SUMMARY

Studies on moderately halophilic bacteria have been increased in the last decade. These studies are mainly focused on their ecology, physiology, taxonomy or phylogenetic relationships. However, it is very important to understand the survival and growth of these microorganisms under long-term stress environments, especially elevated temperatures and hypersaline conditions. Understanding the unique stress tolerance mechanisms of moderately halophilic bacteria will provide novel approaches to science and biotechnology. Unfortunately, very few genomic studies are available about these microorganisms and it is not easy to understand adaptation mechanisms of moderately halophilic bacteria since only limited genetic information is provided. Since proteomics is an important method of gaining data about protein expression levels of these microorganisms, proteome studies of moderately halophilic bacteria possess potent challenge to gain the essential physiological data.

This work was initiated by the new isolation of 11 unknown bacterial strains from Çamaltı Saltern Area near Izmir. The goal of this thesis is to test possible isolation protocols and different proteomic approaches for studying these strains, and to gain information about the type of these new isolates in comparison with related bacteria. On the basis of preliminary biochemical studies, the moderately halophilic bacteria *H. salina* DSMZ 5928 as model organism and one of the new isolates, Isolate No 6, were selected to study large–scale proteomics and to identify variations in expressions and to find homologous proteins without full genome knowledge. In this study, high resolution 2-DE gel separation in combination with high throughput MS analysis and N-terminal Edman sequencing have been applied to identify the interesting proteins and proved to be effective for proteome analysis of these moderate halophiles. Different techniques for peptide fragmentation, such as electrospray tandem quadrupole time-of-flight mass spectrometry (ESI-QqTOF MS/MS), MALDI-quadrupole time-of-flight mass spectrometer (MALDI-QqTOF MS) as well as tandem time-of-flight mass spectrometer (MALDI TOF/TOF MS) were applied. ESI-QqTOF MS/MS of the peptide mixture of selected protein spots gave acceptable results to identify and to find homologous proteins for these microorganisms. Also, N-terminal analysis combined with BLAST searches gave

good matches from the protein databases. As can be expected, the proteins or their homologous correspondence, which were not present in the public databases, did not lead to their identification even though good spectra were achieved.

Annotations of the identified proteins were facilitated by highly automated bioinformatics tools, such as ExPASy Proteomics server, NCBI website, KEGG database (Kyoto Encyclopedia of Genes and Genomes) with e.g. BRITE, BIOCyc and METACyc tools. These sites provided many links to different branches of information and special annotations for individual data. In addition to own extensive literature searches bioinformatic tools helped us to check experimental data by comparison to theoretical data and also to results of other fields, like toxicology, physiology, pharmacology, and industrial applications.

As shown in this thesis, in the current situation, bacterial proteomes of new organisms without any detailed knowledge of genome data can be studied effectively, enlarging data sets in various ways. Although genomic knowledge would build the basic information, differences in protein expression studies under various cultural conditions can be addressed. In these exceptional cases, direct proteomics work can answer many questions in combination with the help of bioinformatics.

Totally 15 proteins of *Halomonas salina* have been identified using mass spectrometric techniques. 2 of these identified proteins, which are aconitate hydratase and hypothetical protein ECA3428 have been studied both from group 1 and group 2 gels. 7 of these identified proteins are important enzymes, which generally take role in energy metabolism, TCA cycle, as well as nucleotide and amino acid metabolism. 2 of the identified proteins take role in transportation. Another 2 are hypothetical proteins. The other individual proteins function in translation, DNA replication, respiration and metabolism of cofactors and vitamins. The codes of the studied protein spots, their identification methods, identified homologous proteins and their probable functions are summarized in tables 5.3 and 6.3.

In the case of Isolate No 6, totally 16 proteins have been identified using both mass spectrometric techniques and N-terminal Edman sequencing method. 11 of the identified proteins are important enzymes that take role in energy metabolism, TCA cycle, amino acid, fatty acid metabolisms, glycolysis, ectoine and osmoprotectant synthesis and cell signalling. Other individual identified proteins take role in solute diffusion, structure rigidity, transportation, defensive against stress and protein biosynthesis. The codes of the studied protein spots, their identification methods,

identified homologous proteins and their probable functions are summarized in tables 5.4 and 6.2.