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The Integration of Omics in Microbiological Risk Assessment

Presented By: Elias Rito, Dr. Heidy den Besten, Prof. Luca Cocolin, Dr. Annemarie Pielaat, Dr. Alejandro Amezquita

Sponsored By: ILSI Europe

Organized by: Microbial Modelling and Risk Analysis PDG


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Introduction to ILSI Europe

Mr Elias Rito

Scientific Project Manager
ILSI Europe

ILSI Europe
International Life Sciences Institute

Sound Science

How ILSI Europe makes the difference

The tripartite approach is a fundamental pillar of ILSI Europe

ILSI Europe in a Nutshell

21 Task Forces

45 Expert Groups

53 Members

ILSI Europe

Microbiological Food Safety Task Force

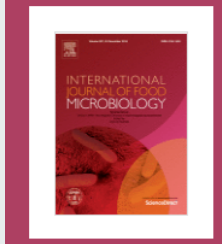
- *“Provides guidance on microbial food safety issues to support society in implementing efficient food safety systems.”*

Microbiological Food Safety Task Force: Objectives and Tools

Ultimate goal is to **investigate microbial issues in foods** that are related to public health risks

Tools:

- Peer-reviewed publications
- Workshops
- Webinars
- European projects



International Journal of Food Microbiology

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Omics in MRA - the integration of omics in microbiological risk assessment

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Edited by Chris W. Michiels
Volume 287,
Pages 1-40 (20 December 2018)

Editorial + 4 papers (open access)

https://www.sciencedirect.com/journal/international-journal-of-food-microbiology/vol/287

'The use of Omics in Exposure Assessment'
Speaker 1 - Dr Heidy den Besten
Wageningen University & Research, NL

'Potential of omics data for Hazard Characterization'
Speaker 2 - Dr Annemarie Pielaat
Unilever, NL

'Meta-omics: The next need for integration'
Speaker 3 - Prof. Luca Cocolin
University of Turin, IT

The use of omics in Exposure Assessment

Heidy den Besten

Alejandro Amézquita, Sara Bover-Cid, Stéphane Dagnas, Mariem Ellouze, Sandrine Guillou, George Nychas, Cian O'Mahony, Fernando Pérez-Rodriguez, Jeanne-Marie Membré

WAGENINGEN
UNIVERSITY & RESEARCH

Heidy.denBesten@wur.nl

Quantitative Microbial Risk Assessment

From Farm to Fork

Hazard identification

potential adverse health effect

Exposure assessment

dose at consumption

Hazard characterisation

P(ill) and severity
as function of dose

Risk Characterisation

P(ill) and severity
with variability

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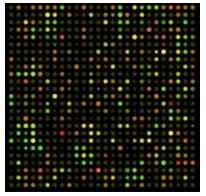
PRINCIPLES AND GUIDELINES FOR THE CONDUCT OF MICROBIOLOGICAL RISK ASSESSMENT CAC/GL-30 (1999)

Omics – extra dimensions

DNA

genomics

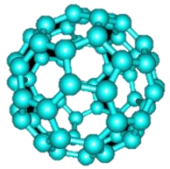


RNA

transcriptomics

proteins

proteomics



metabolites

metabolomics

Who is there and what can happen?

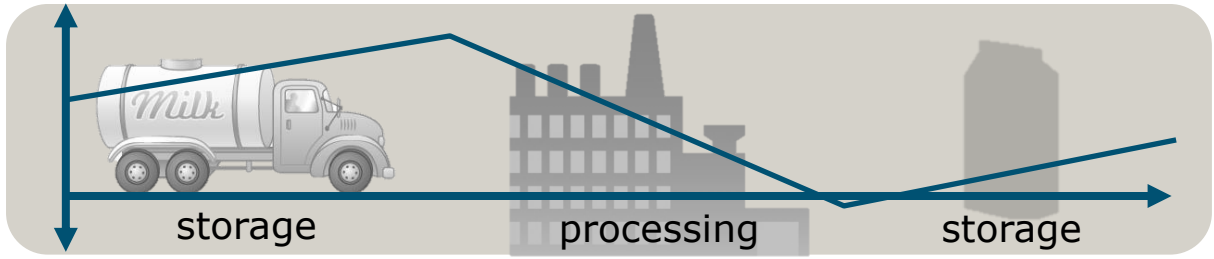
What appears to be happening?

What makes it happen?

What has happened and is happening?

single strain - microbial consortia

How will my troublemaker(s) behave?

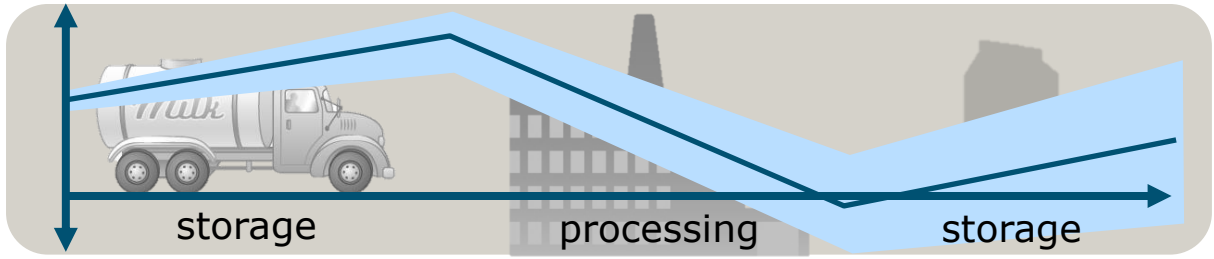


Exposure assessment

Levels and kinetics

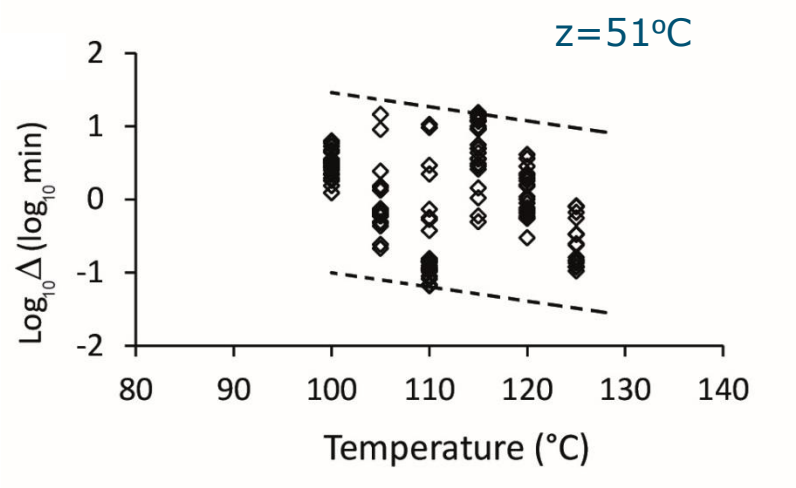
Quantification of growth, inactivation, survival, contamination

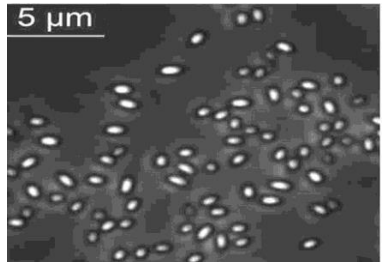
How could omics makes a difference?



- Predicting pathogen behaviour variability
- Understanding dynamics in complex food eco-systems

Strain variability





B. subtilis spores
20 strains

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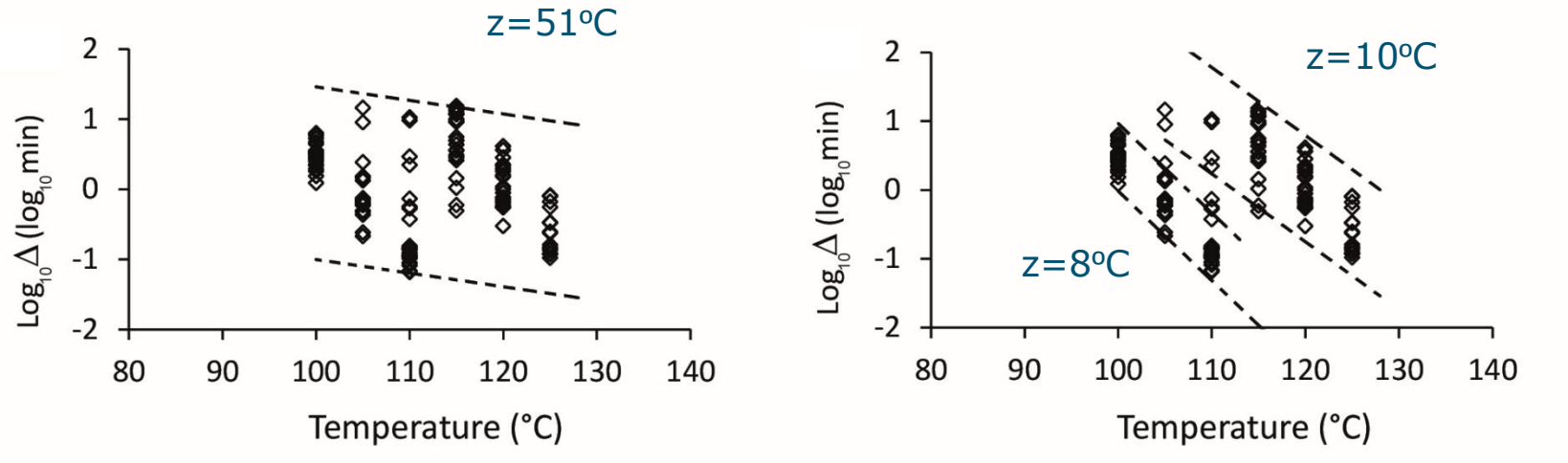
Den Besten et al., 2017
NIZO Food Research, TIFN

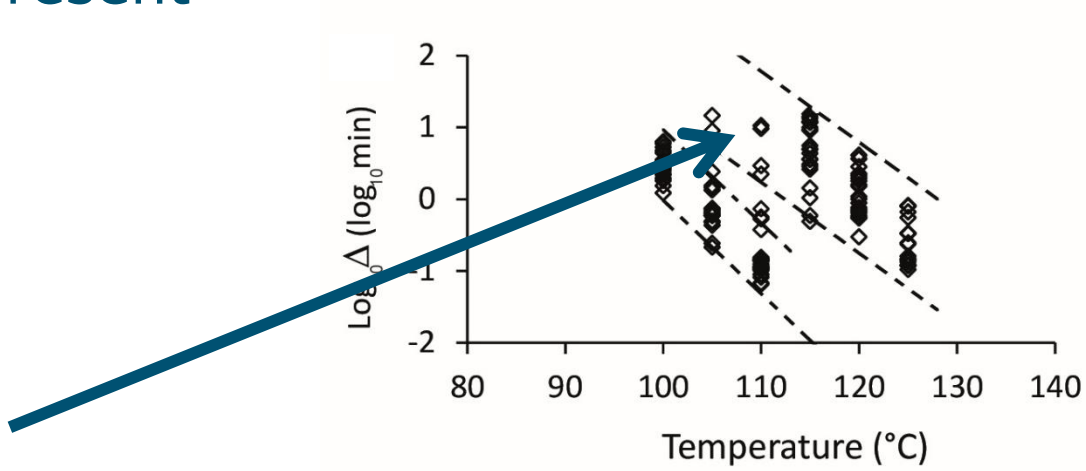
Strain variability

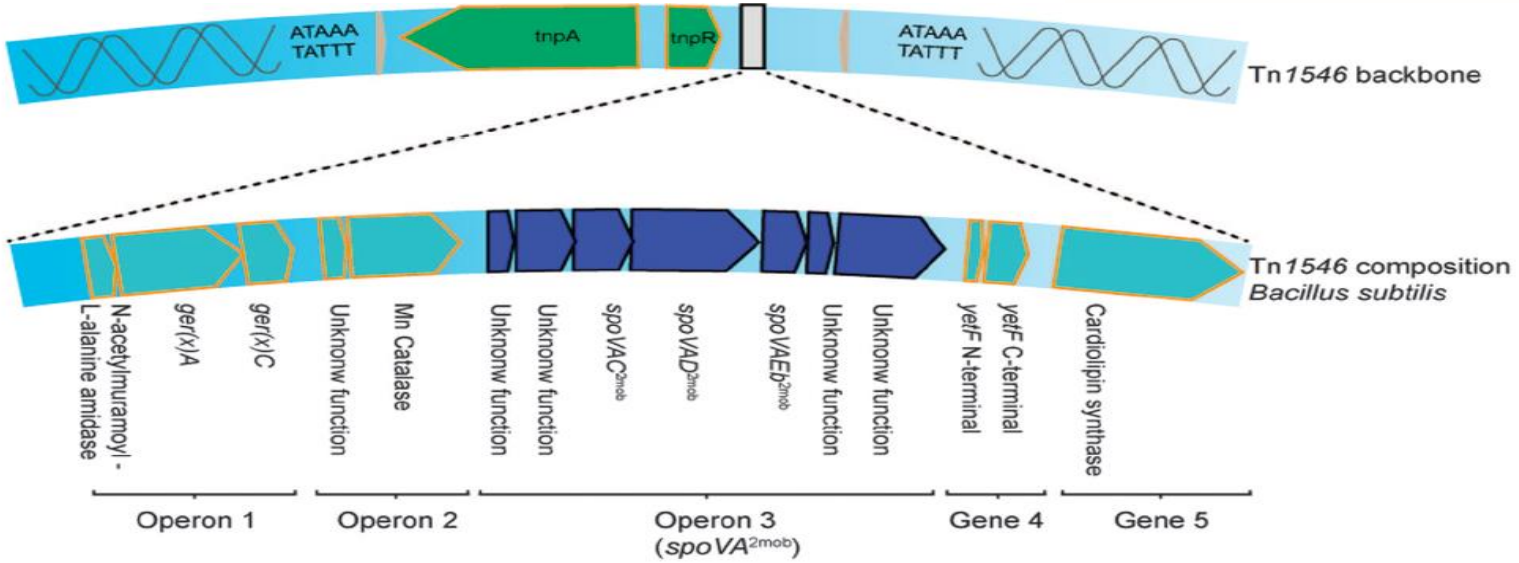


Den Besten et al., 2017
NIZO Food Research, TIFN

Strain variability and biomarker

- Genetic element only present in heat resistant group
- Genetic biomarker for robustness





Berendsen et al., 2016
Den Besten et al., 2017
NIZO Food Research, TIFN

Mechanistic insight to fine-tune EA

- Biomarkers for robustness can be used to make subgroups
- Fine tune EA taking into account phenotypes of the subgroups

Mechanistic insight to fine-tune EA

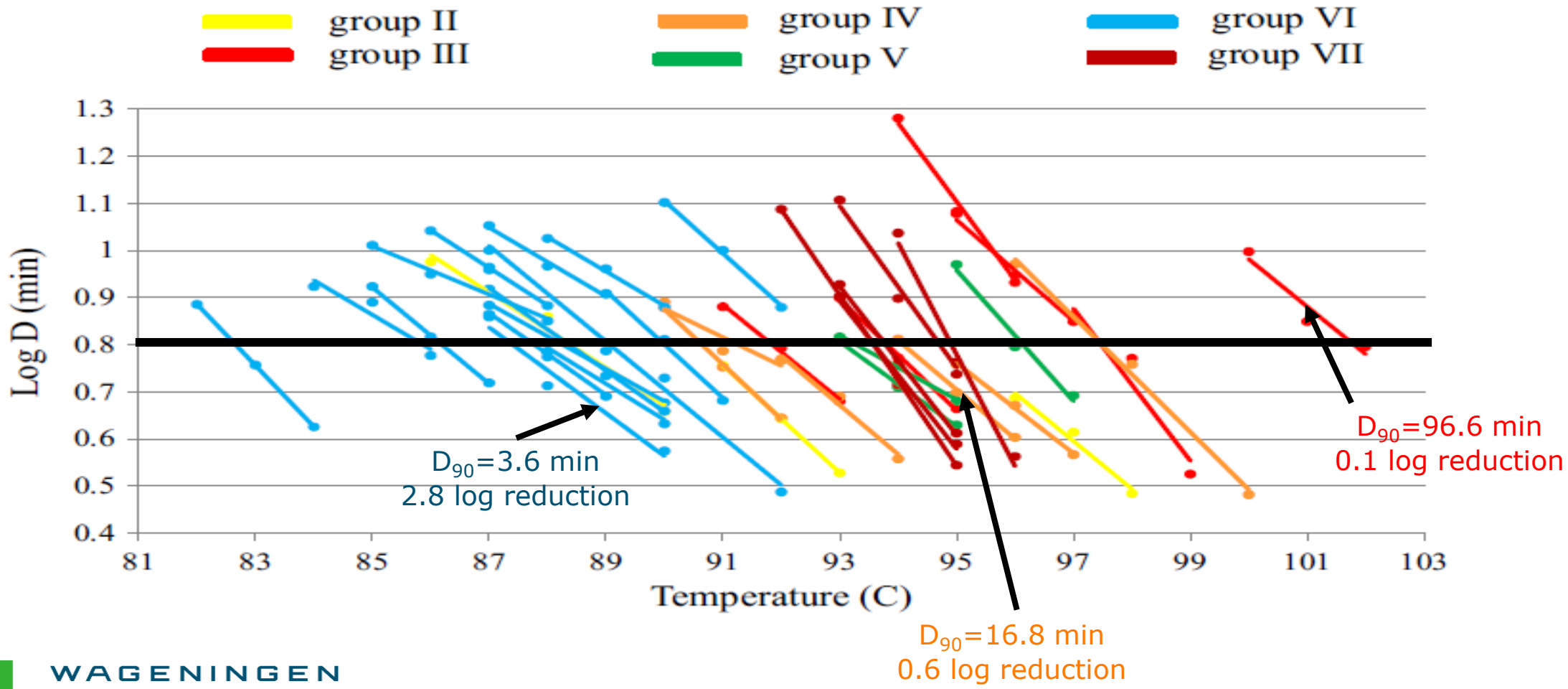
- *B. cereus* group: seven major phylogenetic groups
- Differences in Temp growth ranges between groups

	% strains with growth at:												
Group	4°C	5°C	7°C	8°C	10°C	15°C	20°C	37°C	40°C	43°C	45°C	50°C	55°C
VII	0	0	0	0	0	0	100	100	100	100	100	100	0
III	0	0	0	0	0	100	100	100	100	100	85	0	0
IV	0	0	0	0	100	100	100	100	100	83	58	0	0
I	0	0	0	0	75	100	100	100	100	25	0	0	0
V	0	0	0	14	100	100	100	100	100	0	0	0	0
II	0	0	73	87	100	100	100	100	100	0	0	0	0
VI	0	40	100	100	100	100	100	86	0	0	0	0	0

Guinebretière et al., 2008

Mechanistic insight to fine-tune EA

- Also clear difference in heat robustness



90°C, 10 min treatment

Luu Thi et al., 2013

Mechanistic insight to fine-tune EA

- Also clear difference in heat robustness

Group	$T_{logD=0.8}$ (°C) ± SD	z-value (°C) ± SD
III	96.6 ± 3.5	8.4 ± 1.9
VII	94.3 ± 0.9	5.7 ± 0.9
V	94.1 ± 1.4	11.0 ± 3.8
IV	93.3 ± 2.6	10.5 ± 3.1
II	91.4 ± 2.7	10.2 ± 2.2
VI	88.5 ± 2.4	12.0 ± 2.9

- Subgrouping based on mechanistic insight provides more precision in EA than when taking the group as a whole

Luu Thi et al., 2013

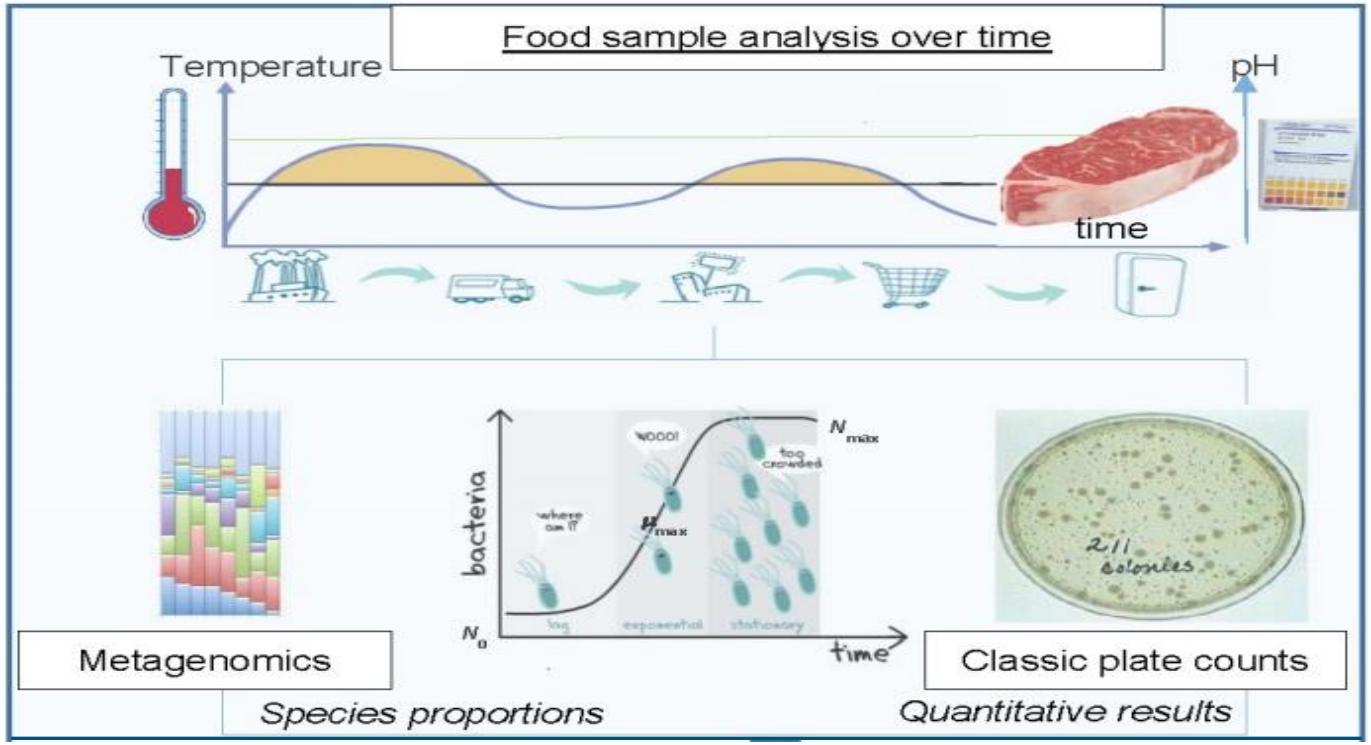
Food ecosystem dynamics

- Microbial communities affect dynamics of pathogens

- Metagenomics to understand ecosystem dynamics
  - Characterise communities
  - Elucidate transmission routes

Food ecosystem dynamics

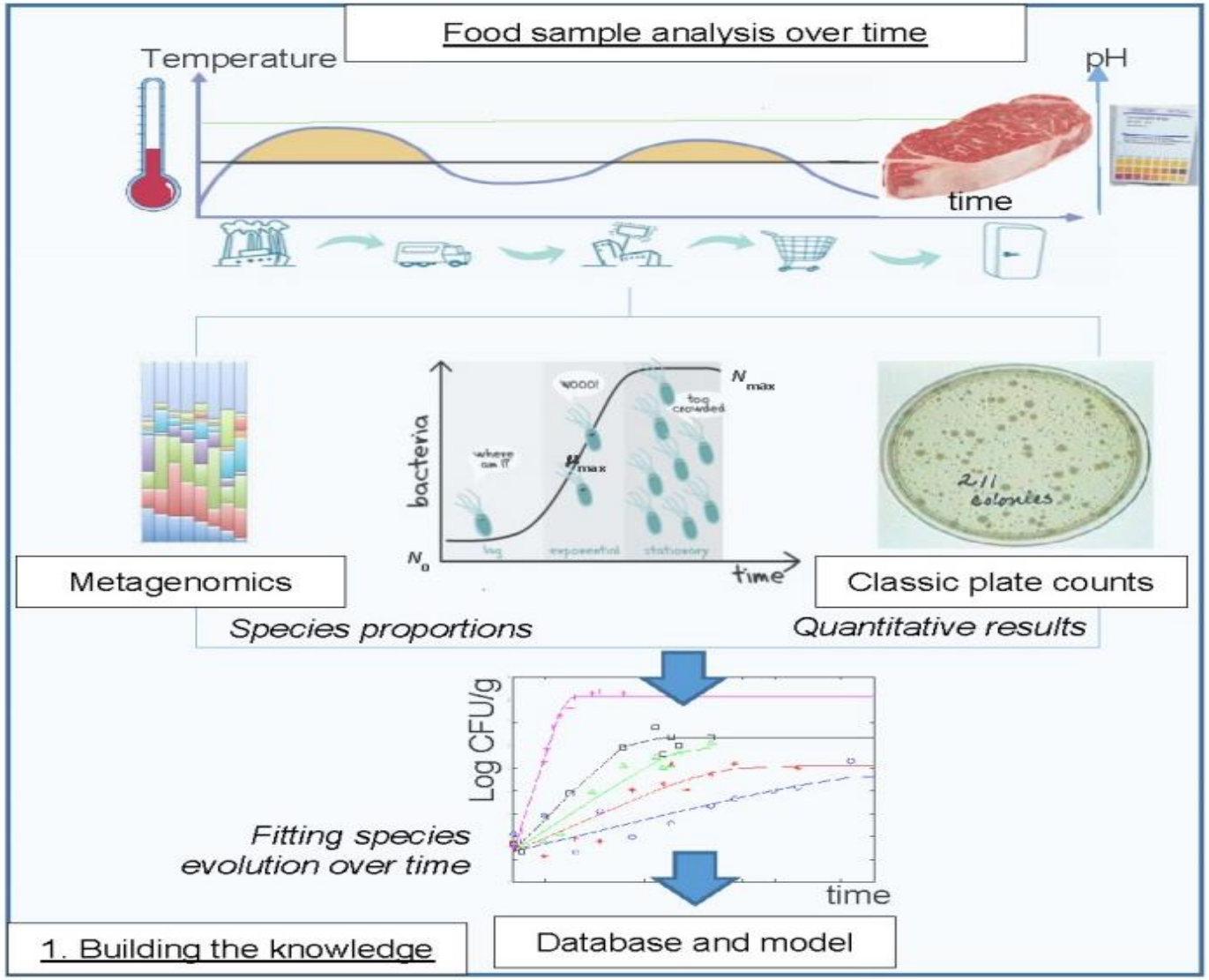
- Meta data collection
  - Food (pH, aw)
  - Chain (Temp)
- Metagenomics
  - relative abundance
- Enumeration
  - counts



Picture from M. Ellouze

Food ecosystem dynamics

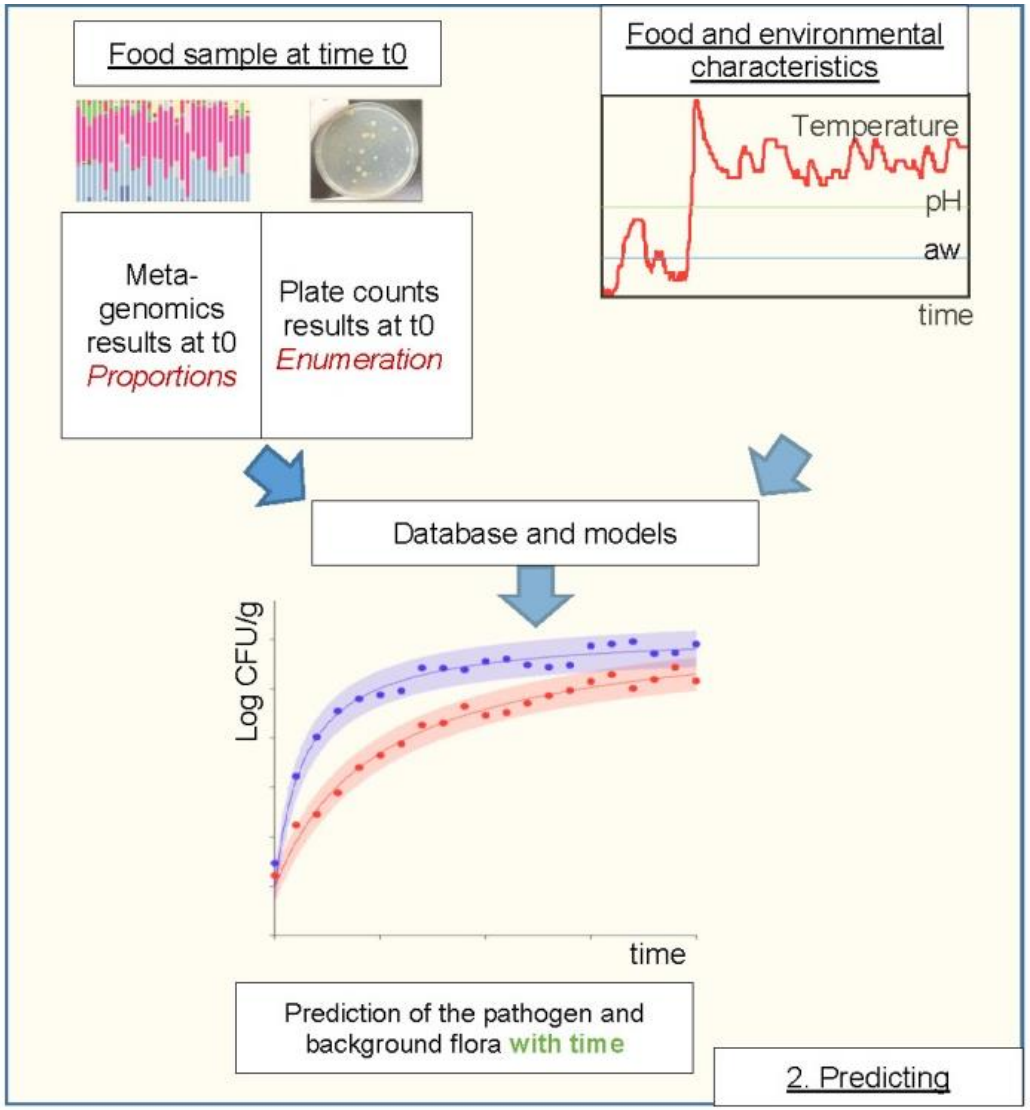
- Meta data collection
  - Food (pH, aw)
  - Chain (Temp)
- Metagenomics
  - relative abundance
- Enumeration
  - counts
- Database of kinetics of species or relevant subgroups



Picture from M. Ellouze

Food ecosystem dynamics

- Prediction
  - Based on database and models
- Challenges: low prevalence of pathogens



Picture from M. Ellouze

Potential of omic data in EA

- Better understanding of biology: behaviour of pathogens, food ecosystems and its dynamics
- Biomarkers help to quantify strain variability
- Help to fine tune EA
- Reducing uncertainty in EA

Joined efforts

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Next generation of microbiological risk assessment: Potential of omics data for exposure assessment

Heidy M.W. den Besten[a], Alejandro Amézquita[b], Sara Bover-Cid[c], Stéphane Dagnas[d], Mariem Ellouze[e], Sandrine Guillou[f], George Nychas[g], Cian O'Mahony[h], Fernando Pérez-Rodriguez[i], Jeanne-Marie Membré[f,*]

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WEBINAR

The Integration of Omics in Microbiological Risk Assessment

27 March 2019

(17.30-18.30 CET – 10.30-11.30 CST)

Upon registration only – Attendance is free!

Potential of omics data for Hazard Characterization

Annemarie Pielaat

Nabila Haddad, Nick Johnson, Sophia Kathariou, Aline Métris, Trevor Phister, Chrysoula Tassou, Marjon H.J. Wells-Bennik, Marcel H. Zwietering

Annemarie.Pielaat@Unilever.com

Unilever

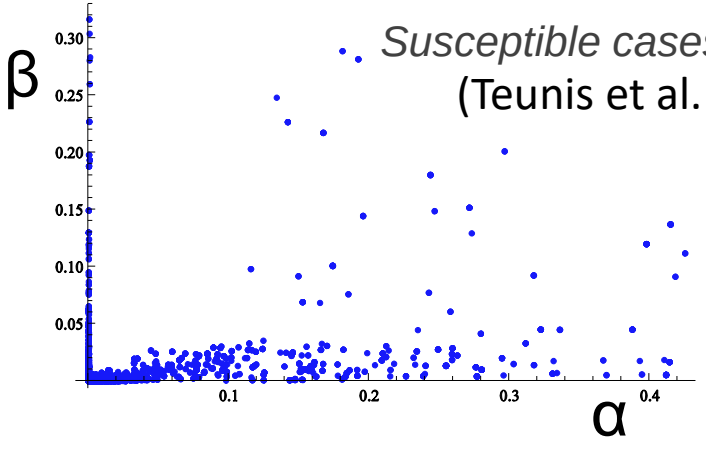
Introduction Hazard Characterization in QMRA

Dose: 10^3 *Salmonella* Typhimurium

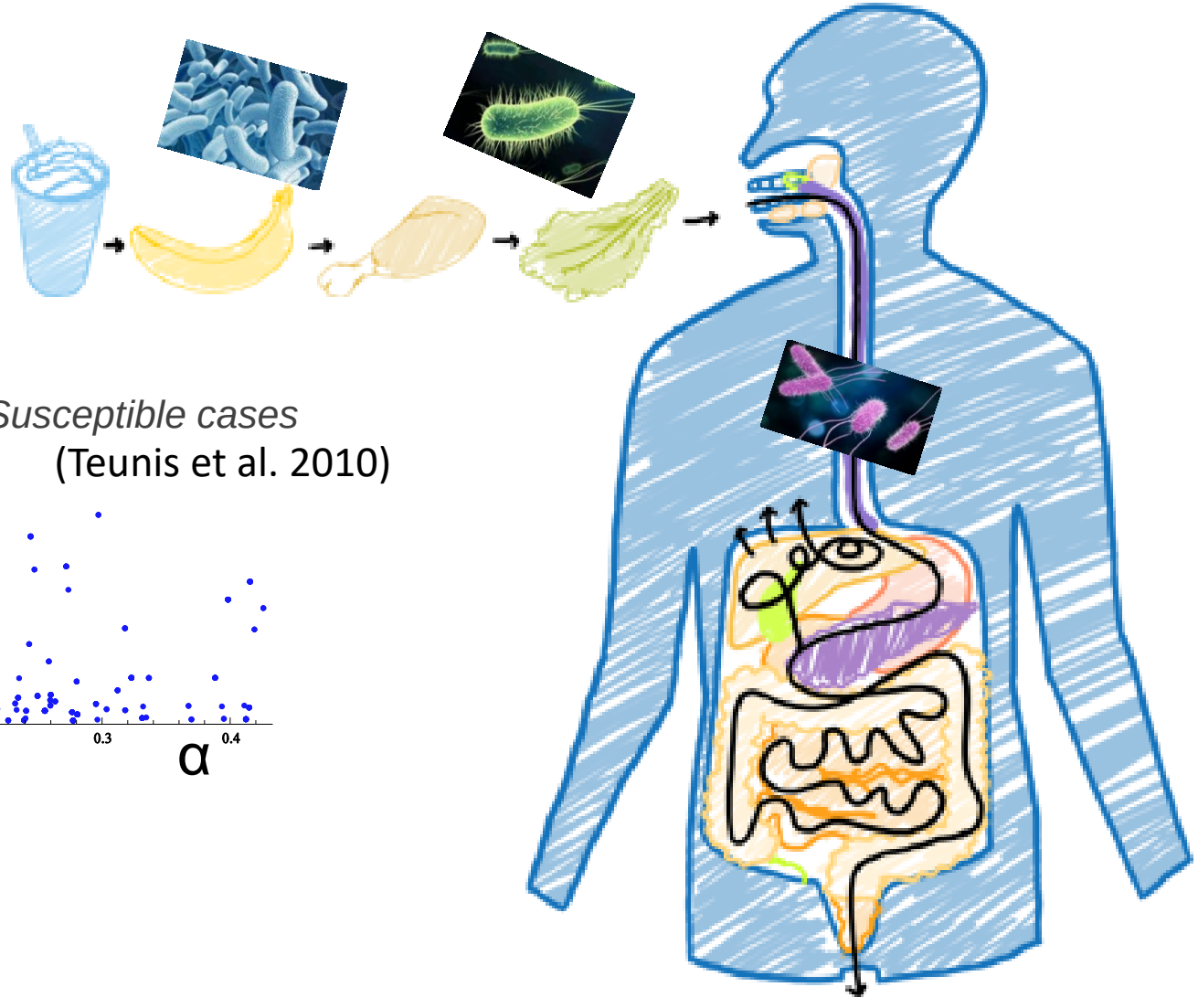
Dose Response Models:

e.g. Exponential

$$P = (a/(a+\beta)) \cdot D$$



Susceptible cases
(Teunis et al. 2010)



- Biological variability
- Experimental uncertainty

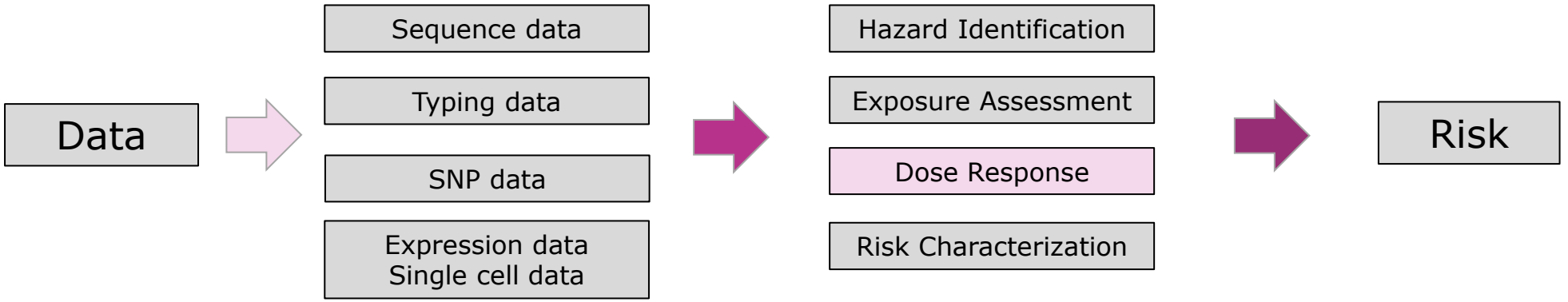
Introduction Hazard Characterization in QMRA

Output: Number of ill cases

		2.5, 50 and 97.5% confidence levels and mean cases of illness			
Mix_ID	Pathogen	2.5%	50%	97.5%	Mean
1	*Salmonella Typhimurium* DT104	0	14	10,016	1,242
2	*Salmonella Typhimurium* DT104	0	20	9,728	1,200
3	*Salmonella Typhimurium* DT104	0	24	10,865	1,365
4	*Salmonella Typhimurium* DT104	0	191	45,241	7,317
5	*Salmonella Typhimurium* DT104	0	135	31,893	5,176
6	*Salmonella Typhimurium* DT104	0	99	53,206	6,459
7	*Salmonella Typhimurium* DT104	0	268	66,276	10,266
8	*Salmonella Typhimurium* DT104	0	128	29,284	4,709
9	*Salmonella Typhimurium* DT104	0	116	26,533	4,263
10	*Salmonella Typhimurium* DT104	0	37	15,571	1,962
11	*Salmonella Typhimurium* DT104	0	20	8,566	1,087
12	*Salmonella Typhimurium* DT104	0	69	29,008	3,691
13	*Campylobacter* spp	0	214	5,147	782
14	*Campylobacter* spp	0	2,247	34,223	6,010
15	*Campylobacter* spp	0	3,256	47,937	8,513
16	*E. coli* 0157	0	1	195	31
	Salmonella Montevideo	0.70	2,510	17,280	4,091

Number of ill cases per year in The Netherlands from the consumption of a portion of mixed salad. *Pielaat et al. (2014) J. Food Protection*

NG-Omics challenge "*The Mapping Problem*"



multidim. genotypic info

$10^{\wedge 3}$ genes

$10^{\wedge 4}$ SNPs

etc

reduced phenotypic info

$10^{\wedge 1}$ characteristics

survival in GIT

growth rate

etc

single risk

$10^{\wedge 0}$

No. ill

NG-Omics challenge "*support decision making*"

Risk assessment is intended to support decision making …
Difficult for new data sets to influence risk assessments directly

There are many questions and even more answers …

- Does a 'new' genotype identify a new hazard? → change policy?
- How does presence/absence of a virulence gene characterise a hazard/non-hazard?
- How does a 'differential' expression characterise a risk?
- How do we use molecular data analysis for probabilistic calculations in QMRA?

NG-Omics challenge "*support decision making*"

- Traditionally viewed/ regulated by serotype
  - Dutch guideline of 2014
    - for high risk ready to eat (RTE) foods, all STEC with (stx1 OR stx2) are considered unacceptable, while for low risk food products (to be cooked), only STEC's that have (stx1 AND/OR stx2) AND [(eae) OR (aaiC AND aggR)] AND belonging to serotypes (O26, O103, O111, O145, O157, O104, O45, O121 en 0174) are considered unacceptable.

- Potential issues of biomarker focused regulation

Table 2: Example of sequence of a more and more stricter definition of pathogenic potential

STEC = (stx1 OR stx2)
STEC = (stx1 OR stx2) AND an attachment factor like genetic element
STEC = (stx1 OR stx2) AND known attachment factor
STEC = (stx1 OR stx2) AND (Eae OR (aaiC and aggR))
STEC = (stx1 OR stx2) AND (Eae)

(Haddad et al. 2018, IJFM)

NG-Omics challenge “The industry”

- The incorporation of omics data that often has little biological meaning into the risk models will require input from the risk manager and will make the decision making process more complex
- There are concerns around the lack of standardization and reproducibility of current methods
- Paucity of relevant data sets

NG-Omics *"The potentials"*

- Can provide greater detail on pathogens
  - Pathogenicity
  - Virulence
  - Stress responses
  - Interaction with other systems (both humans and microbes)
- May be used to identify biomarkers
- How do we translate the biomarkers from human and cell culture or animal model responses into the dose-response models?

Biomarkers, *Haddad et al. (2018) IJFM*

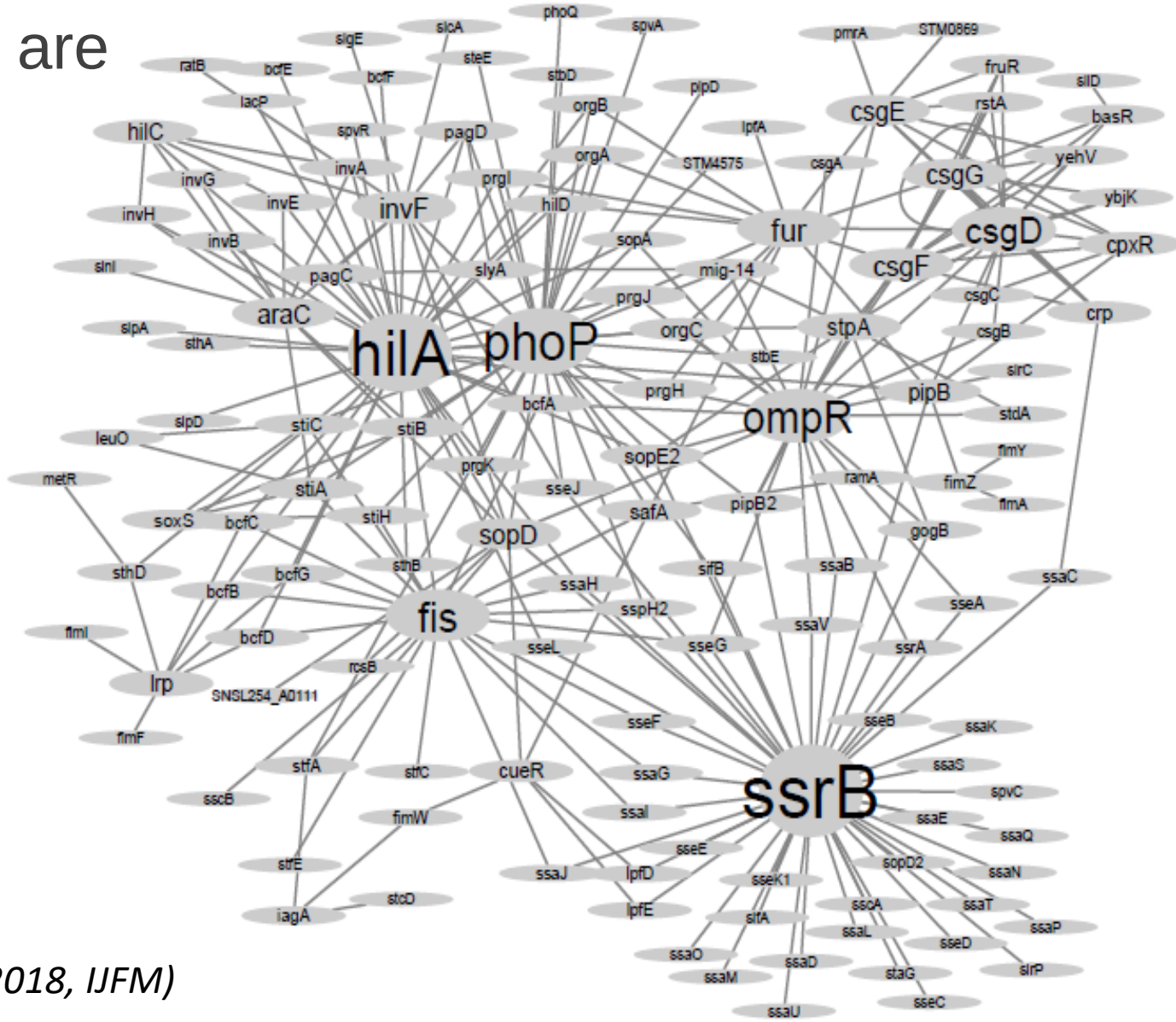
Omic methods	Type of biomarker	Example (from literature)	Type of response: - quantitative value (fold) qualitative response (detection/identification)	Reproducibility	Remarks and references
Genomics	Gene (CDS)	stx of Escherichia coli	Qualitative		Lindsey et al., 2016
	SNP	stx of E. coli	Qualitative		Pielaat et al., 2015
	Multiple copies	Neurotoxin genes of Clostridium botulinum	Qualitative		Peck and van Vliet, 2016
Transcriptomic	mRNA	SPI-1 genes or hil1A of Salmonella enterica	Quantitative	2 biological replicates	Comparison between two different serotypes. Elhadad et al., 2016
Proteomic	protein	TypA of Cronobacter sakazakii	Quantitative	3 technical replicates, but no biological replicate	Comparison between virulent and non-virulent strains. Du et al., 2015
Metabolomic	metabolite	Cereulide toxin of Bacillus cereus	Quantitative		Biesta-Peters et al. 2010 ; Marxen et al., 2015

Biomarkers: Network analysis

The main regulators in this data are

ssrB, hilA, phoP,

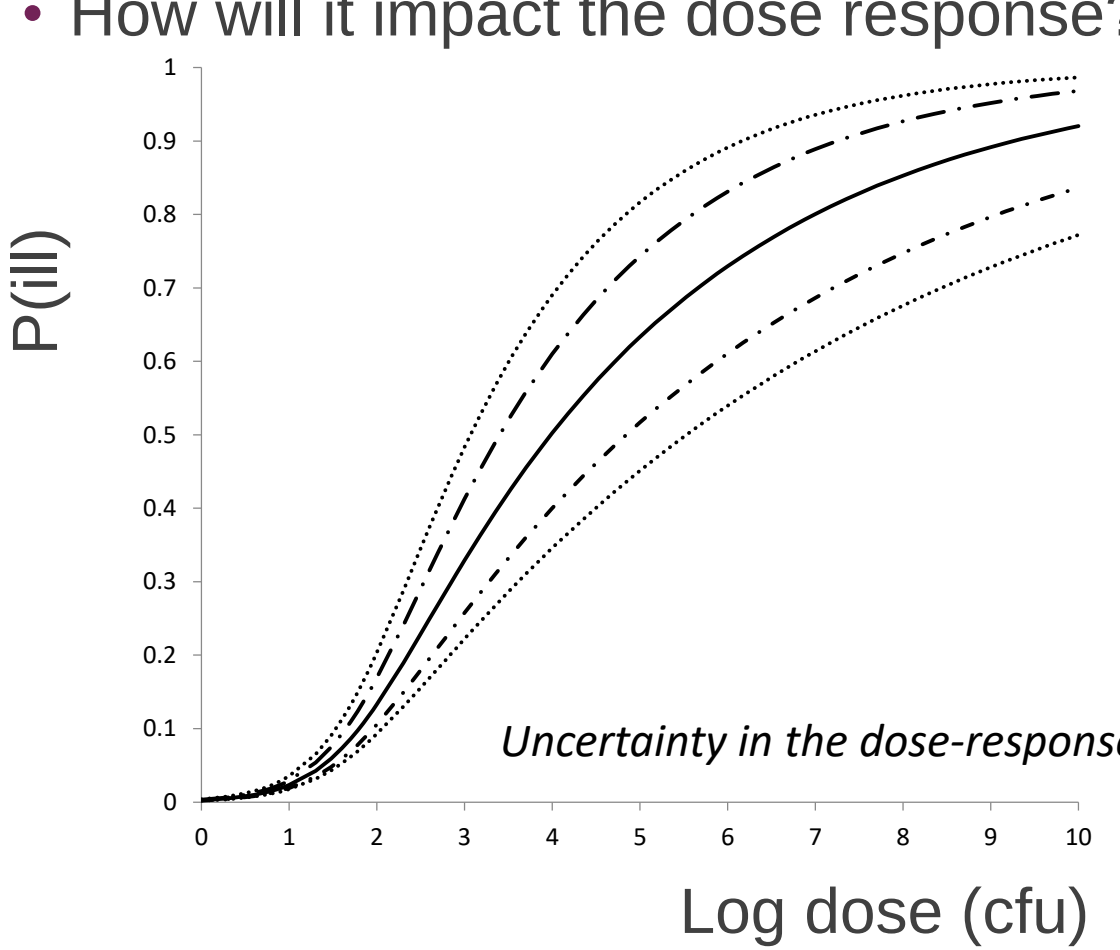
ompR and csgD

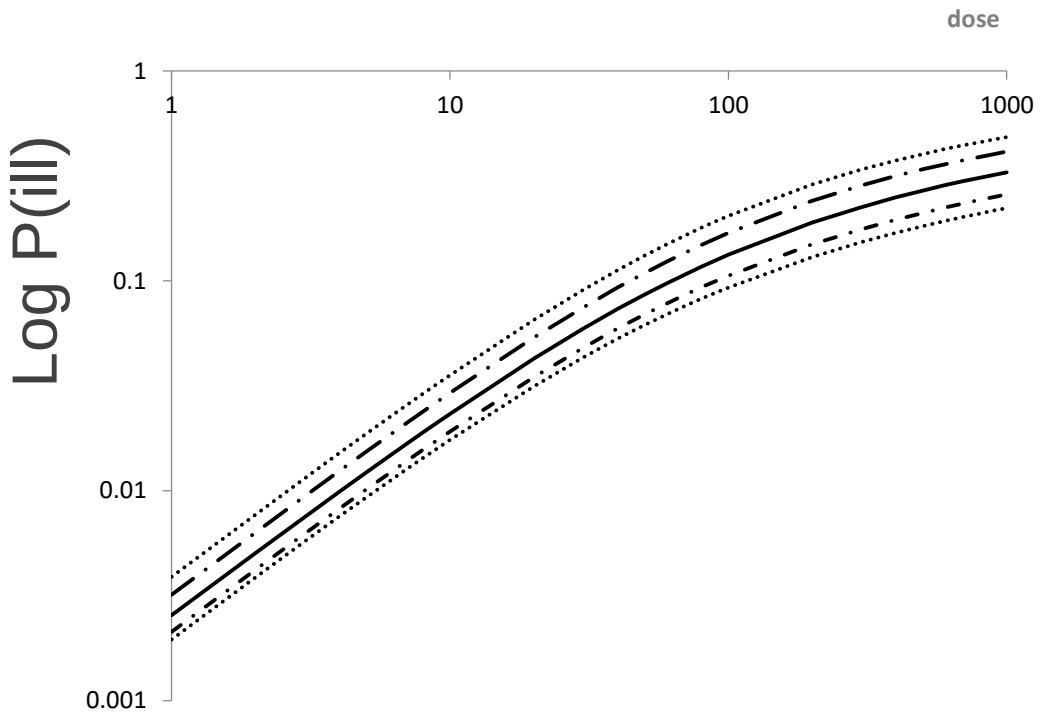


(Haddad et al. 2018, IJFM)

Biomarkers and dose response

- How do we correlate biomakers to responses and illness conditions
- How do we quantify these correlations
- How will it impact the dose response?

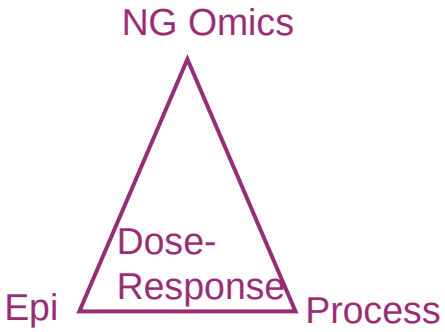


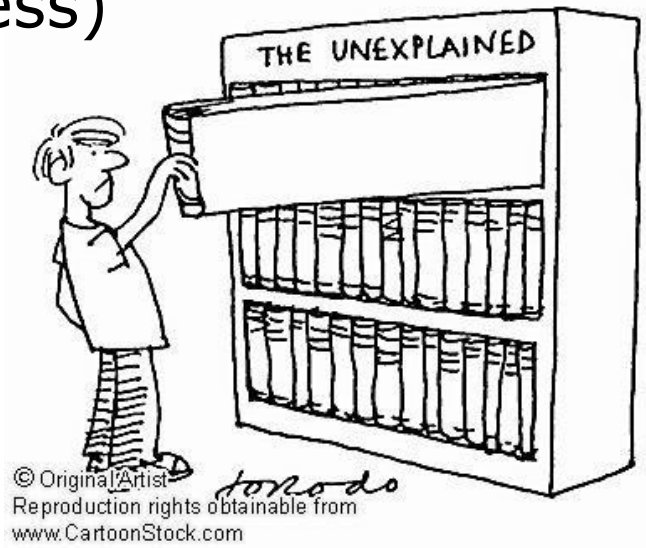


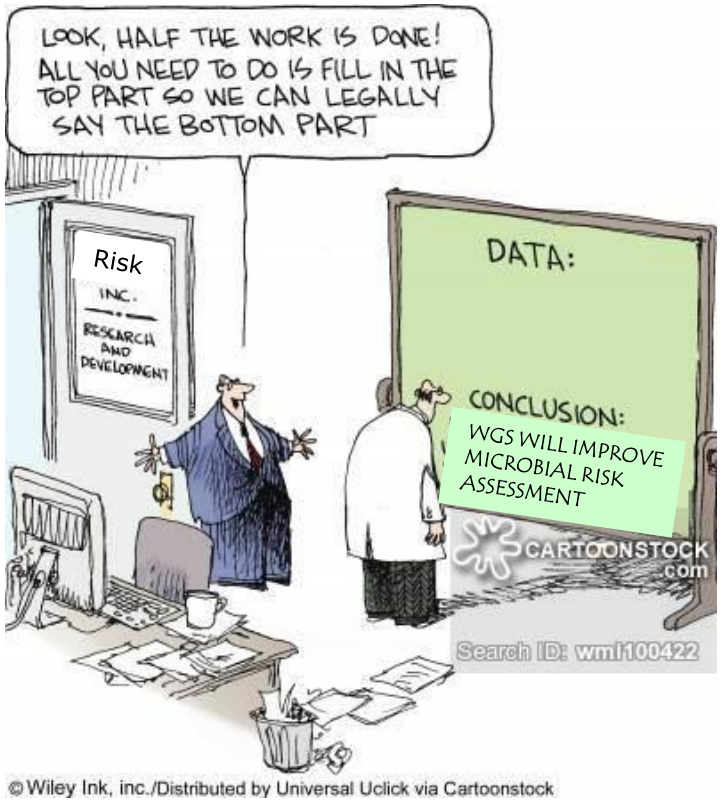
Uncertainty in the dose-response relationship used in the WHO-FAO report, 2002

NG Omics *"Ways forward"*

- Link with other data (eg epidemiological, process)







- Systems biology

The presence of a biomarker (gene, metabolome, protein) may by itself not always be a good predictor, since the expression is influenced by a large variety of (biological) factors & biomarkers are dependent.

Concluding remarks

- Omics is already changing the food industry
- In the next few years it is going to impact industry's ingredient and products specifications, surveillance programs and detection methodologies
- May also increase the challenges for companies distributing products across different regulatory environment
- Ultimately though if we collaborate effectively between academics, regulatory agencies and Industry the impact of Omics on MRA will improve the quality and accuracy of our hazard characterizations

- Finally
  - *The views expressed in this presentation are those of the authors and do not necessarily represent positions or policies of IAFP, ICFMH, ILSI, Nestlé, PepsiCo Inc., Unilever, NIZO or any authors affiliation.*

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Meta-omics: The next need for integration

Luca Cocolin

Marios Mataragas, Francois Bourdichon, Agapi Doulgeraki, Marie-France Pilet, Balamurugan Jagadeesan, Kalliopi Rantsiou, Trevor Phister

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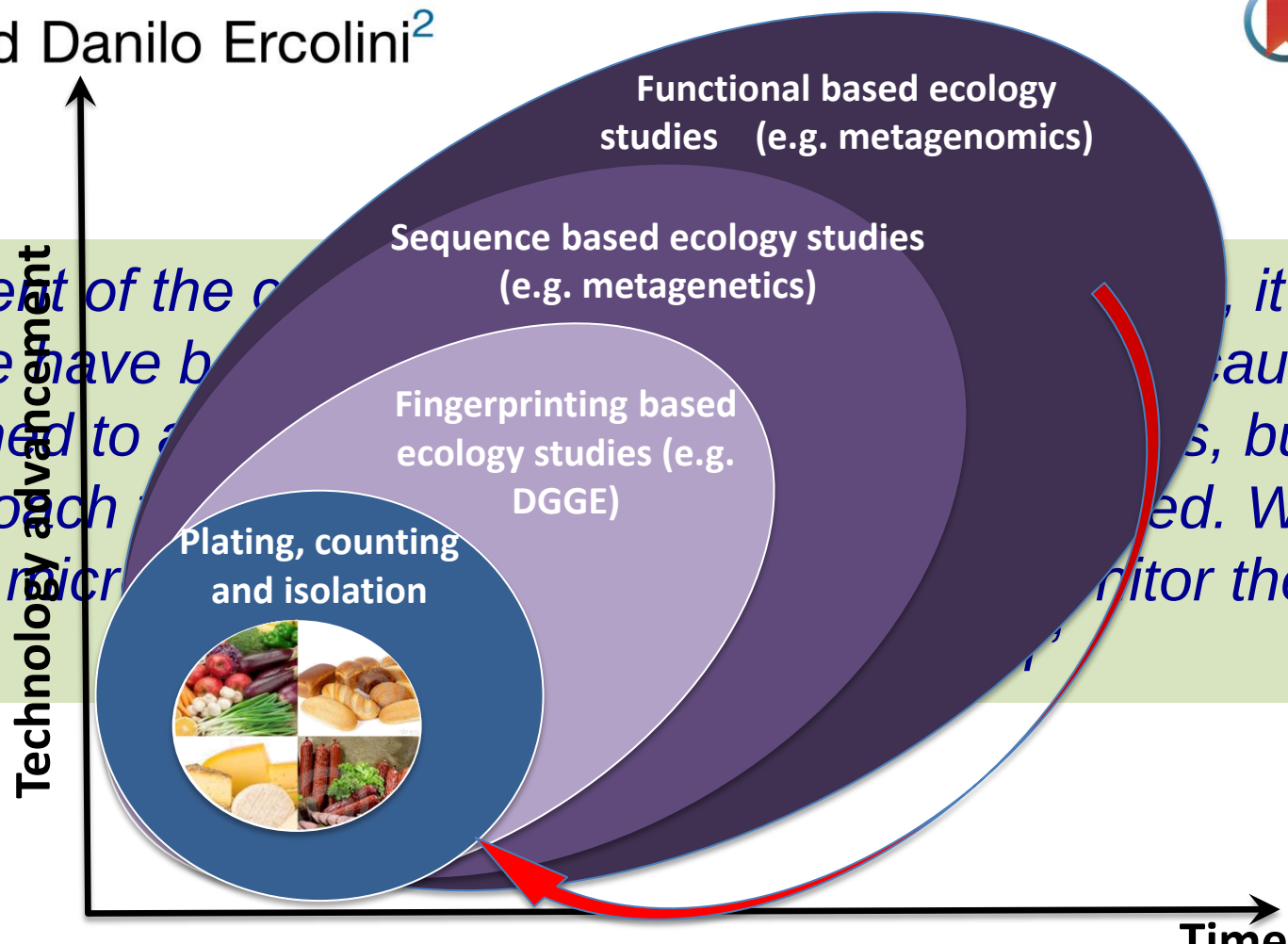
Current Opinion in Food Science 2015, 2:43–50

Current Opinion in Food Science

Zooming into food-associated microbial consortia: a 'cultural' evolution

Luca Cocolin[1] and Danilo Ercolini[2]

"With the advent of the c… , it is a "cultural" evolution we have b… cause we have technically learned to a… s, but also because our mental approach … ed. We have evolved to think at food micr… nitor their occurrence,



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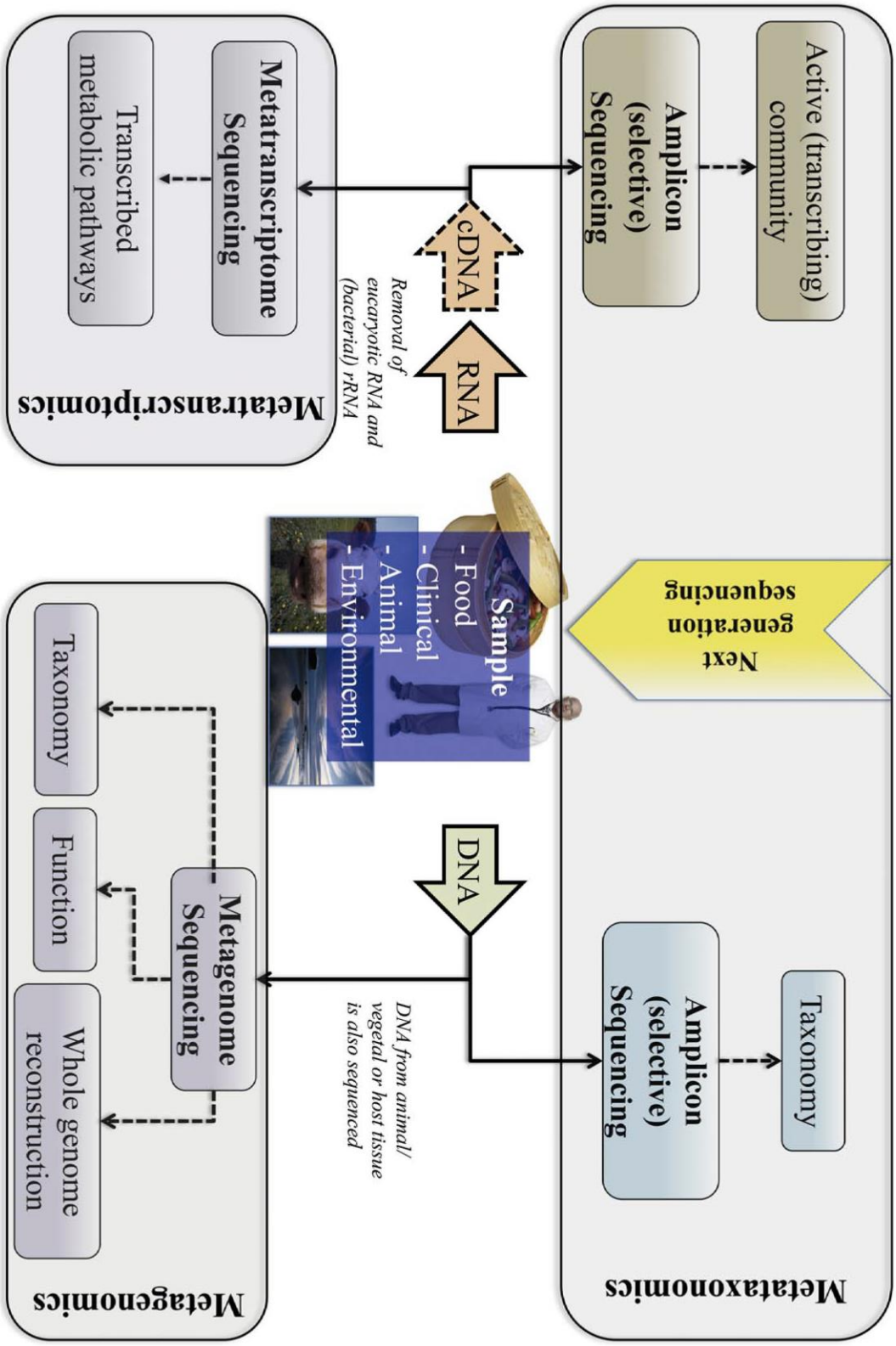
Next generation microbiological risk assessment meta-omics: The next need for integration

Luca Cocolin[a,*], Marios Mataragas[b], Francois Bourdichon[c], Agapi Doulgeraki[d], Marie-France Pilet[e], Balamurugan Jagadeesan[f], Kalliopi Rantsiou[a], Trevor Phister[g]



Opportunities and challenges

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FOOD MICROBIOLOGY

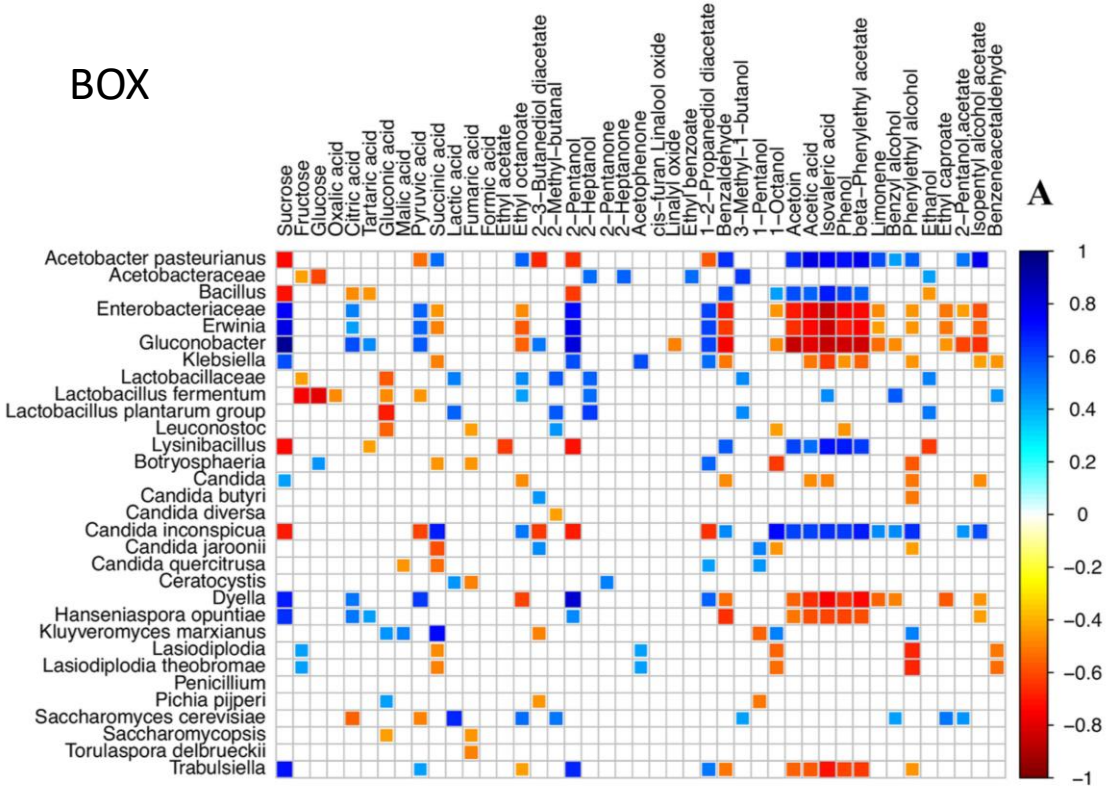
October 2018 Volume 84 Issue 19 e01164-18

Dynamics and Biodiversity of Bacterial and Yeast Communities during Fermentation of Cocoa Beans

Jatziri Mota-Gutierrez,[a] Cristian Botta,[a] Ilario Ferrocino,[a] Manuela Giordano,[a] Marta Bertolino,[a] Paola Dolci,[a] Marcella Cannoni,[b] Luca Cocolin[a]

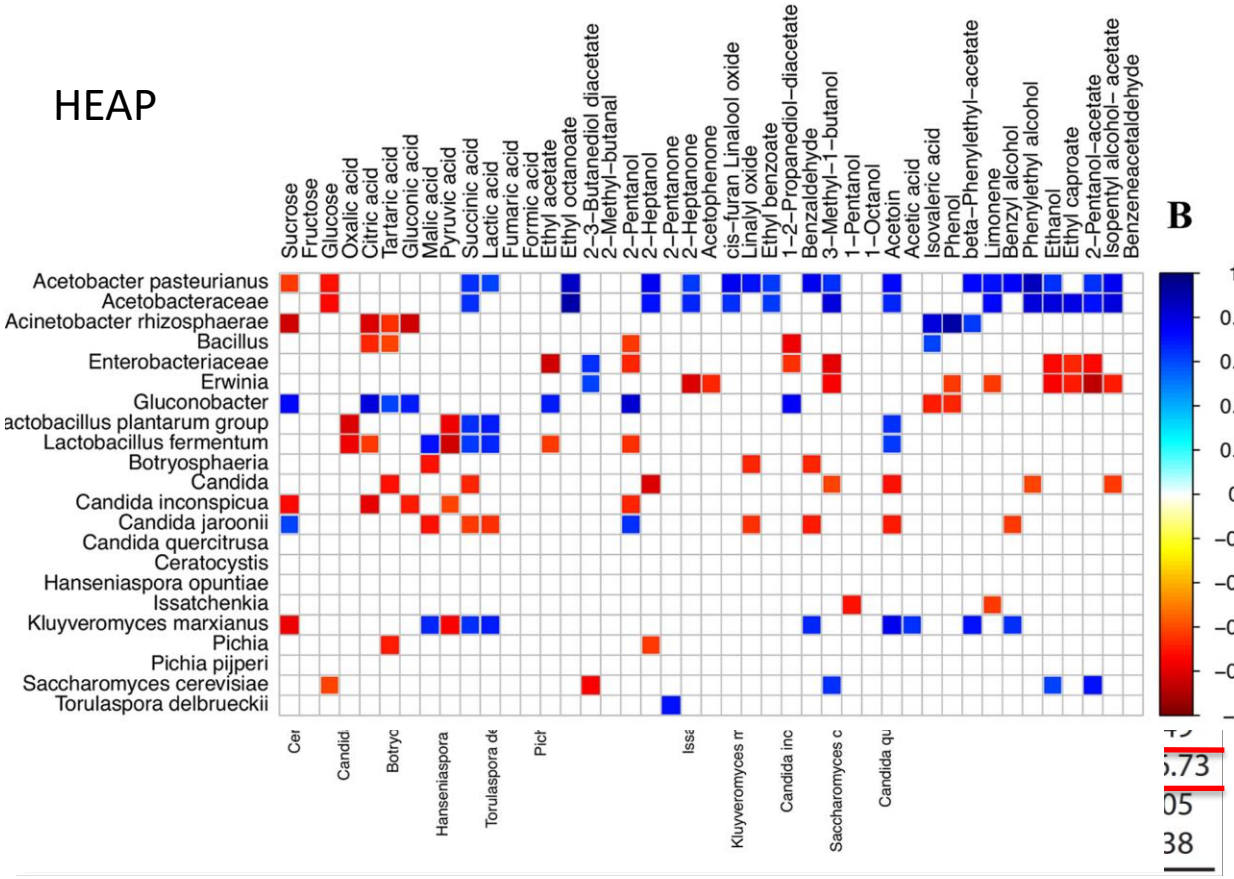
Yeasts

BOX



Torulaspora delbrueckii	0.02	0.07	0.27	0.03	0.12	0.09	0.13	0.09

HEAP



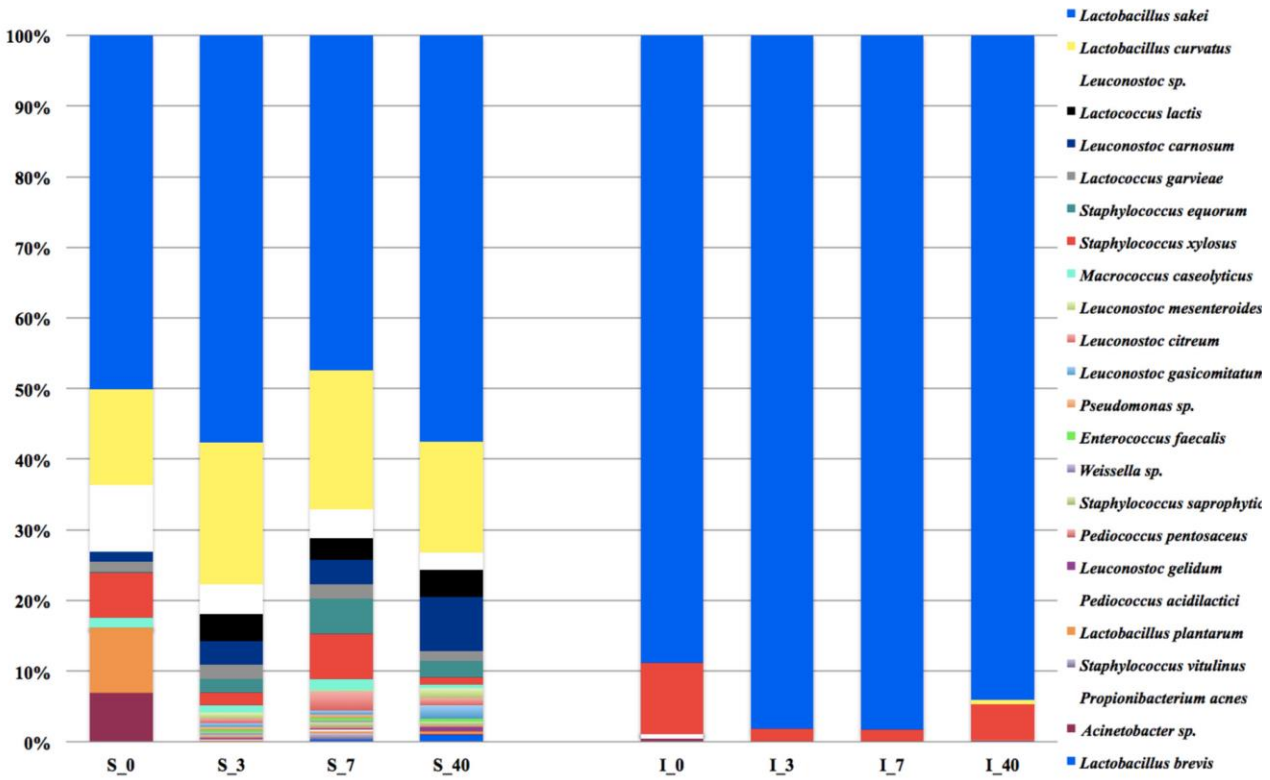
AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

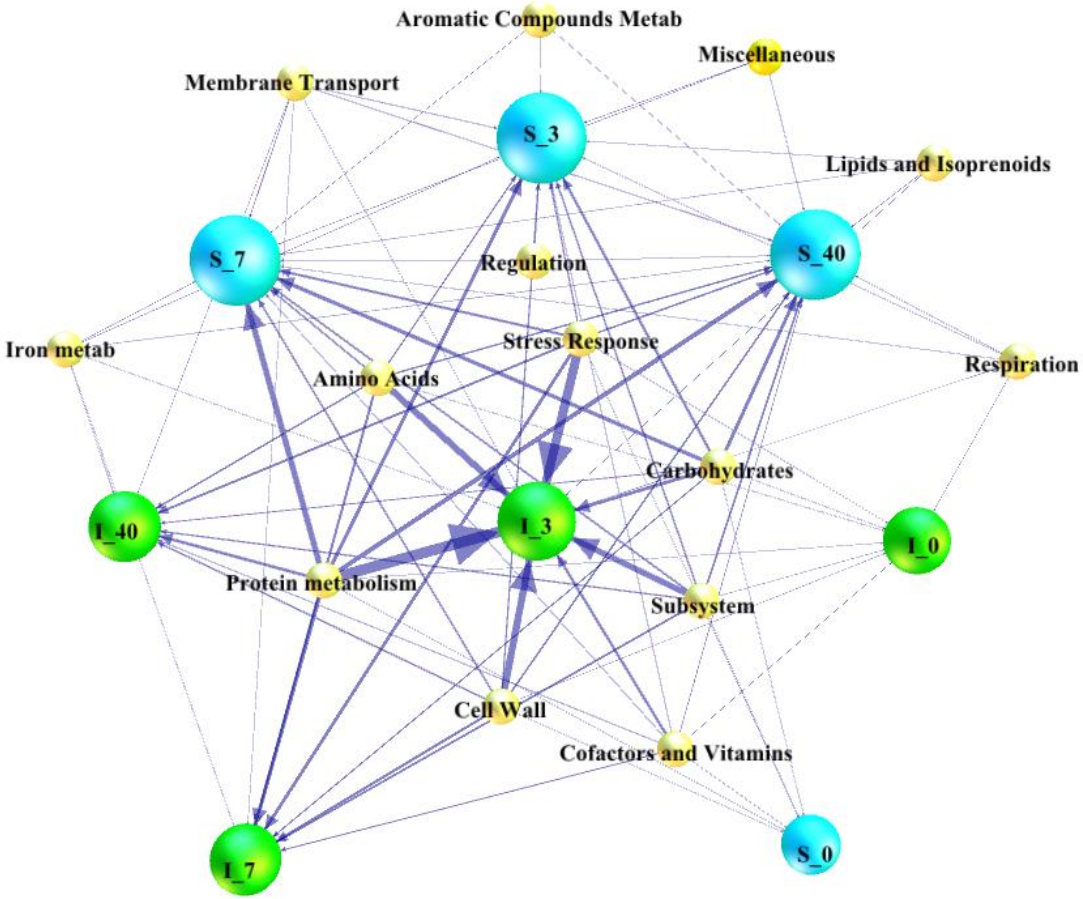
FOOD MICROBIOLOGY

February 2018 Volume 84 Issue 3 e02120-17

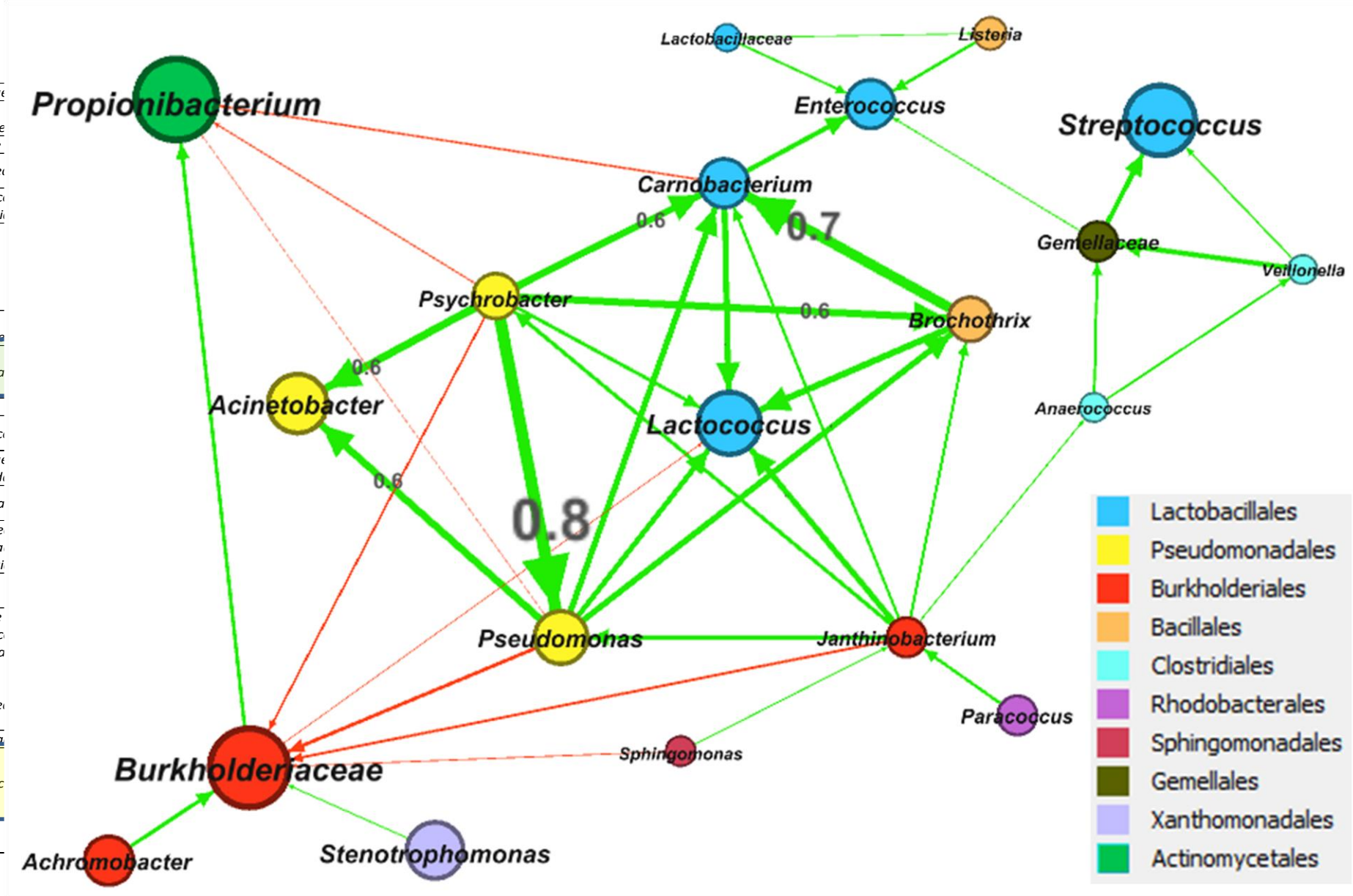
Shotgun Metagenomics and Volatilome Profile of the Microbiota of Fermented Sausages

Ilario Ferrocino,[a] Alberto Bellio,[b] Manuela Giordano,[a] Guerrino Macori,[b] Angelo Romano,[b] Kalliopi Rantsiou,[a] Lucia Decastelli,[b] Luca Cocolin[a]





Benefits for risk assessment: environmental monitoring



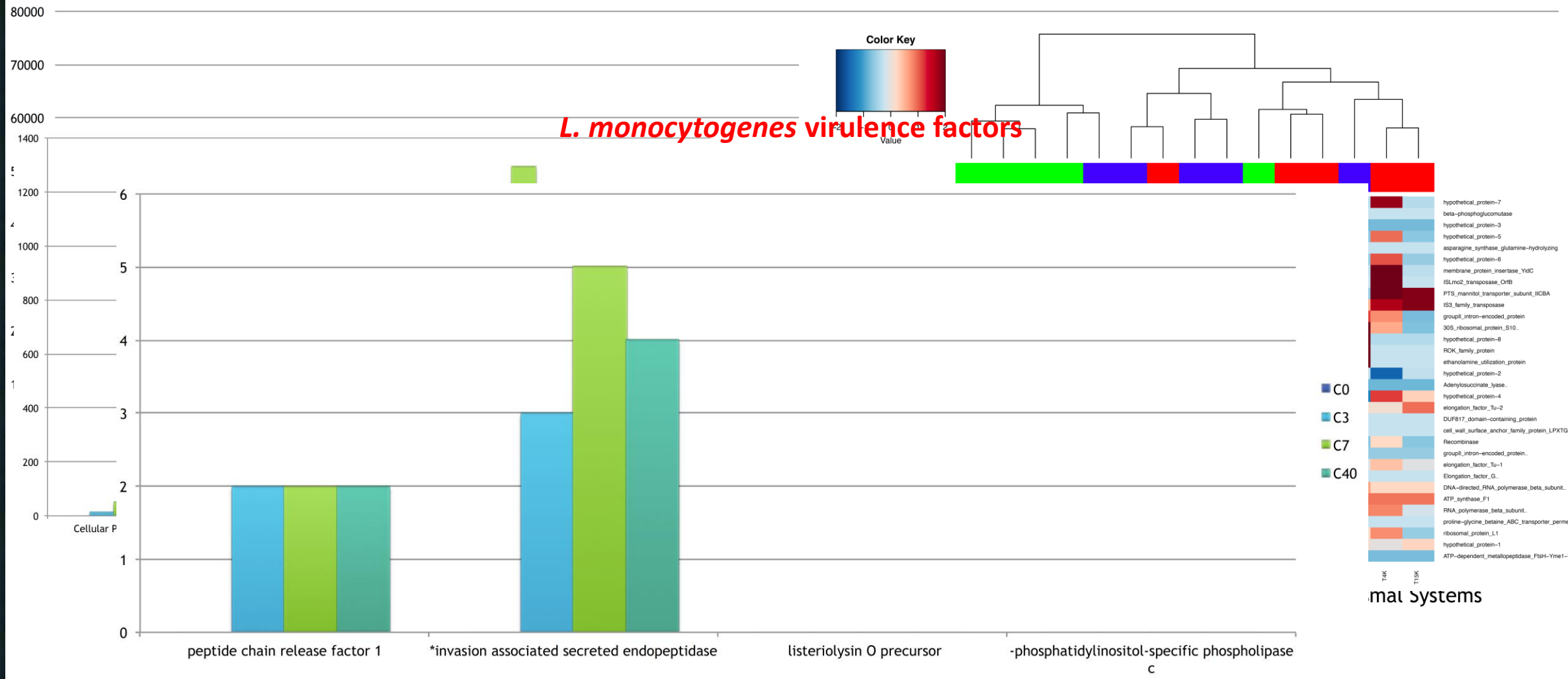
Red –

Green +

Benefits for risk assessment: food pathogens monitoring

L. monocytogenes sequences in the sausage metagenomic libraries

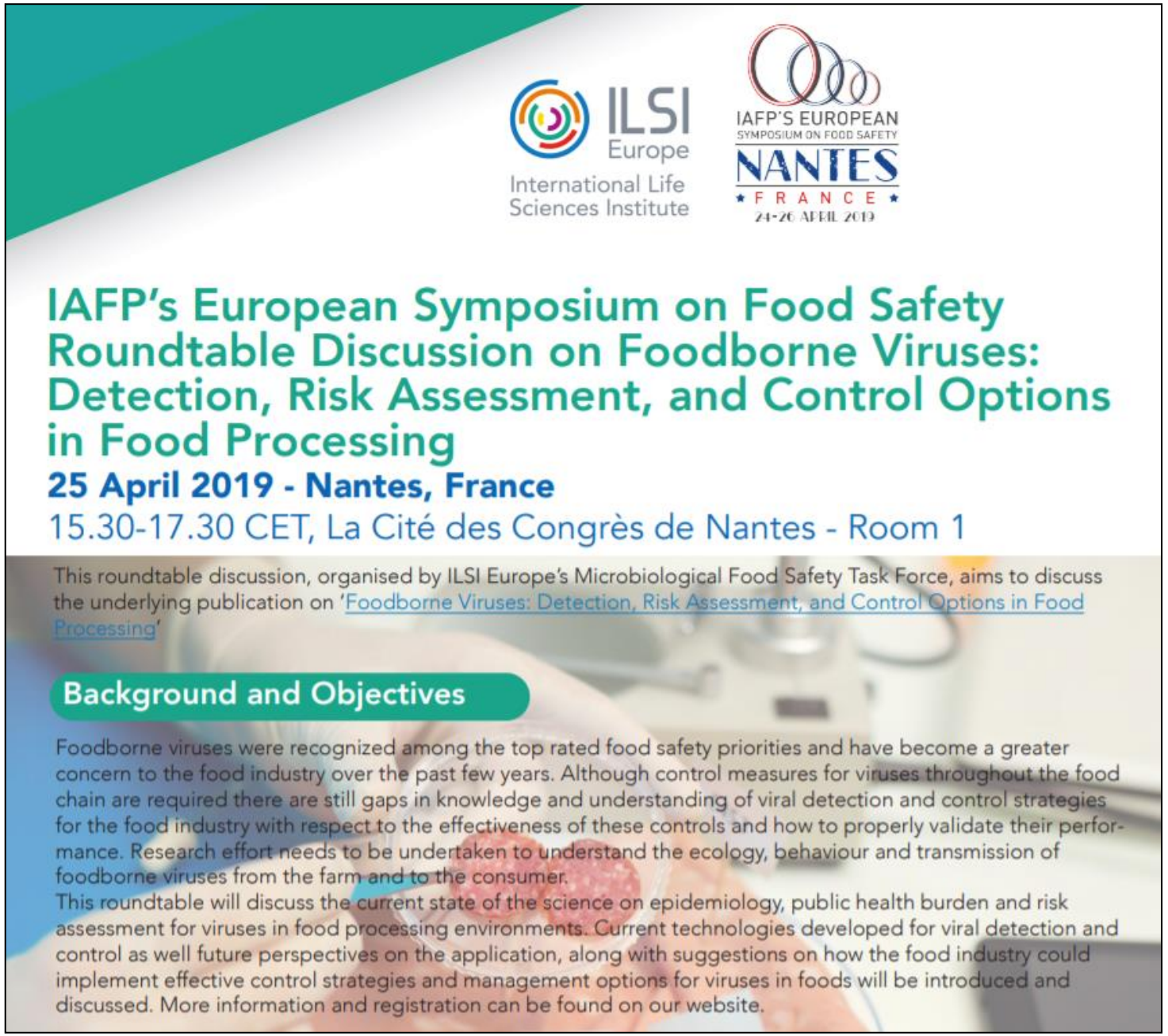
L. monocytogenes virulence factors



Conclusions

"The application of multi-omics in food safety and quality has the potential to answer questions traditional microbiological methods could not address. **Approaching the food ecosystem from different angles (metagenomics, metatranscriptomics, metaproteomics and metametabolomics) allows for a "holistic" representation of which microorganisms are present, how they behave, how they interact and which are the phenotypic manifestations in this complex arena.** The expected outcome may have an invaluable impact in food safety, in order to reduce the risk associated to foodborne pathogens, but also to better control spoilage processes. However, before this becomes reality a number of obstacles and **hurdles have to be overcome.** More specifically **we have to learn how to translate molecular events into practical applications, which will give the food industries concrete solution on how to make food products more safe and stable.**"

Upcoming activity: Roundtable Discussion on Foodborne Viruses



Questions?

Questions should be submitted to the presenters via the Questions section at the right of the screen.

Slides and a recording of this webinar will be available for access by IAFP members at www.foodprotection.org within one week.

International Association for Food Protection