International Association for Food Protection WEBINAR

Processing Environment Monitoring in Low Moisture Foods Production: Setting Up a Meaningful Program

April 21, 2022

Organized by: ILSI Europe

Moderator: Anett Winkler, Cargill Germany

Sponsored by the IAFP Foundation

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ILSI Europe
International Life Sciences Institute

Webinar Housekeeping

- It is important to note that all opinions and statements are those of the individual making the presentation and not necessarily the opinion or view of IAFP.
- All attendees are muted. Questions should be submitted to the presenters during the presentation via the Questions section at the right of the screen. Questions will be answered at the end of the presentations.
- This webinar is being recorded and will be available for access by IAFP members at www.foodprotection.org within one week.

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Microbiological Food Safety Task Force

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- ✓ Investigate microbial issues in foods that are related to public health risks
- ✓ Facilitate the development of harmonised, science-based approaches to predict and prevent microbiological risks

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collaboration

common challenges

science


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Guidance on validation of lethal control measures for foodborne pathogens in foods

Erdogan Ceylan, Alejandro Amezquita, Nathan Anderson, Roy Betts, Laurence Blayo, Francisco Garces-Vega, Elissavet Gkogka, Linda J. Harris, Peter McClure, Anett Winkler, Heidy M. W. den Besten

First published: 07 May 2021 | https://doi.org/10.1111/1541-4337.12746 | Citations: 1


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Food Microbiology

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The use of next generation sequencing for improving food safety: Translation into practice

Balamurugan Jagadeesan, Peter Gerner-Smidt, Marc W. Allard, Sébastien Leuillet, Anett Winkler, Yinghua Xiao, Samuel Chaffron, Jos Van Der Vossen, Silin Tang, Mitsuru Katase, Peter McClure, Bon Kimura, Lay Ching Chai, John Chapman, Kathie Grant


ELSEVIER

International Journal of Food Microbiology

Volume 356, 16 October 2021, 109351

Review

Processing environment monitoring in low moisture food production facilities: Are we looking for the right microorganisms?

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https://ilsi.eu/scientific-activities/food-safety/microbiological-food-safety/

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common challenges

science

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ILSI Europe's Scientific Session on Food Allergen Quantitative Risk Assessment (QRA)

4-6 May 2022

Munich, Germany

International Association for Food Protection

Munich GERMANY 4-6 May 2022

Anett Winkler, Ph.D.
Moderator

Organization: **Cargill Germany**

Function: **EMEA Microbiologist**

Work Experience:

- 20 years at Kraft / Mondelez as microbiologist in various roles (regional / global)
- performed numerous validation studies for nut, dairy & cocoa processing
- global expert for thermal processing within Mondelez International
- joined Cargill in October 2017 in her current role
- also active in ILSI Europe (Microbiology Food Safety), and IAFP being the current chair of the Organizing Committee for the IAFP European Symposium

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François Bourdichon, Ph. D.

Organization: **Università Cattolica Del Sacro Cuore**

Function: **Research Collaborator**

Work Experience:

- 15y in the Food Industry: Savencia (FR), Danone (FR), Nestlé (CH), Barry Callebaut (BE)
- Since January 2017, Principal Consultant at Food Safety Microbiology and Hygiene
- Research Collaborator in DiSTAS, Dipartimento di Scienze e Tecnologie Alimentari per una filiera agro-alimentare Sostenibile, Università Cattolica Del Sacro Cuore, Piacenza, Italy
- Member of the IAFP since 2007

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Presenter: Marcel Zwietering

Organization: **Wageningen University, The Netherlands**

Function: **Professor Food Microbiology**

Work Experiences:

- 19 years professor
- 5 Years Danone Research
- 10 years university
- ICMSF chair
- Active in ILSI Europe (Microbiology Food Safety), and IAFP

Presenter: Séamus Fanning

Organization: **University College Dublin, Ireland**

Function: **Professor of Food Safety & Zoonoses**

Work experience:

- appointed to UCD in 2002 and currently is the Director of the UCD-Centre for Food Safety (20-years as a Full Professor)
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- elected as a Fellow of the American Academy of Microbiology (FAAM) in 2019

Processing Environment Monitoring in Low Moisture Foods Production: Setting Up a Meaningful Program

François BOURDICHON

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ILSI Europe
International Life Sciences Institute

Pathogens in Low Moisture Foods

ILSI Europe: 2010 Dedicated Expert Group

Journal of Food Protection, Vol. 76, No. 1, 2013, Pages 150–172
doi:10.4315/0362-028X.JFP-12-211

ILSI Europe
Report Series

PERSISTENCE AND SURVIVAL OF PATHOGENS IN DRY FOODS AND DRY FOOD PROCESSING ENVIRONMENTS

General Interest

Low–Water Activity Foods: Increased Concern as Vehicles of Foodborne Pathogens

LARRY R. BEUCHAT,[1]* EVANGELIA KOMITOPOULOU,[2] HARRY BECKERS,[3] ROY P. BETTS,[4] FRANÇOIS BOURDICHON,[5] SÉAMUS FANNING,[6] HAN M. JOOSTEN,[5] AND BENNO H. TER KUILE[7,8]

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MS 12-211: Received 15 May 2012/Accepted 17 August 2012

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Pathogens in Low Moisture Foods

A Code of Hygienic Practice (CXC 75-2015)

CODEX ALIMENTARIUS

INTERNATIONAL FOOD STANDARDS

Food and Agriculture Organization of the United Nations

World Health Organization

E-mail: codex@fao.org - www.codexalimentarius.org

CODE OF HYGIENIC PRACTICE FOR LOW-MOISTURE FOODS

CXC 75-2015

Adopted in 2015. Revised in 2016. Amended in 2018.

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Pathogens in Low Moisture Foods

ILSI Europe: a ten year plus initiative

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Pathogens in Low Moisture Foods

ILSI Europe: 2020 – Time for update ?

International Journal of Food Microbiology 356 (2021) 109351

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Pathogens in Low Moisture Foods

2022 related outbreaks:

Abbot, US (*Cronobacter* spp.)

Ferrero, BE (*Salmonella* spp.)

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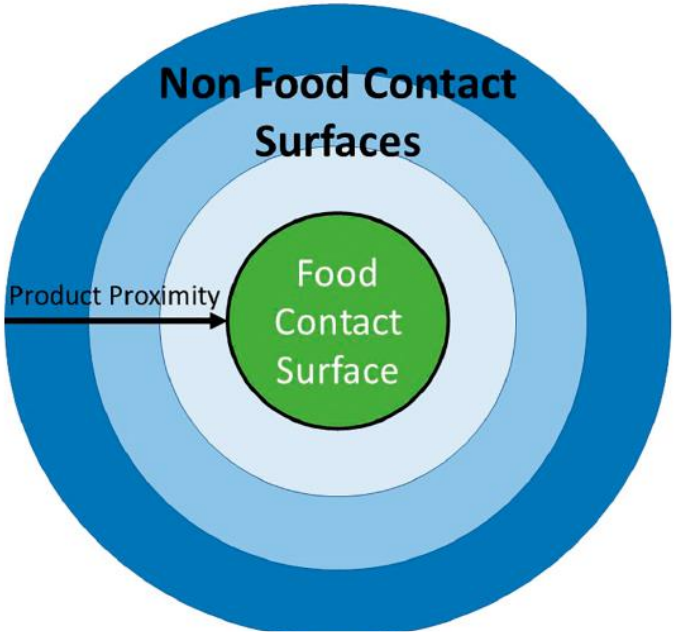
Risk-based approach in setting up a meaningful environmental monitoring program

Marcel Zwietering

WAGENINGEN
UNIVERSITY & RESEARCH



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Presenter: Marcel Zwietering

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Low moisture foods

Do not support growth

But survival (in environment and in product)

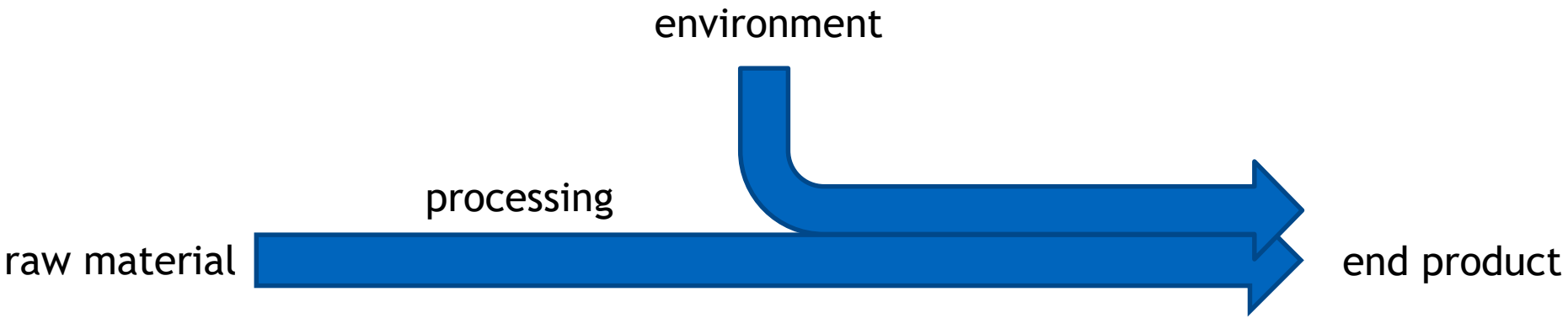
Low levels can already be an unacceptable risk

Relevant pathogens:
Salmonella, *Cronobacter*, pathogenic *E. coli*, *B. cereus*

Milk powder, PIF, cereals, dried meats, spices, nuts, chocolate, peanut butter,

Environmental monitoring

Control: If not in your raw materials (or inactivated) and not in your environment



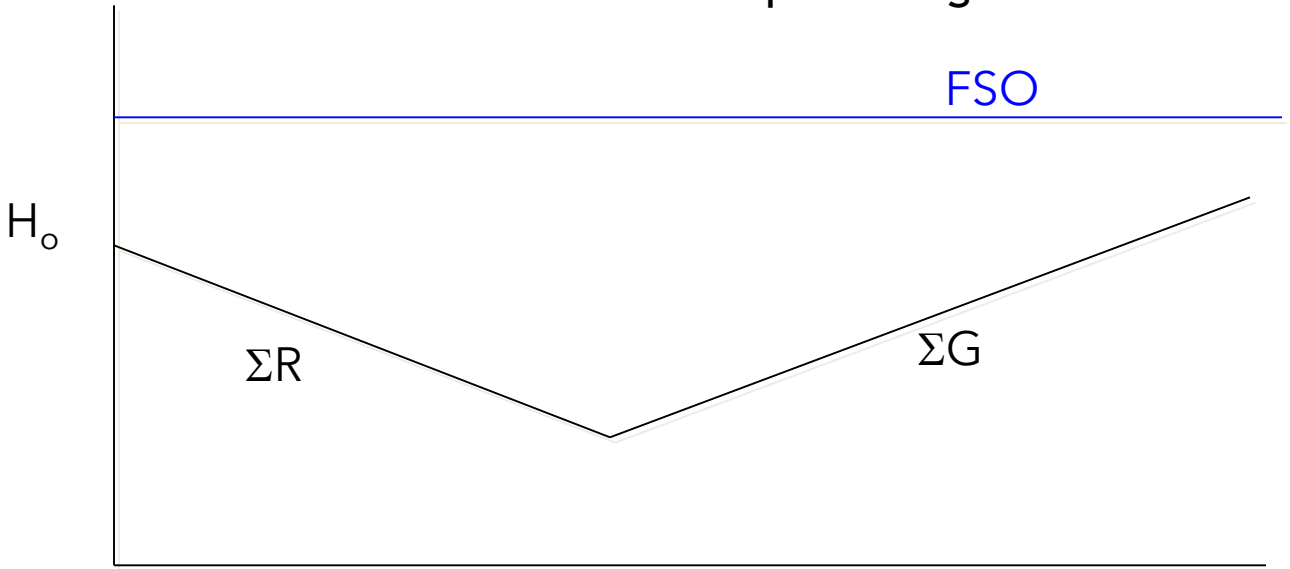
If not in your raw materials (or inactivated) **AND** not in your environment

FSO: Food Safety Objective: norm set by government

- ICMSF

$$H_o - \Sigma R + \Sigma G < FSO$$

Sufficient reduction OR prevent growth



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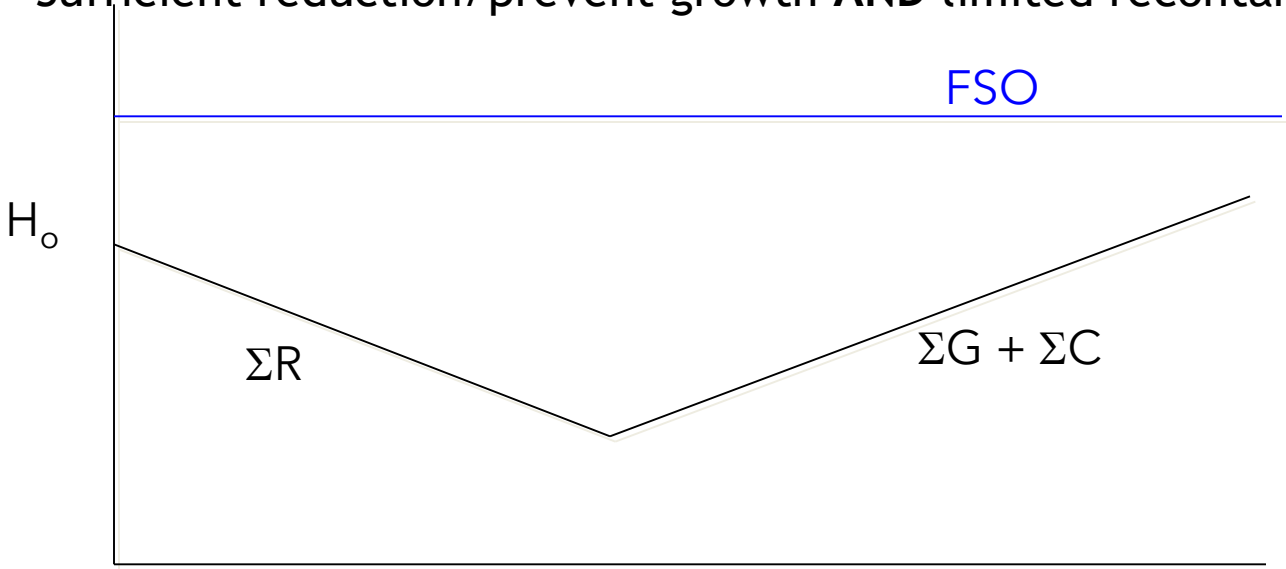
FSO: Food Safety Objective: norm set by government

- ICMSF

$$H_o - \Sigma R + \Sigma G + \Sigma C < FSO$$

Sufficient reduction/prevent growth **AND** limited recontamination



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on log basis

H_o - ΣR + ΣG + ΣC < FSO

H_o = 2 log cfu/g

ΣR = 6D reduction

ΣG = 2 logs growth

2 – 6 + 2 = -2 log cfu/g

4 – 8 + 2 = -2 log cfu/g

ΣR and ΣG not dependant on level

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on log basis

H_o - ΣR + ΣG + ΣC < FSO

Contamination is additive on the linear scale !

100 organisms + 1000 recontaminating = 1100

1000 organisms + 1000 recontaminating = 2000

It is not 3 logs + 3 logs = 6 logs !

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Co (cfu)	C (cfu)	Ho (log cfu)	H_1	ΔH
1	1000	0.00	3.00	3.00
10	1000	1.00	3.00	2.00
100	1000	2.00	3.04	1.04
1000	1000	3.00	3.30	0.30
10000	1000	4.00	4.04	0.04

Serving of 100 g: FSO<1/100g : -2 log cfu/g

Safe ?

If level is -3 log cfu/g, this means 1 organism per 10 bars
1 *Salmonella* has a P illness of 1:400

At FSO=-3 : Pill=1:4000 bars !

Detection probability of C=0.001 cfu/g

n	P-	P+
5	0.88	0.12
10	0.78	0.22
60	0.22	0.78

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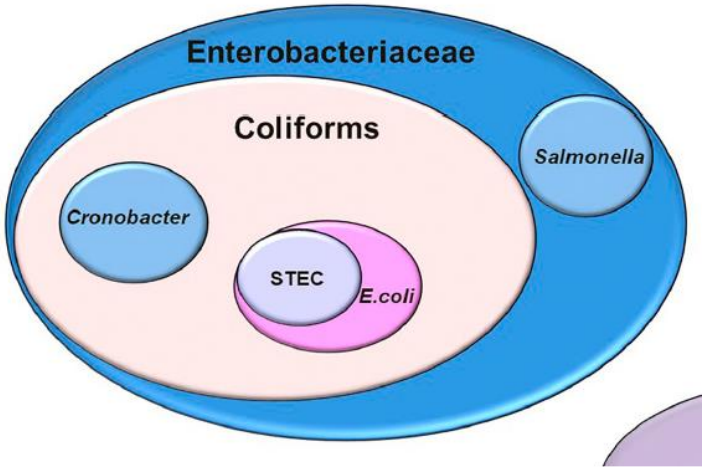
Recontamination difficult to quantify

pathogens: low detection probability
if not detected not there ?
if detected what will be transfer to product



indicators: higher probability
how to relate it to the pathogen
(more Enteros: higher probability of *Salmonella* qualitatively……)

Standardisation difficult:
where to sample
how to sample
how much to sample (quantity and number of samples)

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Verification : testing

Monitor Critical Limits

Validated CCPs

HACCP

PRP (GMP, GHP, ...)

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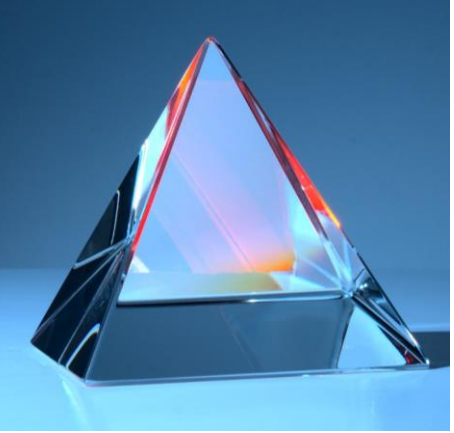

Verification : testing

Monitor Critical Limits

Validated CCPs

HACCP

PRP (GMP, GHP, ...)

Cleaning and desinfecting

Environmental monitoring

Water Management

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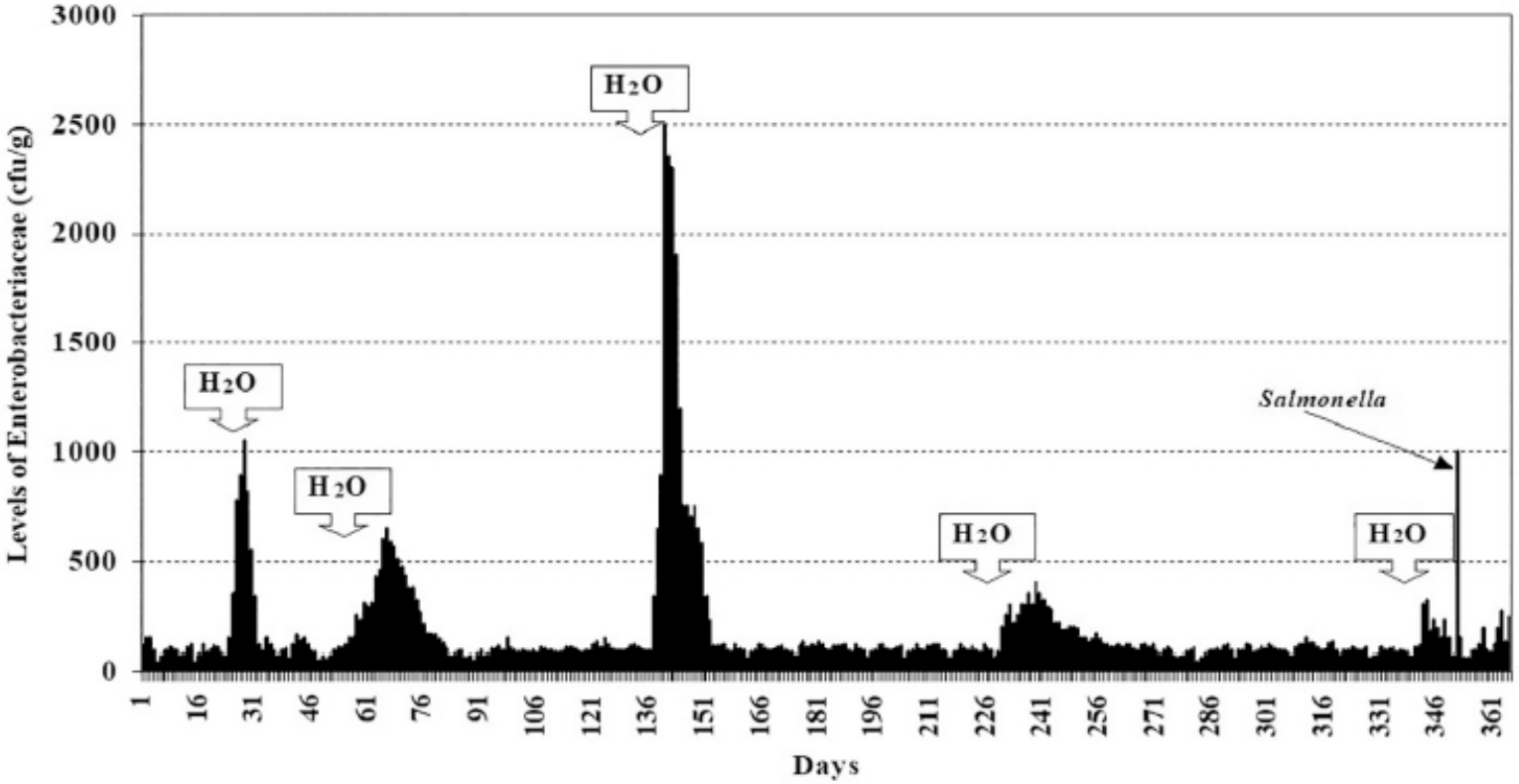
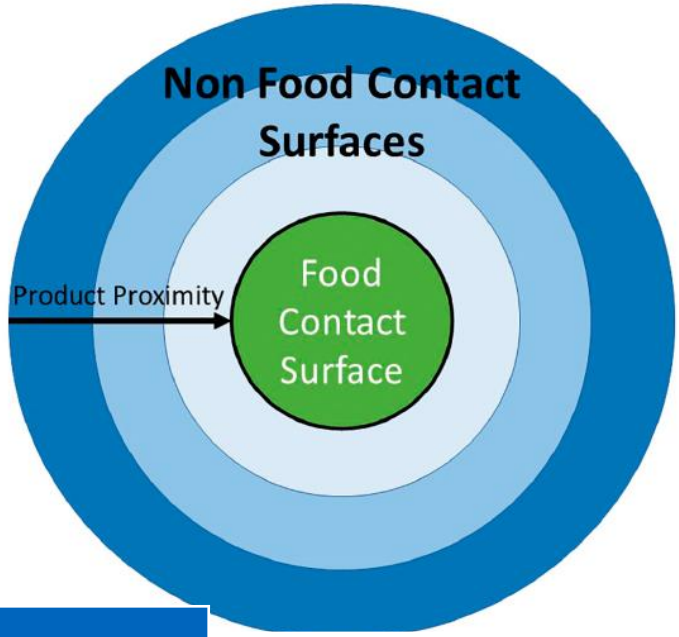


Fig. 5.2. Daily count of *Enterobacteriaceae* on surface swabbing in the processing site. From Cordier (2007).

Sampling

Routine
Investigation
Special events
Following a positive sample

Zoning !



	pathogens	indicators
close to product	60	60
near production	30	40
remote areas	10	0

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Conclusions

LMF outbreaks remain

Environmental monitoring relevant, crucial in low moisture food
….. resident strains and recurrent outbreaks

Combination of control of raw materials, processing, cleaning and disinfecting:
…… and end product and **environmental monitoring**

Indicators…… pathogens
close to product…. remote areas
routine ….. seek and destroy

Not Black and White: 50 shades of Red

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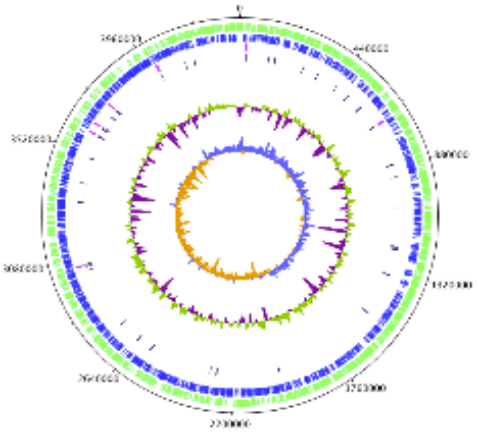
Processing environment monitoring in low moisture foods production-

setting up a meaningful programme



Professor Séamus Fanning,
UCD-Centre for Food Safety,
School of Public Health, Physiotherapy & Sports Science,
University College Dublin,
Belfield, Dublin, Ireland

Presenter: Séamus Fanning

Organization: University College Dublin, Ireland

Function: Professor of Food Safety & Zoonoses

Work experience:

- appointed to UCD in 2002 and currently is the Director of the UCD-Centre for Food Safety (20-years as a Full Professor)
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- served as an expert member of several WHO/FAO missions
- a serving member on editorial boards of learned journals including, *Journal of Food Protection; Foodborne Pathogens & Disease and Research in Microbiology*
- elected as a Fellow of the American Academy of Microbiology (FAAM) in 2019

Preserving food by reducing the moisture content -

- **drying is a traditional method used to preserve food** and low-moisture foods constitute a substantial part of our diet
- **dried foods have a longer shelf-life** and low- and intermediate-moisture foods have a reduced water activity (a_w)
- **low-moisture (LMF) foods include *nuts; cereals; honey*** along with high-moisture foods such as powdered infant formula (PIF) that have been dried
- **although erroneously believed to be a *low risk*, because these food matrices *cannot support microbial growth*, nonetheless these foods remain susceptible to microbial contamination and *therefore pose a risk to consumers* and the *brand***

[Finn, *et al. Frontiers in Microbiol*. (2013) doi: 10.389/fmicb.2013.00331]

How can the moisture content of a food be reduced & what are the microbiological consequences?

- **Freezing -**
- **Physical removal of water** (such as by spray drying) **-**
- **Addition of humectants** (such as NaCl; sugar or others) **-**

Some of the consequences for the (altered) microbial population -

- **metabolism is changed -**
- **spores and vegetative cells ADAPT and remain viable for months and years -**
- **cross-contamination of low-moisture foods can arise from exposure to the production environment or**

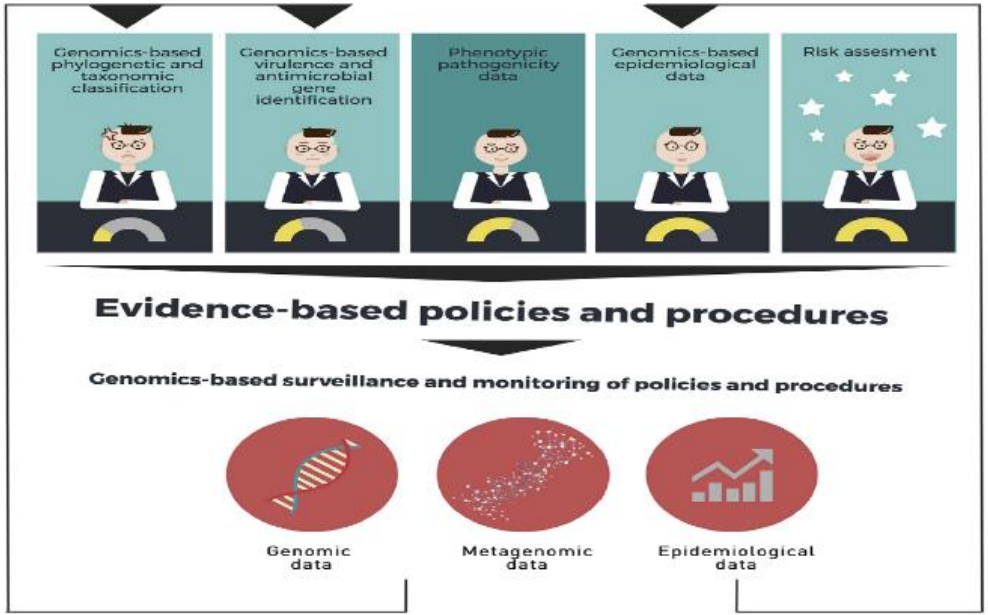
Bacterial adaptation in low-moisture production environments- *can sequencing methods identify how they do it?*

- **Low-moisture foods are NOT sterile and outbreaks of food-borne diseases** associated with the consumption of low-a_w foods and those formulated in part with low-a_w ingredients have increased in frequency
- **Little is known about how bacteria behave** in low-a_w food and dry food processing environments
- Conventional **hygiene protocols** may present a challenge to effective cleaning
- **Manufacturing practices** used for the production of low-a_w foods must be designed to eliminate pathogens
- **Pathogens of concern include mainly *Cronobacter* species [Abbott, USA] and *Salmonella* species [Ferrero, Belgium] that present food safety challenges to low-a_w foods and their production environments**

[Finn, *et al.,* (2013) *Frontiers in Microbiol.* doi: 10.389/fmicb.2013.00331]
[Beuchat, *et al.,* (2013) *J. Food Protect*. **76,** 150-172]
[Bourdichon, *et al.,* (2021) *Int. J. Food Microbiol*. doi: 10.1016/j.ijfoodmicro.2021.109351]

Precision food safety applied to the processing environment; *can protect human health & brand reputation* -

[Kovac *et al.*, *Trends Anal. Chem.* (2017) **96:** 52-61]



identification

interpretation

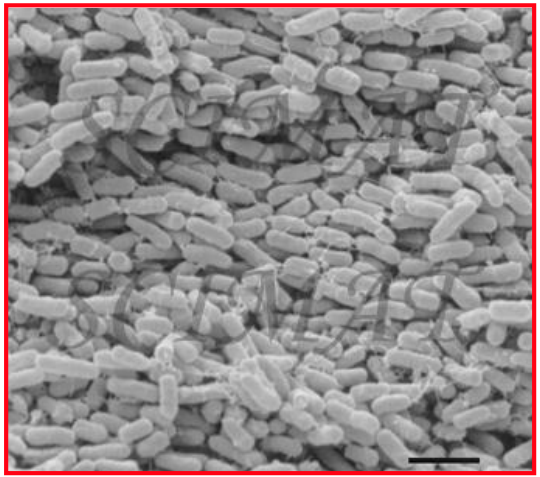
phenotype

epidemiology

risk to health & your brand

GENOME TRAKR

Cronobacter species (formerly known as *Enterobacter sakazakii*) -



General characteristics:

- member of the *Enterobacteriaceae* family
- Gram-negative, motile rods
- facultatively anaerobic
- designated as a genus/species in 1980
- taxonomy revised and a new genus recognised (*Cronobacter* species), now consisting of seven species
- grows readily on laboratory media
- **desiccation resistant**
- rare opportunistic pathogen & causes nosocomial infections

Cronobacter and Powdered Infant Formula Investigation

Español (Spanish)

Updated March 25, 2022

On February 17, 2022, and February 28, 2022, Abbott Nutrition recalled powdered infant formula produced at its manufacturing facility in Sturgis, Michigan, because of possible *Cronobacter* contamination.

Parents and caregivers of newborns should not feed their baby recalled Similac, Alimentum, or EleCare powdered infant formulas.

Fast Facts

- Illnesses: 4
- Deaths: 2
- States: 3 (Minnesota, Ohio, Texas)
- Recall: Yes
- Investigation status: Active

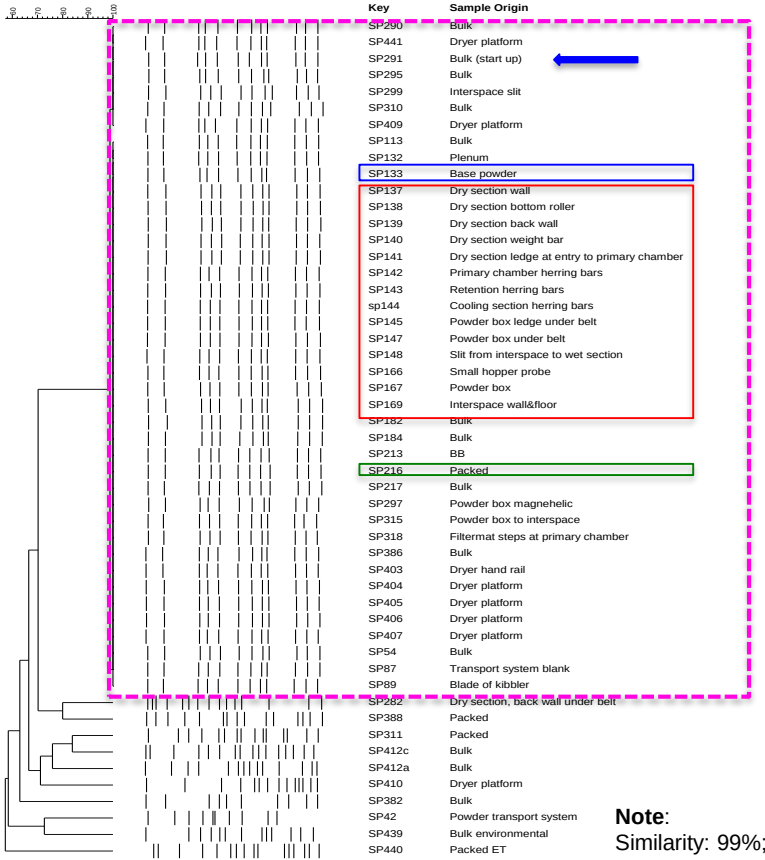
Why do events like this happen?

Cronobacter –in the food production environment

- The minimum a_w value required for growth of *Cronobacter* species has been determined to be 0.94
- Some *Cronobacter* are relatively resistant to heat
- **Heat resistance is greatly increased in low-moisture foods along with those with a high fat content**
- ***Cronobacter* can survive for weeks, months and years in low-moisture foods**

How does exposure of *Cronobacter* to dry environments confer an adaptation phenotype?

[Mullane *et al.*, (2007) *Int. J. Food Microbiol*. **116:** 173-81]
[Brengi *et al.*, (2012) *Foodborne Pathog. Dis*. **9:** 861-867]

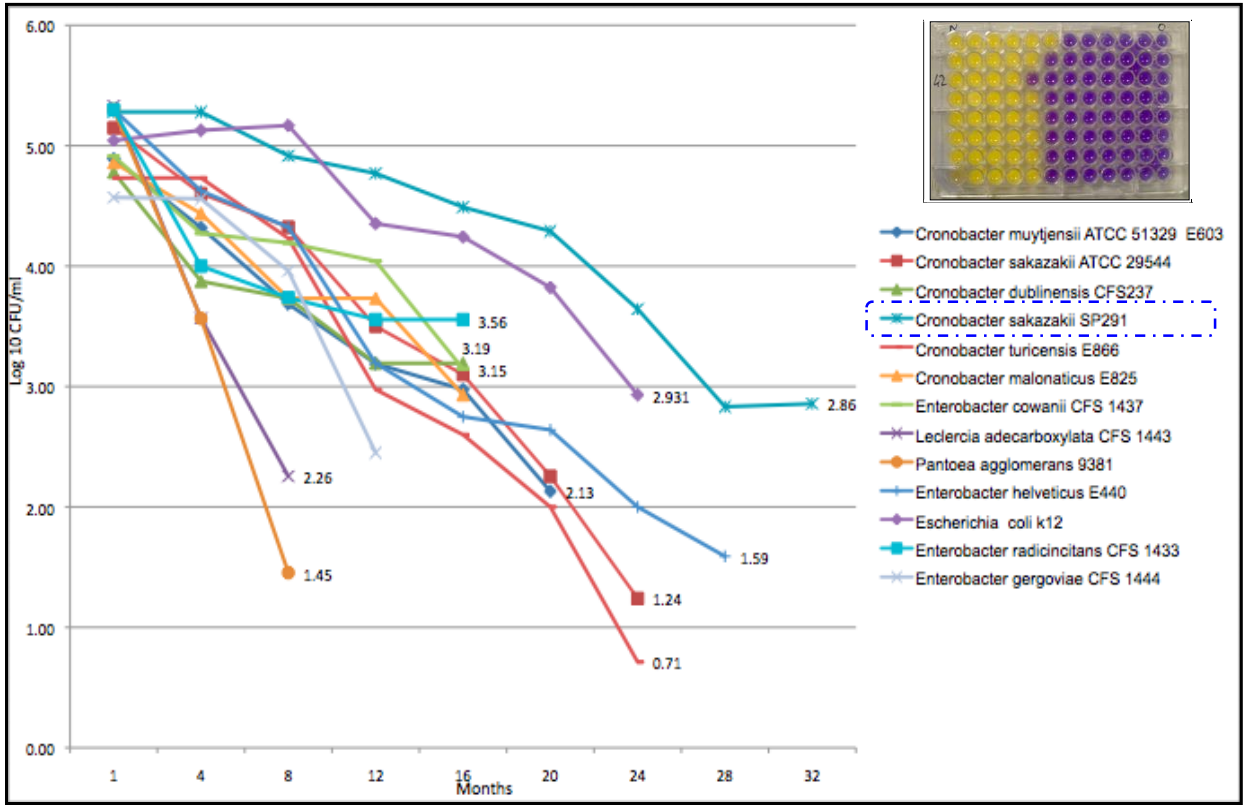


Note:
Similarity: 99%;
Tolerance: 1.5%;
Optimization: 1.5%

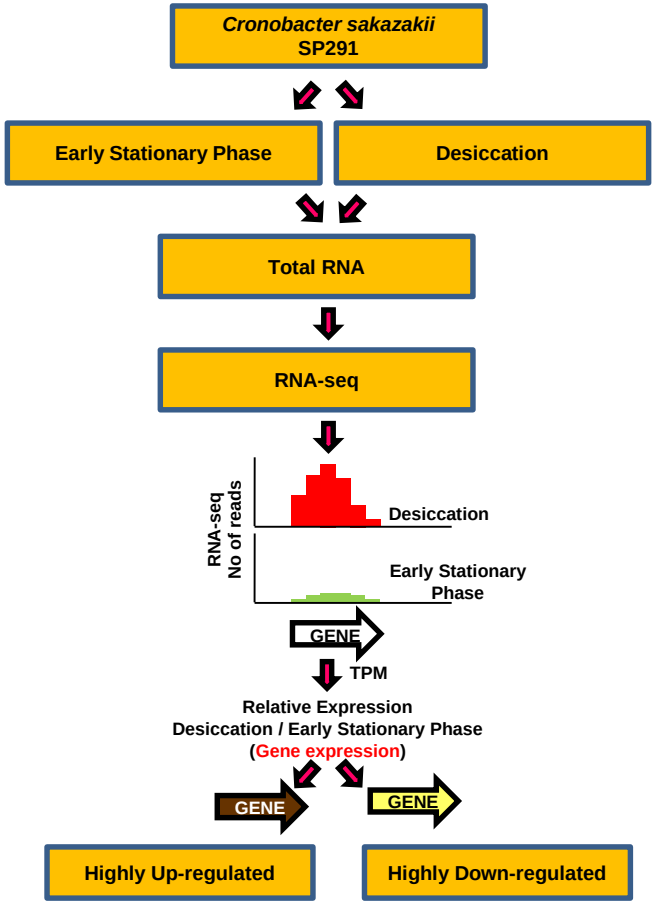
A uniquely adapted *Cronobacter sakazakii* isolate detected in a PIF production environment using PFGE sub-typing

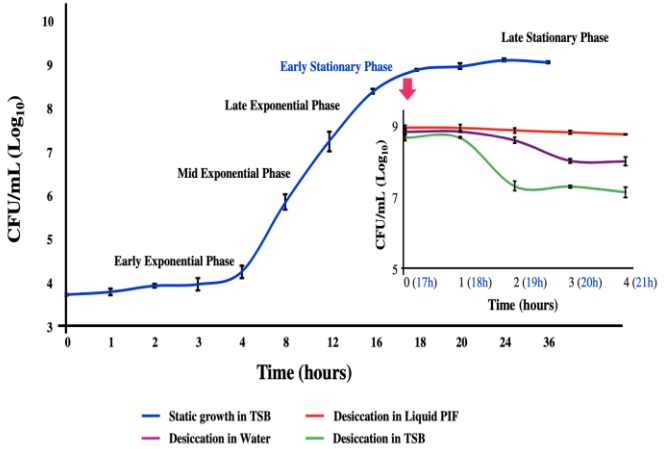
[Cooney, S. (2012) *PhD thesis.* University College Dublin]

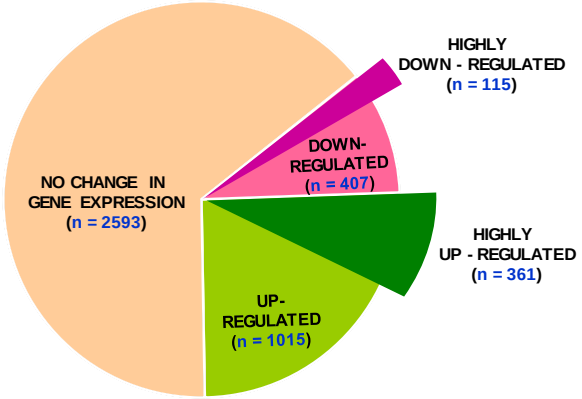
Tolerance to desiccation with time -



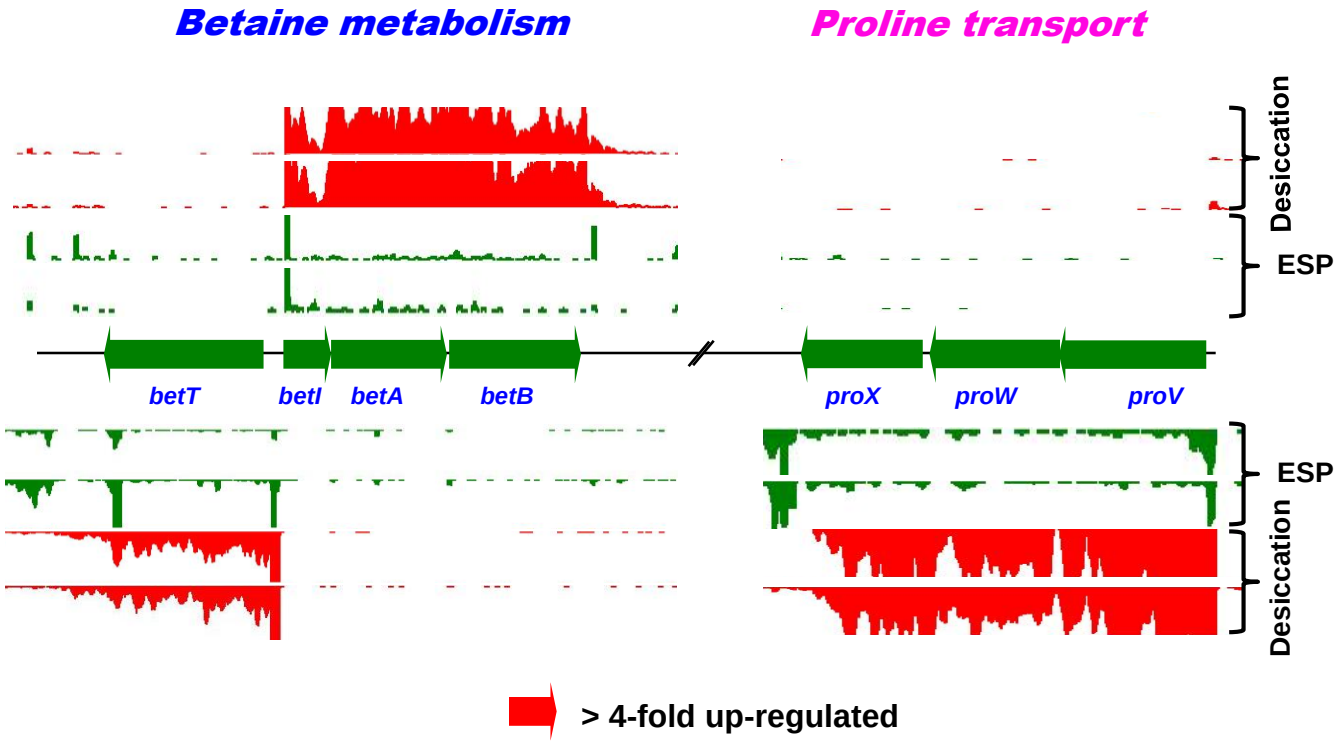
Uncovering bacterial adaptation to low-moisture environments by detecting gene expression using RNA-seq -





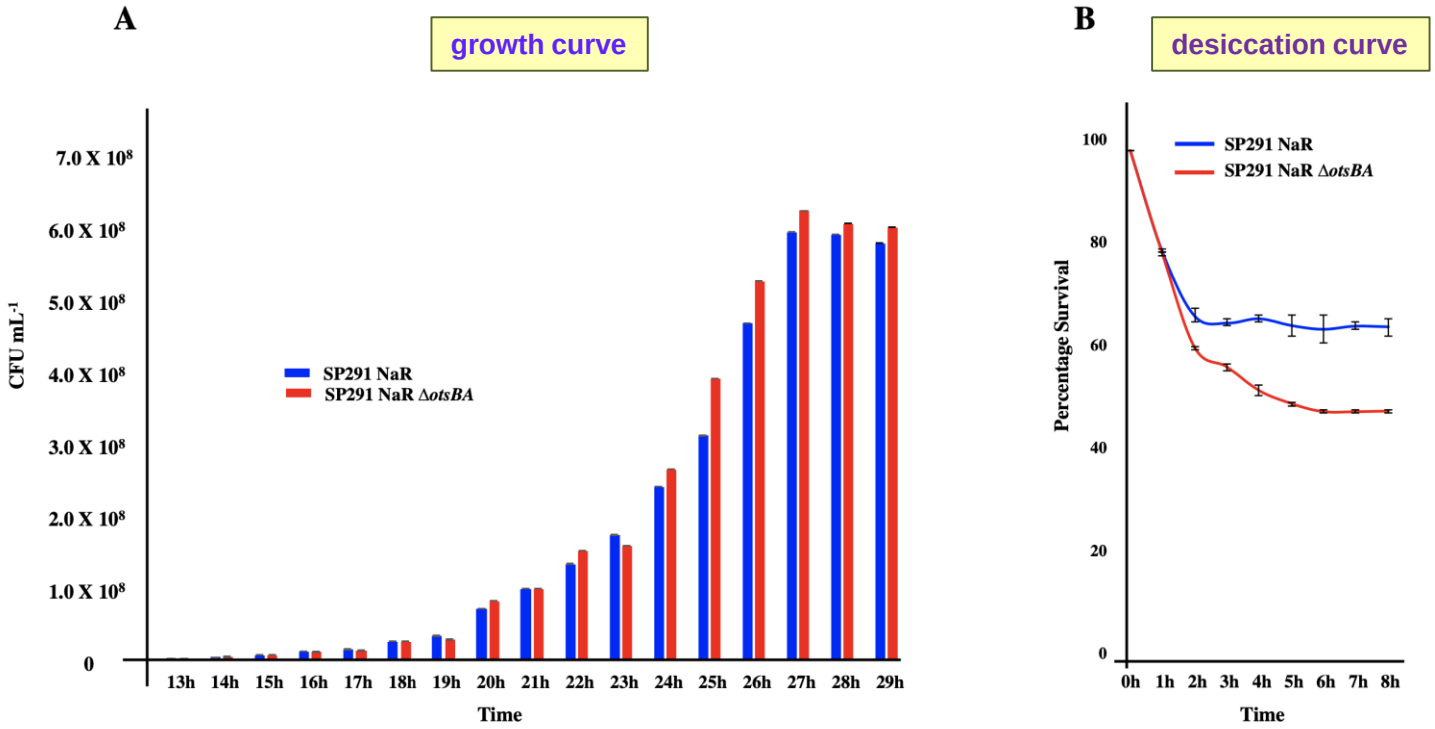


Expression of stress response genes encoding osmoprotectants during desiccation -



[Srikumar *et al.*, (2019) *Appl. Env. Microbiol.* **85:** e019932]

Trehalose metabolism is critical for survival during desiccation -



Do all *Cronobacter sakazakii* respond to low-moisture conditions in the same way?

Comparison between the desiccation curve of *C. sakazakii* ATCC™29544ᵀ (*clinical*) and *C. sakazakii* SP291 (*environmental*)-

- Stage I (Desiccation 0-1 h)

- no obvious change in viable cell count

- Stage II (Desiccation 1 to 2 h)

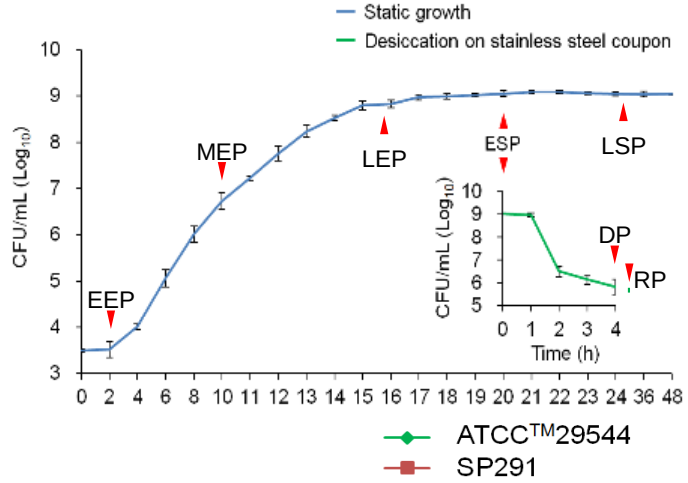
- liquid on the coupon evaporated completely
- a sharp reduction in the viable cell count (~2.5 $\log_{10}$ reduction in 1 h for ATCC™29544)

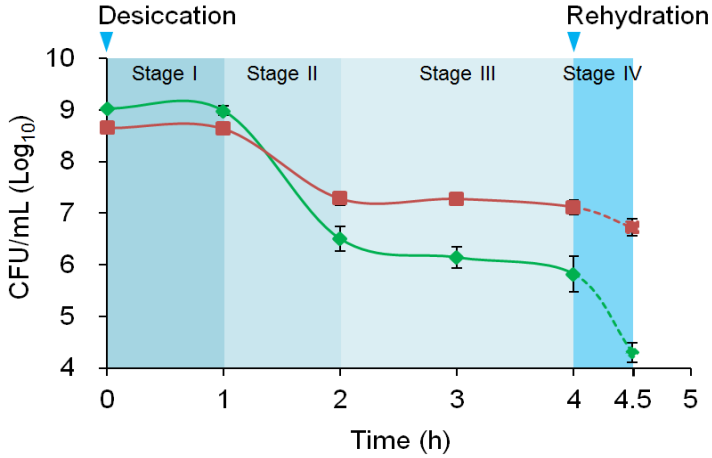
- Stage III (Desiccation 2 to 4 h)

- bacteria were continuously desiccated on the coupon
- decrease in viable cell counts at a much slower rate (~1 $\log_{10}$ reduction in 2 h for ATCC™29544)

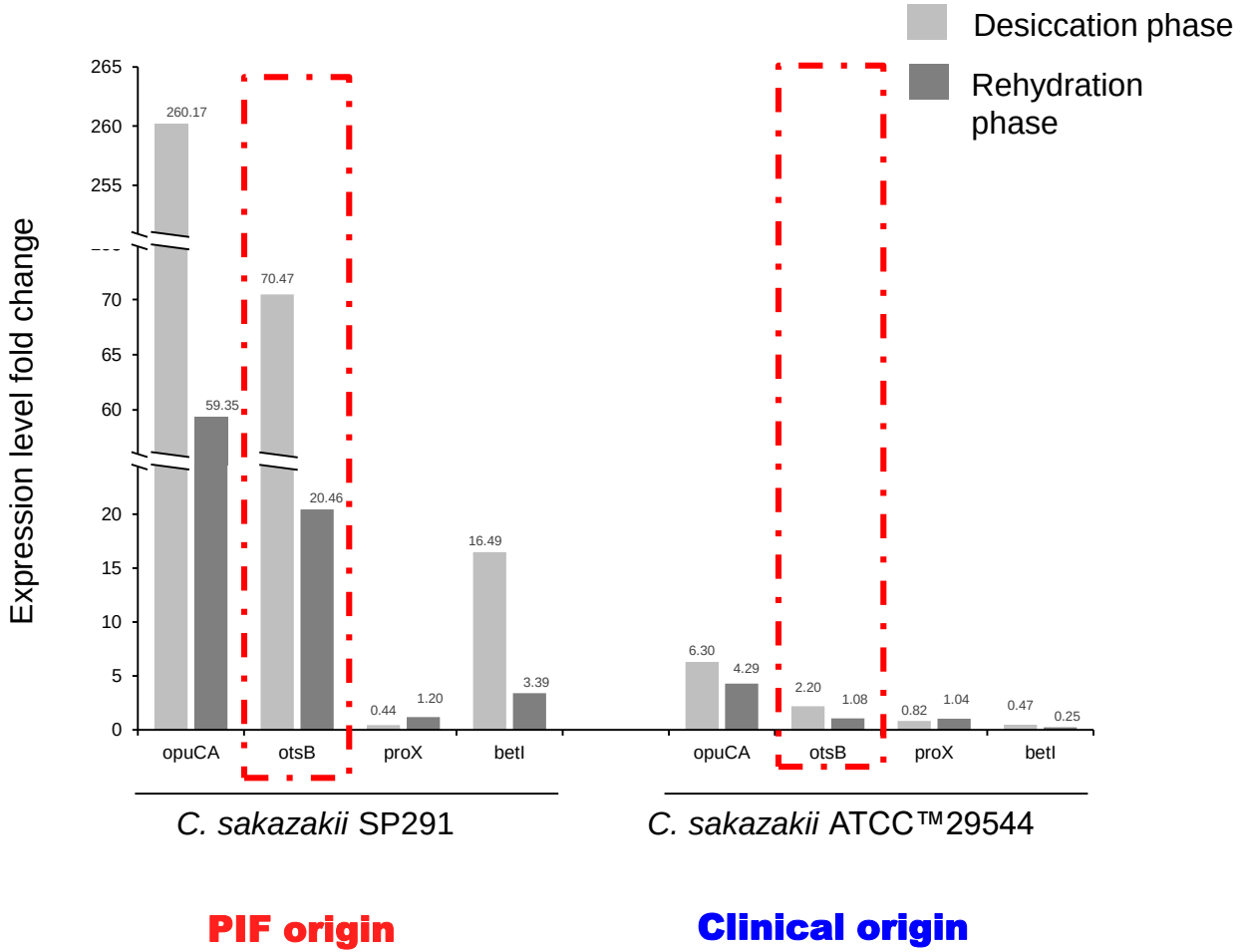
- Stage IV (Rehydration 0-30 min)

- viable cell count change for *C. sakazakii* ATCC™29544 was larger than that for SP291 during each stage

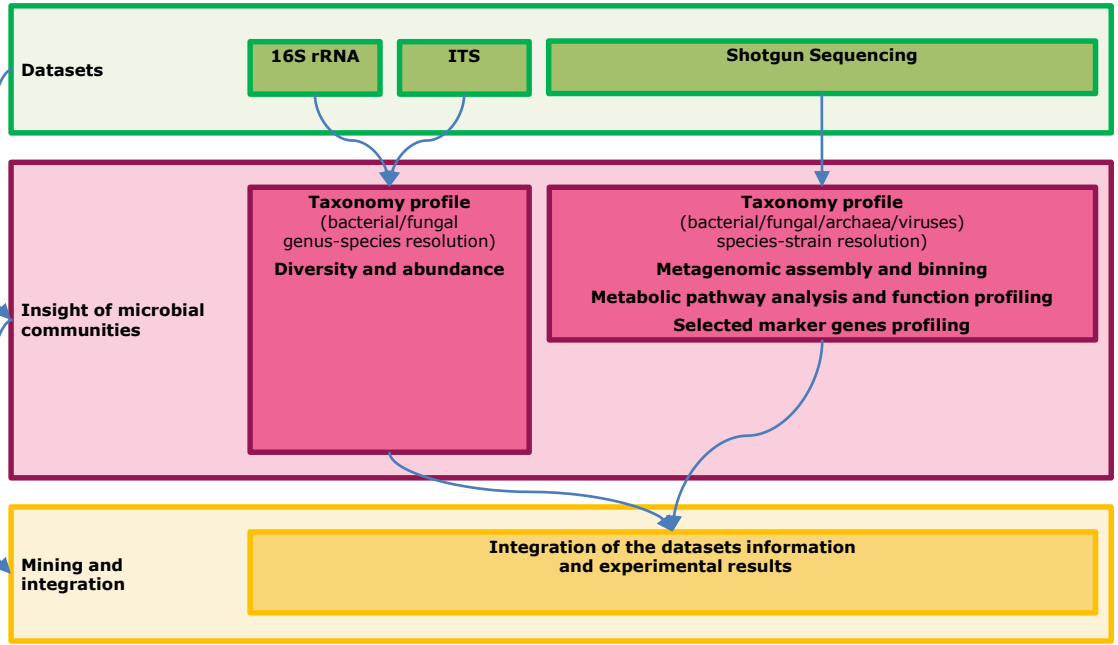




[Cao, *et al. Appl. Env. Microbiol*. (2021) 10.1128/AEM.00820-21]
[Srikumar, *et al. Appl. Env. Microbiol*. (2019) **85**: e01993-18]

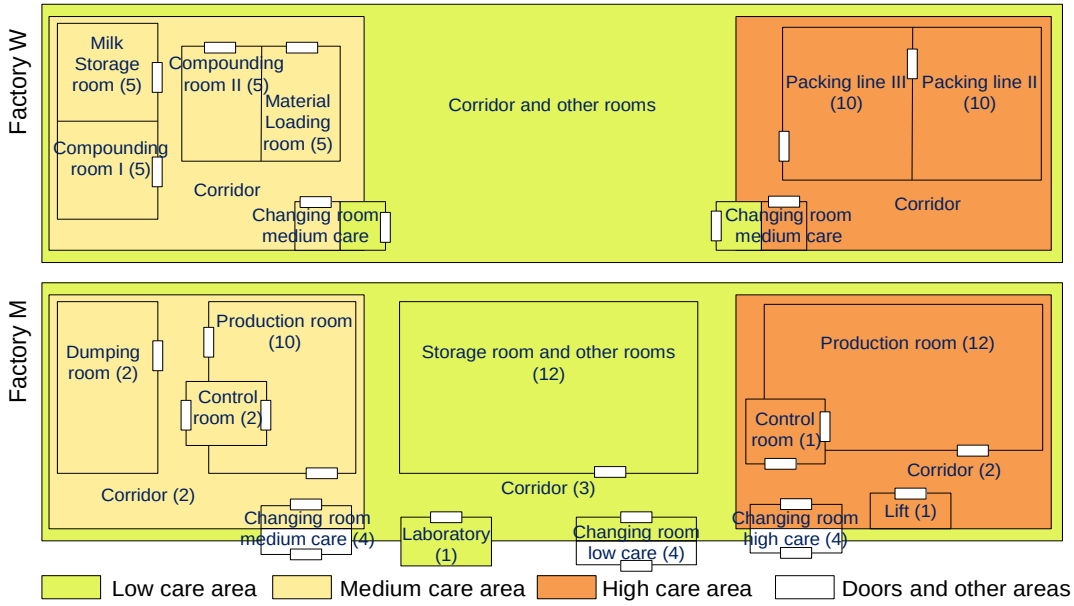

Desiccation phase
Rehydration phase
Expression level fold change
265
260
255
70
65
60
20
15
10
5
0
260.17
59.35
70.47
20.46
0.44
1.20
16.49
3.39
6.30
4.29
2.20
1.08
0.82
1.04
0.47
0.25
opuCA
otsB
proX
betI
opuCA
otsB
proX
betI
C. sakazakii SP291
C. sakazakii ATCC™29544
PIF origin
Clinical origin

16S rRNA sequencing/metagenomics -

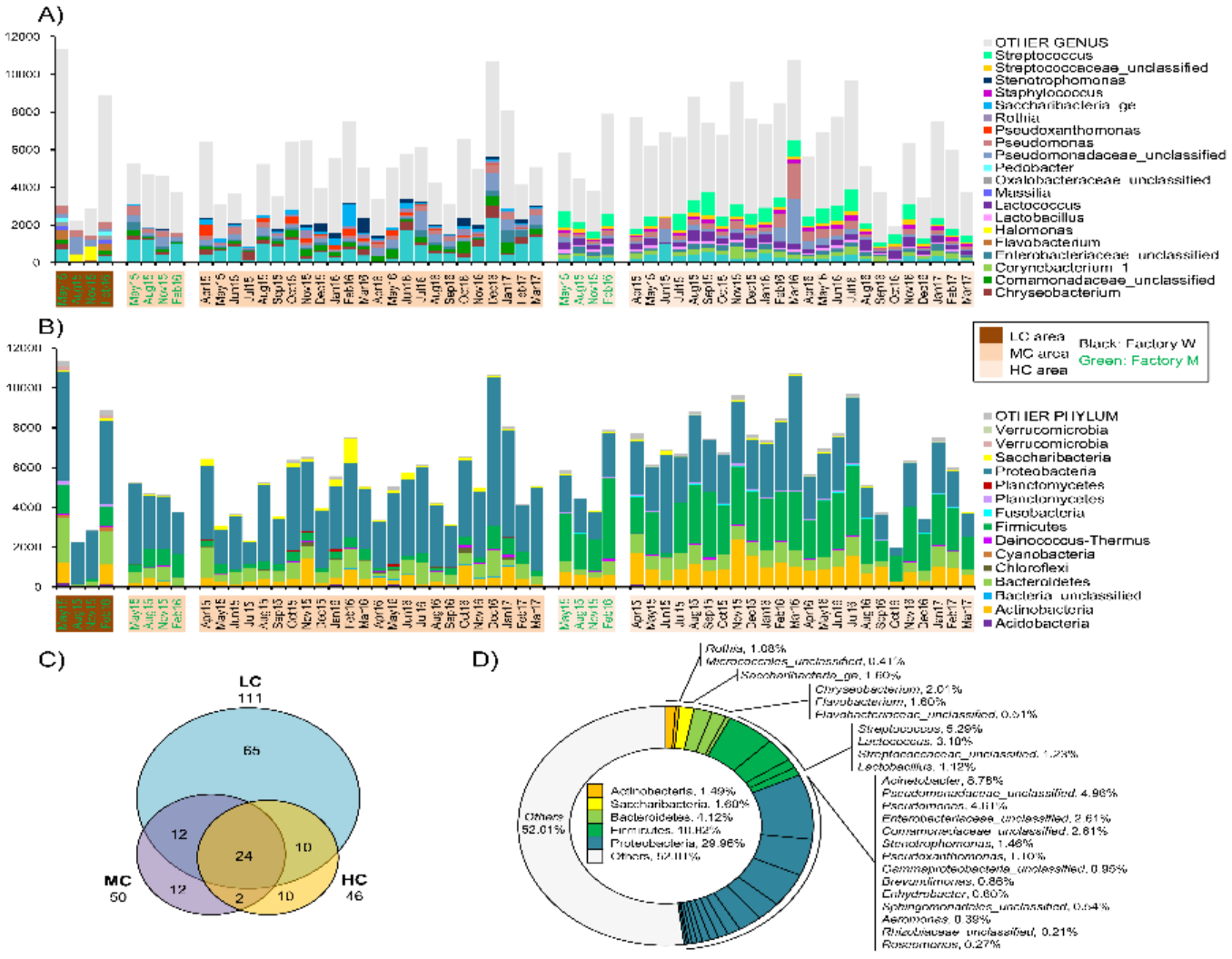


Overall strategy for sequencing a food/environmental sample containing multiple microorganisms

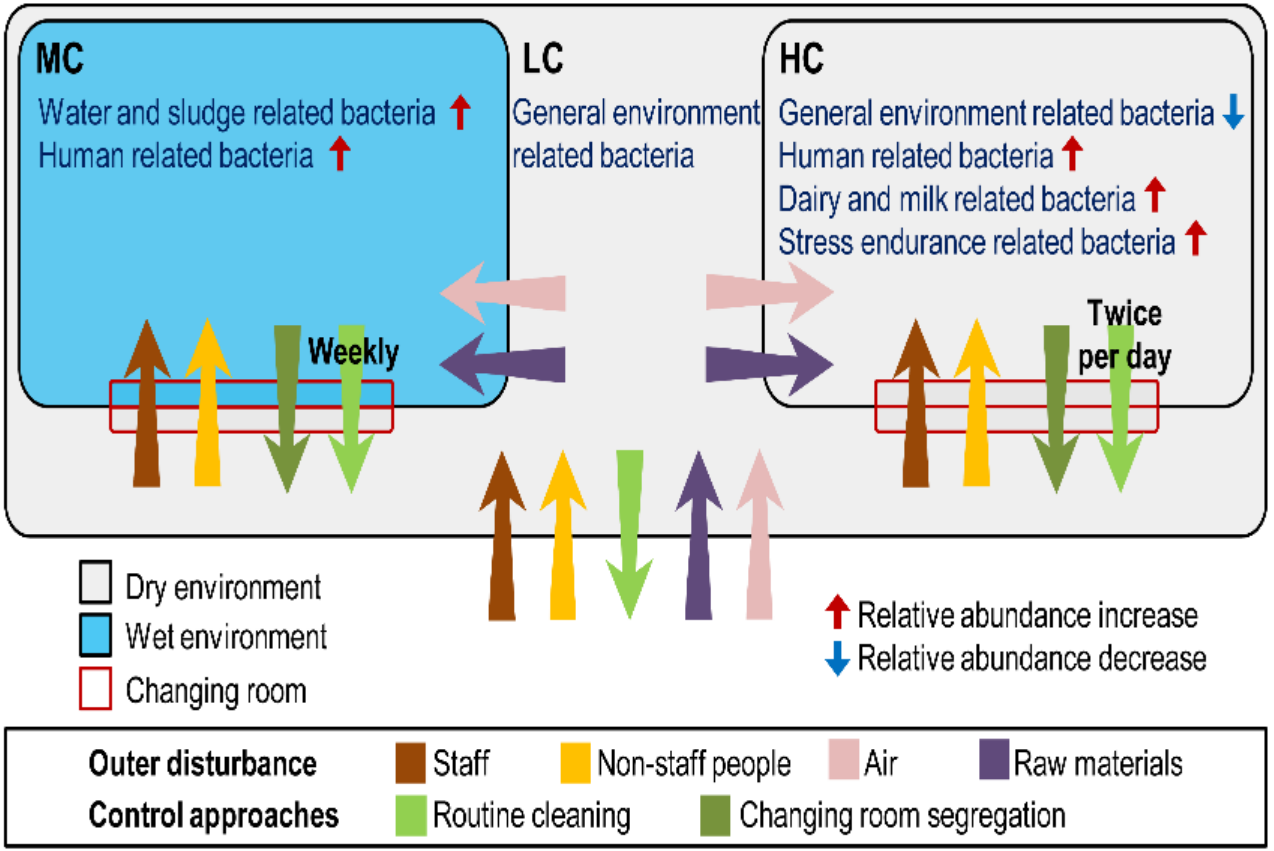
[Bourdichon, *et al.*, (2021) *Int. J. Food Microbiol*. doi: 10.1016/j.ijfoodmicro.2021.109351]



[Cao, *et al.*, (2022) *Foodborne Pathog. Dis*. **In press**]


A)
OTHER GENUS
Streptococcus
Streptococcaceae_unclassified
Stenotrophomonas
Staphylococcus
Saccharibacteria_ge
Rothia
Pseudoxanthomonas
Pseudomonas
Pseudomonadaceae_unclassified
Pedobacter
Oxalobacteraceae_unclassified
Massilia
Lactococcus
Lactobacillus
Halomonas
Flavobacterium
Enterobacteriaceae_unclassified
Corynebacterium_1
Comamonadaceae_unclassified
Chryseobacterium
B)
LC area
MC area
HC area
Black: Factory W
Green: Factory M
OTHER PHYLUM
Verrucomicrobia
Verrucomicrobia
Saccharibacteria
Proteobacteria
Planctomycetes
Planctomycetes
Fusobacteria
Firmicutes
Deinococcus-Thermus
Cyanobacteria
Chloroflexi
Bacteroidetes
Bacteria_unclassified
Actinobacteria
Acidobacteria
C)
LC
111
65
12
24
10
MC
50
12
2
10
HC
46
D)
Rothia, 1.08%
Micrococcales_unclassified, 0.41%
Saccharibacteria_ge, 1.60%
Chryseobacterium, 2.01%
Flavobacterium, 1.60%
Flavobacteriaceae_unclassified, 0.51%
Streptococcus, 5.29%
Lactococcus, 3.10%
Streptococcaceae_unclassified, 1.23%
Lactobacillus, 1.12%
Acinetobacter, 5.78%
Pseudomonadaceae_unclassified, 4.96%
Psychrobacter, 4.11%
Enterobacteriaceae_unclassified, 2.61%
Comamonadaceae_unclassified, 2.61%
Stenotrophomonas, 1.46%
Pseudoxanthomonas, 1.10%
Gammaproteobacteria_unclassified, 0.95%
Brevundimonas, 0.86%
Exiguobacterium, 0.80%
Sphingomonadales_unclassified, 0.64%
Aeromonas, 0.38%
Rhodobacteraceae_unclassified, 0.21%
Roseomonas, 0.27%
Others, 52.01%
Actinobacteria, 1.49%
Saccharibacteria, 1.60%
Bacteroidetes, 1.12%
Firmicutes, 10.82%
Proteobacteria, 29.96%
Others, 52.01%

Evaluating risk associated with the microbiota in the food production environment -



Conclusions -

- **sequencing methods** can support food safety control measures in the food production environment **[WGS]**

- understanding the **microbial ecology** of a food production facility is essential in identifying changes that ***may signal an increased risk*** **[WGS/16S rRNA/metagenomics]**

- **differentiating persistent from non-persistent isolates** recovered is ***important to refine food safety controls*** **[RNA-seq]**

- ***precision food safety measures***, including **whole genome sequencing** of key isolates, **linked to their phenotypes**, will improve our understanding of how bacteria adapt/behave in these hostile environments and provide **novel biomarkers** to aid their rapid detection and ***subsequent risk reduction***

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Thank you

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