Clean Label Anti-microbial Ingredients: How to find them?

Frank Schuren
Our mission

TNO connects people and knowledge to create innovations that boost the sustainable competitive strength of industry and well-being of society.

‘INNOVATION FOR LIFE’

- Based in The Netherlands
- Over 3000 people
- Active world wide
The power of TNO
From idea to innovation

Developing Fundamental Knowledge
- With universities

Knowledge Development
- With partners in the golden triangle

Knowledge Application
- Contract research for and with customers

Knowledge Exploitation
- Embedding in the market (with TNO companies)

Emphasis on technologies/services, no product development
Key technology platforms TNO Microbiology

Conventional Microbiology
- Preservation, Hygienic Processing & design

Genomics technologies
- Genomotyping
- Transcriptomics

Fluorescence assays
- Complex flora analysis
- Viability assessments

SYSTEMS BIOLOGY
Preservation and Food Microbiology

Time consuming
No real understanding of what is happening
Mismatch between technical and consumers demand

Fresh
Short shelf life

Processed
Extended shelf life

→ fresh sensation AND extended shelf life ←

Minimal processing:
optimization of product and process

Need for Innovation!
Our aim: total solutions

- Analyze complex situations based on available molecular analyses
- Identify source(s) of problem
- Identify and rank potential solutions
- Include tailor-made solutions such as strain ID, strain sensitivity, lab simulations
Herbs and spices

- Natural ingredients with potential antimicrobial activity
- However literature data are not always reproducible
- Data only available on selected micro-organisms (mostly not spoilage related)
TNO Screening Platform for ingredients

Natural ingredients

Microorganisms

TNO Screening Platform

Identifying antimicrobial activities

Growth (Q-PCR)

Analysis/Read outs
Identification inhibitory ingredients

Z. bailii; total results screen 1+2
Platform allows for taking many variables into account

Effects of concentration and pH taken into account
Herbs and spices: Natural ingredients with antimicrobial activity

- Single ingredients show antimicrobial activity
- However often high concentrations are needed
- Serious effects on product quality:
  • Taste
  • Colour

- Possible solutions:
  - Separate taste from antimicrobial components
  - Combining ingredients
  - Going for synergistic interactions
TNO Screening Platform for ingredients

Natural ingredients

Microorganisms

Identifying antimicrobial activities

1+1=3 synergy

Analysis/Read outs

Growth (Q-PCR)
## Determining subinhibitory concentrations

<table>
<thead>
<tr>
<th></th>
<th>0,2%</th>
<th>0,1%</th>
<th>0,05%</th>
<th>0,025%</th>
<th>0,0125%</th>
<th>0,00625%</th>
<th>0,00313%</th>
<th>Chosen concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonenkruid</td>
<td>25.0429</td>
<td>25.0031</td>
<td>24.8797</td>
<td>18.218</td>
<td>17.0693</td>
<td>17.2492</td>
<td>16.242</td>
<td>0,025%</td>
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<tr>
<td>Kruidnagel</td>
<td>24.4385</td>
<td>24.8015</td>
<td>24.175</td>
<td>17.0472</td>
<td>16.575</td>
<td>17.2363</td>
<td>16.2855</td>
<td>0,03</td>
</tr>
</tbody>
</table>

**Legend:**
- Growth
- Slight inhibition
- Inhibition
- Selected concentration
Selected concentrations for synergy experiments

<table>
<thead>
<tr>
<th>Species 1</th>
<th>Species 2</th>
<th>Species 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025</td>
<td>0.025</td>
<td>0.075</td>
</tr>
<tr>
<td>0.075</td>
<td>0.13</td>
<td>0.5</td>
</tr>
<tr>
<td>0.00625</td>
<td>0.015</td>
<td>0.0085</td>
</tr>
<tr>
<td>0.075</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>0.02</td>
<td>0.13</td>
<td>0.2</td>
</tr>
<tr>
<td>0.03</td>
<td>0.025</td>
<td>0.07</td>
</tr>
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<td>0.015</td>
<td>0.03</td>
<td>0.07</td>
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<tr>
<td>0.18</td>
<td>0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>0.13</td>
<td>0.25</td>
<td>2</td>
</tr>
<tr>
<td>0.075</td>
<td>0.13</td>
<td>0.4</td>
</tr>
<tr>
<td>0.0125</td>
<td>0.015</td>
<td>0.0185</td>
</tr>
<tr>
<td>0.2</td>
<td>0.25</td>
<td>2</td>
</tr>
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Variation in activity for herb/spoilage organism combinations
The aim of synergy experiments

Observed growth inhibition (sVAB) > Expected growth inhibition (V+A+B)

Indicating synergistic effects between Vanillin, component A and B
Results for one spoilage micro-organism

Synergy: 1+1=3
Additive: 1+1=2
Antagonism: 1+1=0
Synergy or Additive
### Synergy between herbs & spices

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<tr>
<td><strong>Synergy</strong>: 1+1=3</td>
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#### Comments:
- Synergy: When the interaction of two species results in a greater effect than the sum of their individual effects.
- Additive: When the interaction of two species results in a combined effect equal to the sum of their individual effects.
- Antagonism: When the interaction of two species results in a combined effect less than the sum of their individual effects.
Broader application range

- Example shown only focuses on herbs and spices
- In principle every ingredient might have an inhibitory effect, especially in combination with other ingredients
- Furthermore combinations of ingredients and processing conditions may also show synergistic interactions
- All of these examples would fit into a clean label concept
Need for novel approaches

- Synergy approach will be the most straightforward and cost-efficient if successful
- However, certain problems are so complex that more insight is needed to develop novel solutions
- We therefore use genomics technologies to obtain more insight and develop novel approaches
General principle: bacterial cell as a biosensor

- $T$
- $pH$
- $Aw$

Obtain information on bacterial cell AND its environment: *Predictive* !!
We learn that gene expression in cells correlates with external stress factors.

Example 1: temperature
... in helping us understanding the behaviour of cells in food production processes......
Selection of biomarkers
Exploiting the microbial sensor principle

- Identification of model spoilage strains
- Genome sequencing of the strains
- Microarrays or nextgen sequencing
- Assess specific stress response
  Identification and validation of target genes = biomarkers
- Translate data into high-throughput screen (HTS):
TNO Screening Platform for ingredients

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Microorganisms

TNO Screening Platform

Identifying antimicrobial activities

1+1=3 synergy

Analysis/Read outs

Growth (Q-PCR)

Gene profiling
Prevention is better than cure
Hygiene in the food production chain
Using taxonomic profiling of microbial communities

- Barcoded next gen sequencing
  - Bacterial flora (16s/groEL)
  - Archaea
  - Fungi
  - Unicellular eukaryotes
  - plants
- 10-20 million sequences in one run
- 400 samples can be analyzed in one run
Microbiota load in food production environment

Air sampling in bakery

300 l/min

DNA isolation

sequencing

Load of Fungal-amplicon-seqs during Food Processing (seqs/sample)
Microbiota composition in food production environment

Fungal Flora of Air & Product in a Food-processing Factory (% 18S-fungal-classified-seqs / sample)

- Fungi (most Eurotium)
- environmental samples
- Penicillium
- Cladosporium
- Bjerkandera
- Aspergillus
- Pleurotus
- Stereum
- Heterobasidion annosum sp. Complex
- Trametes
- Malassezia
- Alternaria alternata group
- Cryptococcus
- Fomitopsis
- Epicoccum
- Geomyces
- Hyphodontia
- Mucor
- Fomes
- Microdochium
- Basidiomycota
- Vuilleminia
- Epicoccum
- Coprinellus
- Eurotium
- Lentinus
- Mycosphaerellaceae
- Chondrostereum
- Hamigera
- Dioszegia
- Rhodotorula
- Ncortoria
- Schizophyllum
- Alternaria
- Polyporus
- Ascomycota
- Genodema
Complete chain management

› Technology is available to determine spoilage and contamination level at all stages of the food production chain
› Information on both total contamination levels and specific spoilers/pathogens
› Identify weak spots or unexpected entry/transmission routes
› Evaluate efficacy of preventive measures and cleaning
› Focus on preventing problems in stead of treating them

› Future integration in standard QA/QC
Natural preservation = microbial fermentation

- History taught us how to preserve unstable foods
  - Milk > cheese
  - Grain > beer, bread
  - Grapes > wine
  - Meat > sausages

- Microbial fermentation has a very long history of safe use
- Why is it not used on a broader scale for other types of products?
- TNO is exploring possibilities in this field based on combinations of novel and existing technologies
Added values of microbial fermentation

- Unique taste profile/product composition
- Production vitamins
- Production antimicrobial components (antifungal)
- Production fibres/EPS/oligosaccharides (healthier product)
- Specific taste (alternatives for salt)
- Production anti-oxidants (glutathion, thiol components)
- Production organic acids for natural preservation
- Satiety inducing effects by specific organic acids

This could be a very promising shortcut in the clean label discussion
PPS Novel anti fungal solutions

Collective phase
Overall aim: Develop an effective screening platform which enables the consortium to develop novel anti-fungal solutions in a broad range of food applications.

Screening platform development

WP1: Development of stress biomarker screening platform

WP2: Sustainable production processes

WP3: Mode of action, synergy

Individual phase

APPLICATIONS WP’s

Alcoholic beverages
Beverages
Dairy
Bakery

AIM & TARGET MO

t.b.d.
t.b.d.
t.b.d.
t.b.d.
TNO Brings stakeholder chain together under a clean label framework

Critical mass of stakeholders is important to define the clean label framework

Holistic approach
Integrated Project approach

Clean Label Framework

- Stabilizers/Thickeners
- Preservatives
- Colors/Flavors
- Anti-oxidants

Ingredient

Product/process

Consumer

Insight into relationship between ingredient functionality, product quality and consumer acceptance when doing multiple reformulation

Ingredient functionality

Texture, shelf life, ingredient interaction

Taste, acceptance

Product categories:
- Beverages
- Soups and Sauces
- Confections
- Breads and cookies
What if natural products came with a list of ingredients?