Product characterization of pertussis Whole Cell Vaccine by mass spectrometry

Bernard Metz, Joost Uittenbogaard and Arno van der Ark

- Introduction
- Gene and protein regulation during cultivation
- Mass spectrometry
- Antigen composition as measurement of product quality
Reasons for characterization of pertussis vaccines

• Resurgence of pertussis in populations well vaccinated with acellular pertussis vaccines triggers development of new pertussis vaccines

• Polio eradication triggers development of hexavalent DPT-Hib-HepB-IPV$_{sabin}$ vaccines necessitating investigation of compatibility of pertussis component

• Potency release tests are not sensitive and reproducible enough and therefore not suitable for this type of research
B. pertussis culture

- Start of all pertussis vaccine productions
- Undefined intermediate product
- Product characterization required
Process and product characterization

- Genomics (microarrays)
- Proteomics (LC-MS)
- Quick scan key marker antigens with predictive value for efficacy (ELISA)
- Potency (Challenge)
- Immune responses (Antibodies)

In-vitro monitoring

In-vivo testing
Genomics
Bvg-regulated virulence of *B. pertussis*

**Bvg**

- **Bvg**\(^+\) (X mode)
  - Respiratory tract colonization
- **Bvg**\(^i\) (L mode)
  - Respiratory transmission?
- **Bvg**\(^-\) (C mode)
  - Starvation survival
  - *(B. bronchiseptica)*

**B:**

<table>
<thead>
<tr>
<th>Relative expression level</th>
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</thead>
<tbody>
<tr>
<td>100</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
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</table>

- **Bvg**\(^+\)
- **Bvg**\(^i\)
- **Bvg**\(^-\)

- "late" Bvg-activated genes (e.g. *cytA*)
- "early" Bvg-activated genes (e.g. *fhaB, bgAS*)
- Bvg\(^i\) phase genes (e.g. *bipA*)
- Bvg-repressed genes (e.g. *frlAB, flaA*)
Process knowledge = product quality

Culture conditions of *B. pertussis* affect the quality of WCV.

- Bvg regulated virulence factors are in general protective

- Expression of virulence factors affected by e.g. nutrient limitations, culture temperature (artificial factors like nicotine or MgSO4)

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**B. pertussis batch culture**

Score based on activation of 56 vag's

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Consistency = process & product knowledge

Steady state culture of *B. pertussis*
- MgSO4 → *bvg* down regulation → reduction in virulence factors
- **Product characterization**
  - 3 bioreactor runs
  - sampling → T = 0, 2, 6, 12 and 24 hours
  - Plain inactivated WCV A, B, C, D and E
Product characterization

Microarray

ELISA

2D-electrophoresis

LC-MS

Bernard Metz et al, in preparation
Microarrays

Monitoring virulence associated genes

Product quality: good poor
Monitoring key marker antigens

Selection of key marker antigens

- known protective antigens (generally virulence factors)
  - Ptx (excreted → hardly present in WCV)
  - FHA
  - Prn (splitted off → poorly present in WCV)
  - Fimbriae
  - Vag8 (virulence associated gene 8)

ELISA (quick scan)

- Coating serial dilutions of whole cell products
- Detection of specific antigens with MoAb’s
- Goat anti Mouse IgG-HRP

Is this the right selection?

- Gene activation
- Protein production
- Immunoprofiling
Identification of proteins by LC-MS

Nature Reviews | Molecular Cell Biology

Protein database

Peptides predicted from proteolysis

in silico MS/MS pattern from theoretical peptides

m/z

Matching

Identified peptides/proteins

MS/MS spectra of peptides

m/z

Protein (protease fragments)

Protein digestion

Peptide separation

Sample ionization

Spray needle

Mass spectrometry

Mass spectrum

Data analysis

• Trypsin
• Lys-C
• Asp-N
• Glu-C
• HPLC
• Ion exchange
• Electrospray ionization
• MALDI
• Quadrupole
• Time of flight
• Quadrupole ion traps
• FTICR

Peptide ions

Intensity

PeptideSearch

Saquest

Mascot

• PeptideSearch
• Saquest
• Mascot

Nature Reviews | Molecular Cell Biology

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Peptide ions

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Saquest

Mascot

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Quantitation of proteins by LC-MS

3 Cultivations Bordetella (T=0 - T=24)

Total protein isolation

Protein assay
Dilution to equal protein content

Digestion (LysC and Trypsin)

Light dimethyl labeling

Mix Light labeled digest and Heavy CR 1:1

LC/MS Analysis Quantitation (MS1)

Determine ratio peak height (Light/Heavy)

Heavy dimethyl labeling

Mix all heavy labeled digests (=Common Reference)

SCX fractionation

LC/MS analysis Identification peptides and proteins (MS1 and MS2)

SAMPLE 1

Label with light mass tag

Combine labeled samples

LC-MS/MS

Precursor ion spectrum (MS1)

Intensity

Light

m/z

Constructing Extracted Ion Chromatogram (XIC)

Relative quantification (area under curves)

SAMPLE 2

Label with heavy mass tag

Intensity

Heavy

Time

Intensity

Time
Quantitation of peptides and proteins

Ratio at T=6
4.12E6/3.88E6
=1.06

Ratio at T=12
2.38E6/4.90E6
=0.48

Ratio at T=24
5.70E5/3.84E6
=0.15

Up and down regulation of protein expression

<table>
<thead>
<tr>
<th></th>
<th>T=0</th>
<th>T=6</th>
<th>T=12</th>
<th>T=24</th>
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<tbody>
<tr>
<td>Pertactin (P14283)</td>
<td>1.69</td>
<td>1.06</td>
<td>0.48</td>
<td>0.15</td>
</tr>
<tr>
<td>Putative periplasmatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>substrate binding</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>protein (Q7VWX9)</td>
<td>0.11</td>
<td>1.03</td>
<td>1.82</td>
<td>2.47</td>
</tr>
</tbody>
</table>
Gene and protein expression

![Gene and protein expression plots](image)
% virulence proteins in WCV

Vaccine A
good quality (bvg+)

Vaccine C:
Intermediate quality

Vaccine E
Poor quality (bvg-)

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Relation antigen content and protection

![Graph showing the relationship between time (h) after addition of MgSO4 to culture and potency (ic-MPT)]

- Virulence proteins (LC-MS)
- Marker antigens (ELISA)
- Potency (icMPT)

Institute for Translational Vaccinology

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Vaccine protein composition

**LC-MS**

- **omvPV (85.96%)**
  - Description
  - Accession
  - ID

**2DE**

- **wPV (67.20%)**
  - Description
  - Accession
  - ID

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Institute for Translational Vaccinology

Vaccine protein composition

LC-MS
Common reference

Functional analysis
DAVID
GO-terms

omvPV profile:
Enrichment of outer membrane proteins

Absence ribosomal proteins

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# Proteomics whole cell based vaccines

<table>
<thead>
<tr>
<th></th>
<th>WCV</th>
<th>OMVs</th>
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</thead>
<tbody>
<tr>
<td><strong>Peptides identified</strong></td>
<td>7600</td>
<td>-</td>
</tr>
<tr>
<td><strong>Proteins identified</strong></td>
<td>1200</td>
<td>-</td>
</tr>
<tr>
<td><strong>Proteins quantified</strong></td>
<td>332</td>
<td>268</td>
</tr>
<tr>
<td><strong>Proteins differently expressed</strong></td>
<td>151</td>
<td>-</td>
</tr>
<tr>
<td><strong>% virulence factors</strong></td>
<td>≈25%</td>
<td>50-75%</td>
</tr>
<tr>
<td><strong>Top 5 of known antigens</strong></td>
<td>groEL, fhaB, vag8, tcfA, brkA</td>
<td>Vag8, brkA, tcfA, groEL, sphB1</td>
</tr>
</tbody>
</table>
Conclusions

Antigenic composition of whole cell based pertussis vaccines is largely determined by production process conditions

Key marker antigens

- expected: Ptx, FHA, Prn, Fimbriae
- determined: FHA, Vag8, BrkA, TcfA

Efficacy of pertussis vaccine seems to be related to the proportion of specific antigens in the product

LC-MS is a powerful tool to characterize undefined vaccines like WCV
More detailed information can be requested by mail:

marit.holleman@intravacc.nl