Proteomics Course

LECTURE-18
Applications of two dimensional electrophoresis

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Lecture outline

- An overview of 2DE technique
- Applications
  - Case study – 1
  - Case study – 2
**2DE overview: First dimension**

- Isoelectric focusing

- IPG strip

- Increasing pI

- 2DE-GEL
2DE overview: Second dimension

Molecular weight

SDS-Polyacrylamide Gel

Decreasing Molecular Weight

Second Dimension: SDS-PAGE

2DE overview: Representative 2D Gel

A6: Staining

Increasing pl

pH 4

pH 7

Spot analysis: MW and pl of protein

Decreasing molecular weight

Molecular weight

Spot analysis: MW and pl of protein

Increasing pl

pH 4

pH 7

Spot analysis: MW and pl of protein

Decreasing molecular weight

Molecular weight
2DE overview: Data Analysis

Case study-1

Serum proteome analysis of vivax malaria: An insight into disease pathogenesis and host immune response

Malaria – a global view

- Malaria - an epidemic in 103 countries around the globe
- Incidence of malaria worldwide ~300-500 million/ per year and death between 1.1-2.7 million people each year
- *P. vivax* & *P. falciparum* account for 95% of malaria worldwide

AFRICA – single largest cause of death

ASIA – challenge of drug resistant strains

12/6/12
**Plasmodium species which infect human**

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Plasmodium vivax</em></td>
<td>Benign Tertian Malaria</td>
</tr>
<tr>
<td><em>Plasmodium falciparum</em></td>
<td>Malignant Tertian Malaria</td>
</tr>
<tr>
<td><em>Plasmodium ovale</em></td>
<td>Ovale Tertian Malaria</td>
</tr>
<tr>
<td><em>Plasmodium malariae</em></td>
<td>Quartan Malaria</td>
</tr>
</tbody>
</table>

- *P. knowlesi* can also cause acute, severe illness but mortality rates are low

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**An overview of workflow**

1. **Serum samples**
2. **Protein extraction**
3. **Data analysis**
4. **MS analysis**
5. **Gel-based proteomics**
6. **Validation and characterization**
7. **Protein identification** (database search)
8. **Vivax malaria**
9. **Healthy control**
Sample collection

- Patient information
  - Age, Sex, Physiological status, Alcoholic patients
  - Previous history of diseases
  - Treatment [If already treated; treatment information]
- Selection of healthy control
- Pooled versus individual sample
- Process of sample collection
- Sample storage
- Reproducibility

Schematics of serum collection, handling and storage

Blood sample → Serum separation tube → Incubation on ice → Centrifugation → Serum → Collection in small aliquots → Storage at -80°C
Serum collection

5 ml blood collected into butterfly syringe
(kept on ice until the isolation of serum)

Allowed to clot for 1 h

Centrifuged at 2500 rpm at 20°C, 10 min

Serum was collected immediately

Collected serum was divided into aliquots and stored at -20°C

Serum sample processing for proteomic analysis

Classical approach

Alternative approach

High abundance protein removal

Affinity column

Ultrafiltration

Dilution in buffer

High abundance serum proteins

IgG

IgA

Haptoglobin

Albumin

Transferrin

Antitrypsin

Aldotransferrin

Proteomic analysis
Sample preparation optimization

Serum sample

- Crude serum
- Desalting
- Sonication & desalting
- Abundant protein removal, desalting

Two dimensional electrophoresis

Serum sample → IPG strip → 1\textsuperscript{st} dimension [IEF] → 2\textsuperscript{nd} dimension [SDS-PAGE] → Staining → Protein visualization → Software analysis
**IEF Settings**

![IEF Settings image]

**2-DE analysis**

![2-DE analysis images]
2-DE optimization (7 cm)

CRUDE SERUM

DESLATED SERUM

SONICATED-DESLATED SERUM

2-DE optimization (11 cm)

Desalted serum

Sonicated-desalted serum Coomassie

Sonicated-desalted_silver
**Protein extraction**

40 µL serum was precipitated -4 volumes of ice-cold acetone containing 10% w/v TCA

↓

incubated at -20°C for 90 min

↓

Centrifuged at 15 000 X g, 4°C, for 20 min.

↓

1 mL of ice-cold acetone was added to wash the precipitate

↓

incubated on ice for 15 min and centrifuged as above

↓

Acetone-containing supernatant was removed

↓

Pellet dissolved in lysis buffer

[Urea 8M, CHAPS; 4%; 2% IPG buffer; DTT 40mM; 1% BPB]

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**Effect of different sample processing**

**Number of spots [n=3]**

![Graph showing the effect of different sample processing on protein extraction.](image)
Optimization of protein extraction protocols

Serum sample

- Direct crude serum (Scambi et al., PLoS One 2010)
- TCA-Acetone precipitation (Chen et al., Electrophoresis 2005)
- Sonicated desalted serum
- Trizol extraction method (Lee et al., Journal of Microbiological Methods 2008)
- Abundant protein removal, TCA-Acetone precipitation (Modified from Scambi et al., PLoS One 2010 and Chen et al., 2005)

Protein extraction: Acetone vs TCA-Acetone precipitation

Graph showing comparison of spot numbers for Acetone and TCA-Acetone precipitation methods.
Protein extraction: Crude vs Depleted

<table>
<thead>
<tr>
<th>Spot number</th>
<th>Crude serum</th>
<th>Depleted serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Software analysis</td>
<td>533</td>
<td>719</td>
</tr>
</tbody>
</table>

Staining agent: Coomassie vs Silver

<table>
<thead>
<tr>
<th>Spot number</th>
<th>CBB</th>
<th>Silver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Software analysis</td>
<td>719</td>
<td>995</td>
</tr>
<tr>
<td>Parameters</td>
<td>Crude</td>
<td>Sonicated-desalted</td>
</tr>
<tr>
<td>--------------------</td>
<td>-------</td>
<td>--------------------</td>
</tr>
<tr>
<td>1. Sonication</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2. Desalting</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3. Rehydration</td>
<td>Active</td>
<td>Active</td>
</tr>
<tr>
<td>4. Amount of</td>
<td>1200 µg</td>
<td>1200 µg</td>
</tr>
<tr>
<td>protein loaded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Strip</td>
<td>24 cm</td>
<td>24 cm</td>
</tr>
<tr>
<td>6. Staining Soln</td>
<td>Coomassie</td>
<td>Coomassie</td>
</tr>
<tr>
<td>7. Spot Number</td>
<td>513</td>
<td>503</td>
</tr>
<tr>
<td>[Software detected]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Spot Number</td>
<td>351</td>
<td>363</td>
</tr>
<tr>
<td>[After refinement]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Comparative analysis of serum samples**

- **Vivax malaria samples**
- **Healthy control samples**
Representative gel

BSA [66KDa]

600 µg protein

24 cm

Software detected: 539
After refinement: 392 ±10

P. vivax vs healthy controls: representative gels

Healthy control

P. vivax
2D gel analysis: automated matching and refinement

Software detected: 652
After refinement: 441±10

Software detected: 592
After refinement: 372±10

2D gel analysis: differentially expressed spots
2D gel analysis: differentially expressed spots

Down-regulated

Up-regulated

Altered protein expression levels in vivax malaria patients

Data are represented as mean ± SEM where (n=3)
Mass spectrometry analysis

LC-MS Data Analysis

Standard: BSA

Serum Proteins: Spot 1

Mass spectrometry analysis
### Significant differentially expressed proteins identified using 2DE

#### Down-regulated proteins

- Haptoglobin precursor (HP)
- Apolipoprotein A-1 (APO A-1)
- Serum albumin precursor (ALB)
- Clusterin precursor (CLU)

#### Up-regulated proteins

- Serum amyloid A (SAA)
- Ceruloplasmin precursor (CP)
- Leucine-rich α-2-glycoprotein precursor (LRG)
- Alpha-1-antitrypsin precursor (Alpha-1 protease inhibitor)

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### Conclusions

- Few differentially regulated serum proteins identified in this study have not been reported earlier in vivax malaria pathogenesis.

- An important role of serum amyloid A and P, haptoglobin, apolipoprotein A-1 and E proteins elucidated in vivax malaria.
Summary

• Two dimensional electrophoresis can be applied for various applications
• Case studies –
  • Host response to malaria infection
  • Drug treatment to malaria parasite

REFERENCES