S., Heissig, F., Giavalisco, P., Nieselt-Struwe, K., Muchmore, E., Varki, A., Ravid, R., Doxiadis, G.M., Bontrop, R.E., & Pääbo, S., (2002). Intra- and interspecific variation in primate gene expression patterns. *Science*, Vol. 296, No. 5566, pp. 340-343.

Fagerquist, C. K., Miller, W.G., Harden, L.A., Bates, A.H., Vensel, W.H., Wang, G.L., & Manderll, R.E., (2005). Genomic and proteomic identification of a DNA binding protein used in the fingerprinting of Campylobacter species and strains by MALDI-TOF-MS protein biomarker analysis. *Analytical Chemistry*, Vol. 77, No. 15, pp. 4897-4907.

Ferrero, R.L., & Labigne A., (2001). *H. pylori* vaccine development in the post-genomic era: can *in silico* translate to *in vivo*? *Scandinavian Journal of Immunology*, Vol. 53, No. 5, pp. 443-448.

Frantz, J., & Mccallum, R., (1980). Changes in macromolecular composition and morphology of *Bacteriodes fragilis* cultured in a complex medium. *Applied and Environmental Microbiology*, Vol. 39, No. 2, pp. 445 – 448.

Freed, J.K., Smith, J.R., Li, P., & Greene, A.S., (2007). Isolation of Signal transduction complex using biotin and cross linking methodologies. *Proteomics,* Vol. 7, No. 14, pp. 2371 – 2374.

Friedman, D. B., Stauff, D. L., Pishchany, G., Whitwell, C. W., Torres, V. J., Skaar, E. P. (2006). *Staphylococcus aureus* Redirects Central Metabolism to Increase Iron Availability. *PLoS Pathogens*, Vol. 2, No. 8, pp. 0777-0789.

Gallo, G., Alduin, R., Renzone, G., Thykaer, J., Bianco, L., Eliasson-Lantz, A., Scaloni, A., Puglia, A.M. (2010). Differential proteomic analysis highlights metabolic strategies associated with balhimycin production in *Amycolatopsis balhimycina* chemostat cultivations. *Microbial Cell Factories , Vol. 9*, pp. 95-109.

Giot, L., Bader, J.S., Brouwer, C., Chaudhuri, A., Kuang B, Li Y, Hao YL, Ooi CE, Godwin B, Vitols E, Vijayadamodar G, Pochart P, Machineni H, Welsh M, Kong Y, Zerhusen B, Malcolm R, Varrone Z, Collis A, Minto M, Burgess S, McDaniel L, Stimpson E, Spriggs F, Williams J, Neurath K, Ioime N, Agee M, Voss E, Furtak K, *et al.* (2003). A protein interaction map of *Drosophila melanogaster. Science*, Vol. 302, pp. 1727-1736.

Görg, A., Weiss, W., & Dunn, M.J., (2004). Current two dimensional electrophoresis technology for proteomics. *Proteomics*, Vol. 4, No. 12, pp. 3665–3685.

Govorun, V.M., Moshkovskii, S.A., Tikhonova, O.V., Goufman, E.I., Serebryakova, M.V., Momynaliev, K.T., Lokhov, P.G., Khryapova, E.V., Kudryavtseva, L.V., Smirnova, O.V., Toropyguine, I.Y., Maksimov, B.I., & Archakov, A.I. (2003). Comparative Analysis of Proteome Maps of *Helicobacter pylori* Clinical Isolates. *Biochemistry*, Vol. 68, No. 8, pp. 42-49.

Gray, C.P., & Keck, W., (1999). Bacterial Targets and antibiotics: genome-based drug discovery. *Cellular and Molecular Life Sciences*, Vol. 56, No. 9-10, pp. 779-787.

Guidi, F., Magherini, F., Gamberi, T., Borro, M., Simmaco, M., & Modesti, A., (2010). Effect of different glucose concentrations on proteome of *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta*, Vol. 1804, No. 7, pp. 1516-1525.

Gupta, N., Tanner, S., Jaitly, N., Adkins, J.N., Lipton, M., Edwards, R., Romine, M., Osterman, A., Bafna, V., Smith, R.D., & Pevzner, P.A., (2007). Whole proteome analysis of post-translational modifications, applications of mass spectroscopy for proteomic annotation. *Genome Research*, Vol. 17, No. 9, pp. 1362–1377.

Gygi, S.P., Rist, B., Griffin, T.G., Eng, J., & Aebersold, R., (2002). Proteome analysis of low-abundance proteins using multi-dimensional chromatography and isotope-coded affinity tags. *Journal of Proteome Research*, Vol. 1, No. 1, pp. 47-54.

Halligan, B.D., (2009). ProMoSt: a tool for calculating the pI and molecular mass of phosphorylated and modified proteins of two dimensional gels. *Methods in Molecular Biology*, Vol. 527, pp. 283-298.

Halligan, B.D., Rootti, V., Jin, W., Laffoo, S., Twigger, S.N., & Dratz, E.A., (2004). ProMoSt [protein Modification screening tool] a web-based tool for mapping protein modification of two-dimensional gels. *Nucleic Acids Research*, Vol. 32, pp. w638-w644.

Han, M. J., Yoon, S.S., Lee, S. Y. (2001). Proteome Analysis of Metabolically Engineered Escherichia coli Producing Poly(3-Hydroxybutyrate). *Journal of Bacteriology , Vol. 183*, No.1, pp. 301–308.

Han, M. J., Yoon, S.S., Lee, S. Y. (2011). Understanding and engineering of microbial cells based on proteomics and its conjunction with other omics studies. *Proteomics , Vol. 11 No. 4*, pp. 721–743.

Hart, C., Schulenberg, B., Steinberg, T.H., Leung, W.Y., & Patton, W.F., (2003). Detcction of glycoproteion in polyacrglamide gels and on electroblots using pro- Q Emerld 488 dye, a fluorescent periodate schiff-base stain. *Electrophoresis*, Vol. 24, No. 4, pp. 588-598.

Harvey, D.I., (2001). Identification of protein-bound carbohydrates by mass spectroscopy. *Proteomics*, Vol. 1, No. , pp. 311–328.

Hecker, M. and Völker, U. 1998. Non-specific, general and multiple stress resistance of growth-restricted *Bacillus subtilis* cells by the expression of the _B regulon. *Molecular Microbiology*, 29: 1129–1136.

Hecker, M., Reder, A., Fuchs, S., Pagels, M., Engelmann, S. (2009). Physiological proteomics and stress/starvation responses in *Bacillus subtilis* and S*taphylococcus aureus*. *Research in Microbiology*, Vol. 160, pp. 245-258.

Helenius, A., & Aebi, M., (2001). Intracellular functions of N-linked glycans. *Science*, Vol. 291, No. 5512, pp. 2364-2369.

Hipkiss, A.R., (2006). Accumulation of altered proteins and ageing: causes and effects. *Experimental Gerontology*, Vol. 41, No. 5, pp. 464-473.

Ho, L., Gineste, C., & Pompl, P.N., (2002). Expression of Psoriasin and Xystine C in the CSF of early Alzheimer's disease. The 2nd Annual Meeting of the Society of Neuroscience. Orlando FL.

Huang, C.H., Chuang, M.H., Lo, W.L., Wu, M.S., Wu, Y.H., Wu, D.C., & Chiou, S.H., (2011). Alkylhydroperoxide reductase of *Helicobacter pylori* as a biomarker for gastric patients with different pathological manifestations. *Biochimie*, Vol. 93, No. 7, pp. 1115-1123.

Isarankura-Na-Ayudhya, C., Panpumthong, P., Tangkosakul, T., Boonpangrak, S., Prachayasittikul, V. (2008). Shedding Light on the Role of Vitreoscilla Hemoglobin on Cellular Catabolic Regulation by Proteomic Analysis. *International Journal of Biological Sciences* , Vol. 4, No. 2, pp. 71-80.

Jensen, N.B.S., C. R. Melchiorsen, K. V. Jokumsen, and J. Villadsen. (2001). Metabolic behavior of *Lactococcus lactis* MG1363 in microaerobic continuous cultivation at a low dilution rate. *Applied and Environmental Microbiology*, 67:2677–2682.

Jensen, O.N., (2004). Modification - specific proteomics: characterization of post-translational modification by mass spectrometry. *Current Opinion in Chemical Biology*, Vol. 8, No. 1, pp. 33-41.

Jönsson, M., Z. Saleihan, I. F. Nes, and H. Holo. 2009. Construction and characterization of three lactate dehydrogenase-negative *Enterococcus faecalis* V583 mutants. *Applied and Environmental Microbiology*, Vol. 75, pp. 4901–4903.

Jungblut, P.R., Bumann, D., Hass, G., Zimny-Arndt, U., Holland, P., Lamer, S., Seijak, F., Aebischer, A., & Meyer T.F., (2000). Comparative proteome analysis of *Helicobacter pylori*. *Molecular Microbiology*, Vol. 36, No. 3, pp. 710-725.

Jungblut, P.R., & Hecker, M., (2007). *Proteomics of microbial pathogens*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.

Kan, B., Habibi, H., Schmid, M., Liang, W., Wang, R., Wang, D., & Jungblut, P. R., (2004). Proteome comparison of *Vibrio cholerae* cultured in aerobic and anaerobic conditions. *Proteomics*, Vol. 4, No. 10, pp. 3061-3067.

Kint, G., Sonck, K. A. J., Schoofs, G., De Coster, D., Vanderleyden, J., De Keersmaecker, S. C. J. (2009). 2D proteome analysis initiates new Insights on the *Salmonella* Typhimurium LuxS protein. *BMC Microbiology*, Vol. 9, pp. 198-210

Klose, J., (1975). Protein mapping by combined isoelectric focusing and electrophoresis in mouse tissues. A novel approach to testing for induced point mutations in mammals. *Humangenetik*, Vol. 26, No. , pp. 231–243.

Klose, J. and Kobalz, U. 1995. Two-dimensional electrophoresis of proteins: An updated protocol and implications for a functional analysis of the genome. *Electrophoresis*, Vol. 16, pp. 1034–1059.

Konecka, E., Kaznowski, A., Ziemnicka, J., & Ziemnicki, K., (2007). Molecular and phenotypic characterisation of *Bacillus thuringiensis* isolated during epizootics in *Cydia pomonella L. Journal of Invertebrate Pathology*, Vol. 94, No. 1, pp. 56-63.

Krogen, N.J., Cagney, G., Yu, H., Zhong, G., Guo, X., Ignatchenko, A., Li, J., Pu, S., Datta, N., Tikuisis, A.P., Punna, T., Peregrín-Alvarez, J.M., Shales, M., Zhang, X., Davey, M., Robinson, M.D., Paccanaro, A., Bray, J.E., Sheung, A., Beattie, B., Richards, D.P., Canadien, V., Lalev, A., Mena, F., Wong, P., Starostine, A., Canete, M.M., Vlasblom, J., Wu, S., Orsi, C., Collins, S.R., Chandran, S., Haw, R., Rilstone, J.J., Gandi, K., Thompson, N.J., Musso, G., St Onge, P., Ghanny, S., Lam, M.H., Butland, G., Altaf-Ul, A.M., Kanaya, S., Shilatifard, A., O'Shea, E., Weissman, J.S., Ingles, C.J., Hughes, T.R., Parkinson, J., Gerstein, M., Wodak, S.J., Emili, A., & Greenblatt, J.F., (2006). Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. *Nature*, Vol. 440, No. 7084, pp. 637–643.

Kunst, F., Ogasawara, N., Moszer, I., Albertini, A.M., Alloni, G., Azevedo, V., Bertero, M.G., Bessieres, P., Bolotin, A., Borchert, S., et al. (1997). The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis. Nature*, Vol. 390, pp. 249–256.

Kuster, B., Krogh ,T.N., Mortz, E., & Harvay, D.J., (2001). Glycosylation analysis of gel separated proteins. Proteomics, Vol. 1, No. 2, pp. 350-361.

Lahner, E., Bernardini, G., Possenti, S., Renzone, G., Scaloni, A., Santucci A., & Annibale B., (2011). Immunoproteomics of *Helicobacter pylori* infection in patients with atrophic body gastritis, a predisposing condition for gastric cancer. *International Journal of Medical Microbiology*, Vol. 301, No. 2, pp. 125-132.

Link, A.J., Hays, L.G, Carmack, E.B., & Yates III, J.R., (1997a). Identifying the major proteome components of *Haemophilus influenzae* type-strain NCTC 8143. *Electrophoresis*, Vol. 18, No. 8, pp. 1314-1334.

Link, A.J., Robinson, K., & Church, G.M., (1997b). Comparing the predicted and observed properties of proteins encoded in the genome of *Escherichia coli* K-12. *Electrophoresis*, Vol. 18, No. 8, pp. 1259-1313.

Lovrić, J., (2011). *Introducing proteomics, from concepts to sample separation, mass spectrometry and data analysis*, Wiley-Blackwell, pp. 1-20.

Madian, A.G., & Regnier, F.E., (2010). Proteomic identification of carbonylated proteins and their oxidative sites. Journal of Proteome Research, Vol. 9, No. 8, pp. 3766-3780.

Mamone, G., Picariello, G., Caira, S., Addeo, F., & Ferranti, P., (2009), Analysis of food proteins and peptides by mass spectrometry-based techniques. *Journal of Chromatography A*, Vol. 1216, No., 43, pp. 7130-7142.

Mann, M., & Jensen, O.N., (2003). Proteomic analysis of post-translational modifications. *Nature Biotechnology*, Vol. 21, No. 3, pp. 255-261.

Marcotte, E.M., (2000). Computational genetics: finding protein function by nonhomology methods. *Current Opinion in structural Biology*, Vol. 10, No. 3, pp. 359-365.

Marques, M.A.M., Espinosa, B.J., Xavier da Silveira, E.K., Pessolani, M.C.V., Chapeaurouge, A., Perales, J., Dobos, K.M., Belisle, J.T., Spencer J.S., & Brennan, P.J., (2004). Continued proteomic analysis of *Mycobacterium leprae* subcellular fractions. *Proteomics*, Vol. 4, No. 10, pp. 2942–2953.

Martinez-Moya, P., Watt, S. A., Niehaus, K., Alcaíno, J., Baeza, M., Cifuentes, V. (2011). Proteomic analysis of the carotenogenic yeast Xanthophyllomyces dendrorhous. *BMC Microbiology*, Vol. 11, pp. 131-144.

Mattow, J., Schmidt, F., Höhenwarter, W., Siejak, F., Schaible, U.E., & Kaufman, S.H.E., (2004). Protein identification and tracking in two-dimensional electrophoretic gels by minimal protein identifiers. *Proteomics*, Vol. 10, No. 10, pp. 2927-2941.

Mehmeti, I., Jo¨nsson, M., Fergestad, E. M., , Mathiesen, G., Nes, I. F., Holo, H. (2011). Transcriptome, Proteome, and Metabolite Analyses of a Lactate Dehydrogenase-Negative Mutant of Enterococcus faecalis V583. *Applied and Environmental Microbiology* , Vol. 77, No. 7, pp. 2406–2413.

Melchiorsen, C. R., K. V. Jokumsen, J. Villadsen, H. Israelsen, and J. Arnau. (2002). The level of pyruvate-formate lyase controls the shift from homolactic to mixed-acid product formation in *Lactococcus lactis*. *Applied Microbiology and Biotechnology*, Vol. 58, pp. 338–344

Mini, R., Bernardini, G., Salzano, A.M., Renzone, G., Scaloni, A., Figura, N., & Santucci A., (2006). Comparative proteomics and immunoproteomics of *Helicobacter pylori* related to different gastric pathologies. *Journal of Chromatography B*, Vol. 833, No. 1, pp. 63-79.

Msadek, T. 1999. When the going gets tough: Survival strategies and environmental signaling networks in *Bacillus subtilis*. *Trends in Microbiology.* Vol. 7, pp. 201–207.

Neidhardt. (1990). *Physiology of the bacterial cell: A molecular approach.* Sunderland: Sinauer Publishing.

Nyman, T.A., (2001). The role of mass spectroscopy in protein studies. *Biomolecular Engineering*, Vol. 18, No. 5, pp. 221-227.

O'Connor, C.D., Adams, P., Alefounder, P., Farris, M., Kinsella, N., Li, Y., Payot, S., & Skipp, P., (2000). The analysis of microbial proteomes: Strategies and data exploitation. *Electrophoresis*, Vol. 21, No. 6, pp. 1178-1186.

O'Farrell, P.H., (1975). High resolution two-dimensional electrophoresis of proteins. *The Journal of Biological Chemistry*, Vol. 250, pp. 4007-4021.

Osman, K.M., Ali, M.M., Radwan, M.J., Kim, H.K., & Han, J., (2009). Comparative proteomic analysis on *Salmonela gallinarum* and *Salmonela enteritidis* exploring protein that may incorporate host adaptation in poultry. *Journal of Proteomics*, Vol. 72, No. 5, pp. 815 – 821.

Pandey, A., & Mann, M. (2000). Proteomics to study genes and genomes. *Nature*, Vol. 405, No. 6788, pp. 837-846.

Panmanee, W., Gomez, F., Witte, D., Pancholi, V., Britigan, B. E., Hassett, D. J. (2008). The Peptidoglycan-Associated Lipoprotein OprL Helps Protect a *Pseudomonas aeruginosa* Mutant Devoid of the Transactivator OxyR from Hydrogen Peroxide-Mediated Killing during Planktonic and Biofilm Culture. *Journal of Bacteriology,* Vol.190, No. 10, pp. 3658-3669.

Paradela, A., & Albar, J.P., (2008). Advances in the analysis of protein phosphorylation. *Journal of Proteome Research*, Vol. 7, No. 5, pp. 1809-1818.

Parker, J.L., Jones, A.M.E., Serazdinove, L., Sa'albach, G., Bibb, M.J., & Naldrett, M.J., (2010). Analysis of the phosphoproteome of the multicellular bacterium *Streptomyses coelicolor* A3(2) by protein/peptide fractionation. *Proteomics*, Vol. 10, No. 13, pp. 2486-2497.

Pe'er, I., Felder, C.E., Man, O., Silman, I., Sussman, J.L., & Beckman, J.S., (2004). Proteome signatures: amino acid and oligopeptide compositions differentiate among phyla. *Proteins*, Vol. 54, No. 1, pp. 20-40.

Pellitteri-Hahn, M.C., Halligan, B.D., Scalf, M., Smidth, L., & Hickey, W., (2011). Quantitative proteomic analysis of the chemolithoautotrophic bacterium *Nitrosomonas euopaea*: comparison of growing- and energy-starved cells. *Journal of Proteomics*, Vol. 74, No. 4, pp. 411-419.

Phizicky, E., Bastiaens, P.I.H., Zhu, H., Snyder, M., & Fields, S., (2003). Protein analysis on a proteomic scale. *Nature*, Vol. 422, pp. 208-212.

Reinders, J., & Sickmann, A., (2005). State of the Art in phosphoproteomic. *Proteomics*, Vol. 5, No. 16, pp. 4052–4061.

Renzone, G., D'Ambrosio, C., Arena, S., Rullo, R., Ledda, L., Ferrara, L., Scaloni, A. (2005). *Ann Ist Super Sanità* Vol. 41 No. 4, pp. 459-468.

Roth, S., Fromm, B., Gäde, G., & Predel, R., (2009). A proteomic approach for studying insect phylogeny: CAPA-peptides of ancient insect taxa (Dictyoptera, Blattoptera) as a test case. *BMC Evolutionary Biology*, Vol. 9, pp. 50-62.

Rudd, P.M., Elliott, T., Cresswell, P., Wilson, I.A., & Dwek, R.A., (2001). Glycosylation and the immune system. *Science*, Vol. 291, No. 5512, pp. 2370-2376.

Salama, N., Guillemin, k., McDaneil, T.K., Sherlock, G., Tompkins, L. & Falkow, S., (2000). A whole-genome microarray reveals genetic diversity among *Helicobacter pylori* strains. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 97, No. 26, pp. 14668-14673.

Schäffer, C., Graninger, M., & Messner, P., (2001). Prokaryotic glycosylation. *Proteomics*, Vol. 1, No. 2, pp. 248-261.

Schaumburg, J., Diekmann, O., Hagendorff, P., Bergmann, S., Rohde, M., Hammerschmidt, S., Jänsch, L., Wehland, J., & Kärst, U., (2004). The cell wall subproteome of *Listeria monocytogenes*. *Proteomics*, Vol. 4, No. 10, pp. 2991-3006.

Schmidt, F., Donahoe, S., Hagens, K., Mattow, J., Schaible, U.E., Kaufmann, S.H., Aebersold, R., & Jungblut, P.R., (2004). Complementary analysis of the *Mycobacterium tuberculosis* proteome by two-dimensional electrophoresis and isotope-coded affinity tag technology. *Molecular Cell Proteomics*, Vol. 3, No. 1, pp. 24-42.

Seo, J., Lee, K. J. (2004). Post-translational modifications and their biological functions: proteomics analysis and systematic approaches. *Journal of Biochemistry and Molecular Biology*, Vol. 37, No. 1, pp. 35-44.

Seul, K. J., Cho, H. S., Ghim, S. Y. (2011). Characterization of a PyrR-deficient Mutant of *Bacillus subtilis* by a Proteomic Approach. *Korean Journal of Microbioliogy and B iotechnology* , Vol. 39, No. 1, pp. 9-19.

Sharon, N. & Lis, H., (1997). *Glycoproteins: structure and function in Glycosciences: status and perspectives*. Gabius, H. J. & Gabius, S., (eds.). Chapman & Hall, Weinheim, Germany. pp. 133-162.

Shin, N. R., Lee, D. Y., Yoo, H. S. (2007). Identification of quorum sensing-related regulons inVibrio vulnificus by two dimensional gel electrophoresis and differentially

displayed reverse transcriptase PCR. FEMS *Immunology and Medical Microbiology,* Vol. 50, pp. 94-103.

Smithies, O., & Poulik, M.D., (1956). Two-dimentional electrophoresis for serum proteins. *Nature,* Vol.177, No. 4518, pp. 1033.

Stadtman, E.R., (1992). Protein oxidation and aging. *Science*, Vol. 257, No. 5074, pp. 1220-1224.

Steinberg, T.H., Top, K.P.O., Berggren, K.N., Kemper, C., Jones, L., Diwu, Z., Haugland, R.P., & Patton, W.F., (2001). Rapid and simple single nanogram detection of glycoprotains in polyacrylamide gels and on electroblots. *Proteomics*, Vol. 1, No. 7, pp. 841-855.

Thingholm, T.F., Larsen, M.R., Ingrall , C.R., Kassem, M., & Jenson, O.N., (2008). TiO_3 based phosphoproteomics analysis of the plasma membrane and the effects of phosphatase inhibitor treatment. *Journal of Proteome Research*, Vol. 7, No. 8, pp. 3304-3314.

Tong, A.H., Evangelista, M., Parsons, A.B., Hong, X., Bader, G.D., Pagé, N., Robinson, M., Raghibizadeh, S., Hogue, C.W.V., Bussey, H., Andrews, B., Tyers, M., & Boone C., (2001). Systematic genetic analysis with arrays of yeast deletion mutants. *Science*, Vol. 294, No. 5550, pp. 2364-2368.

VanBogelen, R.A., Abshire, K. Z., Moldover, B., Olson, E.R., & Neidhardt, F.C., (1997). *Escherichia coli* proteome analysis using the gene-protein database. *Electrophoresis*, Vol. 18, No. 8, pp. 1243-1251.

VanBogelen, R.A., Schiller, E.E., Thomas, J.D., & Neidhardt, F.C., (1999). Diagnosis of cellular states of microbial organisms using proteomics. *Electrophoresis*, Vol. 20, No. 11, pp. 2149-2159.

VanBogelen, R.A., & Neidhardt, F.C. (1990). Global systems approach to bacterial physiology: Protein responders to stress and starvation. *FEMS Microbiology and Ecology,* Vol. 74, pp. 121–127

Van Vliet, A. H. M., Wooldridge, K. J., & Ketley, J. M., (1998). Iron-Responsive Gene Regulation in a *Campylobacter jejuni fur* Mutant. *Journal of Bacteriology*, Vol. 189, No. 20, pp. 5291-5298.

Varki, A., (1993). Biological roles of oligosaccharides - all of the theories are correct. Glycobiology, Vol. 3, No. 2, pp. 97-130.

Varki, A., Commings, R., Esko, J., Freeze, H., Hart, G., & Marth, J., (1999). *Essentials of Glycobiology*, Gold spring Harbor Press, New York.

Vemuri, G. N ., & Aristidou, A. A. (2005). Metabolic engineering in the -omics era: elucidating and modulating regulatory networks. *Microbiology and Molecular Biology Reviews*, Vol. 69, No. 2, pp. 197-216.

Vlahou, A., & Fountoulakis, M., (2005). Protcomic approach in the search for disease biomarkers. Journal of Chromatography B, Vol. 814, No. 1, pp. 11–19.

Walsh, C., (2006). *Post-translational modification of proteins: expanding nature's inventory.* Englewood, Colo.: Roberts and Co. Publishers. xxi, 490.

Wasinger, V.C., Bjellqvist, B., & Humphery-Smith, I., (1997). Proteomic 'contigs' of *Ochrobactrum anthropi*, application of extensive pH gradients. *Electrophoresis*, Vol. 18, No. 8, pp.1373-1383.

Westermeier, R., (2005). Electrophoresis in practice: *A guide to methods and applications of DNA and protein separations*. (fourth edition). Wiley VCH.

Wilkins, M.R., Pasquali, C., Appel, R.D., Ou, K., Golaz, O., Sanchez, J.C., Yan, J.X., Gooley, A.A., Hughes, G., Humphery-Smith, I., Williams, K.L., & Hochstrasser, D.F., (1996a). From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and arnino acid analysis. *Nature Biotechnology,* Vol. 14, No. 1, pp. 61–65.

Wilkins, M.R., Sanchez, J.C., Gooley, A.A., Appel, R.D., Humphery-Smith, I., Hochstrasser, D.F., & Williams, K.L., (1996b). Progress with proteome projects: Why all proteins expressed by a genome should be identified and how to do it. *Biotechnology and Genetic Engineering Reviews,* Vol. 13, pp. 19–50.

Zhang, J., (2009). Lysine acetylation is a highly abundant and evolutionarily conserved modification in *E. coli. Molecular and Cellular Proteomics*, Vol. 8, No. 2, pp. 215–225.
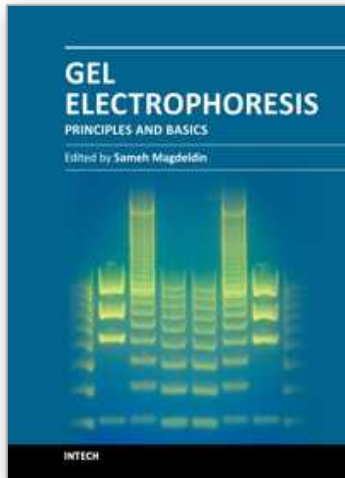
Gel Electrophoresis - Principles and Basics

Edited by Dr. Sameh Magdeldin

Most will agree that gel electrophoresis is one of the basic pillars of molecular biology. This coined terminology covers a myriad of gel-based separation approaches that rely mainly on fractionating biomolecules under electrophoretic current based mainly on the molecular weight. In this book, the authors try to present simplified fundamentals of gel-based separation together with exemplarily applications of this versatile technique. We try to keep the contents of the book crisp and comprehensive, and hope that it will receive overwhelming interest and deliver benefits and valuable information to the readers.

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