DISCOVERING BIOACTIVE SUBSTANCES IN THE FOOD CHAIN

Dr. Joseph Schwager
DSM Nutritional Products
Basel, Switzerland

Joseph.Schwager@dsm.com

Elements of the presentation

- Bioactives and their importance in nutrition
- Concepts of screening and its application in nutrition science
- Identifying bioactives present in the food chain
- From identification to application in the real world
Nutrition and health

- (Pharmaceutical approach to bioactives)
- Food components condition and impair/improve our health
- Ethnobotanical evidence
  - Accumulation of empirical knowledge at the food/health interface
  - Different patterns of nutrition in different societies
  - Association of types of nutrition and benefits on health conditions
- "Du bist, was du isst" – (you are what you eat‘)
- Rise (and fall) of functional food / nutraceuticals

Identification of bioactives in the food chain

The screening cascade
- Selection of health indication
- Definition of target (molecular, cellular)
- From primary screening assays to lead compounds
- Efficacy and safety
- Steps from bench to product: molecular target → humans
Joseph Schwager, DSM. Winter School
December 1st 2009

Health indications and screening approach

Definition and selection of molecular target
- Indication Diabetes: Receptor, ligand to receptor
- Indication Joint Health - Arthritis: enzymes
- Indication Neurodegenerative diseases – mood/mental performance: re-uptake of neurotransmitters

Directional positive nutritional screening

Natural ‘pure’ compound library

Screening

Natural compound databases

Selection of candidate extracts in food chain

Fractionated extract

Bio-assay guided fraction identification

Modified extracts enriched for bio-actives
Complex (high contents) screening

- 'Pure' compound library of natural substances
- Natural compound databases
- Selection of candidate extracts in food chain
- Modified extracts enriched for bio-actives

Multiparametric platform

- Cell morphology and differentiation
- Physiological parameters (production of proteins, metabolites)
- Molecular parameters (gene expression profiling)

Reverse nutritional screening

- Ethno-botanical database
- Natural substances database
- Indication-driven target selection

Pre-selected fractionated food extracts

- Bioassays
- Efficacy testing of extract
- Modified extract with improved efficacy
**Negative nutritional screening**

Pure natural substances → Panel of relevant toxicity and early safety assays → Natural substance database → Fractionated food extracts → Corroborate safety profile → Select ‘safe’ extracts for further analyses

**Screening cascade (in an environment of nutrition industry)**

Primary screening assays → Secondary screening assays → Lead substances

Evaluation/Filters:
- Efficacy
- Safety
- Chemistry
- Intellectual property
- Regulatory issues

Lead substances → Developing relevant substances → Marketing of product

Molecular/Cellular in vitro → in vivo Animal models → Human studies (Clinical studies)
Example for Nutritional Screening: Rose hip (*Rosa canina*)

**Rationale of REVERSE SCREENING** (adapted from Schwager et al. Curr Op Biotech 2008)

- Ethno-botanical database
- Natural substance database

Indication-driven target selection → Selected fractionated food extracts → Bioassays

- Efficacy testing of extract
- Modified extract with improved efficacy

**Bio-assay guided fractionation**

Testing fractions in bioassays (*i.e.* enzyme inhibition, receptor binding, production of proteins in cellular response to stimuli, metabolites)

- High degree of automatization/robotization
- Adaptation to high throughput screening platforms
Bioactives identified in *Cajanus cajan* (pigeon pea)

Rationale and approach
- Fractionation of *Cajanus cajan* extracts by standard procedures
- Individual testing of inhibitory potential of each fraction (in NO and PGE₂ production)
- Identifying of ‘hot spots’
- Structure elucidation of material contained in active wells

Outcome
- Numerous ‘hot spots’ in NO inhibition profile; this is consistent with the overall activity of the extract
- Some hot spots identified in the PGE₂ inhibitory profile
- The two profiles are not overlapping

Rationale of bio-assay guided fractionation

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Assays</th>
<th>Cellular Systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTBE/MeOH</td>
<td>Nitric oxide</td>
<td>Raw 267.4</td>
</tr>
<tr>
<td></td>
<td>PGE₂</td>
<td>Human PBL</td>
</tr>
<tr>
<td></td>
<td>Interleukins, chemokines</td>
<td>Chondrocytes</td>
</tr>
<tr>
<td></td>
<td>Quantitative PCR</td>
<td></td>
</tr>
<tr>
<td>Methanol Gradient</td>
<td>Nitric oxide</td>
<td>Raw 267.4</td>
</tr>
<tr>
<td></td>
<td>PGE₂</td>
<td>Chondrocytes</td>
</tr>
<tr>
<td></td>
<td>Quantitative PCR</td>
<td></td>
</tr>
<tr>
<td>Preparative HPLC</td>
<td>Nitric oxide</td>
<td>Raw 267.4</td>
</tr>
<tr>
<td></td>
<td>PGE₂</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quantitative PCR</td>
<td></td>
</tr>
<tr>
<td>Multi-well fractions</td>
<td>Selected purified substances</td>
<td></td>
</tr>
<tr>
<td>Structure Elucidation</td>
<td>Global activity profile established with different assays</td>
<td></td>
</tr>
</tbody>
</table>
Anti-inflammatory effects of Rose hip constituents: Nitric oxide production

Table 3: Target and Isolated Peaks:

<table>
<thead>
<tr>
<th>Peak-No.</th>
<th>RT [min]</th>
<th>Mass (m/z)</th>
<th>contained in fraction</th>
<th>MW [g/mol]</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28,01</td>
<td>2708</td>
<td>C-0273-S-051</td>
<td>0</td>
<td>I [Tarpenoid]</td>
</tr>
<tr>
<td>2</td>
<td>30,40</td>
<td>2645</td>
<td>C-0273-S-022</td>
<td>278</td>
<td>Limonene</td>
</tr>
<tr>
<td>3</td>
<td>31,92</td>
<td>3078</td>
<td>C-0273-S-032</td>
<td>280</td>
<td>Limonene</td>
</tr>
<tr>
<td>4</td>
<td>31,93</td>
<td>3078</td>
<td>C-0273-S-056</td>
<td>466</td>
<td>I [Tarpenoid]</td>
</tr>
<tr>
<td>5</td>
<td>32,05</td>
<td>3099</td>
<td>C-0273-S-044, -07, -08</td>
<td>286</td>
<td>Palmitic acid</td>
</tr>
<tr>
<td>6</td>
<td>33,18</td>
<td>3202</td>
<td>C-0273-S-061, -07, -08</td>
<td>906</td>
<td>III (Oxycarboxyl)</td>
</tr>
<tr>
<td>7</td>
<td>33,74</td>
<td>3245</td>
<td>C-0273-S-044, -05</td>
<td>774</td>
<td>III (Oxycarboxyl)</td>
</tr>
<tr>
<td>8</td>
<td>37,70</td>
<td>3014</td>
<td>C-0273-U-001</td>
<td>0</td>
<td>III [Oxycarboxyl]</td>
</tr>
<tr>
<td>9</td>
<td>38,05</td>
<td>3002</td>
<td>C-0273-U-103</td>
<td>0</td>
<td>III [Oxycarboxyl]</td>
</tr>
</tbody>
</table>
Many thanks to:
- Hasan Mohajeri
- Ann Fowler
- Peter Weber
- Nathalie Richard

Additional reading:
- Current Opinion in Biotechnology Volume 19, issue 2 (April 2008)

Questions *(ex officio)*

- What is the state of the art within the field in question?
- What are the hypotheses?
- Which results have been achieved?
- What does future work focus on?
Questions \textit{(ex officio) /} Answers \textit{(quoque ex officio)}

- What is the state of the art within the field in question?
  - High level of tools in analytical and preparative platforms in natural product chemistry, large databases
  - For pharmaceutical approaches: mature
  - For nutraceutical approaches: expanding
- What are the hypotheses?
  - Not unlimited universe of the bioactives. Redundances (dependent on indications)
  - Bioactives contribute to therapeutic approaches; prevention of diseases
- Which results have been achieved (by described screening approaches)?
  - Limited additional results to what is already published \rightarrow virtual screenings
  - Corroboration of previous observations and screening results
- What does future work focus on?
  - Interactions between bioactives
  - Marketing potential of bioactives (dietary supplements, food ingredients)
  - Revival of interest of pharmaceutical industry in bioactives?