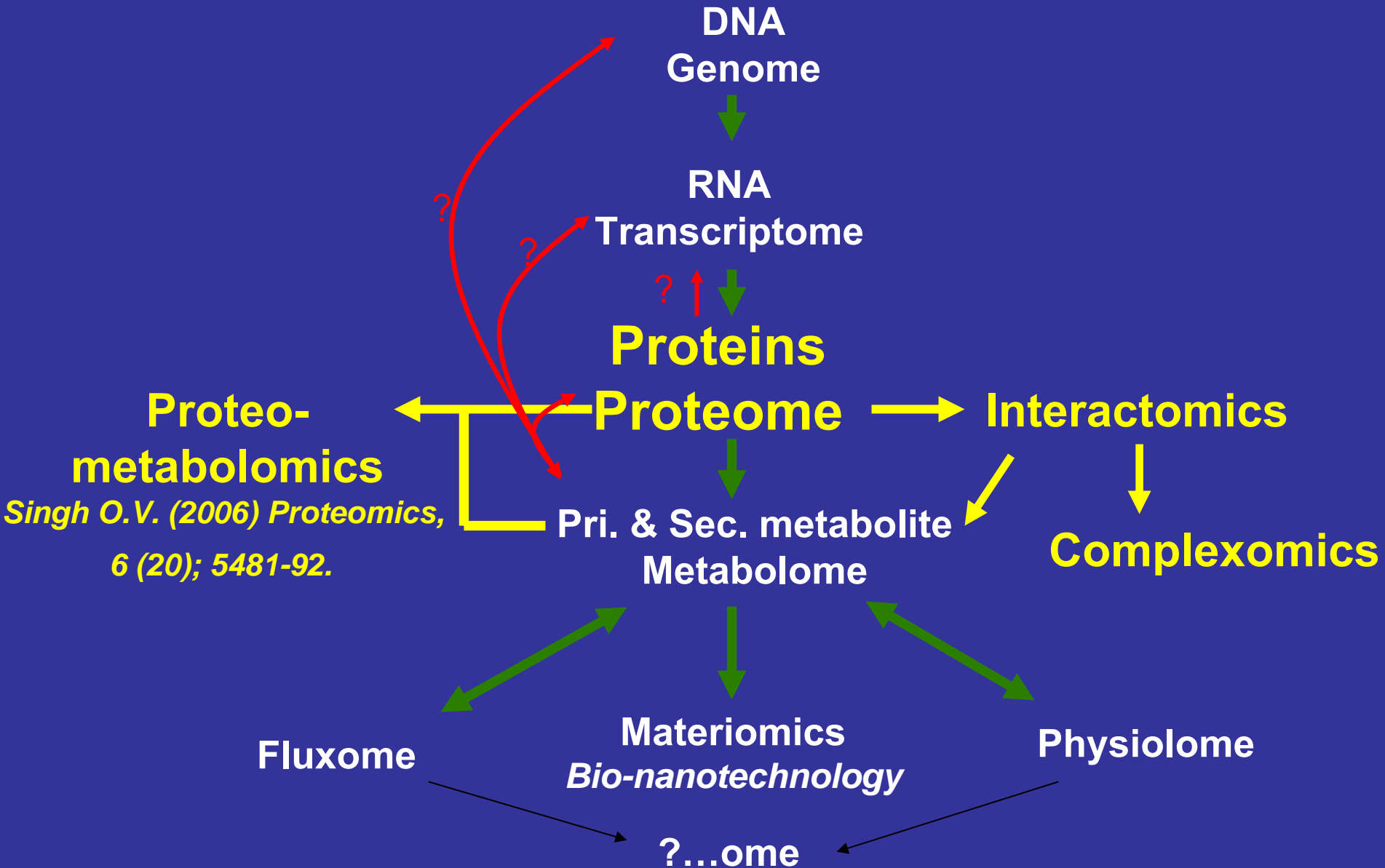


“OMICS”
Systems Biology Sensors
of the Microbial Environment

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Systems Biology & OMICS?



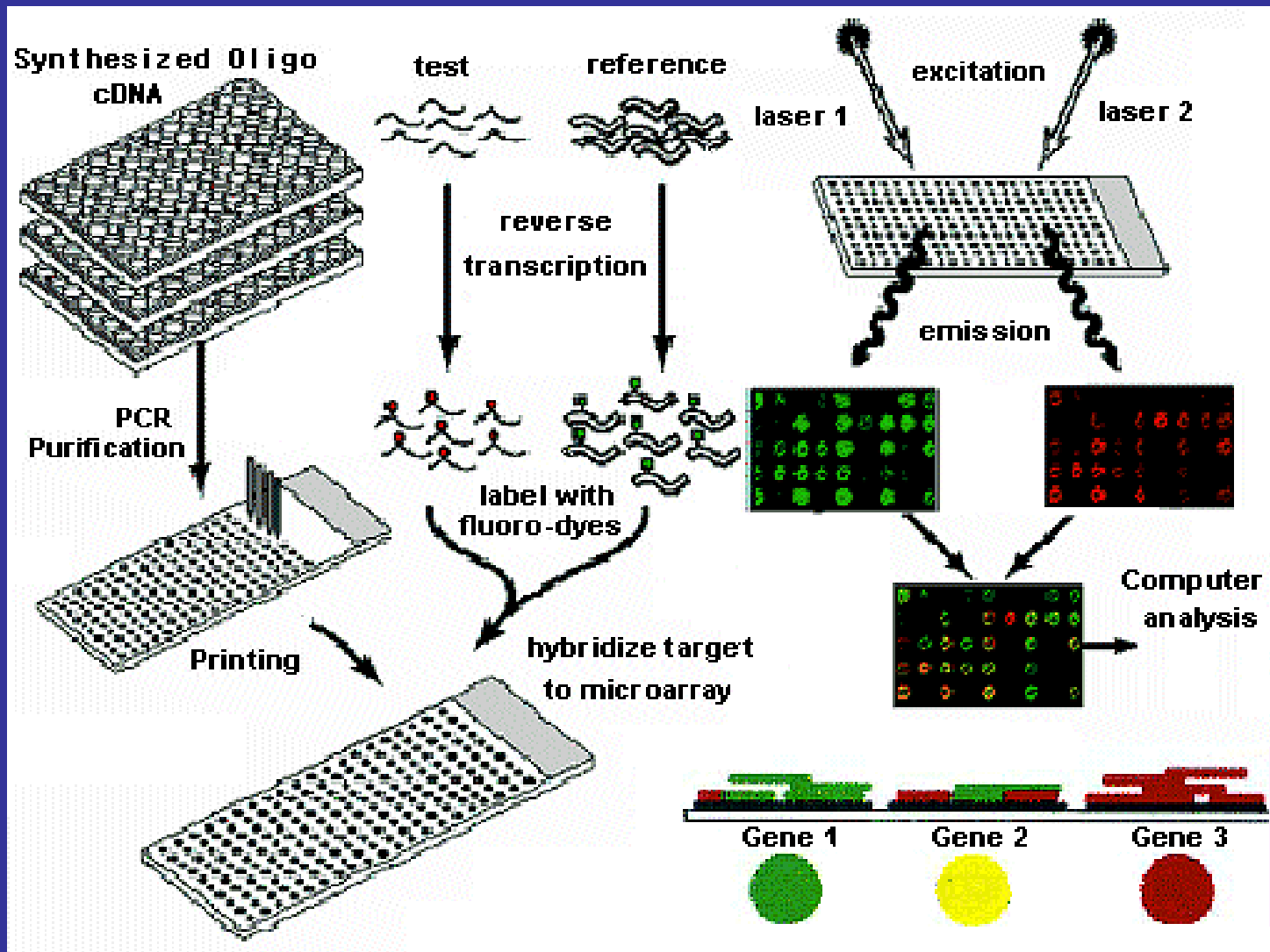
Genomics

- Ability to sequence the entire genome of an organism has given rise to the field of genomics
- Study of the entire composition of the genetic instruction and capabilities that are contained on the chromosomes from a particular cell.
- Methods available for sequencing DNA
 - Chain termination
 - Automated Sequence

Transcriptomics

- Ability to analyze the gene-expression profile.
- Measure the relative changes in mRNA in the cells.
- Genes can be probed with an array – up to 100 thousand different genes.
- Types of microarrays:
 - cDNA ; DNA chips

Basics of cDNA Microarray



Microarray Data Processing

- Commercially available software can be used to process microarray data. Basics remain same:
 - Image Processing
 - Normalization
 - Identifying differentially expressed genes
 - Statistics-based data analysis
- Using gene expression profiling, it is now possible to:
 - Differentiate cells
 - Classify microbial state – stress response, and virulence factor etc.

Proteome

- Proteome - the entire PROTEin complement expressed by a genOME of a cell or organism
- PROTEOMICS – Any large scale or systematic characterization of the proteome of a cell, tissue, or simple organism

Sounds like simply a new name for an old discipline, i.e. protein biochemistry ??

Why study proteins?

- Genes contain information that translates into proteins
- Genes expression can result in expression of multiple proteins
- Unique proteins participate in environmental sensing and cell signaling
- Cell specific proteins avoids problems with gene homology
- Understand cellular functions to predict virulence stage, stress response

Proteomics – Study Scales

Global or targeted proteomics?

- **Global** – Attempts to analyze all proteins present in a cell from given environmental site
 - Most practical for prokaryotes
- **Key challenge**- Differences will ALWAYS be observed, but challenge is to interpret biologically significance changes

Targeted Proteomics

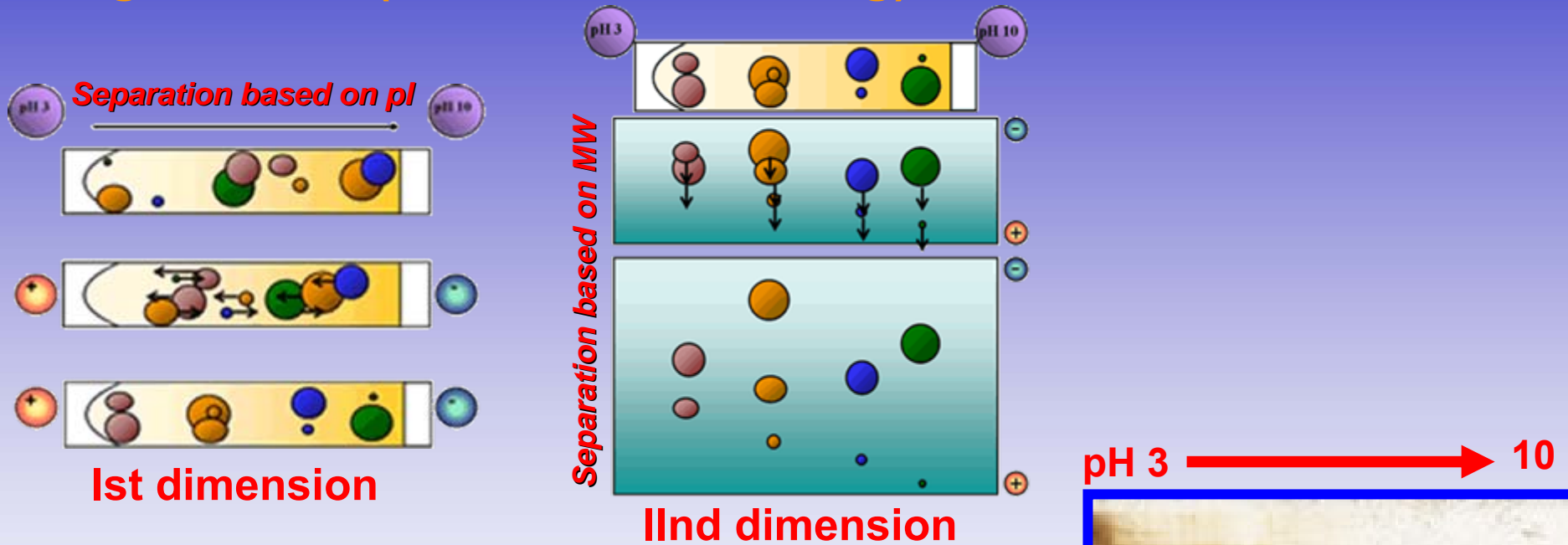
- Characterize a well-defined sub-proteome
- Usually within range of analytical capacities even for bacterial cells

Examples:

- **Organelles**
- **Macromolecular complexes and cellular machines**
- **Protein groups / classes – phosphoproteins, glycoproteins, cell surface proteins, oxidative modifications etc.**
- **Key challenge** – Reproducible isolation of sub-proteome & distinguishing specific from non-specific contaminants

Tools for Proteome Analysis

2D gels – IEF (Isoelectric focusing) → SDS-PAGE



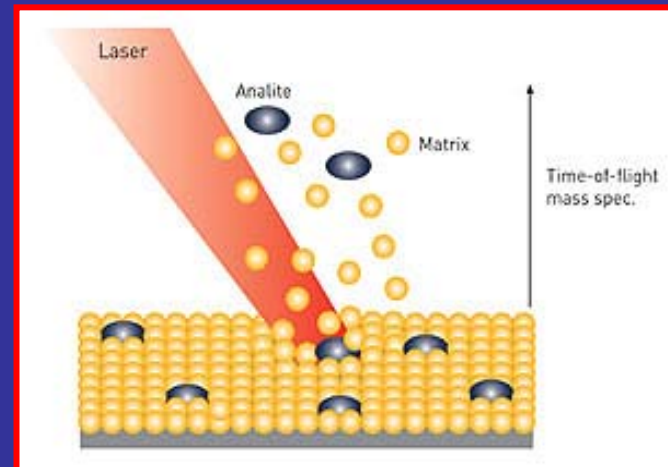
Protein Identification By Mass Spectrometry

- Peptide Mass Fingerprinting – use tryptic peptide masses only (MS)
- MS/MS – use tryptic peptide masses followed by fragmentation with an inert gas (helium) and measure masses of daughter ions
- Antibody arrays
 - Not discovery based
 - Most sensitive

MS Instrument Design

- Initially – two flavors
 - MALDI – TOF (masses only)
 - ESI – quadrapoles (masses or MS/MS)

- Now – with new flavors (all possible combs)
 - MALDI TOF/TOF
 - MALDI + ESI – qTOF
(quadrapole + time of flight) (+FT)
 - MALDI + ESI – 3-D ion traps,
linear ion traps (+FT)



Metabolomics

- **Characterization of low-molecular-weight primary and secondary metabolites.**
- **Key issue:** How to exploit the hidden information that exists in different metabolites compositions?

Metabolomics and system biology



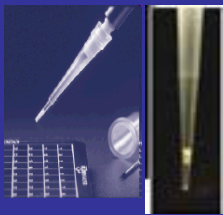
Stress vs growth dependent selection of microorganism; sampling, and quenching

Transcriptome
Proteome

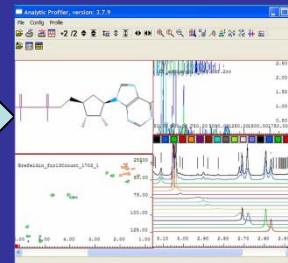
MS based
Raw data



Systematic Data
Management



Filtration
Derivatization

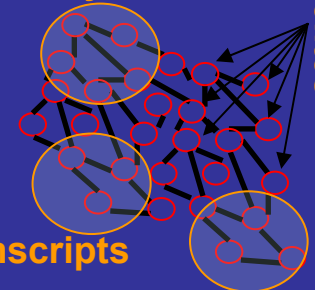


Data
analysis



Enzymes

Metabolites



Transcripts

Mapping of OMICS data

Extraction:
Non polar
Polar

Sample analysis:
Nano-LC; GC MS

Metabolome

Environmental stressors: Pollutants & 'OMICS'

- Environmental stressor: **Phenol**
 - Toxic, mutagenic, carcinogenic aromatic pollutants are posing serious threats to human health and the environment
- Site specific microorganisms have ability to sense toxicant, initiate stress responses and initiate bioremediation
- **OMICS Tools:** Possible to explore new catabolic pathways to study the regulatory control of primary and secondary metabolites

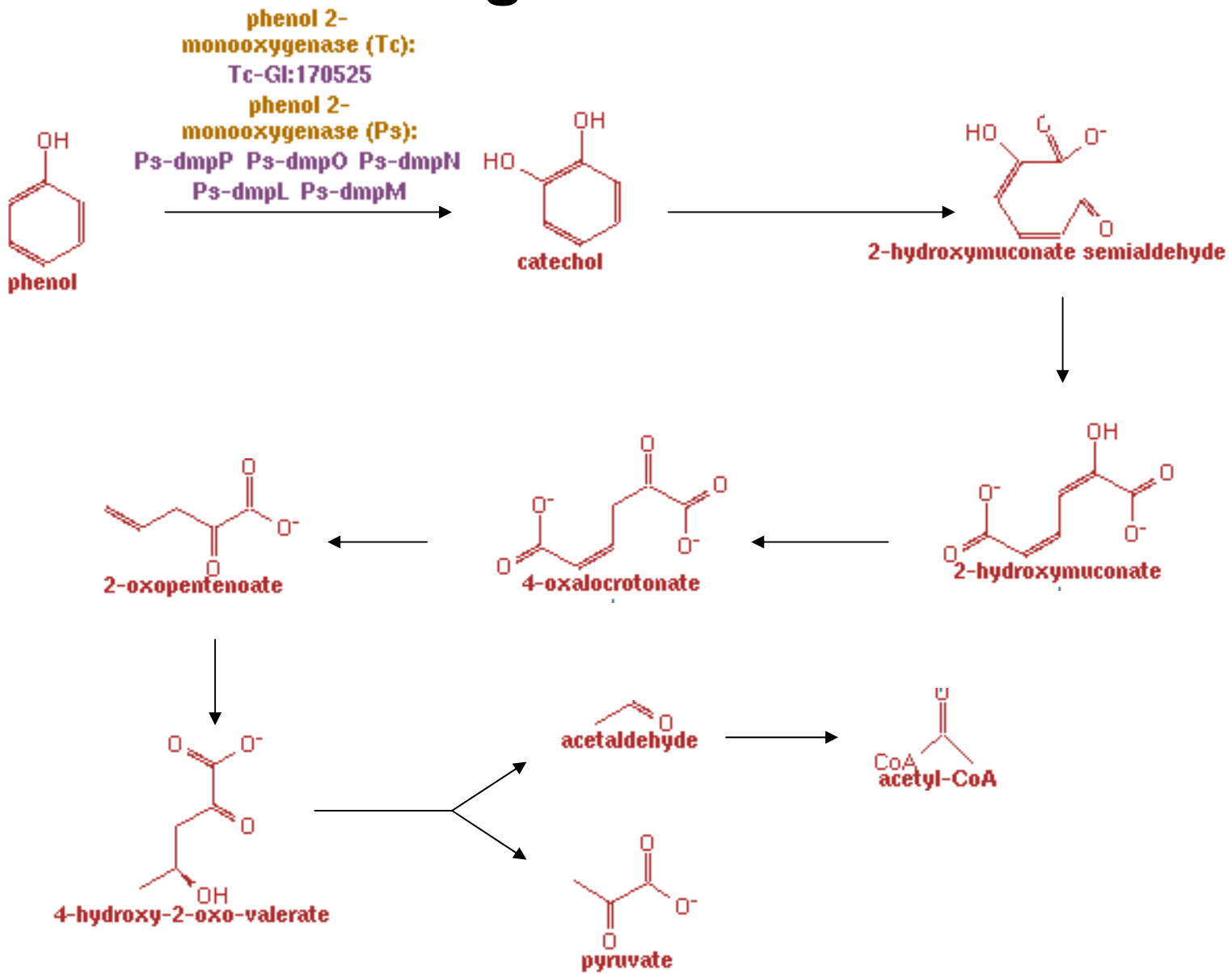
Phenol

- Toxic white crystalline solid – C_6H_5OH
- Properties: Production of drugs, herbicides, and synthetic resins
- Health hazards:
 - skin exposure may cause dermatitis, corrosive to eyes, skin, and the respiratory tract, lung edema.
 - Substance may also effect on the central nervous system, heart and kidneys, resulting in convulsions, coma, cardiac disorder or respiratory failure

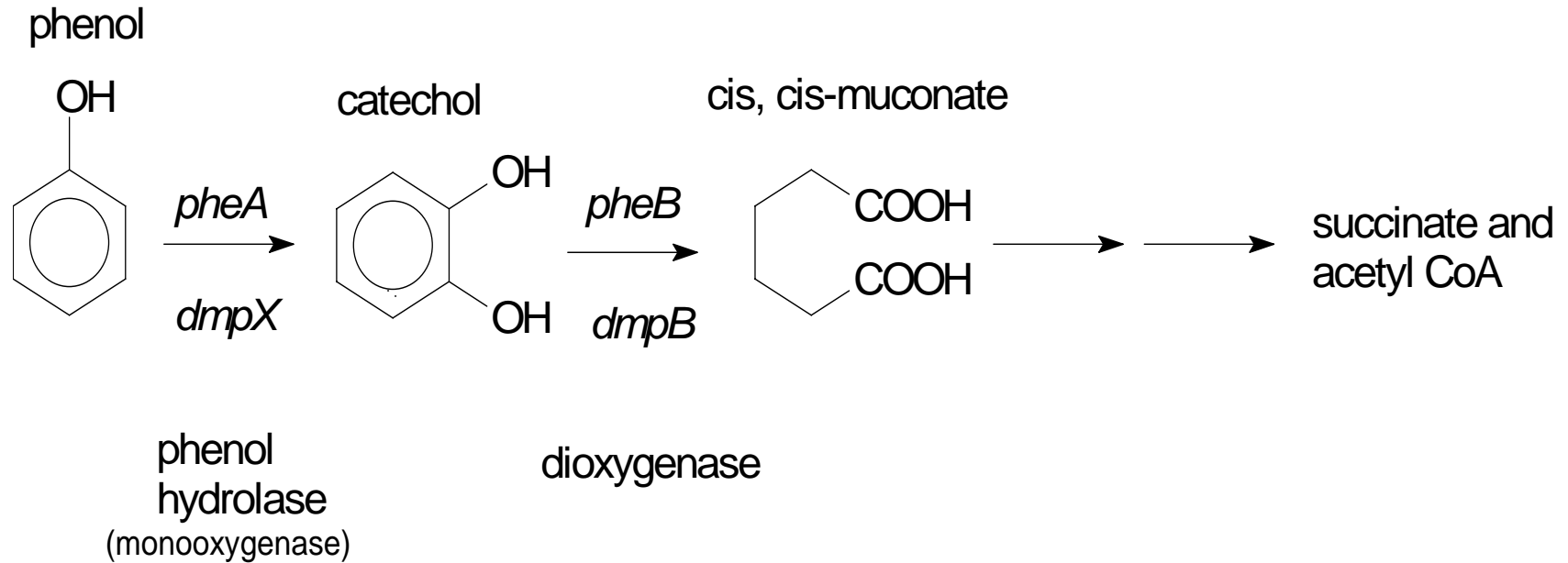
Challenges remains for bioremediation of Phenol

- ❑ May exist at high, toxic concentrations
- ❑ Degradation may depend on another nutrient that is in limiting supply
- ❑ Microbes may not yet have evolved biochemical pathways to degrade compounds
- ❑ May require a consortium of microbial populations
- ❑ Entire biochemical pathways involving many enzymes coded by many genes are not known yet.

PHENOL Degradation, and 'OMICS' Signature?



Unique Gene Signature



- Phenol-degrading *dmp* operon is regulated by *DmpR*, a *NtrC*-like positive regulator.

Unique Gene Signature in Phenol degradation

- The most common first step in the aerobic degradation of phenol is hydroxylation to a catechol.
- Reaction encoded by following major gene:
 - dmpP: phenol hydrolase reductase
 - dmpO: phenol hydrolase gamma subunit
 - dmpN: phenol hydrolase alpha subunit
 - dmpL: phenol hydrolase beta subunit
 - dmpM: phenol 2-monooxygenase

Hypothesis

- Proteomics based characterization will detect unique stress responsive biomolecules of *P. putida* during phenol exposure

'Functional OMICS': Phenol Specific Microbial Response

- *Pseudomonas putida*:
 - Ideal candidate for genetic engineering, applications in biotechnology, bioremediation, and agriculture due to its metabolic versatility, degradative potential, and ability to colonize bulk soil and the rhizosphere.
- *Pseudomonas putida*-11:
 - Gram-negative, rod-shaped Non-motile, non-spore forming, rod shaped bacterium

Unique proteomics signature- Methods

- The phenol stress in *P. putida*-11 was induced at 500 mg/l along with the control (no phenol) in parallel for 5 hrs.
- Cells were pelleted, washed (PBS) and sonicated at 4°C in 8M urea, 4% CHAPS to solubilize total protein.
- The protein content was determined by BIO- RAD RC-DC kit

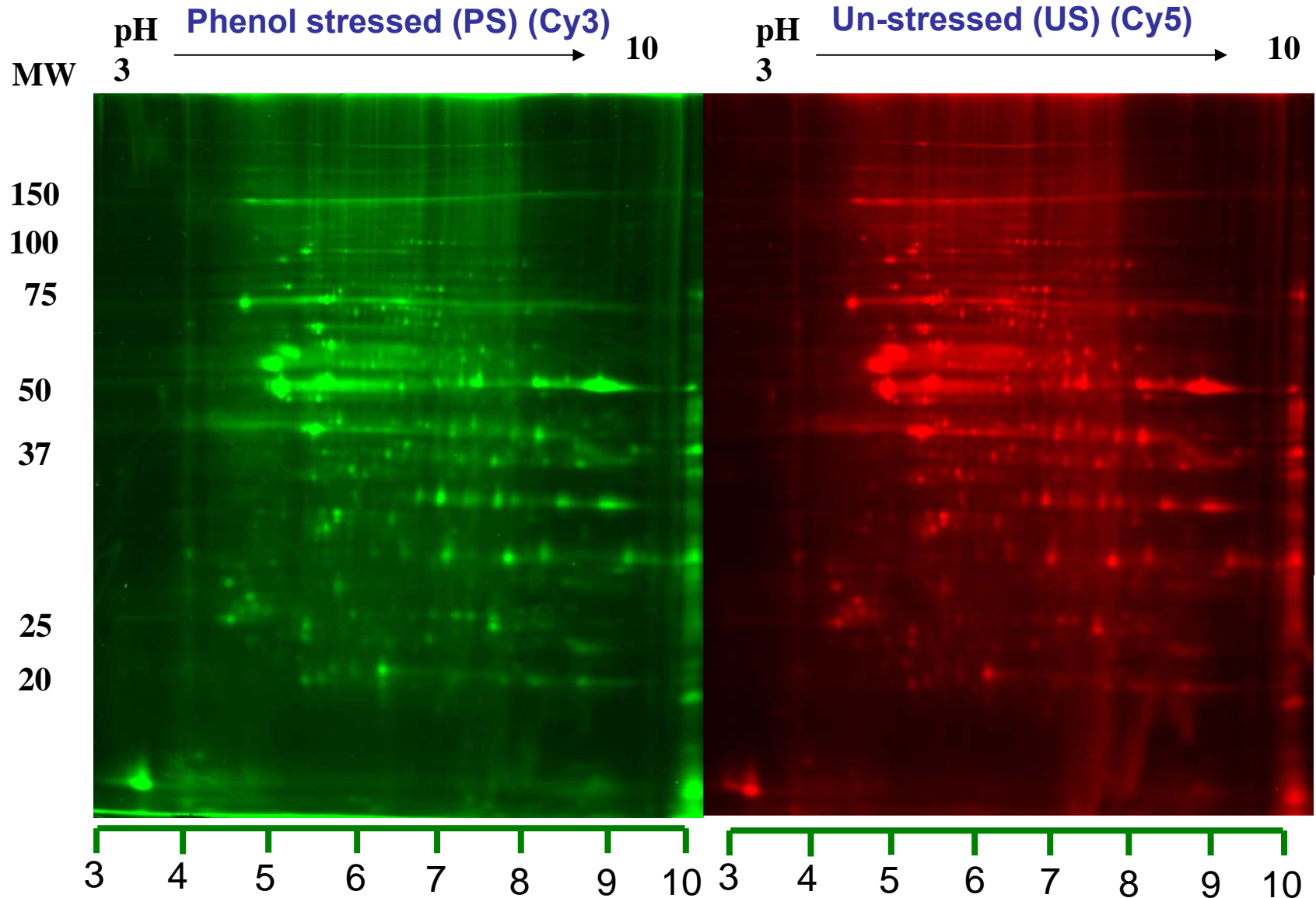
Unique proteomics signature- Methods

- Total protein (100 ug) was laid on 2-DE using IPG strip pH 3-10 followed by 10% SDS-PAGE (2-DE)
- Highly reproducible DIGE images were identified for protein differential expression from phenol stressed (PS) and Un-stressed (US)

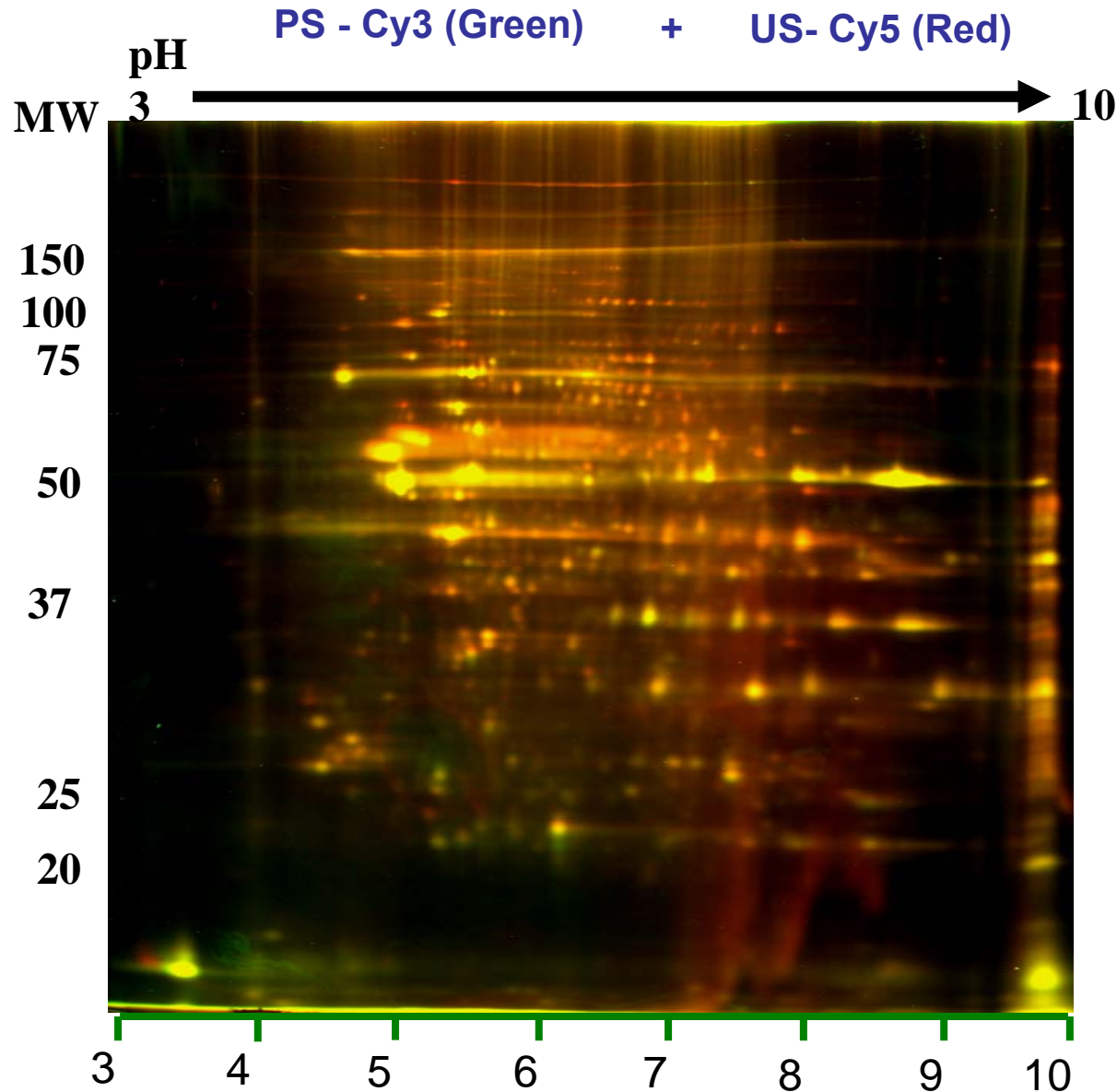
Protein Identification:

- Peptide Mass Finger printing using MALDI-TOF
- MS-Fit and Mascot search engine
- Equipped with NCBI and SwissProt data base

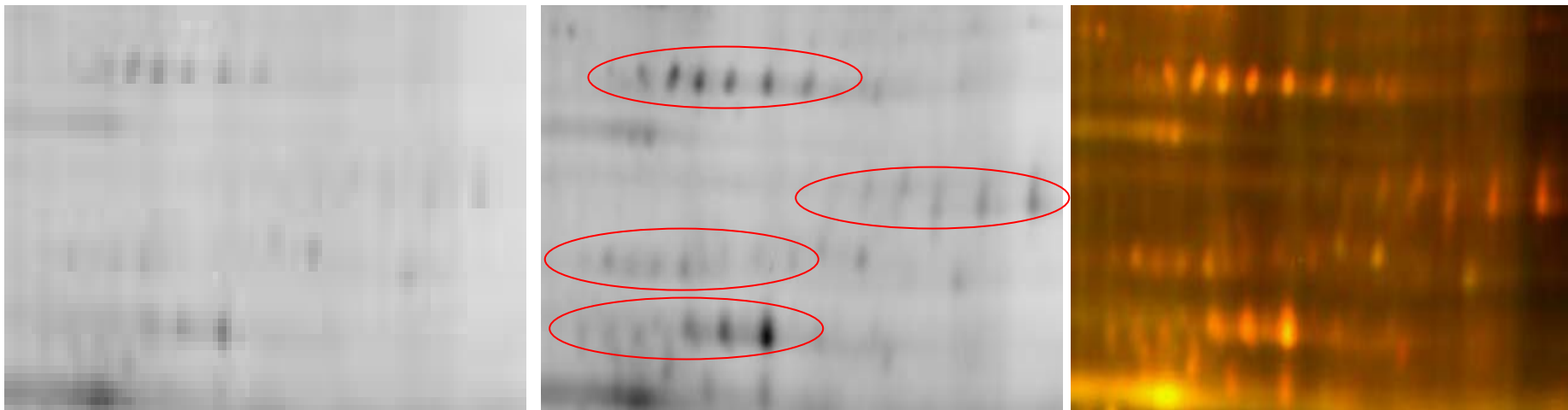
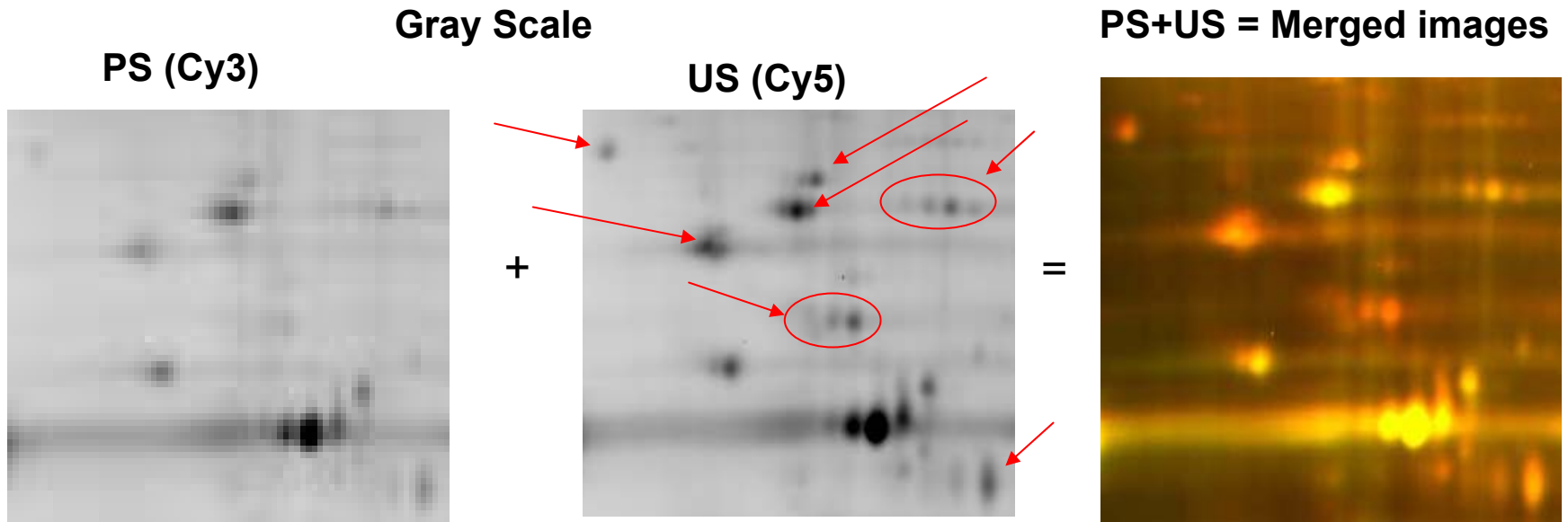
2D Fluorescence Difference Gel Electrophoresis (2-D DIGE) of *P. putida*-11



Merged images of differentially expressed proteins of *P. putida*-11 under PS and US



PS responsive differential expression in proteins of *P. putida*-11 at Gray and pseudo-color images

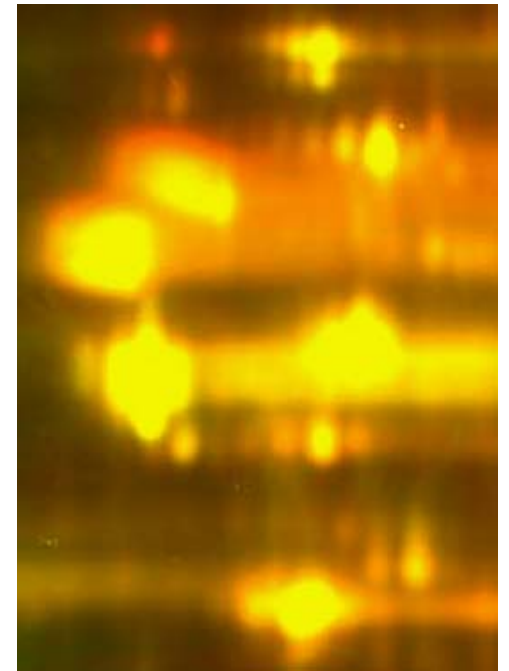
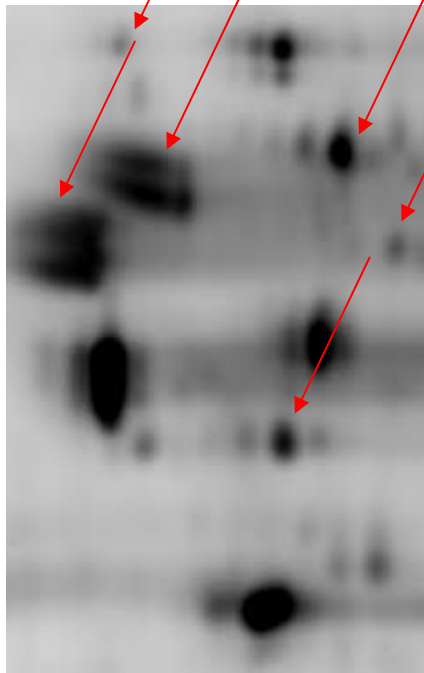
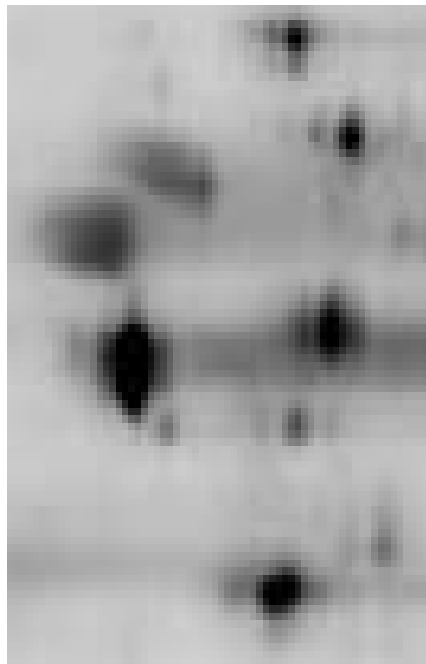
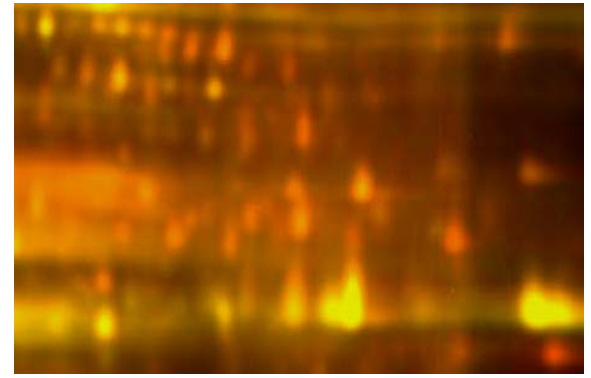
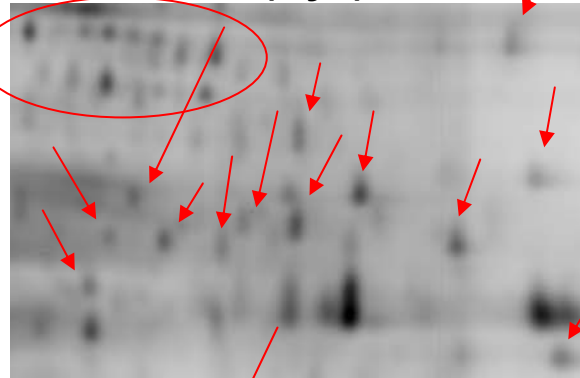
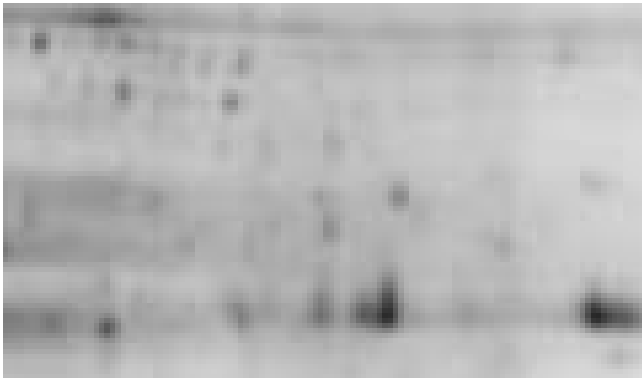


PS (Cy3)

Gray Scale

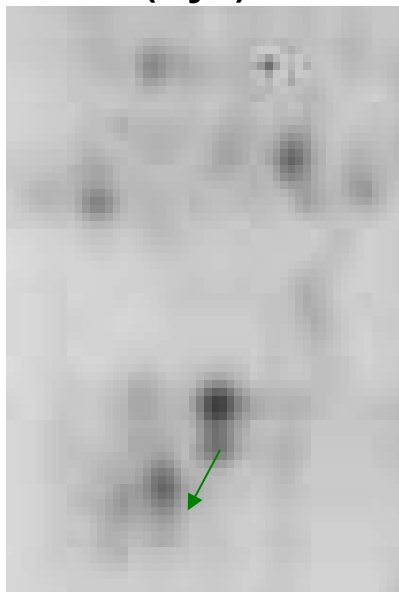
US (Cy5)

PS+US = Merged images

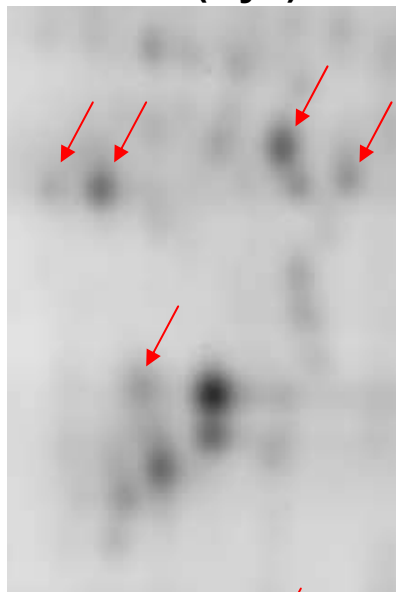


Gray Scale

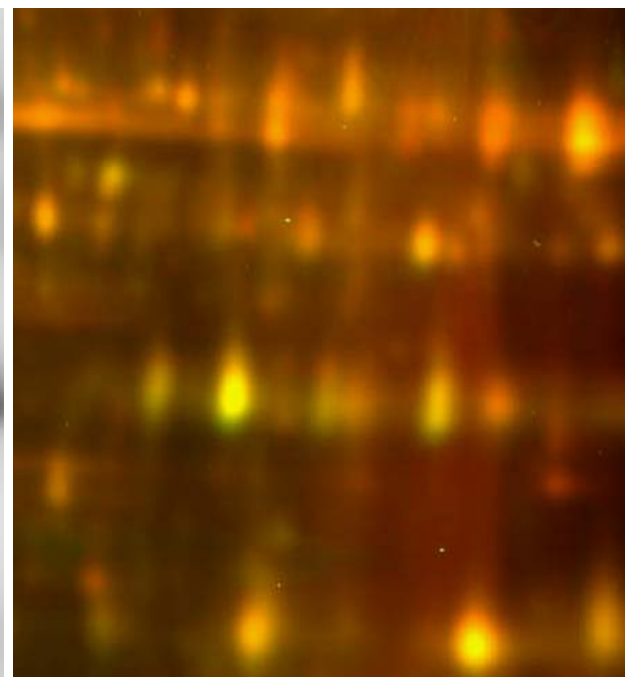
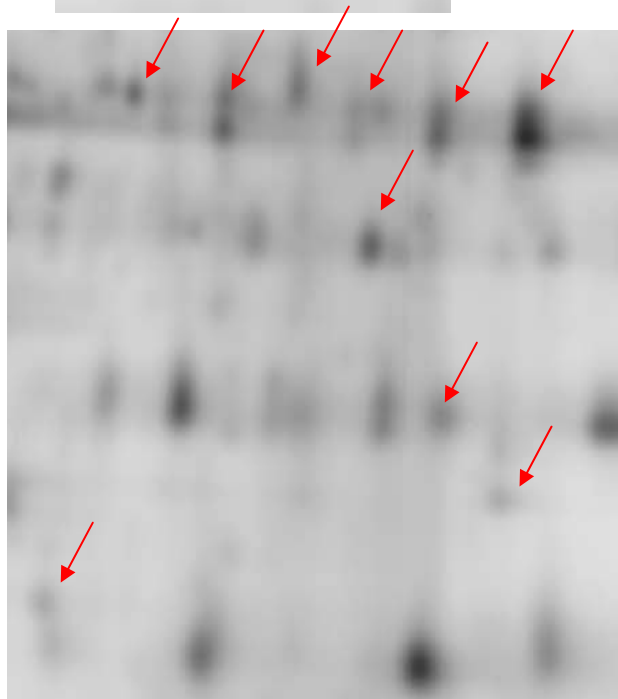
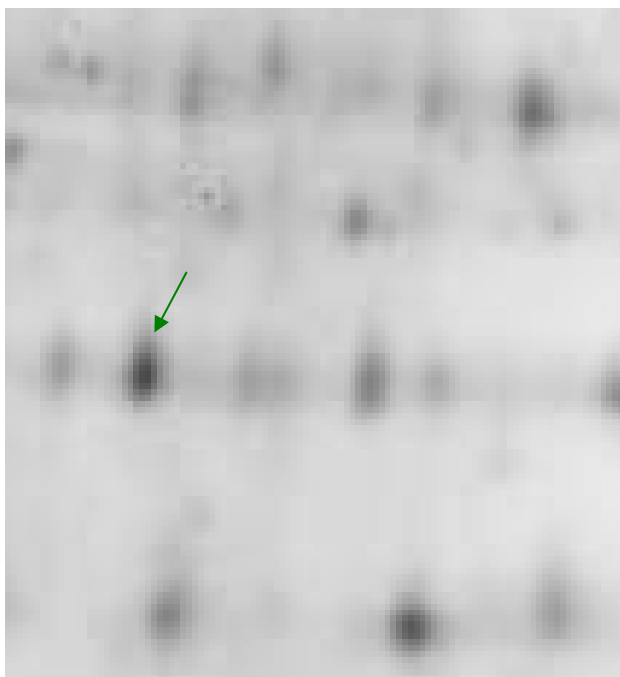
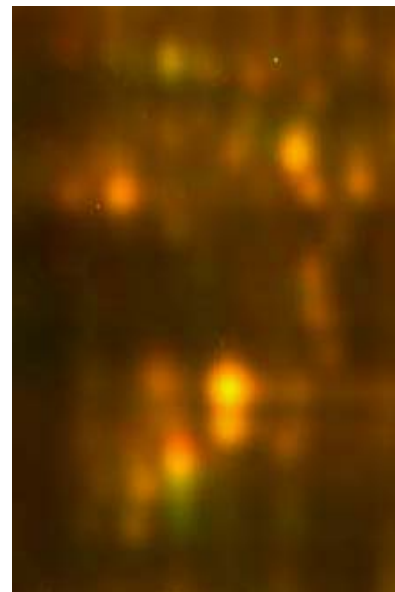
PS (Cy3)



US (Cy5)



PS+US = Merged images



Unique features of differentially expressed proteins under PS of *P. putida*-11

Protein description	Accession No.
1. Periplasmic beta-glucosidase	Q88N13
2. Chaperone protein dnaK	Q88DU2
3. Isocitrate dehydrogenase	A5W024
4. CTP synthase	Q88MG1
5. Phosphoenolpyruvate carboxykinase	B0KI05
6. ATP synthase subunit alpha	B0KRB0
7. AtpD beta synthase chain	Q8VMD8
8. Chaperonin GroEL	P48216
9. 3-isopropylmalate dehydratase	Q99LE8
10. Dihydrolipoyl dehydrogenase	Q31046
11. 3-ketoacyl-CoA thiolase	Q88L01
12. 3-oxoacyl-(acyl-carrier- protein) synthase I	Q88FC3

➤ Analysis of proteins was based on Mascot search

Summary

- A set of catabolic proteins, enzymes, and heat shock molecular chaperones associated with the regulatory network was found to be differentially regulated under phenol-stressed (PS) condition
- Major stress responses:
 - Chaperone protein dnaK
 - Chaperonin GroEL

Future approaches

- **Microarray Chip**

- A system biology based bio-molecular chip can be developed to sense microbial physiological response to the environment

- Gene; Protein and Enzyme Microarray

- Understanding gene, protein and to be explored potential metabolites based bio-molecules may improve understanding stress responsive system in an early warning.

Acknowledgements

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Mr. Anuj Chandel

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