12.1 Antihelicobacter of a Property Protein of Substations Lactobacillus of a Gastric Origin

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It is known, that Lactobacillus spp. produce of substance protein of a nature rendering antimicrobial an operation on various microorganisms. By the purpose of the present work was the study antihelicobacter of activity protein of substations gastric Lactobacillus. For this purpose from a stomach practically of healthy faces and patients with gastroduodenal the diseases isolate the strains Lactobacillus (25 strains) and are investigated antimicrobial of a property them protein of substations in relation to Helicobacter pylori (HP) (14 strains). For want of it we use a medium because of hydrolyse of milk with adding yeast autolysat, containing a gang necessary amynoacids and vitamins and promoting to of accumulation of substances protein of a nature. Microbial of a suspension Lactobacillus subjected autolyse. Autolysats inoculated the test culture HP and through the certain spaces of time (at once after crop, through 15, 30, 45 and 60 minutes) made crop on optimum for HP of a medium. For control was crops the test culture HP in hydrolyse a broth. Is established, that all investigated strains Lactobacillus with a different degree of activity exhibited antagonism in relation to the test culture HP. 32% of autolysats inhibition growth HP already at once ambassador inoculation, and of remaining 68% within 15 minutes. Whereas in control tests the viability HP was saved within 30–60 minutes depending on the strain HP. Thus, among Lactobacillus, chosen from a stomach, there are strains, produce high active antimicrobial of substations protein of a nature with antihelicobacter by an operation, which can be used for creation effective antihelicobacter probiotic of a preparation.

12.2 A Proteome Reference Map for Gluconacetobacter diazotrophicus PAL5.

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Gluconacetobacter diazotrophicus is a nitrogen-fixing bacteria found as an endophyte in roots, stems and leaves from sugar cane, coffee plant, sweet-potato and pineapple. G. diazotrophicus is of great interest not only due its capacity to assimilate N₂, but because it is a good model to study symbiosis of the bacterium with plant. Moreover, besides its economical importance to increase crop productivity, a better understanding of its physiology will allow the development of more efficient methods for its inoculation into vegetal species and others biotechnological approaches. Physiological proteomics requires 2-D reference maps, on which most of the main proteins are identified. We present a reference map for G. diazotrophicus proteins, which contains about 500 spots and more than 100 identified proteins (through MALDI-TOF) with an isoelectric point between 3 and 10. The results were obtained from twelve gels run on 18 cm immobilized pH gradient strips and 12–14% precast SDS polyacrylamide gels, representatives of six different conditions analyzed: logarithmic and stationary phases in nitrogen fixing and non-fixing conditions and logarithmic and stationary phases of a pH controlled growth curve.

Most of identified proteins were categorized as members of energy and aminoacid metabolism and translation, including a glutathione synthetase, an important enzyme of aminoacids metabolism, and ModC, an ATPase of the molybdate transport system, which may helps couple of ATP hydrolysis to active molybdate transport. Nitrogen fixation regulatory proteins, a transketolase and a bacteriocin were also found.

Computer-aided analysis of 2D gels, revealed complex proteomes, with a differential expression of about 40 proteins between the logarithmic and stationary phases of growth, both in fixing and non-fixing conditions. Moreover, at least 15 proteins were specifically induced in nitrogen fixing condition and 20 proteins in the non-fixing condition, in logarithmic phase and stationary phases of growth. The pH control regulated other 16 proteins. Membrane associated processes appears to be of major importance for the bacterial metabolism, because, in all samples tested, many outer membrane proteins were detected by SDS-PAGE.

It is important to note that the determination of Gluconacetobacter diazotrophicus complete genome sequence (project in progress) will contribute to a better proteome annotation and study of proteins from the point of view of their structure and expression.
12.3

Yeast 2D Gel-Based Proteomics:
The State of the Art

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The yeast Saccharomyces cerevisiae is a particularly favourable organism for proteomics investigation: the number of protein species is limited to a few thousand (6000), there is little post-translational modification and its genome is entirely sequenced. This makes yeast a reference organism for proteomic analysis. This is true in particular for 2D gel-based proteomics. More than twenty years after the publication of the first reference map of yeast proteins (1), the high potential of 2D gel electrophoresis for investigating the yeast proteome is fully demonstrated and yeast continues to be a leader organism for 2D gel-based proteomics. In this presentation I will present the current state of the art of the 2D gel-based proteomics of Saccharomyces cerevisiae.

I will also present an example of the application of 2D gel proteomics to the study of a complex biological process, the proteome remodelling of yeast cells occurring during the diauxic shift. The diauxic shift is corresponding to a transition between the fermentative and the oxidative metabolism. At the proteome level, it is associated with drastic changes in protein synthesis. We used strains carrying mutations in regulatory genes to investigate the involvement of different regulatory factors in the control of these changes. The role of the transcriptional factors Msn2p, Msn4p, Cat8p, and of the protein kinases Snf1p and PKAs (cAMP dependent protein kinases) were considered. The data obtained from the analysis of the proteins synthesized in the mutant strains during the diauxic shift show that 2D gel electrophoresis is a powerful tool for dissecting regulatory networks controlling a complex biological process.


12.4

Analysis of Differently Expressed Membrane Proteins Between Male and Female Worm of Schistosoma japonicum After Pairing

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Schistosomiasis is a major public health problem in China and Southeast Asia. Identification of differently expressed proteins between male and female worm Schistosoma japonicum may provide new insights to the development of schistosomes, especially the molecular mechanism of female worm maturation induced by male worm after pairing. Membrane proteins, which take important functions in cells, were isolated by reducing and alkylating with TBP and iodoacetamide from eggs, schistosomulum, male worm and female worm in adult schistosomes and analyzed by two dimensional electrophoresis. The 2D maps of eggs, schistosomulum, female worm and male worm were shown 78, 67, 108, 122 spots respectively. There were 35 spots with specific display in female worm as compared with male worm, but 48 spots in male worm, respectively. The map of female worm displayed 4 unique spots, while the male worm showed 5 unique spots. The unique proteins spots that show between male and female worm after pairing help us to more understand the female worm of development and maturation. The unique spots between male and female worm were primarily identified by the database of SWISS 2D-PAGE. The result showed Calreticulin 1, methyltransferase, Outer membrane protein tolC and Oxygen-evolving enhancer protein in male worm. Enolase, Outer membrane protein X, Ferrienterobactin receptor and Heat shock cognate 70 kDa protein 3 were in female worm. The results showed that the main functions of those differentially expressed proteins were involved in signal transduction, metabolism and hormone reception. In future, MALDI-TOF-MS and ESI-MS were employed to analyze the peptide mass fingerprint and sequence of protein. The deeply research on the differently expressed proteins between male and female worm may provide us with a way of founding candidate vaccine molecules for control the schistosomiasis.
12.5 Discovery and Characterization of Novel Post-translational Modifications of PilE, the Pilin Subunit of Neisseria gonorrhoeae

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Type IV pili of Neisseria gonorrhoeae (Ng) and Neisseria meningitidis (Nm) are filamentous cell surface protein structures that are critical colonization factors for their human hosts. In addition to its renowned capacity to undergo antigenic variation by alterations in primary structure, the pilin subunit PilE is subject to post-translational modifications (PTMs). The Ng disaccharide attached to Ser-63 was recently characterized and, contrary to previous reports, was found to be linked via 2,4-diacetamido-2,4,6-trideoxyhexose. Furthermore, a novel O-linked phosphoethanolamine at Ser-68 was identified [1]. However, the full spectrum of neisserial pilin PTMs has not been characterized yet.

Therefore, a complementary approach including mass spectrometric analyses of whole intact protein PilE (Ng) and of peptides from in-gel digestions was performed to fully identify all major PTMs. A direct-infusion ESI-MS analysis following a two step precipitation procedure of PilE digestions was performed to fully identify all major PTMs. Furthermore, a novel O-linked phosphoethanolamine at Ser-68 was identified [1]. However, the full spectrum of neisserial pilin PTMs has not been characterized yet.

Therefore, a complementary approach including mass spectrometric analyses of whole intact protein PilE (Ng) and of peptides from in-gel digestions was performed to fully identify all major PTMs. A direct-infusion ESI-MS analysis following a two step precipitation procedure of PilE digestions was performed to fully identify all major PTMs. Furthermore, a novel O-linked phosphoethanolamine at Ser-68 was barely detectable in the tryptic digests and not during whole protein MS analysis. Therefore, only a minor fraction of PilE carries this modification.


12.6 Analysis of Autoantigens in the Proteomes of Human Epidermis and Streptococcus pyogenes: A Route to Understanding the Pathogenesis of the Autoimmune Skin Disease, Psoriasis

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Accumulating evidence suggests that the common inflammatory and scaling skin disease, psoriasis, is mediated by autoimmunity, however convincing disease-specific lesional autoantigens have yet to be demonstrated. Some cases are provoked by pharyngeal infections with Streptococcus pyogenes (Sp), suggesting that molecular mimicry plays a pathogenic role. In this study, total protein extracellular and cytoplasmic extracts were obtained from ammonium sulfate precipitates of culture supernatants from large volume Sp cultures grown in chemically defined, protein-free medium, and from ultrasonic lysates of pelleted bacteria from the same cultures, were subjected to 2D gel electrophoresis. Parallel 2D Westerns show that psoriatic serum IgGs recognize fewer protein spots than native psoriatic serum, suggesting immunological mimicry. These experiments are being extended to exclude effects of Sp extracellular and cytoplasmic preparations reproducibly showed that IgG in pooled sera of psoriatics and controls recognizes many Sp extracellular proteins but not proteins from cytoplasmic lysates of Sp. The ability of IgG from normal, non-convalescent sera to recognise Sp proteins has apparently not been reported previously. Although it might have been considered to be predictable, the finding that most antigenic material is in the Sp secreted and not cellular fraction is an apparently novel finding. Although psoriatic and control serum IgGs recognized a similar profile of proteins on 2D Western blots, the amounts of IgG bound were consistently higher with sera obtained from psoriatic patients. This enhanced cross-reactivity to Sp antigens by psoriatic patients was confirmed by ELISA comparison of normal and psoriatic sera using proteins from the total Sp secreted protein fraction (patients and healthy controls n = 9). Subsequently, the proteome of lesional psoriatic epidermis was explored by 2D electrophoresis, epidermal material being obtained by removal of the rapidly accumulating surface scales from a volunteer patient. Numerous psoriatic epidermis proteins were resolved, with 2D Western blots of these extracts showing that IgGs in pooled psoriatic serum recognize at least 18 lesional protein spots, whereas normal serum IgGs recognize only 4. Preliminary “competition” Westerns show that psoriatic serum pre-incubated with total Sp secreted proteins cross-reacts with fewer protein spots than native psoriatic serum, suggesting immunological mimicry. These experiments are being extended to exclude effects of Sp virulences factors e.g. proteases such as pyrogenic exotoxin B. MALDI-TOF/MS of selected epidermal protein spots recognized by psoriatic serum IgG but not normal serum has so far revealed the structure of 4 different proteins. One has homology with Sp proteins but none has previously been reported as a putative autoantigen in inflammatory skin disease. These experimental approaches are providing novel insights into the pathogenesis of psoriasis.
12.7
Protein Expression During Biofilm Formation in *Rhizobium etli* and *Sinorhizobium meliloti*

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Most microbes grow in the rhizosphere as organised biofilm communities on surfaces, sessile bacteria (biofilm), constitute a major component of the bacterial biomass in many environments. Bacterial cells attached to, and growing on, surfaces in mature biofilms are physiologically distinct from their planktonic counterparts. We are analysing with proteomics methodology the phenotypic changes that take place when planktonic cells of *R. etli* CE3 and *S. meliloti* make the transition to the biofilm mode of growth. High-resolution two-dimensional gel electrophoresis (2DE) was used to demonstrate phenotypic differences between 6, 12, 24, 72, 120 hours-old biofilm cells and planktonic cells grown in glass-pearls. 900 distinct protein spots were observed in the pH range from 3 to 10 after silver staining. Comparison of the 2DE protein patterns obtained indicated specific protein spots expressed in the biofilm mode of growth. This finding supports several recent observations that a number of functions are required de novo in the initial stages of biofilm development. 100 protein spots were selected and subjected to identification by Mass Spectrometry to define the role of these in the biofilm metabolism in *R. etli* and *S. meliloti*. Ours results indicated that the biofilm proteome differed from the planktonic and sessile proteomes. The sessile proteome differed from the more developed biofilm (10 days) proteome, and the observed difference was not due to a single factor; rather, it was due to a multitude of up- and down-regulated proteins, and posttranslational modification of proteins may also have been involved.

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12.8
Molecular Characterization of *Helicobacter pylori* Clinical Isolates from Early Cancer and from Normal Mucous Membrane from the Same Human Stomach

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*Helicobacter pylori* is a widely distributed human pathogen which is associated with both chronic duodeno-gastro inflammation process, ulcers and cancers. Recently, it was shown that *H. pylori* is highly variable genetically even within the same patient stomach.

The aim of our study to describe biodiversity of *H. pylori* clinical isolates obtained from early cancer and from normal mucous membrane extracted from the same human stomach. Biodiversity was characterized by proteomic, transcriptomic and the related techniques.

The main methods: We used different endoscopic and in vivo staining methods for the cancer tissue observation, diagnosis and resection. After resection we confirmed the diagnosis by different immunohistochemical and genetic tests. *H. pylori* isolation and cultivation was followed by preparation of micro- and macro-arrays for the whole genome scan procedure and expression profiling. In case of the proteomic study we used 2D gel electrophoresis with different staining methods for the quantification procedures and proteome profiling, peptide fingerprinting and MALDI-TOF MS and nano-ESI MS for the protein identification. In all our experiments two reference strains J99 and 26695 were used as control samples.

Results: We examined two patients with early gastric cancer (adenocarcinoma). Two pairs of *H. pylori* isolates were obtained. For each patient, we extracted one *H. pylori* isolate from not further than 0.5 cm from cancer tissue and the other from normal mucous membrane. We describe many differences in the m-RNA expression levels and some variations in the protein expression levels. In contrast, there were no variations in the genome content. Besides, we found out some polymorphisms in the genes which display variations on the m-RNA and protein level.

Conclusion: This is the first observation which confirmed the correspondence between the metabolically altered tissue and the metabolic profiling of the *H. pylori*. Some specific features of the *H. pylori* molecular variability within the same stomach should clarify the basic details of host-pathogen interactions.
12.9 Functional Characterization of Halophilic Thioredoxin from *Halobacterium salinarum*: TRX Defense Against Oxidative Stress

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*Halobacterium salinarum*, an extremely halophilic archaea, grows in the environment containing more than 25% of NaCl. The enzymes and proteins from this archaea organism have thus been adapted to be active and stable in the hyper-saline and hyper-oxidation stress conditions. Thioredoxin, a family of redox proteins, has been found in both prokaryotes and eukaryotes. It is a small ubiquitous protein that participates in various intracellular redox reactions. The amino acid sequence of this protein around the redox-active disulfide bond seems to be highly conserved. Therefore, they are important factors in regulation of oxidative stress by the interaction with target proteins. The typical function of these enzymes is to break the disulfide bonds so that to change the activity of target proteins. To analyze the antioxidant activity of Archaeon Thioredoxin (ArcTRX), we evaluated the antioxidant activity by in vivo *H₂O₂* tolerance bioassay into complementary mutant *E. coli* system and by in vitro MCO (metal-catalyzed oxidation) assay. Supercoiled DNA is prone to nicking when exposed to oxidative radicals such as those generated by the MCO system. The protein of ArcTRX profile of *H. salinarum* cultured under different *H₂O₂* concentrations was investigated with the two-dimensional gel electrophoresis (2-DE). After culturing in the presence of 0.5 mM and 0.75 mM of *H₂O₂*, the soluble proteins were extracted using ultraconcentration and TCA/acetone precipitation. Proteins, which showed different expression level in *H₂O₂* treated cells would be further characterized using the ESI-Q TOF MS/MS spectrometry. ArcTRX protected supercoiled DNA form degradation induced by the MCO system in the presence of FeCl₃ and DTT.

12.10 Proteomic Analysis of Halophilic Archaeon *H. salinarum* and Identification of Phenol Degradation

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Phenol and its derivatives are common constituents of wastewater originating from many industries including pharmaceutical, petroleum and coal refining. Additionally phenol is a common pollutant due to its presence in the effluents of the agricultural activities. Phenol is a toxic and hazardous substance even at low concentrations and efficient treatment methods are necessary to reduce phenol concentration in wastewater to acceptable levels. Among methods available for treatment of phenol, the biological treatment is especially attractive because it has the potential to degrade phenol almost completely with producing innocuous end products and minimum secondary waste generation. Biological degradation of phenol has been extensively investigated, and several studies have shown that phenol can be aerobically degraded by a wide variety of microorganisms, including pure bacterial cultures. In this study, the response of *Halobacterium salinarum*, an extremely halophilic archaea, exposed to phenol were studied for the alteration of proteins at the high concentrations of salt. *H. salinarum* degraded phenol at the concentration of 1 mM as the carbon source under 4.3 M NaCl. The protein expression profile of *H. salinarum* cultured in different phenol concentrations was investigated with the two-dimensional gel electrophoresis (2-DE). We further identified and confirmed the proteins using the MALDI-TOF analysis and ESI-Q TOF mass spectrometry. We identified up-regulated proteins under phenol stress conditions, spots identified as hypothetical proteins, permease, GvPL protein and HR proteins. In addition down-regulated proteins were identified as translation initiation factor, succinate dehydrogenase and heat shock proteins.

12.11 Comparative Proteomic Analysis of *Thiobacillus ferrooxidans* Cultivated with Fe²⁺ and Elemental Sulphur Separately

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*Thiobacillus ferrooxidans* is a gram-negative, acidophilic, chemolithotrophic bacterium which has been described as one of the most important microorganisms involved in metal solubilization in mining operations and coal desulfurization. It can fix atmospheric carbon dioxide, obtaining its energy through the oxidation of ferrous iron as well as from the reduction of inorganic sulfur compounds in ores. With regard to Fe²⁺ utilization, which has been extensively investigated, several oxido-reductase proteins involved in its oxidation have been identified and functionally characterized. Conversely, no clear cut pathway has emerged for the biological oxidation of elemental sulfur and inorganic sulfur compounds. 2-DE technique for *Thiobacillus ferrooxidans* proteomic study which involves conditions for sample preparation, hydration, isoelectric focusing, equalization, dry strips’ selection etc has been optimized, through which high resolution ratio and reproducible 2-DE images were successfully obtained. In three experiments with the same sample, the total number of protein spots is 640 ± 20, the average declination of protein position at IEF orientation is 1.58 ± 0.31 mm and1.45 ± 0.15 mm in SDS-PAGE orientation, the relative standard deviations for protein volume is 5.87 ± 2.50%. About 55 protein spots show different expression level between Fe²⁺ cultivated and sulphur cultivated *Thiobacillus ferrooxidans* cells through analyzing by Image Master 2-D Elite software. 16 protein spots have been identified by MALDI-TOF/MS which including ATP synthase beta chain etc. Further research will focus on these identified proteins’ function in utilizing Fe²⁺ or sulphur as substrate.

12.12 The Preparation of Protein G-2 of Herpes Simplex Virus Type-2 (HSV-2) in Prokaryotic System to Show Type Specific Antibody Against This Virus

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Herpes Simplex Virus type-2 is the major cause of genital ulcer diseases and also cause encephalitis and other disorders in newborns that their mothers afflicted of this virus. Identification of active and latent HSV-2 infection in serological test is based on G-2 protein and this protein is a novel antigen potentially suitable for type discriminating serodiagnosis. In this study HSV-2 was propagated in “HeLa” cell line and viral genome was extracted by phenolchloroform, then G-2 coding gene was amplified by nested-PCR. The amplified gene was cloned into “pTZ57R/T” as a cloning vector. The transformed white colonies was selected in the presence of ampicillin, x-gal and IPTG. The plasmid was extracted and purified and subjected to restriction enzyme and gel electrophoresis. The gene of interest was extracted and purified from the gel, subcloned into Bam-H1 and HindIII sites of pTrc as an expression vector and transformed to E. coli DH5α. The protein production was induced by IPTG and confirmed by SDS-PAGE. The recombinant protein was purified using Ni-NTA purification system, that could utilized in serological diagnostic test such as ELISA.
12.13 Proteomic Analysis of Proteins in Epidemic Strain of Vibrio Cholerae

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Vibrio cholerae O1 biotype EL Tor (VCET), a Gam-negative bacterium, is the aetiological agent of the seventh cholera pandemic. The variability of growth properties, biochemical characteristics and gene types resulted in the great difference of pathogenicity and its pandemic potential. To understand comprehensively the evolution of seventh pandemic VCET strains we selected the epidemic strain (99–128) causing cholera epidemics and the non-epidemic strain (02–002) causing only sporadic infections respectively and investigated the difference of basic biological characteristics and the pattern of protein expression between two strains. The results showed that only in the epidemic strain the test of sorbitol-Fermentation was negative. By PCR analysis toxin genes including tcpA, tcpI, ctxA, ace, cep, tcpA, tcpI, hlyA, rtxA, rtxC, ompU were found in the epidemic strain, but only hlyA, rtxA, rtxC, toxR were detected in non-epidemic strain.

Whole proteins of VCET were isolated in turn by solubilizing bacteria in the lytic buffer and precipitated in analysis medium. The proteins were analyzed by two-dimensional electrophoresis, using immobilized pH gradient strips and approximately 243 proteins were identified. Either in the epidemic strain or in the non-epidemic strain the about 95% proteins were shown in region of pl 4.0–7.0 with molecular masses from 15 to 60 kDa, which their abundant protein spots had acidic pl regions. After comparing the protein profiles of VCET between the two strains by computer-assisted image analysis, the difference of protein expression pattern was observed. The 16 protein spots were found altered in epidemic strain, of which 9 proteins increased and 7 proteins decreased. And the other 8 protein spots were found only in the profile of epidemic strain. The identification of protein spots was performed by trypsin, and subjected to peptide mass fingerprinting. C-terminus of those spots, coomassie brilliant blue G staining was performed. By coomassie staining, 65 spots were visualized, was excised from gel, digested by trypsin, and subjected to peptide mass fingerprinting. C-terminus of those protein spots were compared with that of eleven proteins that have anchoring motif, LPXTG, at their C-terminus in complete IL1403 genome sequence. The research is supported by the science ground of Tianjin Health Bureau (01KY49).

12.14 Purification of Cell Wall and Identification of Cell Wall-anchored Proteins in Lactococcus lactis

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Lactococcus lactis is a Gram-positive bacteria, which belongs to the group of lactic acid bacteria among which several genera play an essential role in the manufacture of food products. L. lactis is a potent candidate as vehicles for the delivery of bioactive proteins in gastrointestinal tract of man and animals. So, exposure of proteins on bacterial surface is of great interest in many medical issues, especially oral vaccine development. The aim of this work was to figure out proteins that are anchored on the cell wall of L. lactis. For identification of the cell wall anchored proteins, a protocol for purification of cell wall of L. lactis was established. Proteins attached covalently to the cell wall well released by digestion with the cell wall-degrading enzyme, Mutanolysin and released proteins were separated two-dimensional gel electrophoresis, and stained with silver nitrate. By silver nitrate staining, 130 spots were visualized. For identification of those spots, coomassie brilliant blue staining was performed. By coomassie staining, 65 spots were visualized, was excised from gel, digested by trypsin, and subjected to peptide mass fingerprinting. C-terminus of those protein spots were compared with that of eleven proteins that have anchoring motif, LPXTG, at their C-terminus in complete IL1403 genome sequence. Of those protein spots, it was confirmed three protein spots had same C-terminus. We first found the three proteins were cell wall anchoring proteins of L. lactis IL1403.

12.15 Lessons Learned from Microbial Proteomics

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Pathogenic bacteria are a major cause of death worldwide. Together with the completed genomes of the pathogens proteomics allows the identification of proteins important for diagnosis, therapy and vaccination against the bacteria. The Mycobacterium tuberculosis and Helicobacter pylori proteomes with about 4000 and 1600 predicted genes were investigated over several years. About 500 predicted proteins were confirmed by two-dimensional electrophoresis/MALDI-mass spectrometry and liquid-chromatography/mass spectrometry for each bacterium. Detailed peptide mass fingerprint analysis of spots from 2-DE gels revealed that many spots contain more than one protein species of different proteins. Proteins of not-predicted genes were identified and predicted N-termini were corrected. Prefractionation of bacteria and different biological situations were investigated. However, the most effective complementarities may be obtained by a combination of 2-DE/MS, LC/MS and SDS-PAGE/MS. Immuno-proteomics revealed protein species of H. pylori, which may be used as diagnostic markers to distinguish between patients suffering from ulcer and gastric carcinoma. The first vaccine candidates for both bacteria were promising results. Storage of data in a proteome database system was established (www.mpiib-berlin.mpg.de/2D-PAGE/). The next steps in proteomics are to analyse the proteome at the protein species level and to understand the network of the proteins and their interactions, which can only be reached if a high completeness of the proteome is experimentally accessible.
Pseudomonas exist with respect to the metabolic pathways of aromatic compounds in pathways, including that of biodegradation, and that interrelationships presence of benzoate or aniline. These results suggests that proteome when combined, rapidly than others (benzoate /H11022 that 
dation pathway induced. The NMR analysis of substrates demonstrated 
at, the protocatechuate 4,5-dioxygenase pathway was the major degra-
dary pathway induced by aniline (aniline analogues) exposure. On the other 
hand, the catechol 1,2-dioxygenase pathway was the major pathway 
induced by benzene exposure for the degradation of p-hydroxybenzo-
ate, the protocatechuate 4,5-dioxygenase pathway was the major degra-
dation pathway induced. The NMR analysis of substrates demonstrated 
that Pseudomonas sp. K82 metabolizes some aromatic compounds more 
rapidly than others (benzoate > p-hydroxybenzoate > aniline) and that 
when combined, p-hydroxybenzoate metabolism is repressed by the 
presence of benzene or aniline. These results suggests that proteome 
analysis can be useful in the high throughput study of bacterial metabolic 
pathways, including that of biodegradation, and that interrelationships 
exist with respect to the metabolic pathways of aromatic compounds in 
Pseudomonas sp. K82.

**12.17**

**Proteome Analysis of the High Light Treated Cyanobacterium Synechocystis sp. PCC 6803**

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Light is an important role in cyanobacterial growth not only as an energy source but also as a stimulus. Study on gene expression profile during acclimation from low to high light intensity has been done using DNA microarrays by Hihara et al. (Plant Cell, 2001, 13, 793). Two dimensional gel electrophoresis (2-DE) was performed to investigate proteins related with acclimation against high light in Synechocystis sp. PCC 6803. Wild-type cells were exposed to high light (350 μmol/m²/s) condition for 24 hrs. For 2-DE, samples were prepared in various time intervals 15 min, 1 hr, 6 hrs, 12 hrs, and 24 hrs. More than 800 proteins were displayed on the SDS-PAGE gels stained with silver nitrate. For quantitative analysis, Progenesis workstation software was used. Several proteins showing different expression levels under high light conditions were identified with MALDI-TOF mass spectrometer. In this study more than 80 proteins were identified and classified into distinct groups. Proteins whose quantities were influenced by high light were classified into 3 categories such as increase, decrease and no difference depending on exposure time. Unlike Hihara’s data, many of proteins had late responsive expression patterns. Only some proteins had shown quantitative increase or decrease to short term high light treatment. Our results may help to elucidate the photo-acclimation mechanism of Synechocystis sp. PCC 6803.

**12.18**

**Isolation and Characterization of a Bacterial Laccase and Its Application in Biobleaching of Wood Pulp**

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Laccases (EC 1.10.3.2) have been found exclusively in plants and fungi, however, only the fungal laccases are subjects of current biotechnological applications. Therefore, identification of bacterial laccases for which genetic tools and biotechnological processes are well established would be of significant importance. Because of their capabilities of catalyzing the oxidation of phenols, laccases are receiving increasing interest as potential industrial enzymes in various applications such as delignification and detoxification. Present work reports the laccase activity in a novel bacterial isolate, which is isolated from a forest site (Roorkee, India). This bacterium produced laccase as the predominant extra cellular phenoloxidase. Among 2,2’-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), syringaldazine, veratryl alcohol and vanillic acid-syringaldazine (0.20μM) was found as the best inducer for laccase induction. Laccase activity in the crude extra cellular medium and purified sample was assayed by monitoring the oxidation of 2,2’-azinobis-(3-ethylbenzthiazoline-6-sulfonate) at 420 nm (ε = 432 cm² μmol⁻¹). This enzyme was also found capable to oxidize vanillic acid, catechol and syringaldazine. Purified enzyme was identified as a glycoprotein with a molecular mass of 54 kDa. In order to check the delignification activity, extra cellular medium containing enzyme was concentrated 20 times and unbleached wood pulp was incubated for 2 hours at 37°C. Secreted enzyme was found capable to bleach the wood pulp by 2% as measured spectrophotometrically.
12.19

Functional Analysis of the Two Putative Sortase Genes SrtA and SrtB in *Lactococcus lactis*

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Lactic acid bacteria (LABs) are potent candidates as vehicles for the delivery of bioactive proteins both in food product and in the gastrointestinal tract of men and animals. Cell wall anchoring of antigens at the surface of LABs is particularly promising for live vaccine development. Surface proteins of Gram-positive bacteria are covalently linked to the bacterial cell wall by sortase, an enzyme that cleaves polypeptides at the threonine residue of the LPXTG motif and anchors to the pentaglycine of the peptidoglycan. Based on the recent genome sequence of the model LAB *L. lactis*, two sortase homologues-SrtA, SrtB-were identified. Our object is to analyze the function of two putative sortase genes, SrtA and SrtB of *L. lactis*. Single and double SrtA and SrtB deletion mutants were constructed using allelic exchange technique. The protein fraction released from purified cell walls by digestion with N-acetylmuramidase was profiled by two-dimensional gel electrophoresis. Cell wall protein profiles of the wild type and single and double mutants were compared and analyzed with each other. The number of total protein spots in wild type were 150. More than 16 of the protein spots were missing in the profile of the SrtA mutant compared with that of the wild type strain, and 9 protein spots were missing in SrtB mutant. About 5 among the missing protein spots disappeared both in SrtA and SrtB mutant. These genes seem to be responsible for the covalent anchoring of different proteins. Three protein spots among 16 that were missing in SrtA mutant were identified and found to contain LPXTG motif in their predicted amino acid sequences. So, we reason that putative sortase genes SrtA and SrtB are related to the anchoring of the cell wall proteins in *L. lactis*. We are characterizing genes involved in cell wall anchoring of proteins by comparing the surface protein profiles of wild type and mutant strains. This should help us to better understand and control cell wall anchoring of proteins in *L. lactis*.

12.20

New Approach for the Validation of Cell Banks Using the 2-DE Gel Analysis

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Two-dimensional electrophoretic (2-DE) analyses of cell banks of recombinant *Escherichia coli* for human growth hormone (hGH) were conducted for the cell validation. The hGH is a pituitary-derived polypeptide, which has therapeutic applications in the treatment of dwarfism, bone fractures, skin burns and bleeding ulcers. The aims of this study are to present the expression patterns of proteins in cell banks of recombinant *E. coli* producing hGH and to characterize Master Cell Bank (MCB), Working Cell Bank (WCB) and End of Production Cell (EPC). The 2-DE analyses were performed to compare 2-DE gel of recombinant *E. coli* producing hGH with that of wild type *E. coli*. Protein expression map of MCB was compared with that of WCB cultivated to the mid-exponential growth phase and those of WCB and EPC cultivated to the stationary phase were compared. To identify and compare the proteins from recombinant *E. coli* producing hGH and wild type *E. coli* expressed in same conditions, cells were grown in LB media containing ampicillin and then proteins from the whole cell lysates were separated on the 2-DE gel. The proteome profiles of cell banks were similar on the 2-DE gel. A few spots which showed differences in the expression level were identified with matrix-assisted laser desorption ionization/time-of-flight (MALDI-TOF). The proteome profile of recombinant *E. coli* can help to validate each cell bank and would be a valid approach for the cell validation particularly in the biopharmaceutical industry.
12.21
Study on the Resistibility of SARS Coronavirus

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Since the World Health Organization (WHO) announced interdiction of the transmission chain of severe acute respiratory syndrome virus (SARS-Cov) on July 5, 2003, there is a continuous concern on a new outbreak in winter or in spring as other respiratory epidemics will do. However, only lab infection cases and related cases, and 4 separate patients have been reported since then. This study observed the survival probability of SARS-Cov in excrement, urine and waters, and inactivation in sewage with sodium hypochlorite and chlorine dioxide. It’s shown that in vitro the virus could only survive for 2 days in hospital sewage, domestic sewage and dechlorinated tap water; while 3 days in excrement, 14 days in PBS and 17 days in urine at 20°C. However, at 4°C, the SARS-Cov could survive for 14 days in all above water bodies and at least 17 days in excrement and urine. Resistibility of SARS-Cov to disinfectants in sewage is lower than that of E. coli and f2 phage. With same concentration of disinfectants or free residue chlorine, chlorine has better inactivation effect than chlorine dioxide. Free residue chloride over 0.5mg/L for chlorine or 2.19mg/L for chlorine dioxide in sewage ensures completely inactivation of SARS-Cov while unable to completely inactivate E. coli and f2 phage.

SARS-Cov lives a very short time in vitro environment and is highly sensitive to conventional disinfectants. Given that large amount of various disinfectants were used for environment disinfection in China’s mainland during the SARS epidemic in 2003 and the high temperature of summer-time, except animal-to-human transmission or cross infection within labs, there is little possibility for another outbreak caused by SARS-Cov existing in the environments.

12.22
Constructed 2D Gel Electrophoresis Map of M. tuberculosis Proteome

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Tuberculosis is one of several infection diseases. It is important to find out protein components of M. tuberculosis correlation with pathogenesis. M. tuberculosis H37Rv strains were cultured in the late-log phase. Adding ammonium sulfate precipitated short filtration protein and sonicating staved M. tuberculosis H37Rv strains. All samples were separated using first dimension 18 cm IPG strips, and 12% polyacrylamide gel in the second dimension. Figure 1 show pH 4–7 separations of M. tuberculosis SFP, and Figure 2 show pH 4–7 separation of M. tuberculosis strain (figures were omitted). The result will be analyzed by mass spectrometry and discussed.
Mass Spectrometric Analysis of Proteins Associated with Purified Human Cytomegalovirus

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Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that typically causes asymptomatic infections in healthy individuals but may lead to serious complications in newborns and immunodeficient individual. For example, this virus accounts for one of the most common opportunistic infections in AIDS patients (e.g. CMV retinitis). Moreover, HCMV infection is the leading viral cause of birth defects in newborns and a major cause of morbidity and mortality in bone marrow and solid organ transplant recipients. Understanding the composition of HCMV infectious particles is critical in developing new drugs and novel strategies for treatment and prevention of HCMV infection. Possessing a large linear double-stranded DNA genome of 230 kbps, HCMV is capable of encoding more than 220 open reading frames (ORFs). Here we report a systematic identification of proteins associated with purified HCMV particles using MALDI-TOF. We purified HCMV infectious virions using potassium-tartrate gradient centrifugation method and isolated virion proteins. 1D-GE or 2D-GE-separated proteins were subjected to PMF using MALDI-TOF. All known virion proteins, previously identified using amino acid sequencing using Edman degradation, were confirmed using library-based database search. Most of these proteins (such as UL25, UL26, UL32, UL47, UL48, UL55, UL82, UL83, UL85, UL86) are suspected to have structural roles, consistent with the idea that they are discovered on the surface of the viral envelope. Interestingly, a cellular protein, annexin V, was found to be associated with virion particles. A homologous protein (annexin II) has been previously shown to directly interact with HCMV, suggesting that annexin V may be involved in a similar interaction as well. Our study demonstrates a rapid and straightforward method of viral proteomics and could be extended to study of other enveloped viruses as well.

Analysis of the Metabolic Network of Lactococcus lactis

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Lactococcus lactis is widely used as a starter for the fermentation of milk and the production of dairy products intended for human consumption. In order to select strains well adapted to the industrial constraints and preserving the safety of the consumer, it is highly desirable to know the detail of the physiological adaptation of L. lactis to milk. The proteomic analysis makes it possible to rebuild the potential metabolic activity of micro-organisms. This approach was implemented to characterize the physiology of L. lactis at the time of its development in dairy media (whey and milk). The comparison of the E-2D profiles in synthetic and dairy media made it possible to identify around fifty of proteins whose intracellular concentration varies positively in the dairy environment. The analysis shows a broad redeployment of the metabolism towards the biosynthesis of nitrogenous compounds like amino acids and purine bases. We also observe the activation of the arginine deiminase pathway: the ATP and ammonia produced at the time of the arginine catabolism thus take part in the adaptation to this environment. The concentration of enzymes implied in the metabolism of amino sugars also increases in a systematic way. Lastly, we note the increase in several enzymes intervening in the response to the oxidative stress. This work thus enabled us to establish a signature of the metabolic activity of L. lactis in dairy media. The essentiality of certain proteins in milk was further confirmed through the inactivation of the corresponding genes. In the course of this work, we made an intensive use of the PARIS software (see poster by J. Wang et al.). We also exploited the recent results on the structure of the theoretical metabolic networks in order to characterize the experimental metabolic networks deduced from the proteomic data.
12.25
Proteomic Studies of Staphylococcus Epidermidis Using Two-dimensional Gel Electrophoresis and Tandem Mass Spectrum

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In nature, the majority of bacteria live in close association with surfaces, as complex communities referred to as biofilms. AT12228 and RP35984 are two bacteria belonged to staphylococcus epidermidis, the latter can produce biofilms while the other can not. We can use proteomic to study this objective. Proteomics refers to the comparative identification of all proteins expressed under various conditions. This technique is an essential complement to transcriptome analysis because it allows the detection of proteins, the functional entities of a cell, and of PTMs, which cannot be predicted by mRNA expression analysis.

A proteomic analysis of staphylococcus epidermidis AT12228 and RP35984 was carried out by 2-DE and MALDI-TOF-MS and database searching. Using 2-DE, the overall protein map was obtained, in which 150 spots were matched with the control gel (match ratio: 70%). Compared with AT12228, 35 spots significantly changed in the RP35984 (p < 0.05), among which the expression levels in 10 spots increased and 12 spots decreased. Especially, 3 spots in AT12228 were absent in the RP35984; while 10 spots in the RP35984 were absent in AT12228. Using MALDI-TOF-MS and database searching, we have identified changed expression proteins and some high abundant proteins. According to their functions, the proteins identified could be classified into several categories. The application of proteomics to the study of staphylococcus epidermidis makes it possible to characterize global alterations in protein expression in biofilm formation.

12.26
Modelling the Mammalian Cellular Proteome During Invasion by an Intracellular Pathogen

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Toxoplasma gondii is an apicomplexan, intracellular parasite that can cause disease and mortality in humans and livestock. Inside the cell it forms a parasitophorous vacuole in the cytoplasm where it resides and replicates until it causing the cell to lyse. We are using proteomic methods to model the protein expression changes in Toxoplasma infected cells in order to gain a better understanding of the host-pathogen interaction. We have generated 2D maps of infected and mock-infected cells, and identified proteins differentially expressed between the two cell populations using mass spectrometry. Several functional categories of differentially expressed proteins have been identified including upregulation of anti-apoptotic proteins and a down regulation in mitotic proteins, consistent with the hypotheses that Toxoplasma inhibits host programmed cell death and induces cell senescence.

We are investigating the protein expression changes over a variety of time-points post invasion. In addition to analysing steady-state protein levels we are also profiling the phosphorylated protein component of the cell.

To investigate whether the cell may have a common response to invasion we plan to use several intracellular pathogens, including Leishmania. We also discuss the use of DIGE (Differential gel electrophoresis) to investigate further the host cell response to invasion.

12.27
In Depth Analysis of Halobacterium Species NRC-1 Proteome

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Halobacterium sp. NRC-1 is an extremely halophilic archaeon known to contain a highly acidic proteome. Previous analysis predicted approximately 2,400 unique protein coding genes in the 2,571,010 bp genome (Ng et al., 2003). Among these, 165 (54.5% contained TMHMM predicted membrane spanning domains) were only found in the membrane. Recently we applied an offline 2-dimensional (SCX and C18) separation approach to reduce the complexity of peptide prior injected into the mass spectrometer. In 64 tandem MS runs, more than 850 proteins were identified from the soluble proteome. These data provide us the essential information to reconstruct the physiology of this organism grown in laboratory culture conditions. In additions, these data allow us examine the physical and biochemical properties of the identified peptides and proteins and determine the logistics to tackle more complex proteomes.

12.28
Rapid Screen of Highly Efficient Vaccine Candidates by Immunoproteomics

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Diseases caused by microorganisms can be controlled by vaccines, which require neutralizing antigens. Therefore, it is very important to determine highly efficient immunogens for immune prevention. Combining immunoproteomics and bacterial challenge after immunization, we developed a rapid method for screen of protected antigens of pathogenic bacteria in aquaculture in the current study. Our approach may be divided into three consecutive steps. First, dominant immunogens of OMPs were screened by immunoproteomics. Second, proteins with ability inducing production of neutralizing antibodies were identified from the immunogens with the method of virulent bacterium challenge following vaccination. Third, vaccine candidates were determined by the evaluation of the neutralizing abilities. Information on the candidates has been provided for further gene cloning by MS. Our results indicated that highly efficient protected antigens were identified from outer membrane proteome of Aeromonas hydrophila, in which an immunogen showed 71.4% protective ability with multivalent functions to Aeromonas hydrophila and Aeromonas sobria. In summary, the methodology developed in the current study is high throughput and hence approached rapid, highly efficient and accuracy aims, which will play active role in immune prevention for microbiological diseases.
Temperature, Nitrogen and Phosphorus Affect Protein Profile of Microcystis aeruginosa: A Comparative 2-Dimensional Gel Electrophoresis (2-DGE) Analysis

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Cyanobacterial blooms are potential health hazards and Microcystis aeruginosa is the vital specie. When environmental elements change, such as higher temperature, higher concentration of phosphorus and nitrogen, bloom blossoms. We report here a comparative analysis of the effect of different temperature (21°C, 37°C, 42°C, 60°C), different concentration of nitrogen (1.6 mg/L, 16.3 mg/L, 81.7 mg/L, 245.1 mg/L, 490.2 mg/L), while phosphorus is 6.9 mg/L and phosphorus (0.3 mg/L, 1.4 mg/L, 6.9 mg/L, 20.7 mg/L, 62.1 mg/L while nitrogen is 81.7 mg/L) on the protein profile of Microcystis aeruginosa. The expression profiles of cellular proteins in response to the different cultivated environment were analyzed by two-dimensional gel electrophoresis (2-DGE) within pH ranges of 3–10. Twenty-three protein spots express differently. Among these, two proteins (pl 4.6, 7.5 kD; pl 9.2, 55 kD) were expressed only at 42°C and nineteen proteins have a large amount expression. Two out of nineteen differential spots were identified as hsp60 and hsp70 that have been found to serve as an adaptive and protective mechanism against environmental stresses. In contrast, more than seven proteins were down-regulated at 20°C. Variety of phosphorus and nitrogen concentration in the growth medium revealed significant differences in the 2-DE gel. Compared to the normal controlled condition (nitrogen 81.7 mg/L and phosphorus 6.9 mg/L), sixteen protein spots were up-regulated at lower concentration of phosphorus (0.3 mg/L) while eight protein spots were down-regulated. And nine proteins were unique to higher phosphorus concentration (20.7 mg/L). Limitation of nitrogen (1.6 mg/L) in growth affected the synthetization of protein in a large degree. In contrast, there are fifteen protein spots were up-regulated while six protein spots were down-regulated under the rich nitrogen (245.1 mg/L to 490.2 mg/L) environment.

Use of Microbial Protein Substitutions for Treatment of Oncological Diseases

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For the last years the researches of a role of various microorganisms in a regression malignant neoplasm were sharply actuated in the world. The study of microbial substitutions, analogues of “Coley’s vaccine” is one of the most perspective among them. This vaccine for the first time offered by W. Coley in XIX century, represents the association of bacteria prepare in the special manner. The main acting factors of such preparations are high-power stimulation of an immune system; direct killer effect of microbial toxins on atypical cells and others. Analogic effect and accelerated healing of operational wounds is observed during use of them in parallel with a regression of tumours. The developed medicines are standard on the structure and are stables during long storage, they do not contain living microorganisms, are accessible in preparation, little toxic. We studied efficiency of analogous preparation CV-1 on white mice (line Balb/c) with carcinoma of small intestine. The mice (32 pieces) were divided into 4 groups: 1 - control (placebo); 2 –into tumour injection 0.5 ml of a working solution (WS) of the preparation (10⁻² ml of an initial solution); 3 –intraperitoneal injection of 0.5 ml WS; 4 - intraperitoneal injection of 1.0 ml WS. Injections were made an every second day for at least 2 weeks. All animals of 1 group perished. In groups 2 and 3 from 50 up to 60% of mice survived. In 4 group 87.5% of animals survived. The morphological re-searches confirmed absence of atypical cells at the survived mice. Thus, the preliminary outcome testify high efficiency of developed anticancer drugs based on microbial protein substitutions and their perceptiveness.

Proteomic Approach to the Pathogenesis of Mycoplasma hyopneumoniae

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Mycoplasma hyopneumoniae is a highly prevalent pathogen which colonizes the ciliated epithelial lining of porcine respiratory tract and induces damage to the cilia. Though a few pathogincity-associated proteins had been studied, the pathogenic mechanism remains elusive. The high passage strain J originated from a pig with mycoplasma pneumoniae and had been used for studies on the pathogenesis of the disease. However, the J strain lost virulence through successive passage in vitro. Previous EM studies have shown that the strain J does not adhere to the cilia and induce no clinical disease. Recent studies of the P97 adhesin molecule of M. hyopneumoniae demonstrated that a size shifting of P97 for strain J. The effect of size variation of P97 on the adherance ability of the mycoplasma remains to be determined.

In the present studies, proteomic approach was taken to look for all the possible virulence traits. The M. hyopneumoniae strain J appears to bind much less than the virulent strain 232 as revealed by the MPAA experiments. Two-dimensional gel electrophoreses were performed to compare the strain J and the virulent strain 232. The 2D gels were also blotted and the protein profiles confirmed with the rabbit hyperimmune serum against M. hyopneumoniae. Western analysis and MALDI-TOF analysis reveal that the major differences between the two strains include the aminotransferase-like protein and the P97 adhesin molecule (pl 8.6, MW 124 kD; 97 kD on SDS-PAGE gels). The protein spots may represent the virulent factors or the variation of antigens and deserve further identification and characterization.

Analysis of Protein Expression in the Early Stage of Conidia Germination in Aspergillus nidulans FGSC4

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Aspergillus nidulans has been used as a well-established nonpathogenic laboratory model organism to investigate the eukaryotic cell cycle, regulation of metabolism and differentiation. Many genetic researches have been conducted on the asexual spore production, development, germination, and morphological change of Aspergillus nidulans. For a global analysis of the proteins involved in the process of germination in Aspergillus nidulans FGSC4, we investigated the changes in protein expression profiles during the early stages of conidia (asexual spore) germination. The proteomes of dormant and germinating conidia were analyzed by 12% homogenous two-dimensional polyacrylamide gel electrophoresis using an immobilized pH gradient system (pH 4–7). About 1700 protein spots were visualized by silver staining and analyzed using PDQuest image analysis software. We found that protein profile of germinating spores was greatly different from that of dormant spores. During germination, the intensities of 32 spots increased over three-fold, and those of 31 protein spots decreased significantly. Also, we observed that 448 protein spots appear newly on the profile of germinating spores. The proteins expressed differentially during germination were identified by MALDI-TOF mass spectrometry. We have identified 19 protein spots of which intensities increased during germination, 9 decreased protein spots and 28 protein spots which appeared newly upon germination.
Preliminary Proteomics Analysis of Yersinia pestis Strain 91001

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Yersinia pestis, the causative agent of plague, is thought to be one of the most deadly pathogenic bacteria in the world. Although numerous information provided by three finished genomes of Y. pestis have shed lights on the mysterious pathogenicity of this deadly bug, extensive proteomics analysis will undoubtedly be of great value to better understand these puzzles. In this study, three different methods (Shotgun-LC-MS-MS, 1D-LC-MS-MS and 2D-MS) were used to profile the proteome a human-avirulent Y. pestis strain, 91001. For Shotgun-LC-MS-MS, the proteins from strain 91001 grown at 28°C were extracted into three parts fractionally according to their solubility, and then analyzed by LC-MS-MS directly. While for 1D-LC-MS-MS analysis, the fractioned proteins were separated by 15% SDS-PAGE, followed by full-gel slicing, enzyme digestion and LC-ESI-MS/MS identification. All the raw data was identified as individual proteins by searching the theoretical protein database of strain 91001. In this study, Shotgun-LC-MS-MS method identified 971 proteins, accounting for 23.4% of the predicted proteins of strain 91001 (971/4143); 1D-LC-MS-MS method identified 915 proteins, accounting for 22.1% of the predicted proteins of strain 91001 (915/4143); while 2D-MS revealed 5.62% of the predicted proteins (233/4143). All the three methods jointly uncovered 1193 proteins, which sum up to 28.7% of all the predicted CDS in 91001 (1193/4143). The identities and numbers of proteins identified by different proteomics methods differed from each other dramatically; therefore it is necessary to utilize several methods to get more reliable protein profiles of Y. pestis.

Antibacterial Drug Discovery

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There is growing concern that we will very soon enter a post-antibiotic age—an age when death from bacterial infections will be prominent. Despite the obvious need for new antibiotics, many pharmaceutical companies have downsized or eliminated their antibacterial programs. Although commercial opportunity is the leading reason for the downsizing of antibacterial drug discovery, another reason is the difficulty developing antibiotics that hit novel targets. So might proteomics assist with this secondary issue?

Much of the antibacterial drug discovery currently underway in the pharmaceutical industry is focused on two strategies. First, a strategy to find new chemotypes for proven targets, like Gyrase and the ribosome. The second strategy is focused on selecting new targets from the genome information. This approach relies heavily on bioinformatics, high throughput screening, structural biology and chemistry. About the same hundred targets have been selected by all groups. Both strategies desire broad-spectrum antibiotics—one chemotype works for all or most pathogens.

Proteomics has been used for more than a decade on the first strategy, using pattern matching to find new chemotypes. Proteomics more currently has been used for the 2nd strategy. For this strategy, proteomics is used to determine the cellular response when the expression of the target is knocked down genetically. The assumption is that this response will be similar to the response to inhibitors of the target. This approach also seems to work.

Looking forward, what can proteomics do to help the pharmaceutical industry work on narrow spectrum antibiotics? What can proteomics do to help find pairs of targets whose inhibition will results in a synergistic antibacterial effect and potentially a therapy that will be less prone to development of resistance by micro-organisms?
12.35
Physiological and Proteome Characterizations of Oxidative-stress Response in Rhizobium etli During Free Life and Symbiosis with Phaseolus vulgaris.

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Cellular metabolism of molecular oxygen produces reactive and potentially toxic oxygen species such as superoxide, hydroxyl radicals and hydrogen peroxide. For defense against reactive oxygen species, organisms contain antioxidants and enzymes that prevent or repair oxidative damage. In R. etli katG encodes a plasmid-borne dual-function catalase-peroxidase (KatG) that was inducible and heat-labile. In contrast to other Rhizobia, katG was shown to be solely responsible for catalase and peroxidase activity in R. etli and in not essential for nodulation and nitrogen fixation (Vargas et al., 2003). Upstream of the catalase promoter we found another gene transcribed divergently from katG and putatively encoding the regulator protein OxyR. In addition we observed that oxyR is essential for nitrogen fixation in symbiosis with P. vulgaris. Proteome analysis showed that some proteins are presents only in the wild type protein patterns, which suggest a putative regulation in a OxyR-dependent manner. In free life, protein expression maps were constructed in the presence of organic and inorganic peroxides. Some proteins are being examined by Mass spectrometry and with this result we will suggest the putative role of these proteins. As we mentioned, KatG protein is not essential for nodulation and nitrogen fixation in symbiosis with P. vulgaris, however, our proteome analysis suggest that in symbiosis the oxidative stress response includes some newly identified elements as a peroxiredoxin protein which is expressed only in symbiotic conditions. The substrates of peroxiredoxin family proteins include hydrogen peroxide, alkyl hydroperoxides, and peroxyxynitrite. To define the role of this component in symbiosis we obtained the mutant strain in this gene, our preliminary results indicated that this protein is indispensable during the nitrogen fixation in symbiosis. With this aim we suggest alternatives protection systems for oxidative protection of R. etli in free life and symbiosis. Part from this work is supported by CONACYT 40046-Z and DGAPA 203003–3 grants.

12.36
The Proteomic Studies of Glycolytic Pathway in Thermoanaerobacter tengcongensis

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Thermoanaerobacter tengcongensis (T. tengcongensis) is an extremotolerant eubacterium found in China. Although this bacterium grows in a glucose-required culture medium, interestingly, the analysis of T. tengcongensis genome has suggested that hexokinase or glucokinase does not exist, but all of other glycolytic enzymes are found in this genome. How can the conflict between the function observation and genomic data be addressed? We have employed proteomic approach to profile the bacterial proteins expressed at the optimal culture condition and to re-screen all of the identified proteins against the catalytic domains of glucose kinase. TTE0090 was found to have an acceptable sequence homology (>54%) with glucokinase. Not only in the homology of amino acid sequence, does TTE0090 also contain the conserved motifs for ATP and glucose binding. The TTE0090 gene was inserted into an E.coli expression vector and was expressed in BL-21 strain. The recombinant TTE0090 catalyzed the phosphorylation of glucose and mannose with the apparent K_m values about 500μM. Importantly, TTE0090 performed its enzyme activity at high temperature, an optimal point around 70°C. In conclusion, by combining proteomic analysis, homology comparison of enzyme domains, and molecular cloning, we have discovered a new gene which can efficiently convert glucose to glucose-6-phosphate at high temperature. Moreover, identification of TTE0090 could contribute an elucidation for the adaptable mechanism of T. tengcongensis surviving at the extreme environment.

12.37
Proteomic Research of Bacillus Anthracis

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Bacillus anthracis has always been an important pathogen because it can cause lethal inhalational anthrax and may be used as a bioweapon or used by bioterrorists. Its whole genome sequences have been published by TIGR last year, so a comprehensive proteomic study based on it may result in more information about pathogenesis and physiology. In this study, a reference map of Bacillus anthracis A16R from mid-exponential phase was constructed and the protein expression patterns during spore formation were analyzed. The silver-stained 2-DE maps with various IPG strips such as pH 3–10, pH 4–7, pH 4.5–5.5, pH 5–6 and pH 5.5–6.7 were constructed. In total, 450 proteins were processed for MALDI-TOF-MS analysis and 348 proteins representing 259 genes were identified. At the same time, in order to reveal the mechanism for spore formation, we selected 6 representative phases from mid-exponential phase to mature spore through Gram-stain and subsequently by transmission electron microscope. 2-D electrophoresis of the whole-cell and extracellular proteins from these 6 phases were done by use of IPG strips pH 4–7. Consequently, 136 differentially expressing whole-cell and 57 extracellular proteins were found and analyzed.
12.38
Amino Acid Substitutions at Thumb-Palm Interface of Reverse Transcriptase Affect Replication Competency of Genotype C HBV
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Based on the nucleotide sequence homology of full-length HBV strains, seven genotypes of HBV have been conclusively identified, among which genotype B and C are prevalent in China and South-Eastern Asia. Analysis of HBV database (constructed by our laboratory and Shanghai Center for Bioinformatics Technology) showed that genotype C is more common than genotype B in HCC cases. The analysis study also revealed that, in the viral polymerase, multiple unique amino acid substitutions were present in the both HBV genotypes, whereas proline 652 is always conserved. In our previous study, a putative thumb-palm interface of the reverse transcriptase domain in HBV polymerase, where proline 652 is located, was observed important in the regulation of genotype B HBV replication. To study the importance of the conserved proline 652 in genotype C HBV replication, full-length DNA of a wild-type genotype C HBV strain (97–34) isolated from an HCC patient was used to generate amino acid substitutes. Residue 652 (proline) was replaced by 7 amino acids separately, including alanine, aspartic acid, glutamic acid, glycine, leucine, serine, and valine. All mutants showed decreased virus replication in cultured hepG2 cell line, confirming that the residue located at the thumb-palm interface was of importance in regulating the replication of HBV. Since the nucleotides encoding residue 652 overlap with the cis-regulatory element enhancer I, the possible effects of these nucleotide substitutions on the function of Enhancer I and replication are under investigation.

12.39
Structural and Functional Analysis of Hepatitis B Virus Polymerase
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HBV DNA polymerase is composed of four domains and starting from the N-terminus they are the terminal protein (TP), a spacer region which has not been associated with a specific function, the reverse transcriptase (RT) and the RNase H, which digests the RNA in RNA-DNA hybrids. By comparing full-length HBV genomes with high homology in nucleic acid sequences (98.7%) but replicated at high and low virus loads (97–34) isolated from an HCC patient was used to generate amino acid substitutes. Residue 652 (proline) was replaced by 7 amino acids separately, including alanine, aspartic acid, glutamic acid, glycine, leucine, serine, and valine. All mutants showed decreased virus replication in cultured hepG2 cell line, confirming that the residue located at the thumb-palm interface was of importance in regulating the replication of HBV. Since the nucleotides encoding residue 652 overlap with the cis-regulatory element enhancer I, the possible effects of these nucleotide substitutions on the function of Enhancer I and replication are under investigation.

12.40
Human Endogenous Opioid Peptide and Heat Shock Protein Involve in the Response to Mycobacterium tuberculosis Infection: Revealed by a Combination of Transcriptomic and Proteomic Approach
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Two-dimensional electrophoresis was used to compare the proteome of macrophage cell line U937 before and after Mycobacterium tuberculosis clinical isolates infection. Two spots upregulated markedly after infection was subjected to peptide fingerprint and bioinformatics analysis. The two spots were identified as heat shock protein 105 beta and preproenkephalin respectively. Upregulation of heat shock protein 105 beta was revealed by a combination of proteomic and transcriptomic approach. This was the first report on the upregulation of host endogenous opioid peptide and heat shock protein 105 beta by M. tuberculosis clinical isolates infection. Opioid peptide stimulate the phagocytosis of M. tuberculosis. This study help to explain the high incidence of tuberculosis among drug abusers. Neuroimmunology is another clue to further investigation of Mycobacterium tuberculosis pathogenesis and host immune strategy.
Genomic and Proteomic Identification of a Global Regulatory Network in the Lyme Disease Spirochete, *Borrelia burgdorferi*

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Spirochetes are phylogenetically distinct from other major bacterial groups. Little is known about the molecular biology of spirochetes because of the inability to cultivate and genetic manipulate them. In the past few years, genome sequences for several spirochetes have been decoded, including *Borrelia burgdorferi*, *Treponema pallidum*, *Treponema denticola*, and *Leptospira interrogans*, which are the causative agents of Lyme disease, syphilis, periodontitis, and leptospirosis, respectively. This genomic information provided an unprecedented opportunity for studying molecular biology of this unique, yet medically important, group of bacteria. We chose *B. burgdorferi* as a model system. *B. burgdorferi* transmits to humans via tick bites (genus *Ixodes*), resulting in Lyme disease, one of the most common arthropod-borne diseases in the United States. Previously, using genomic, genetic and biochemical approaches, we demonstrated that there is a novel regulatory network, the Rrp2-RpoN-RpoS network, present in *B. burgdorferi*. Herein, we present further characterization of this network using two-dimensional gel electrophoresis (2D-PAGE) coupled with matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS). We showed that the Rrp2-RpoN-RpoS network controls expression of many infection-associated immunogens of *B. burgdorferi*, most of which are known or predicted lipoproteins.

Some of these proteins are essential for borreliar transmission and infection (OspC, DbpA). In addition, many of these proteins (OspC, DbpA, Mips, Erps) exhibit temperature and pH-dependent expression. Furthermore, our results reveal that RpoS is the main target controlled by Rrp2 and RpoN. Thus, Rrp2, RpoN and RpoS constitute a global regulatory network that modulates *B. burgdorferi*’s adaptive responses in both ticks and mammalian hosts and plays a pivotal role in *B. burgdorferi*’s virulence.

Progress in Microbial Proteomics

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Proteomics, analysis of proteins in a high-throughput mode, has begun to provide profound insight into the gene function and biological process directly at the protein level. Progress in microbial proteomics focuses on five aspects as follows: (1) Proteome analysis of microorganisms under various conditions of environmental stress. A number of experiments have shown that bacteria develop an adaptive response when grown under various conditions, this helps to elucidate the molecular mechanisms of microorganisms adapting to changing environment. The heat-shock response has been studied extensively in several Gram-positive bacteria and Gram-negative bacteria. Study on response of *Bradyrhizobium japonicum* to heat shock resulting in the discovery of 19 Hsp, including several shsp. Proteome analysis of *Escherichia coli*, *Listeria monocytogenes*, *Bacillus subtilis*, *Brucella melitensis*, *Agrobacterium tumefaciens*, *Acinetobacter baumannii* and *Hansenula polymorpha* show that the stress proteins induced by various stress such as salt, ethanol, heat, heavy metals, disulfide and nutritional starvation can overlap with each other to different extents. (2) Proteomics of bacterial pathogen. Analysis of pathogenic microorganisms proteins can greatly help us to better understand the molecular mechanisms of diseases, to identify novel drugs and drug targets, as well as to perform clinical diagnostics and develop new vaccine. The comparative study of *Salmonella typhimurium* result in the discovery of pathogenicity genes and SPI2 regulon, proteome analysis of *Mycobacterium tuberculosis* H37Rv and H37Ra reveals virulence genes, and also, 32 antigens were found in *Helicobacter pylori* relevant to gastric cancer. (3) Microbial sub-proteomics mainly study cell membrane proteins, ribosome, regulators, stimuli, chaperon, nucleic acid-binding proteins at present. (4) Quantitative proteomics. The approaches applied to quantitative analysis of microbial proteomics include isotope ([15]C and [15]N) tracing, isotope-code affinity tags (ICAT), fluorescence 2-dimensional differential gel electrophoresis (DIGE) and mass spectrometry approaches. (5) Proteome analysis provides a sensitive method to examine plant-microbe interactions. By identifying differentially displayed proteins expressed between the rhizobia and the legume, novel symbiosis proteins were isolated and characterized and how the two symbiotic partners alter their respective metabolisms were determined.

In addition, proteome analysis complement the sequence of the genome of strain *Sinorhizobium meliloti*, combining gene knock-out and mutant with proteomics methods is becoming effective strategies to study gene function of microorganisms, studies of protein–protein interactions using techniques such as the yeast two-hybrid system and microarray help us to probe cellular metabolism pathways related to important biological processes.
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Proteomic Research of Shigella flexneri 2457T
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Shigella infection is the leading cause of dysentery among children in developing countries. Based on our previously established a 2-DE reference map of Shigella flexneri 2457T, we focus our study on immunoproteomics and comparative proteomics. Considering the mechanism of the bacterial infection, we describe the whole map of it’s outer membrane proteins first (85 points in pH3–10 and 122 points in pH4–7, totally 45 points identified), then we combined 2-DE techniques with immunoblotting as a basis for the development of sensitive and specific diagnostic tests. The investigation of 2457T outer membrane proteins immunoproteome revealed 18 antigens, 14 of them were identified, representing 9 proteins. Two of them were confirmed from other similar studies and 7 were newly identified. One hypothetical protein with strong immunoreactivity was identified by MALDI-TOF and Q-TOF. Because of insufficient spatial resolution, we introduce narrow IPGs in 2457T whole cell proteins immunoproteome study. Within one 18cm×18cm gel 120 and 131 antigens were detected in pH 4.7 and pH 4.5–5.5 respectively. The results show a high diversity of antigens. To gain insights into the mechanism by which Shigella flexneri in response to a given environmental shift, we studied the protein profiles of response to cold-shock, heat-shock and acid. Several changes were observed. Additionally, to understand why it is avirulent under incubation in 30°C, we introduced DIGE technique in our study. In the proteome of 2457T grown at 30°C expression of 15 proteins were significantly increased, and expression of 31 proteins decreased. One of the upregulated proteins which can be nakedly seen on the coomassia brilliant blue stained gels was identified as ArgT. It was still unclear about the relationship between the upregulation of ArgT and the inactivation of invasion-related operons.