*Review paper*
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# MICROBIAL OMICS FOR FOOD SAFETY

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## Abstract

Fungi, bacteria and other organisms secrete into the extracellular environment numerous compounds that are required for their survival. The secreted components can be scrutinized by proteomic and other "omic" tools. Many of these secretion systems are involved in pathogenic processes and indicate mechanisms of pathogenesis and could as well be of great interest for the applications in food technology and biotechnology. Further improvements in "omic" strategies and techniques will enable studies of pathogen secretomes in order to build data sets of proteins and other metabolites. Network of these components will lead to the increased understanding of interactions between the host and pathogen. The identification of proteins and small molecules that are produced by a still unknown pathogen will be the first step on the way of detection of food borne diseases.

We investigated the Gram positive *Bacillus subtilis* and *Listeria monocytogenes*, as well as the Gram negative *Escherichia coli* and *Yersinia enterocolitica*. Changes of proteome in these bacteria grown under stress conditions were identified. By the application of a new method for sample preparation, followed by LC–MS/MS, both characterization and comparison of proteomes of these food pathogens were achieved.

It was shown in all investigated bacteria that some of the proteins of key importance for protein turnover and metabolism are down regulated. Some stress proteins involved in protein folding and degradation were up regulated. Most of both up and down regulated proteins belong to the group of proteins with high abundance. Flagellin is the only protein of lower abundance that is down regulated in two strains, *B. subtilis* and *E. coli*.

Presented results give better view into the proteome of food pathogens, and pave the way for further investigation of their virulence, pathogenicity and detection of biomarkers for tracing the ways and sources of microbial food contamination.

***Key words**: Food microbial safety, Foodomics, Sample preparation, Mass spectrometry.*

## 1. Introduction

Outbreak of food-borne diseases has always been a severe health risk in developing countries, but these diseases are also a problem in industrial countries. Microbial pathogens, mostly bacteria and fungi, are frequently responsible for both food spoilage and food-borne illnesses that cause enormous commercial and health damage around the world. Consequently, protection against spoilage and prevention of food-borne diseases is a task of enormous social and economic importance [1]. E.g. only in the U.S.A. each year are registered about 325,000 hospitalizations and 5,000 deaths that are caused by food poisoning [2].

In food production and storage, careful monitoring of microbial contamination in the final product as well as monitoring of the production process and cleaning and sanitation are one of the most essential factors of the manufacturing process. The tracing, identification and quantification of microbial contaminants and their toxins in food are important analytical problems. The most common bacteria that cause food poisoning are *Staphylococcus aureus, Campylobacter jejuni,* some *Salmonella, Staphylococcus* and *Bacillus spp.* and *Escheria coli*, most frequently the O157:H7 strain, as well as toxin-producing fungi such as *Aspergillus, Penicillium and Claviceps spp.* [2-4]. There are well-established and sensitive methods for detection of food-borne pathogens and their toxins available, mostly based on immunochemical analyses. "Omics" technologies, if applied in food analysis and technology are

recently named "foodomics" [3]. These techniques are often more sensitive and specific and sensitive alternatives for identification of microbial food contaminants and their toxins, for monitoring of cleaning and sanitation, and for further validation of already existing methods for analysis of food safety and quality [2-5]. Some of "foodomic" methods and strategies for monitoring of the quality and microbial safety of food of plant and animal origin are summarized in Table 1.

**Table 1. Summary of on-line available data for use of "foodomic" technologies for monitoring of food quality and microbial safety {according to Ref. [6] with permission}**

| Data types | Online resource | Description | URL |
|---|---|---|---|
| Genomics | Genomes OnLine Database (GOLD) | Repository of completed and ongoing genome projects | http://www.genomesonline.org |
| | Microbial Genome Database (MBGD) | MBGD is a database for comparative analysis of completely sequenced microbial genomes (ortholog identification, paralog clustering, motif analysis and gene order comparison) | http://mbgd.genome.ad.jp/ |
| | National Microbial Pathogen Data Resource (NMPDR) | The NMPDR provided curated annotations in an environment for comparative analysis of genomes and biological subsystems, with an emphasis on the food-borne pathogens | http://www.nmpdr.org/FIG/ wiki/view.cgi |
| Transcriptomics | Gene Expression Omnibus (GEO) | Microarray and SAGE-based genome-wide expression profiles | http://www.ncbi.nlm.nih.gov/geo |
| | Stanford Microarray Database (SMD) | Microarray-based genome-wide expression data | http://smd.princeton.edu/ |
| | ArrayExpress -functional genomics data | Functional genomics experiments include gene expression data from microarray and high throughput sequencing studies | http://www.ebi.ac.uk/ arrayexpress/ |
| | ExPASy – Bioinformatics Resource Portal | Links to transcriptomics | http://www.expasy.org/ transcriptomics |
| Proteomics | World-2DPAGE | Links to 2D-PAGE data | http://us.expasy.org/ch2d/ 2d-index.html |
| | ExPASy – Bioinformatics Resource Portal | Link to protein sequences and identification | http://www.expasy.org/ proteomics/protein_sequences_and_ identification |
| | ExPASy – Bioinformatics Resource Portal | Links to mass spectrometry and 2-DE data | http://www.expasy.org/ proteomics/mass_spectrometry_ and_2-DE_data |
| | BIOBASE | BKL PROTEOME™ is a database and data analysis platform containing manually curated details from the PubMed literature in a highly structured and easily searchable format | http://www.proteinscience.com/ databases.htm |

| | | | |
|---|---|---|---|
| | PRIDE – Proteomics Identifications Database | The PRIDE PRoteomics IDEntifications database is a centralized, standards compliant, public data repository for proteomics data, including protein and peptide identifications, post-translational modifications and supporting spectral evidence | http://www.ebi.ac.uk/pride/ |
| Metabolomics | MetaCyc | MetaCyc is a database of non-redundant, experimentally elucidated metabolic pathways | http://metacyc.org/ |
| | The Molecular Ancestry Network (MANET) | MANET database traces evolution of protein architecture onto biomolecular networks | http://www.manet.uiuc.edu/ |
| | Kyoto Encyclopedia of Genes and Genomes (KEGG) | KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large scale molecular datasets generated by genome sequencing and other high throughput experimental technologies | http://www.genome.jp/kegg/pathway.html |
| | Metabolite and Tandem MS database (METLIN) | The METLIN Metabolite Database is a repository of metabolite information as well as tandem mass spectrometry data | http://metlin.scripps.edu/index.php |
| Lipidomics | Lipid Metabolites and Pathways Strategy (LIPID MAPS) | Genome-scale lipids database | http://www.lipidmaps.org |
| | The official database of Japanese Conference on the Biochemistry of Lipids (JCBL) | Lipid class database | http://www.lipidbank.jp/ |
| | Avanti®,Polar Lipids,Inc. | Services for research and pharmaceutical scientists with the highest quality phospholipids, sphingolipids and sterols | http://avantilipids.com |
| | The AOCS Lipid Library | The AOCS Lipid Library | http://lipidlibrary.aocs.org/index.html |
| | Cyberlipid | Lipid databases and encyclopedia | http://www.cyberlipid.org |
| | LipidHome | LipidHome theoretically generates lipid molecules and useful metadata | http://www.ebi.ac.uk/apweilersrv/lipidhome |

* This table presents some of the databases that store and distribute omics data sets through publicly accessible Web sites. Some omics technologies such as glycomics do not yet have associated data-dissemination resources, and are therefore not included.

## 2. Use of omics methods for identification of microbial contaminants

Sensitive routine methods are available for identification of microbial contaminants. They are mostly based on immunochemical techniques for detection of microbial antigens and their products, mostly secreted metabolites. Omics technologies, mostly proteomics, genomics, glycomics, lipidomics and metabolomics are further, more sensitive and specific ways for identification of microbial food contaminants and their toxins, and for monitoring of cleaning and sanitation [2, 3]. Among others, DNA microarray technology, GC-MS based metabolomics, LC-MS/MS based proteomics and lipidomic methods were applied [3, 7 - 9]. To follow changes during food processing, omics investigations of model microorganisms such as both bacteria and fungi under stress conditions such as cold and heat influence, osmotic pressure, high pressure, availability of nutrients and other environmental factors are essential in order to follow their adaptation and reaction to extreme conditions [7]. Their adaptive stress response is a crucial mode of cellular protection towards environmental and food relevant stress. Cellular and metabolic biomarkers are correlating to the adaptive stress and can also predict the impact of the environmental changing on microbial ability to resistance and survival. Minimally processed, so called "ready to eat" food that have become very popular in recent years, and antimicrobial washing by using different disinfectants and natural antimicrobial agents are other important factors that drastically change populations of microorganisms in fresh food. Such food can still retain some potential pathogenic bacteria, such as *Aeromonas* spp. and *Yersinia* spp. and even some unexpected contaminants [10]. It is also documented that the recent serious outbreak of food poisoning in Germany, caused by a novel strain of *E. coli* appeared after consuming of such minimally processed food [4, 11].

### 2.1. Use of omics for following the microbial stress adaptation

The adaptive stress response of microbial population is a crucial mode of cellular protection towards environmental and food-relevant stresses. Some cellular components, mostly specific proteins and metabolites, are quantitatively correlating to adaptive stress and can also predict the impact of changing environments on microbial ability to resistance and survival [11]. Several molecular biomarkers for stress adaptive behavior at transcriptome, proteome and metabolome level were already identified. In *Bacillus cereus* as a model microorganism, potential candidate biomarkers to stress response were following proteins: transcriptional regulator $s^B$, catalases involved in $H_2O_2$-scavenging, chaperones and ATP-dependent Clp proteases involved in protein repair and maintenance [12]. In an overview, Abee *et al*. [13] integrated three different «omics» strategies, namely use of information from transcriptomic and proteomic data, as well as determination of activity of marker enzymes. This approach has led to the identification of biomarkers important for prediction of the robustness level of adaption of the microorganism towards the lethal stresses.

The investigations using a wide variety of environmental changes were extended to the broad range of food-borne contaminants [13-16]. Such quantitative approaches by use of several omics methods lead to prediction of microbial performance using molecular biomarkers for the early detection of food pathogens and to the control of their adaptive behavior that results in enhanced resistance [4, 11-16]. The strategy for use of «omics» method in order to enhance food quality and safety is presented in Figure 1 [17]. Most frequently investigated were changes after:

1. Temperature shock (heating or freezing, References [18] and [19])
2. Osmotic stress and high hydrostatic pressure [18, 20]
3. High hydrostatic pressure [20]
4. Other stress factors such as oxidizing agents and other disinfectants [16, 21].

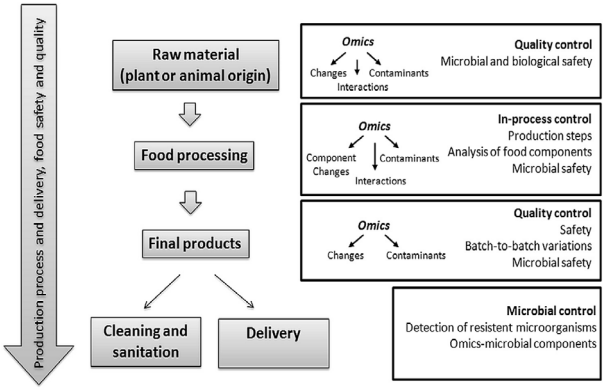


**Figure 1. Use of foodomics in the development pathway for food production, and assessing food safety, origin and quality. {Adapted from [2], with permission}**

### 2.2. Food toxins

Food toxins are cellular components or metabolic products of food pathogens, and they are severe treat to human health. Bacterial and fungal toxins (mycotoxins) may be acutely toxic, but especially the mycotoxins may also cause chronic damage, such as teratogenic, immunotoxic, nephrotoxic and estrogenic effects [11]. Most intracellular and secreted bacterial toxins that cause food poisoning are intensively studied together with bacterial food poisoning, and they are discussed above.

In addition, there have been considerable public safety concerns, thus interest for the investigation of fungal

toxins, toxic chemicals in food and possible changes during the food processing [22]. These events may contaminate the soil, water and air with mycotoxins. Sometimes it is difficult to link poisonings caused by toxic components to a particular food; the onset of the effects may be gradual and not be detected until chronic or permanent damage occurs. Surveillance studies showed that mycotoxin contamination is a worldwide problem, especially in developing countries, where suitable cultivation, processing and storage technologies are implemented with difficulty [23]. Identification of biomarkers of the most-relevant toxins that have been detected in this type of environment and their monitoring will yield a more accurate risk assessment.

Around 400 mycotoxins have been registered until today. They can be found in a variety of foods, ranging from cereals, peanuts, spices, fruits and vegetables. In most cases the mycotoxins that are found in products of animal origin, mostly in meat, milk and eggs are originated from animal feeds.

The classes of mycotoxins are aflatoxins, ochratoxins, trichtothecenes, zearalenone, fumonisins, tremorgenic toxins and ergot alkaloids. After suitable sample preparation, even low concentration of mycotoxins can be detected by use of metabolomic techniques [11, 17, and 23].

New safety risks have to be taken into consideration because of the continuous adaptation of the relevant food borne pathogens and other potentially pathogen microorganisms, changing production methodologies, changes in the environment and increase of global trade of foodstuffs. Increased demand for traditional food in industrial countries, and with growing living standard in some developing countries has to be taken into consideration [11, 13].

### 2.3 Model system - inhibitory activity of pyridinium oximes to Gram-positive and Gram-negative food pathogens

Pyridinium oximes have been firstly demonstrated to be potent re-activators of organophosphate-inhibited enzyme acetylcholine esterase (AChE). Pyridoxal oxime, a derivative of vitamin $B_6$ can be used for synthesis of compounds structurally similar to common antidotes, and their derivatives have been tested as re-activators of AChE after inhibition of neural poisons [24]. Quaternary ammonium salts of heterocyclic bases of dimethylpyridine (see Figures 2 and 3) have been already evaluated for antimicrobial activity against both Gram-positive and Gram-negative microorganisms [16]. Quaternary salts bind to the cell wall as well as other cellular compounds of the microorganisms causing inhibition of bacterial growth and cell death [25]. These activities make them suitable for use as model substances for proteomic and other "omic" studies to find changes in both cell surface and intracellular proteins of four investigated food borne pathogens, Gram-positive bacteria *Bacillus subtilis* and *Listeria monocytogenes*, as well as Gram-negative bacteria *Escherichia coli* and *Yersinia enterocolitica*.

Methods for cellular destruction, fractionation and identification of selectively solubilized proteins that were separated according to their hydrophobicity were developed. In the next step, proteins were identified by semi-quantitative LC-ESI-MS/MS, and changes in the bacterial proteomes were investigated.

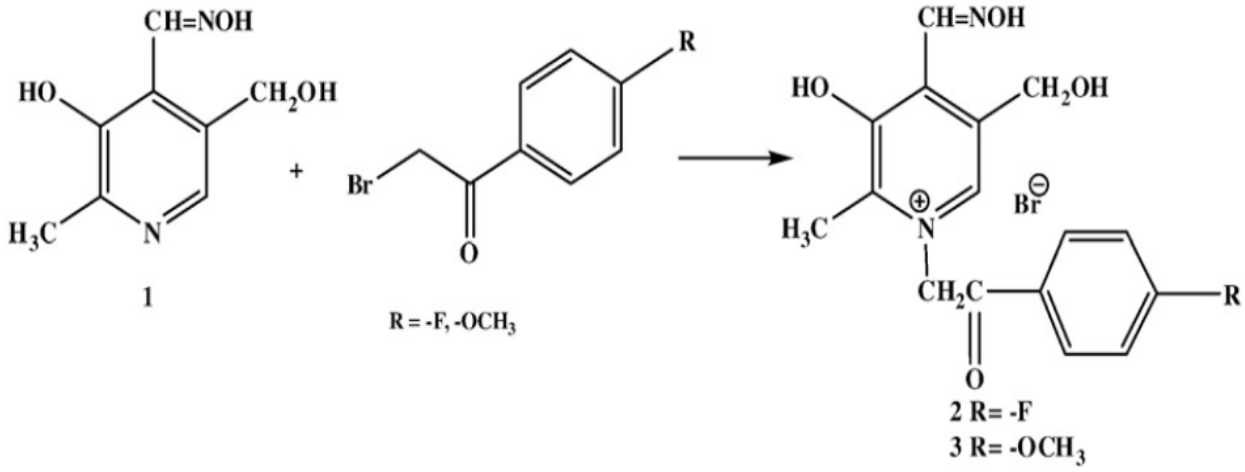


**Figure 2. Synthesis of the oximes 2 and 3 from pyridoxal oxime**

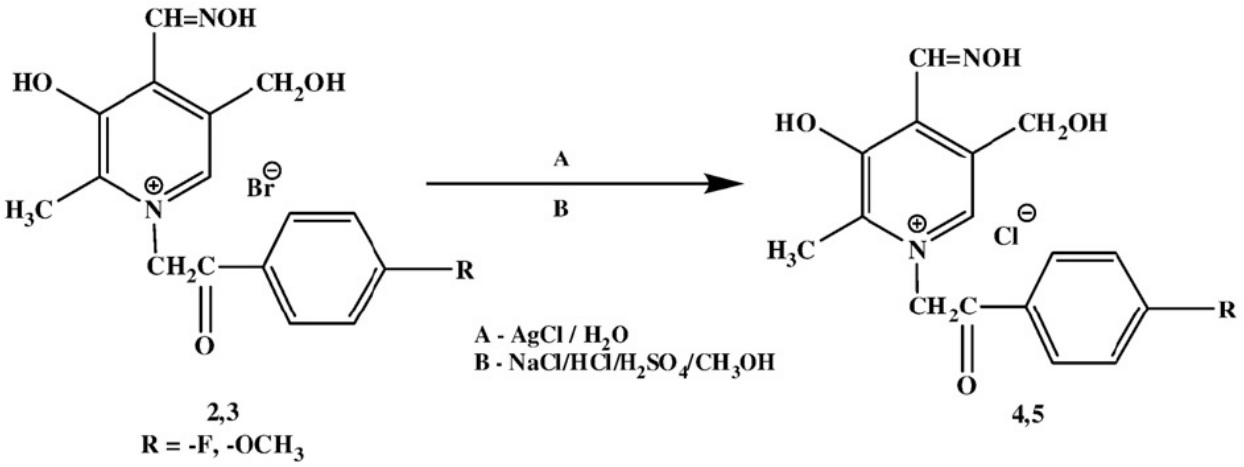


**Figure 3. Anion exchange reaction (adapted from Ref. [16] with permission)**

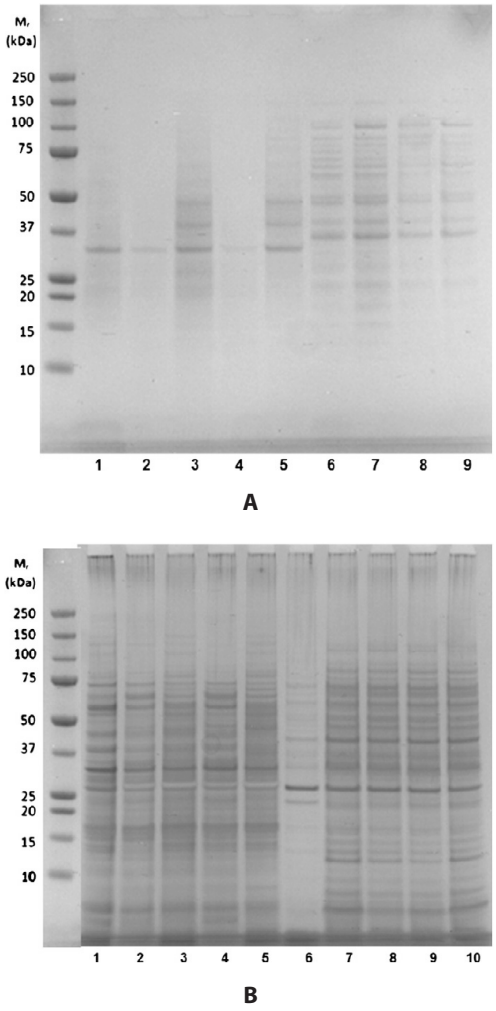


**Figure 4. A - SDS-PAGE of cell extracts of Gram positive bacteria obtained after sonication (only) and subsequent extraction with lysis buffer. Lanes 1-5 - *Bacillus subtilis*, 6-9 - *Listeria monocytogenes;* B - SDS -PAGE of cell extracts of Gram positive bacteria obtained after sonication, mechanical destruction of cells and subsequent extraction with lysis buffer. Lanes 1-5 - *Bacillus subtilis*, 6-9 - *Listeria monocytogenes* (Adapted from Reference [16] with permission)**

*2.3.1 Results*

As shown in Figures 4 A and B, destruction of bacterial cells and proper extraction of proteins according to their hydrophobicity are the key steps in the sample preparation before their identification by LC-MS/MS. Sonication alone is not sufficient as a method for cell destruction, additional mechanical treatment is necessary for optimal disintegration and optimal extraction of cellular components.

In the first step, gel bands of interest after SDS-PAGE (see Figure 5) were excised and subjected to tryptic digestion as previously described [26]. The proteins were subsequently identified by LC-ESI-MS/MS [16].

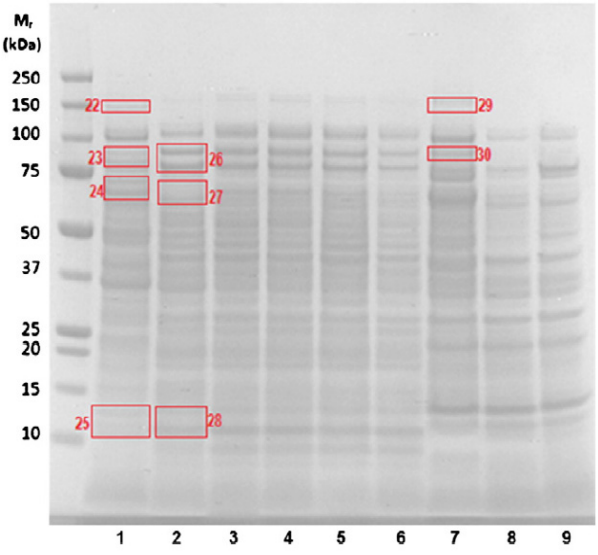


**Figure 5. SDS-PAGE of cell extracts of Gram negative bacteria obtained after sonication (only) and subsequent extraction with lysis buffer. Lanes 1-6 - *Escherichia coli*, 6-9 - *Yersinia enterocolitica*. Differently expressed proteins were recognized according to the band intensity (see band numbers), excised, digested with trypsin and identified by LC–MS/MS**

Especially for Gram positive bacteria (*B. subtilis* and *L. monocytogenes*) this treatment was not sufficient for complete cell destruction (see Figure 4A), and before sonication, the cells had to be destroyed mechanically with micro glass beads in order to obtain sufficient amount of extracted proteins (see Figure 4B).

In order to trace differently expressed proteins, we separated 40 samples of four bacteria treated with pyridinium oximes and control samples of untreated bacteria. After SDS-PAGE, up- and down-expressed proteins were recognized as bands of different intensity (see Figure 5). They were excised, extracted, trypsinized and identified by LC-MS/MS. The lists of differently expressed proteins are shown in Tables 2-5. In these tables, the names of proteins that are up or down-regulated in more than one bacterium are in bold.

**Table 2. The list of differently expressed proteins after treatment with pyridinium oximes in *Bacillus subtilis***

| Down regulated | Up regulated |
|---|---|
| **1. Flagellin** | **1. Chaperonin GroEL** |
| **2. Glyceraldehyde-3-phosphate dehydrogenase** | 2. Glycerol-3-phosphate dehydrogenase |
| 3. Phosphoglycerate kinase | 3. Glucosamine-fructose-6-phosphate aminotransferase |
| 4. Elongation factor Tu | 4. Succinate dehydrogenase |
| 5. Serine hydroxymethyltransferase | 5. Class III stress response-related ATPase |
| **6. DNA-directed RNA polymerase** | **6. Elongation factor Tu** |
| 7. Hypothetical protein BSn5_18325 | 7. D-alanyl-D-alanine carboxypeptidase |
| 8. Manganese ABC transporter manganese binding lipoprotein | 8. Aconitate hydratase |
| 9. Molecular chaperone lipoprotein | 9. DNA gyrase subunit A |
| 10. Hypothetical protein BSn5_18325 | 10. ATP-dependent Clp protease proteolytic subunit |
| 11. Elongation factor G | 11. Oxalate decarboxylase |
| 12. Chemotactic two-component sensor histidine kinase | |
| 13. Peptidoglycan glycosyltransferase ((penicillin-binding protein) | |
| 14. Penicillin-binding lipoprotein 3 | |

**Table 3. The list of differently expressed proteins after treatment with pyridinium oximes in *Escherichia coli***

| Down regulated | Up regulated |
|---|---|
| **1. Glyceraldehyde-3-phosphate dehydrogenase** | **1. Chaperonin GroEL** |
| 2. Outer membrane protein C | **2. Heat shock protein 60 family chaperone GroEL** |
| 3. Outer membrane protein COmpc | 3. Lysine tRNA synthetase |
| 4. Outer membrane protein A | 4. Transaldolase B |
| 5. Outer membrane protein F | 5. Malate dehydrogenase |
| 6. **Flagellin** | 6. Protein Chain elongation factor EF-Ts |
| 7. Putative global regulator | 7. Phosphoribosylpyrophosphate synthetase |
| 8. tRNA-modifying protein ygfZ | 8. ADP-L-glycero-D-mannoheptose-6-epimerase |
| 9. Glycerophosphodiester phosphodiesterase | 9. 50S ribosomal subunit protein L2 |
| 10. RNA polymerase | 10. 6-phosphofructokinase |
| 11. **DNA-directed RNA polymerase** | 11. Hydroxymethylbilane synthase |
| 12. Heat shock protein htrC | 12. DNA starvation/stationary phase protection protein Dps |
| | 13. Putative 30S ribosomal subunit protein S7 |
| | 14. 30S ribosomal subunit protein S5 |
| | 15. Putative nucleotide-binding protein |
| | 16. 50S ribosomal protein L10 |

For the "in-solution" digestion extracted proteins were precipitated, digested with trypsin and identified by LC-MS/MS. In each of analyzed three fractions that were selectively extracted, between 400 and 600 proteins were identified, and altogether, in bacteria more than 1800 proteins each were identified. In Figure 6, the numbers of down and up-regulated proteins in each bacterium after treatment with pyridinium oximes are shown. These inhibitors seem to be mostly effective in *B. subtilis* cell, and in *Y. enterocolitica* the lowest number of proteins were up or down-regulated. The lists of twenty most abundant proteins that are up or down-regulated in each of investigated bacteria are shown in supplement, Tables S1 - S1.

**Table 4. The list of differently expressed proteins after treatment with pyridinium oximes in *Yersinia enterocolitica***

| Down regulated | Up regulated |
|---|---|
| 1. Protein hdeB | **1. Heat shock protein 60 family chaperone GroEL** |
| 2. Integration host factor subunit beta | 2. Urease subunit alpha |
| **3. DNA-directed RNA polymerase** | **3. Glyceraldehyde-3-phosphate dehydrogenase A** |
| 4. Heat shock protein 90 | 4. PTS system mannose-specific EIIAB component |
| 5. Chaperone protein dnaK | 5. UPF0194 membrane protein YE2891 |
| 6. Chaperone protein clpB | 6. Heat shock protein 90 |
| 7. Formate acetyltransferase 1 | 7. Chaperone protein dnaK |
| 8. 1,4-alpha-glucan-branching enzyme | **8. Elongation factor Tu** |
| 9. Pyruvate dehydrogenase subunit E1 | |
| 10. Threonyl-tRNA synthetase | |
| 11. Formate acetyltransferase | |
| 12. Elongation factor G | |
| 13. Outer membrane protein assembly factor yaeT | |
| 14. Glycogen phosphorylase | |
| **15. ATP-dependent Clp protease ATP-binding subunit** | |
| 16. Chaperone protein cipB | |

**Table 5. The list of differently expressed proteins after treatment with pyridinium oximes in *Listeria monocytogenes***

| Down regulated | Up regulated |
|---|---|
| **1. DNA-dependent RNA polymerase subunit beta** | **1. Class I heat-shock protein (chaperonin) GroEL (Hsp60 complex)** |
| **2. DNA-directed RNA polymerase** | 2. Acetolactate synthase |
| **3. ATP-dependent Clp protease ATP-binding subunit** | 3. Pyruvate kinase |
| 4. Transketolase | 4. Oligopeptide ABC transporter |
| 5. Alcohol acetaldehyde dehydrogenase | 5. Pyridine nucleotide-disulfide oxidoreductase |
| 6. Translation elongation factor G | 6. Arginyl-tRNA synthetase |
| 7. Formate acetyltransferase | 7. Phosphoenolpyruvate-protein phosphotransferase |
| 8. Polynucleotide phosphorylase/ polyadenylase | 8. Fumarate reductase |
| 9. Polyribonucletide nucleotidyltransferase | 9. Alcohol acetaldehyde dehydrogenase |
| 10. Methionyl-tRNA synthetase | 10. DNA-binding protein HU |
| 11. L-lactate dehydrogenase | |
| 12. Clp endopeptidase ATP-binding subunit | |

## 2.4 Discussion

These investigations of four model bacteria clearly show that destruction of Gram-positive bacteria for proteomic investigation is a pretty difficult task. Therefore, the detection of intracellular proteins coming from this type of food-borne pathogens will be practically impossible in most of the cases. The investigation should be focused instead on detection of secreted proteins and other extracellular components.

Here, we investigated changes resulting from chemical insult, specifically by pyridinium oximes. The twenty

most abundant proteins that are up or down-regulated in each of the investigated bacteria (see Tables 1S-8S) give a representative overview about the changes in each microorganism. As shown in Figure 6, *B. subtilis* and *E. coli* are mostly prone to proteome changes after inhibition, and *Y. enterocolitica* is a less sensitive microorganism.

Proteins that are differently expressed in more than 1 bacterium are summarized in Table 6. Most of them are enzymes and co-factors involve energy metabolism, heat shock proteins and some membrane- and cell wall associated proteins (see also Tables S1-S8). A change of expression of proteins that are involved in energy metabolism and protein synthesis could be expected because all the investigated substances were added in concentrations that inhibit bacterial growth.

As expected, the influence of inhibitory agents also causes induction of the expression of stress proteins in the cell. Chaperon GroEL, e.g., a heat shock protein, is up-regulated in all four bacteria (see Tables 2-6).

Flagellin was the only protein with lower abundance that was down-regulated in both *B. subtilis* and *E. coli* in the presence of inhibitory agents (see Table 6). Down-regulation of this protein may significantly reduce bacterial virulence. On the other hand, this protein is also an important antigen that is frequently recognized by the human immune system, and anti-flagellin antibodies are used as non-specific protection against bacterial pathogens [27].

**Table 6. Summary of proteins that are up or down regulated in more than one bacterium, and detected after "in-gel". The names of these proteins are in bold in Tables 2 to 5**

| Down regulated | Up regulated |
|---|---|
| Flagellin (*B. subtilis, E. coli*) | Elongation factor Tu (*B. subtilis, Y. enterocolitica*) |
| Glyceraldehyde-3-phosphate dehydrogenase (*B. subtilis, E. coli*) | Chaperonin GroEL (*B. subtilis, E. coli*) |
| DNA-directed RNA polymerase (*B. subtilis, E. coli, Y. enterocolitica, L. monocytogenes*) | Heat shock protein 60 family chaperone GroEL (*E. coli, Y. enterocolitica, L. monocytogenes*) |
| ATP-dependent Clp protease ATP-binding subunit (*Y. enterocolitica, L. monocytogenes*) | |

## 3. Conclusions

- Application of the presented method for sample preparation follow by in-gel and in-solution digestion and Lc-MS/MS enables the investigation and quantitative comparison of both Gram negative and Gram positive food pathogens.

- Destruction of Gram-positive bacteria requests an additional, mechanical step, and it is an indication that detection of their intracellular proteins in samples of contaminated food may be difficult.

- Following the fact stressed above, in the case of Gram-positive bacterial contaminants their detection shall be performed through detection of extracellular components (secretome).

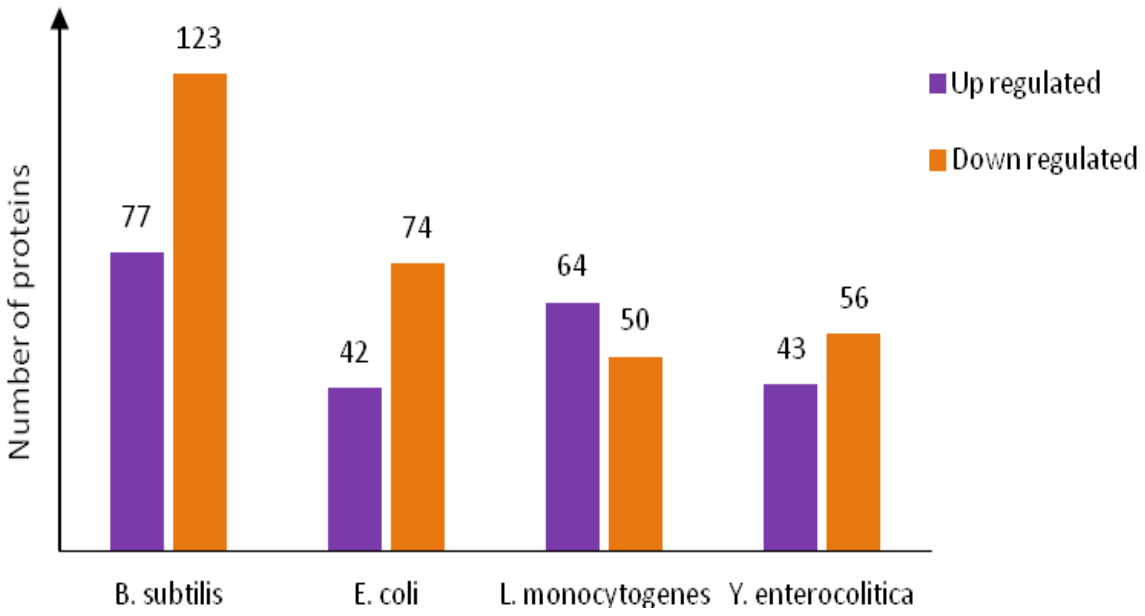


**Figure 6. Number of down- and up-regulated proteins in model microorganisms after treatment with pyridinium oximes**

- Furthermore, it was shown that in all investigated food borne pathogens many proteins of key importance for protein turnover are down-regulated after incubation in the presence of pyridinium oximes.

- Some stress proteins involved in protein folding and degradation are up-regulated.

- Flagellin is the only low abundance protein that was found to be down regulated in two strains, the Gram positive bacterium *B. subtilis* and the Gram negative one *E. coli*.

- Flagellin is a protein that plays an important role in bacterial virulence and induction of host immunity [28].

- The Gram positive bacterium *B. subtilis* is the mostly sensitive to changes induced by applied inhibitors.

- The changes in Gram negative bacterium *Y. enterocolitica* proteome induced by pyridinium oximes are lowest comparing to other investigated bacteria.

## Supplement

**Table S1. *Bacillus subtilis* - down regulated proteins**

| | Protein Name | Total Score | Total Peptides |
|---|---|---|---|
| 1. | Lon protease 1 | 1086,44 | 23 |
| 2. | Ribose-phosphate pyrophosphokinase | 993,67 | 16 |
| 3. | Glycyl-tRNA synthetase beta subunit | 952,82 | 21 |
| 4. | DNA polymerase III subunit beta | 941,92 | 18 |
| 5. | NADH dehydrogenase | 808,26 | 17 |
| 6. | Peptide chain release factor 1 | 760,48 | 15 |
| 7. | D-alanine--D-alanine ligase | 743,61 | 13 |
| 8. | Methionyl-tRNA synthetase | 741,86 | 15 |
| 9. | 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase | 731,78 | 12 |
| 10. | Transcription elongation protein nusA | 713,82 | 13 |
| 11. | Aspartyl-tRNA synthetase | 704,44 | 14 |
| 12. | Sensor protein degS | 636,04 | 12 |
| 13. | DNA mismatch repair protein mutS | 604,1 | 13 |
| 14. | DNA polymerase I | 601,25 | 15 |
| 15. | UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-2,6-diaminopimelate ligase | 600,67 | 10 |
| 16. | UPF0042 nucleotide-binding protein yvcJ | 570,97 | 10 |
| 17. | Lipoate-protein ligase LplJ | 568,67 | 12 |
| 18. | Enoyl-[acyl-carrier-protein] reductase [NADPH] FabL | 565,93 | 11 |
| 19. | Uncharacterized protein ydcI | 553,24 | 14 |
| 20. | Ferrochelatase | 459,2 | 12 |

**Table S2. Bacillus subtilis - up regulated proteins**

| | Protein Name | Total Score | Total Peptides |
|---|---|---|---|
| 1. | Penicillin-binding protein 1A/1B | 524,66 | 10 |
| 2. | Uncharacterized protein yjlC | 430,95 | 7 |
| 3. | L-cystine-binding protein tcyA | 287,34 | 5 |
| 4. | Penicillin-binding protein 3 | 283,03 | 7 |
| 5. | Disulfide bond formation protein D | 226,66 | 5 |

| | | | |
|---|---|---|---|
| 6. | Manganese-binding lipoprotein mntA | 225,33 | 5 |
| 7. | Oligopeptide-binding protein oppA | 215,17 | 5 |
| 8. | D-alanyl-D-alanine carboxypeptidase dacA | 205,95 | 6 |
| 9. | L-lactate permease | 184,05 | 3 |
| 10. | Uncharacterized protein yrhD | 177,94 | 4 |
| 11. | Membrane protein oxaA 1 | 175,02 | 3 |
| 12. | Cell cycle protein gpsB | 169,54 | 4 |
| 13. | ATP synthase epsilon chain | 166,14 | 5 |
| 14. | Methionine-binding lipoprotein metQ | 163,59 | 4 |
| 15. | Uncharacterized membrane protein yhaH | 163,04 | 5 |
| 16. | Dihydrolipoyl dehydrogenase | 148,53 | 4 |
| 17. | L-cystine transport system permease protein tcyB | 148,21 | 3 |
| 18. | 50S ribosomal protein L29 | 143,54 | 3 |
| 19. | Phage-like element PBSX protein xkdF | 137,79 | 3 |
| 20. | Putative carboxypeptidase yodJ | 135,07 | 4 |

**Table S3. *Listeria monocytogenes* - down regulated proteins**

| | Protein Name | Total Score | Total Peptides |
|---|---|---|---|
| 1. | aconitate hydratase | 740,16 | 16 |
| 2. | ribonucleotide-diphosphate reductase subunit alpha | 659,96 | 13 |
| 3. | hypothetical protein LM5578_0641 | 637,50 | 12 |
| 4. | NAD-dependent DNA ligase LigA | 577,53 | 12 |
| 5. | hypothetical protein LM5578_0028 | 463,39 | 10 |
| 6. | hypothetical protein LM5578_1921 | 427,80 | 10 |
| 7. | hypothetical protein LM5578_2322 | 410,10 | 7 |
| 8. | hypothetical protein LM5578_1657 | 390,43 | 7 |
| 9. | hypothetical protein LM5578_2234 | 370,03 | 6 |
| 10. | phosphoribosylaminoimidazole carboxylase ATPase subunit | 366,66 | 4 |
| 11. | glutamate-1-semialdehyde aminotransferase | 347,05 | 8 |
| 12. | hypothetical protein LM5578_1392 | 342,47 | 10 |
| 13. | hypothetical protein LM5578_0853 | 336,61 | 6 |
| 14. | hypothetical protein LM5578_2136 | 294,92 | 5 |
| 15. | ribonuclease PH | 290,09 | 5 |
| 16. | azoreductase | 284,02 | 5 |
| 17. | hypothetical protein LM5578_2088 | 263,86 | 6 |
| 18. | hypothetical protein LM5578_2668 | 244,41 | 5 |
| 19. | two-component response regulator | 221,27 | 5 |
| 20. | prephenate dehydratase | 218,56 | 4 |

**Table S4. *Listeria monocytogenes* - up regulated proteins**

| | Protein Name | Total Score | Total Peptides |
|---|---|---|---|
| 1. | listeriolysin O precursor | 918,80 | 19 |
| 2. | pyridoxine biosynthesis protein | 466,85 | 9 |
| 3. | leucyl-tRNA synthetase | 308,80 | 8 |
| 4. | hypothetical protein LM5578_0105 | 295,27 | 6 |
| 5. | transmembrane protein | 282,99 | 5 |
| 6. | hypothetical protein LM5578_1493 | 252,53 | 5 |
| 7. | hypothetical protein LM5578_1007 | 249,40 | 4 |
| 8. | hypothetical protein LM5578_0157 | 238,36 | 4 |
| 9. | hypothetical protein LM5578_0460 | 237,70 | 4 |
| 10. | hypothetical protein LM5578_1302 | 225,59 | 5 |
| 11. | hypothetical protein LM5578_0144 | 200,29 | 3 |
| 12. | hypothetical protein LM5578_2051 | 178,03 | 5 |
| 13. | hypothetical protein LM5578_0323 | 177,14 | 5 |
| 14. | hypothetical protein LM5578_2030 | 149,29 | 4 |
| 15. | hypothetical protein LM5578_1815 | 145,46 | 4 |
| 16. | glutamate dehydrogenase | 143,91 | 4 |
| 17. | hypothetical protein LM5578_0538 | 143,63 | 3 |
| 18. | hypothetical protein LM5578_1673 | 139,62 | 3 |
| 19. | hypothetical protein LM5578_2049 | 136,15 | 4 |
| 20. | LacI family transcription regulator | 97,56 | 3 |

**Table S5. *Escherichia coli* - down regulated proteins**

| | Protein Name | Total Score | Total Peptides |
|---|---|---|---|
| 1. | alcohol dehydrogenase (adhE) | 3081,22 | 50 |
| 2. | trimethylamine N-oxide reductase subunit | 871,38 | 19 |
| 3. | IrgA-like protein | 741,08 | 14 |
| 4. | DkgA | 725,40 | 16 |
| 5. | Chain A, E. Coli Ferric Hydroxamate Uptake Receptor (Fhua) In Complex With Bound Ferrichrome-Iron | 644,45 | 14 |
| 6. | trimethylamine-N-oxide reductase | 623,75 | 14 |
| 7. | Chain A, Crystal Structure Of N-Acetyl-D-Glucosamine-6-Phosphate Deacetylase Liganded With Zn | 511,82 | 10 |
| 8. | glyceraldehyde 3-phosphate dehydrogenase C | 446,76 | 7 |
| 9. | outer membrane protein C OmpC | 439,61 | 9 |
| 10. | osmC | 386,76 | 7 |
| 11. | pyruvate oxidase | 377,26 | 8 |
| 12. | Chain C, The Crystal Structure Of Unliganded Phosphofructokinase | 376,18 | 8 |

| | | | |
|---|---|---|---|
| 13. | hypothetical protein ECs2150 | 372,05 | 7 |
| 14. | Chain B, Complex Of Enzyme Iiaglc And The Histidine-Containing Phosphocarrier Protein Hpr | 360,57 | 5 |
| 15. | Chain A, Phosphate-Binding Protein With Ala 197 Replaced With Trp | 354,82 | 7 |
| 16. | ABC transport system, periplasmic binding protein SitA | 311,86 | 8 |
| 17. | SitA | 304,64 | 8 |
| 18. | cold shock protein (cspA) | 288,23 | 7 |
| 19. | threonine dehydratase 2 (EC 4.2.1.16) | 285,55 | 5 |
| 20. | Mannonate dehydratase | 276,11 | 7 |

**Table S6. *Escherichia coli* - up regulated proteins**

| | Protein Name | Total Score | Total Peptides |
|---|---|---|---|
| 1. | type-1 fimbrial major subunit | 416,04 | 5 |
| 2. | type 1 fimbrin | 365,26 | 5 |
| 3. | phoP | 332,27 | 7 |
| 4. | NADH dehydrogenase I chain G | 329,67 | 8 |
| 5. | tryptophanase | 320,73 | 7 |
| 6. | carbamoyl-phosphate synthetase small subunit | 304,91 | 6 |
| 7. | unnamed protein product | 265,08 | 8 |
| 8. | hypoxanthine phosphoribosyltransferase | 251,99 | 5 |
| 9. | hypothetical protein ECs3654 | 207,72 | 6 |
| 10. | Chain B, Structural Genomics, Protein Ybgi | 169,36 | 4 |
| 11. | Chain A, Thymidylate Synthase Complexed With Dgmp And Folate Analog 1843u89 | 164,66 | 3 |
| 12. | hypothetical protein ECs3953 | 161,99 | 4 |
| 13. | heat shock protein IbpA | 146,49 | 3 |
| 14. | hypothetical protein ECs1478 | 146,18 | 4 |
| 15. | hycF gene | 145,39 | 3 |
| 16. | putative ATP synthase beta subunit | 134,05 | 3 |
| 17. | uracil DNA glycosylase | 133,76 | 3 |
| 18. | hypothetical protein ECs4371 | 125,68 | 4 |
| 19. | phosphoglycolate phosphatase | 103,31 | 2 |
| 20. | cysB regulatory protein | 95,40 | 3 |

**Table S7. *Yersinia enterocolitica* - down regulated proteins**

| | Protein Name | Total Score | Total Peptides |
|---|---|---|---|
| 1. | N-ethylmaleimide reductase | 1023,73 | 19 |
| 2. | threonine synthase | 611,50 | 12 |
| 3. | ATP-dependent RNA helicase DeaD | 553,06 | 13 |
| 4. | hypothetical protein YE2259 | 503,17 | 11 |

| | | | |
|---|---|---|---|
| 5. | tRNA modification GTPase TrmE | 480,77 | 9 |
| 6. | cyclopropane fatty acyl phospholipid synthase | 471,52 | 11 |
| 7. | arginine-binding periplasmic protein 1 precursor | 401,75 | 9 |
| 8. | putative D-isomer specific 2-hydroxyacid dehydrogenase | 379,07 | 7 |
| 9. | alcohol dehydrogenase | 369,46 | 7 |
| 10. | 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase | 363,82 | 7 |
| 11. | thymidine phosphorylase | 351,34 | 9 |
| 12. | adenylosuccinate lyase | 344,78 | 8 |
| 13. | dihydroorotate dehydrogenase 2 | 330,40 | 6 |
| 14. | putative global regulator | 325,74 | 5 |
| 15. | thioredoxin-dependent thiol peroxidase | 312,29 | 5 |
| 16. | ubiquinone/menaquinone biosynthesis methyltransferase | 308,94 | 6 |
| 17. | flavodoxin FldA | 299,91 | 6 |
| 18. | azoreductase | 298,30 | 6 |
| 19. | hypothetical protein YE0442 | 277,65 | 5 |
| 20. | fumarate reductase flavoprotein subunit | 277,25 | 7 |

**Table S8. *Yersinia enterocolitica* - up regulated proteins**

| | Protein Name | Total Score | Total Peptides |
|---|---|---|---|
| 1. | 30S ribosomal protein S17 | 335,39 | 9 |
| 2. | putative arsenate reductase | 315,04 | 6 |
| 3. | 30S ribosomal protein S15 | 276,14 | 8 |
| 4. | leucyl aminopeptidase | 228,15 | 5 |
| 5. | 1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase | 216,83 | 5 |
| 6. | peptidyl-prolyl cis-trans isomerase C | 205,92 | 5 |
| 7. | putative ribosome maturation factor | 186,16 | 5 |
| 8. | hypothetical protein YE3017 | 167,35 | 4 |
| 9. | aspartate alpha-decarboxylase | 159,98 | 3 |
| 10. | DNA-binding transcriptional repressor PurR | 144,82 | 3 |
| 11. | pyrrolidone-carboxylate peptidase | 141,47 | 4 |
| 12. | malic enzyme | 139,44 | 4 |
| 13. | formyltetrahydrofolate deformylase | 131,05 | 4 |
| 14. | hypothetical protein YE3671 | 130,87 | 4 |
| 15. | aromatic amino acid aminotransferase | 125,62 | 3 |
| 16. | Maf-like protein | 120,62 | 3 |
| 17. | glycine cleavage system aminomethyltransferase T | 117,54 | 2 |
| 18. | long-chain fatty acid outer membrane transporter | 110,68 | 2 |
| 19. | 23S rRNA pseudouridine synthase D | 101,09 | 2 |
| 20. | DNA-binding transcriptional regulator HexR | 98,78 | 2 |

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