International Association for Food Protection WEBINAR

Cronobacter in the Spotlight: New Insights Into a Known Organism

July 13, 2022

Organized by: ILSI Europe With support from IAFP's Low Water Activity Foods PDG, Microbial Modelling and Risk Anaysis PDG, and International Food Protection Issues PDG

Moderator: Anett Winkler, Cargill Germany

Sponsored by the IAFP Foundation

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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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Cronobacter in the spotlight:
New insights into a known organism

Moderator: Anett Winkler, Cargill Germany

Delivers science-based solutions

that improve public health & safeguards the environment

collaboration

common challenges

science

communicate & disseminate

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International Association for Food Protection

Microbiological Food Safety Task Force

Cargil
Mondelēz International
Institut Mérieux
General Mills
Wageningen University
Campden BRI

Microbiological risk-based decision tool for use of dried spices and herbs, dried vegetables, and dried fruits in foods

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Campden BRI
Wageningen University
University of California
University of Gent
Catholic University of the Sacred Heart

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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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Delivers science-based solutions that improve public health & safeguards the environment

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Anett Winkler

Cargill Germany

EMEA Microbiologist

Moderator

- 20 years at Kraft/Mondelez as microbiologist in various roles
- 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 (Microbiological Food Safety), and IAFP

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Séamus Fanning

University Collage Dublin, Ireland

Professor of Food Safety & Zoonoses

Speaker

- Appointed to UCD in 2002 and currently is the Director of the UCD-Centre for Food Safety (20 years as a Full Professor)
- More than 30 years research experience, applying molecular methods to food safety challenges
- 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

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François Bourdichon

Universitá Cattolica Del Sacro Cuore

Research Collaborator

Panellist

- 15 years in the Food Industry: Savenica (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, Universitá Cattolica Del Sacro Cuore, Piacenza, Italy
- Member of the IAFP since 2007
- Elected as a Fellow of the American Academy of Microbiology (FAAM) in 2019

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Marcel Zwietering

Wageningen University, The Netherlands

Professor Food Microbiology

Panellist

- 19 years professor
- 5 years Danone Research
- 10 years university
- ICMSF chair
- Active in ILSI Europe (Microbiological Food Safety Task Force) and IAFP

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Roy Betts

Campden BRI, UK

Head of Microbiology

Panellist

- Head of Microbiology at Campden BRI and independent international food research organisation based in the UK
- Originally managed a research team at Campden BRI concentrated on research, development and validation of microbiological test methods.
- Currently his interests moved to the assessment of the microbiological quality and safety of foods, advising industry on techniques and procedures to produce and market high quality safe foods.
- Active member of ILSI Europe (microbiological Food Safety, the UK Food and Drink Federation Food Hygiene Sub Committee and the UK Advisory Committee on the Microbiological Safety of Foods as well as British Standards Institute and ISO committees dealing with microbiological test methods

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Stephen Forsythe

Guest lecturer at the University of Hong Kong and Advisor Center for Food Safety, South Africa

Professor of Microbiology

Panellist

- ‘Retired’ Professor of Microbiology from Nottingham Trent University (NTU), UK
- Guest lecturer at the universities of Hong Kong (HKU), Kuopio (Finland), BOKU (Vienna), Stellenbosch, as well as an external advisor to ‘Center for Food Safety’, Stellenbosch, South Africa
- Previously he was external advisor to the UK’s Food Standards Agency (2010-2019), European Food Safety Authority (2003-2007) and World Health Organisation (2004-2014).
- He was external advisor to all three FAO-WHO risk assessments on Cronobacter in infant formula (2004, 2006 & 2008), and ready to eat infant foods in famine relief (2012 & 2014)
- >110 publications are on Cronobacter and related organisms

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- Please ask your questions in the question box, not in the common chat box – thank you.

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WEBINAR

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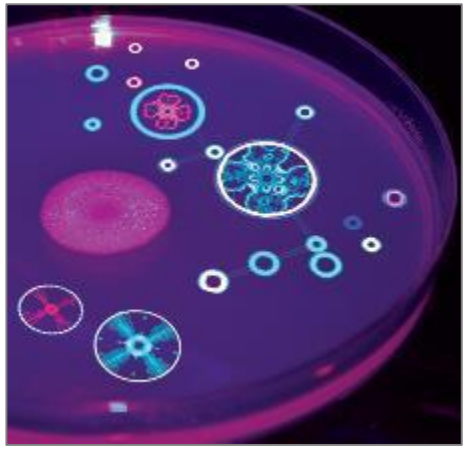
IAFP Webinar-
***Cronobacter* in the spotlight-**
new insights into a known organism

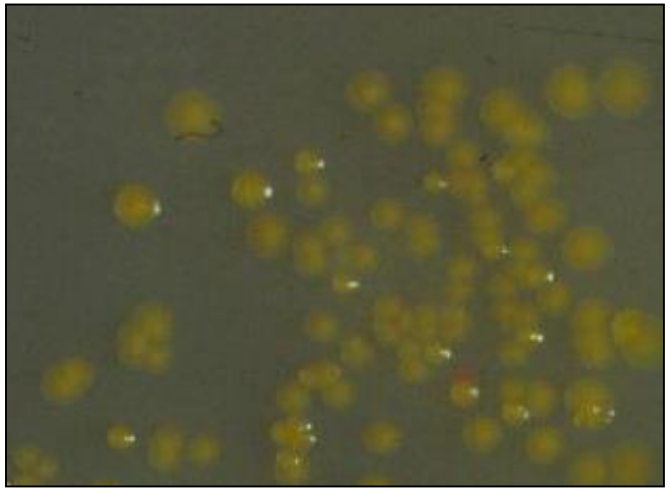
Wednesday 13th July, 2022

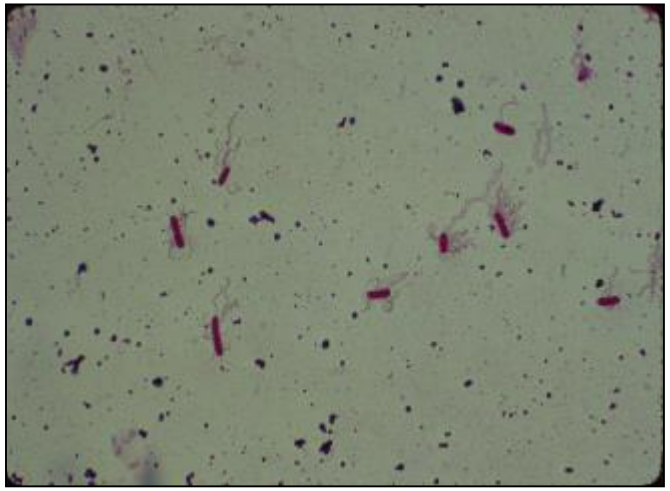


An introduction to *Cronobacter* species
–a food-borne pathogen of concern to PIF manufacturers

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

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

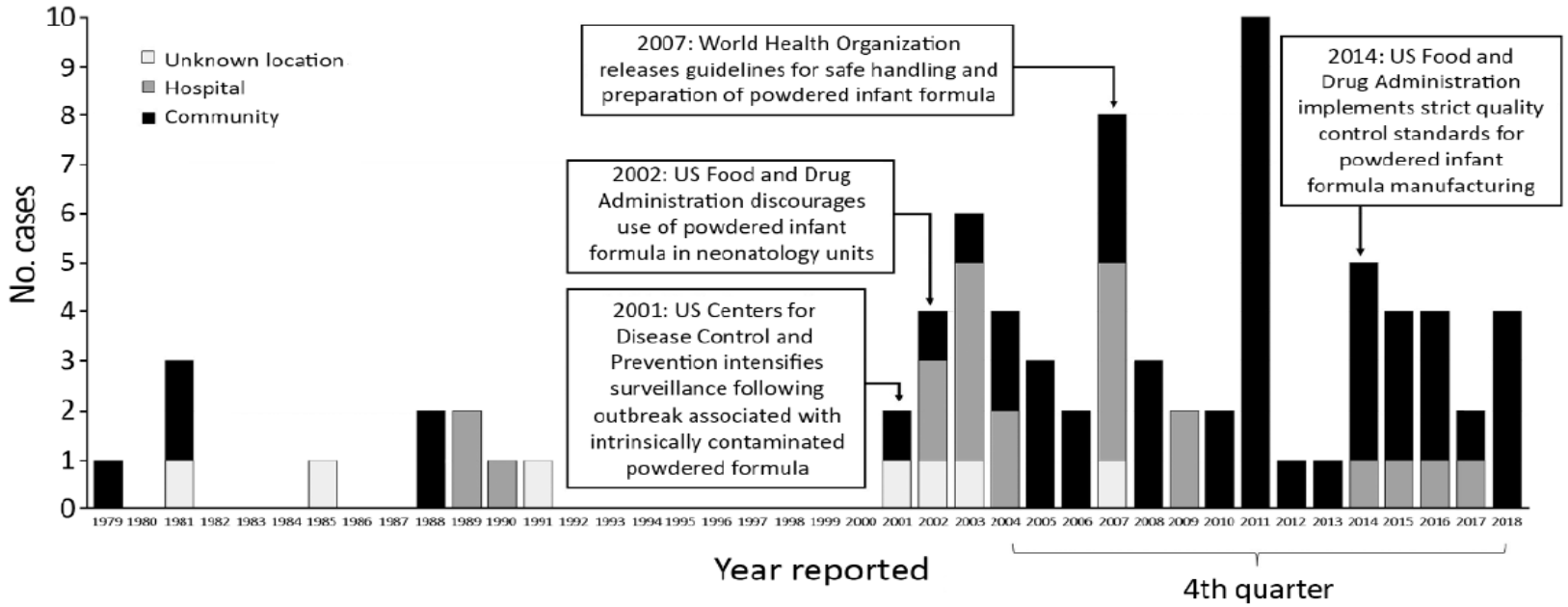




General characteristics-

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

Reported invasive *Cronobacter* infections recorded among infants in the US & recent global cases/outbreaks -



Recent global cases/outbreaks-

Location	Year	Number of cases
USA	2022	4
USA	2016	1
Australia	2015	1
USA	2011	4
Mexico	2010	2
Spain	2007	1
India	2006	1
USA	2005	1

Cronobacter and Powdered Infant Formula Investigation

Español (Spanish)

Updated March 25, 2022

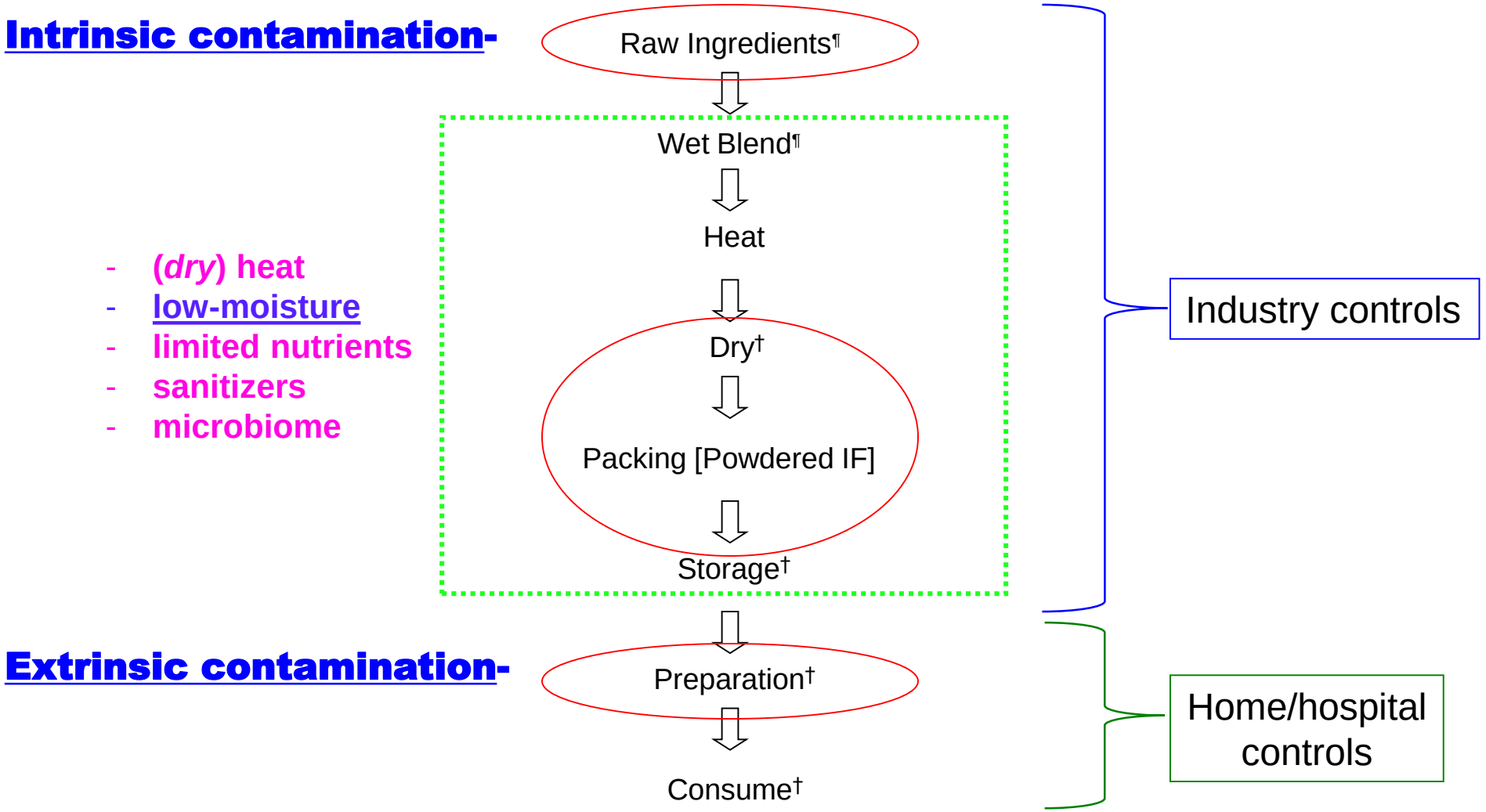
On February 17, 2022, and February 28, 2022, Abbott Nutrition recalled powdered infant formula produced at its manufacturing facility in Sturgis, Michigan, because of possible *Cronobacter* contamination.

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

[Nazarowec-White and Farber (1997) *Int. J. Food Microbiol*. **34**: 103-113]

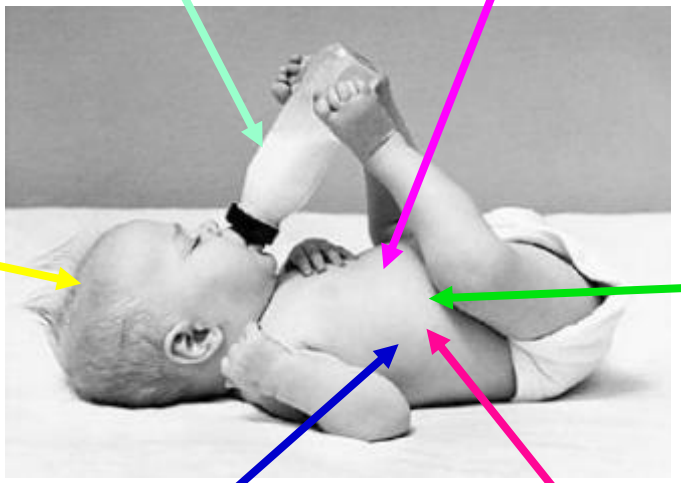


Routes of contamination in reconstituted formula -

Ingestion

Survive acidic pH of stomach

*acr*AB-*tolC*



Cross BBB

Meningitis!

*omp*A

Attach to intestinal epithelia

(NEC?)

Survive in vascular system

(Bacteraemia/Sepsis)

Antibiotic susceptible testing
Macrophage survival assay *phoP/Q*

Cross into vascular system (translocation)

Invasive *E. sakazakii* disease in infants -

Infection is linked to infant formula

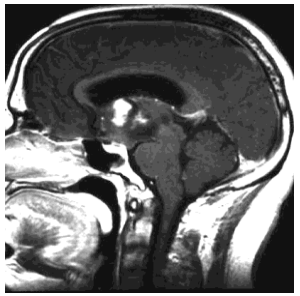
Neonates < 1 month are at most risk

46 cases

33 meningitis

- birth weight, 2.5 kg
- 37 weeks gestation
- infection developed at 6 days

- 11 (33%) seizures
- 7 (21%) brain abscess
- 14 (42%) died



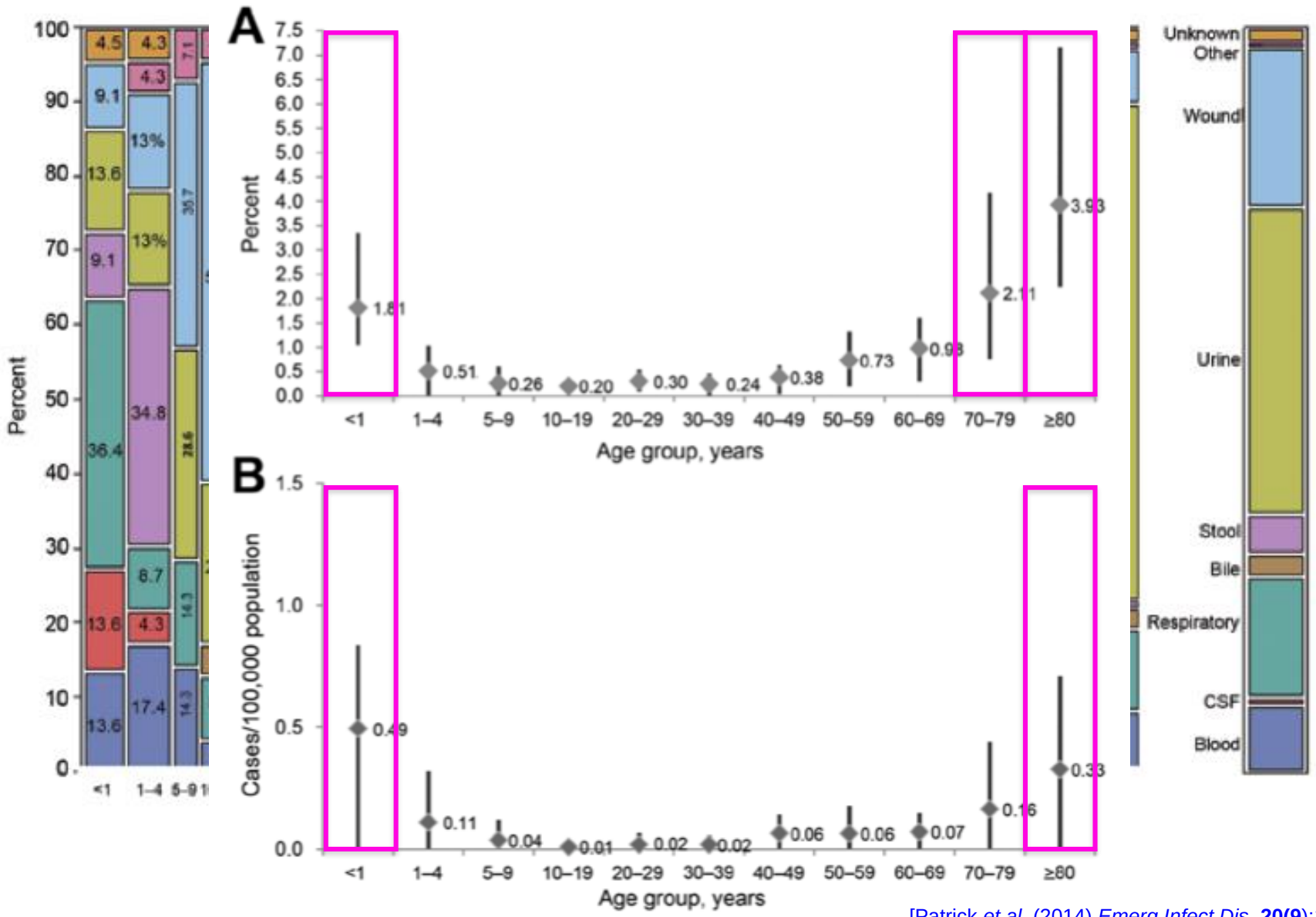
1 UTI

12 bacteremia

- birth weight 850 g
- 27.8 weeks gestation
- infection developed at 35 days

- 24 of 26 infants consumed infant milk formula
- 15 (68%) of 22 cases yielded *Enterobacter sakazakii*
- 13 of the 22 formula strains were identical

[Bowen and Braden (2006) *Emerg. Infect. Dis*. **12**: 1185-90]

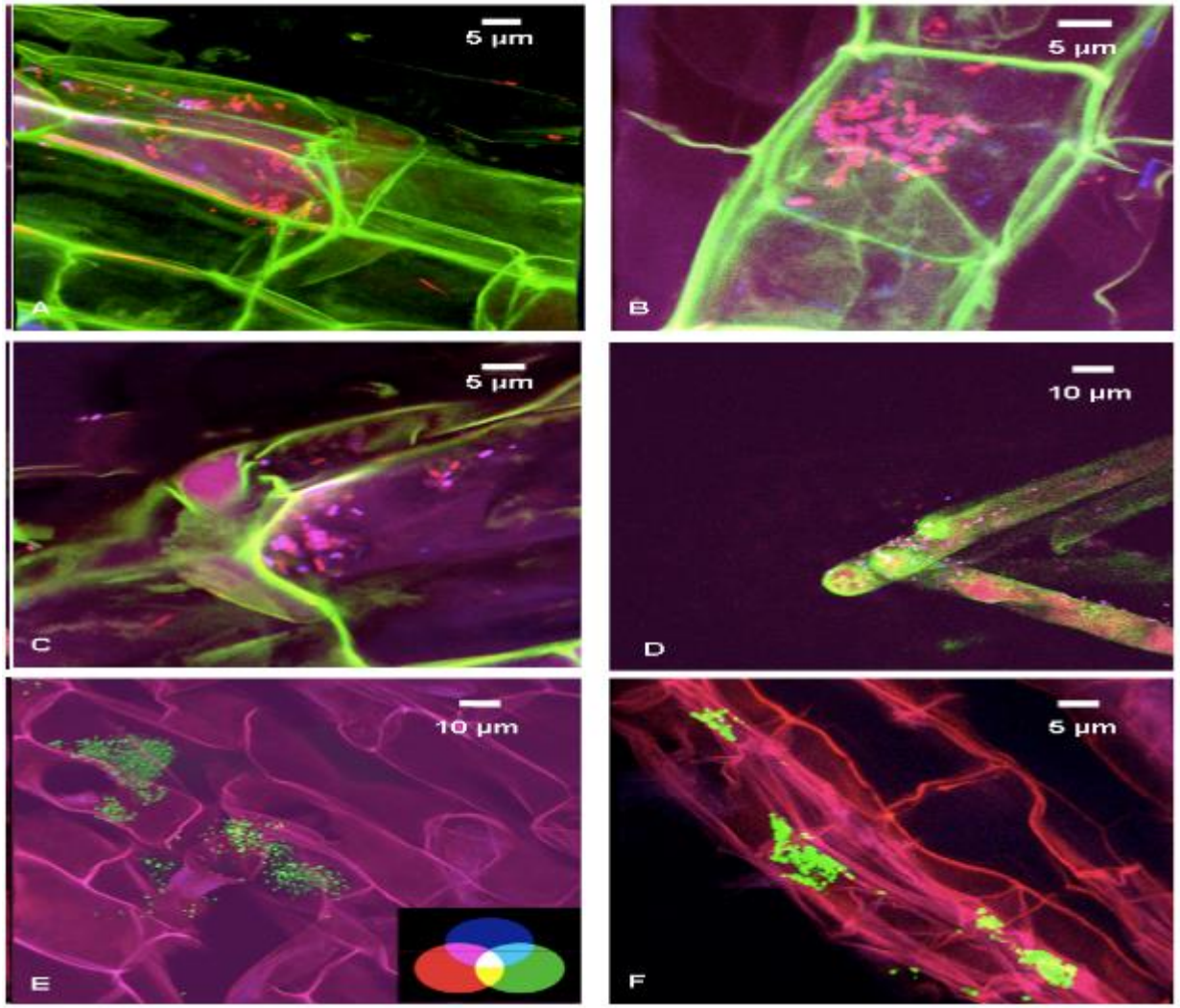


[Patrick *et al*. (2014) *Emerg Infect Dis*. **20(9)**:1536-9.]

Characteristics of food-borne illness (in infants) following *Cronobacter* infection -

Properties	Description
Symptoms	- Necrotizing enterocolitis - Bacteraemia - Meningitis
Long term complications	- Delayed neurological development - Hydrocephalus - Permanent neurological damage
Infectious dose	Not known
Infectious period	Not known
Duration of illness	Not known
Food most frequently implicated	**Powder Infant formula**

What is the source of *Cronobacter*?

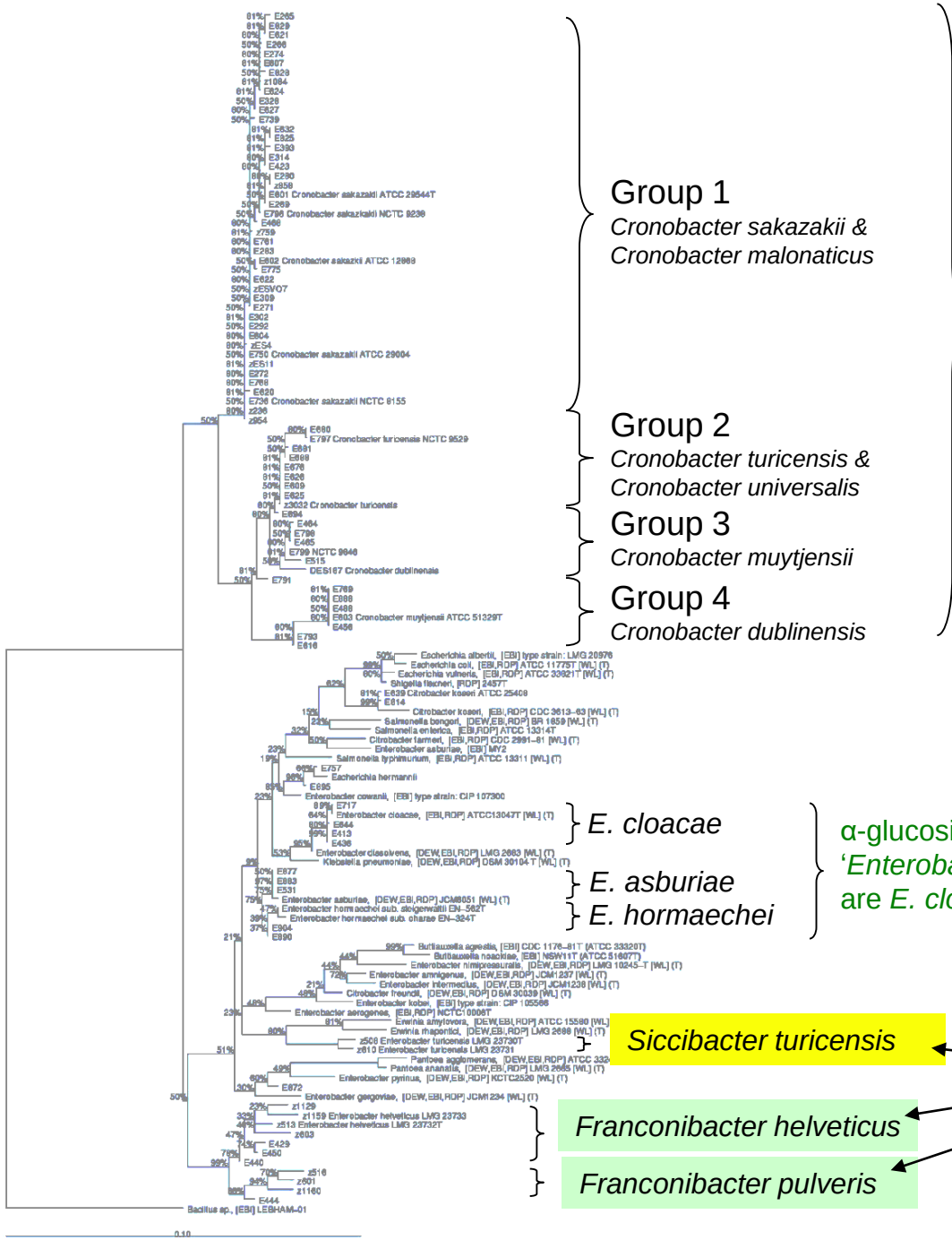


Plants may be the natural habitat for *Cronobacter* species-

- physiological features such as yellow pigmentation; EPS; and persistence in a desiccated state suggest that the bacterium may have an environmental niche
- *Cronobacter* have been isolated from plant roots
- **the bacterium can be found in association with the rhizosphere**
- ***Cronobacter* can endophytically colonize the roots of tomato and maize plants**

[Schmid, *et al* (2009) *Res. Microbiol*. **160**: 608-614]

[Iversen, *et al.*, (2004) *J. Clin. Microbiol*. **42:** 5368-5370]
[Iversen, *et al.*, (2007) *BMC Evol. Biol*. **7:** 64]
[Iversen *et al.*, (2008) *Int. J. Syst. Evol. Microbiol*. **58:** 1442-1447]
[Stephan *et al.*, (2014) *Int. J. Syst. Evol. Microbiol*. **64**:3402-3410]



Enterobacter sakazakii

***Cronobacter* gen. nov.**

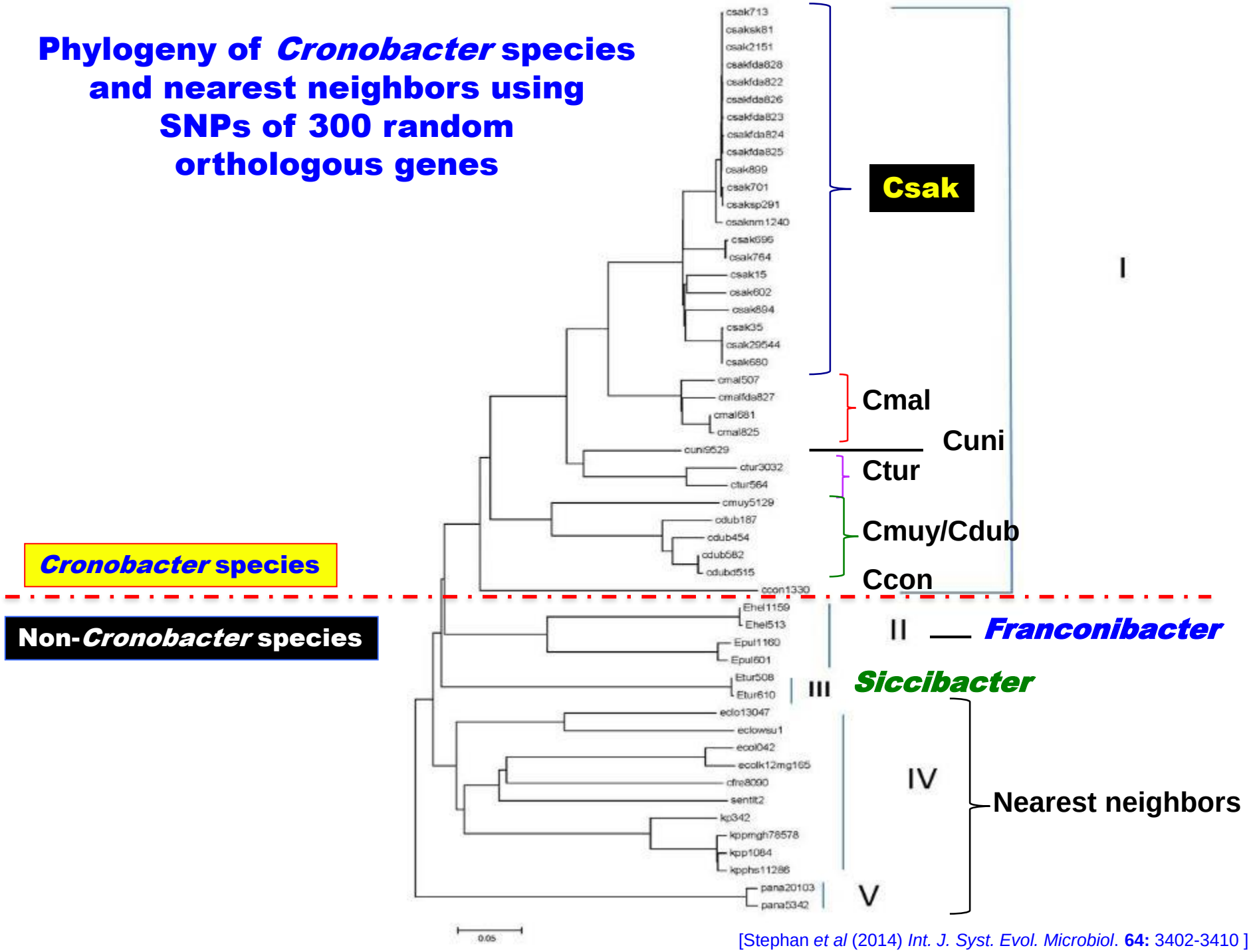
α-glucosidase negative
'*Enterobacter sakazakii* '
are *E. cloacae*-complex spp.

Mis-identified as *E. sakazakii*

α -glucosidase positive
environmental species

Mis-identified as *Pantoea, Escherichia vulneris* and *Buttiauxella*.

Phylogeny of *Cronobacter* species and nearest neighbors using SNPs of 300 random orthologous genes



[Stephan *et al* (2014) *Int. J. Syst. Evol. Microbiol*. **64:** 3402-3410]

Comparing the phenotypes between *Cronobacter* and *Enterobacter* species?

Phenotype reaction	*Cronobacter* species							*Enterobacter*		
	C. sak	C. mal	C. dub	C. muy	C. tur	C. con	C. uni	E. tur	E. pul	E. hel
Voges-Proskauer (VP)[h]	V[b]	+[b]	+	+	+	+	+	-	-	-
Methyl Red[g]	V	-[b]	-	-	-	-	-	+	+	+
Ornithine decarboxylation	V	V	+	+	+	+	V	-	-	-
Arginine dihydrolation	+	+	+	+	+	+	+	-	-	-
Acid[d] from:										
D-cellobiose	+	+	+	+	+	+	+	-	+	-
D-arabitol	-	-	-	-	-	-	-	-	+	-
Sucrose	+	+	+	+	+	+	+	-	+	-
L-rhamnose	+	+	+	+	+	+	+	+	+	-
Malonate utilization[f]	-	+	V	+	+	+	V	+	-	+
Palatinose	+	+	+	+	+	+	+	-	-	-

Enterobacter helveticus
Enteronacter pulveris

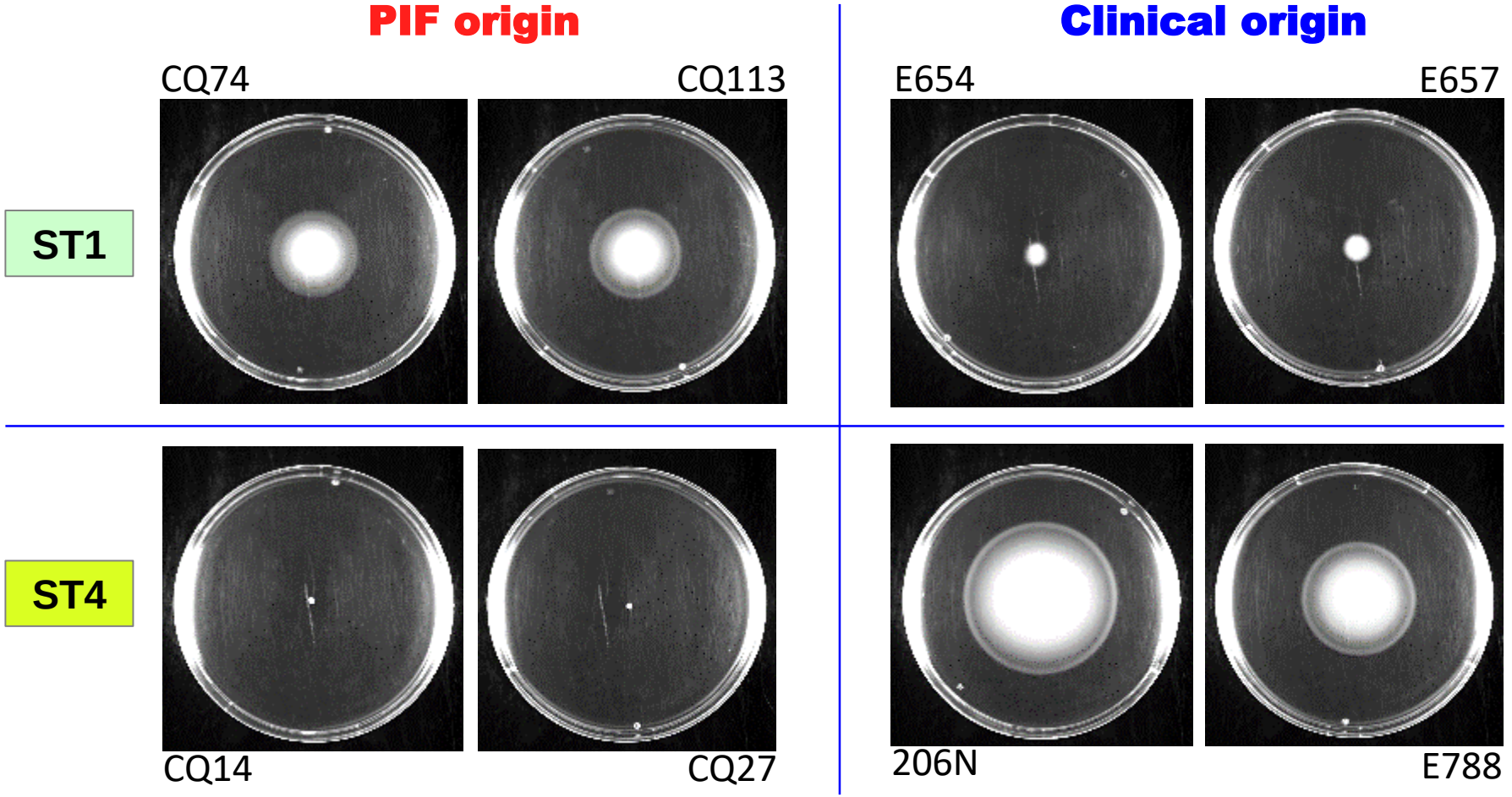
⇨ *Franconibacter helveticus*
Franconibacter pulveris

Enterobacter turicensis

⇨ *Siccibacter turicensis*

[Stephan *et al* (2014) *Int. J. Syst. Evol. Microbiol*. **64:** 3402-3410]

Motility –swim assay

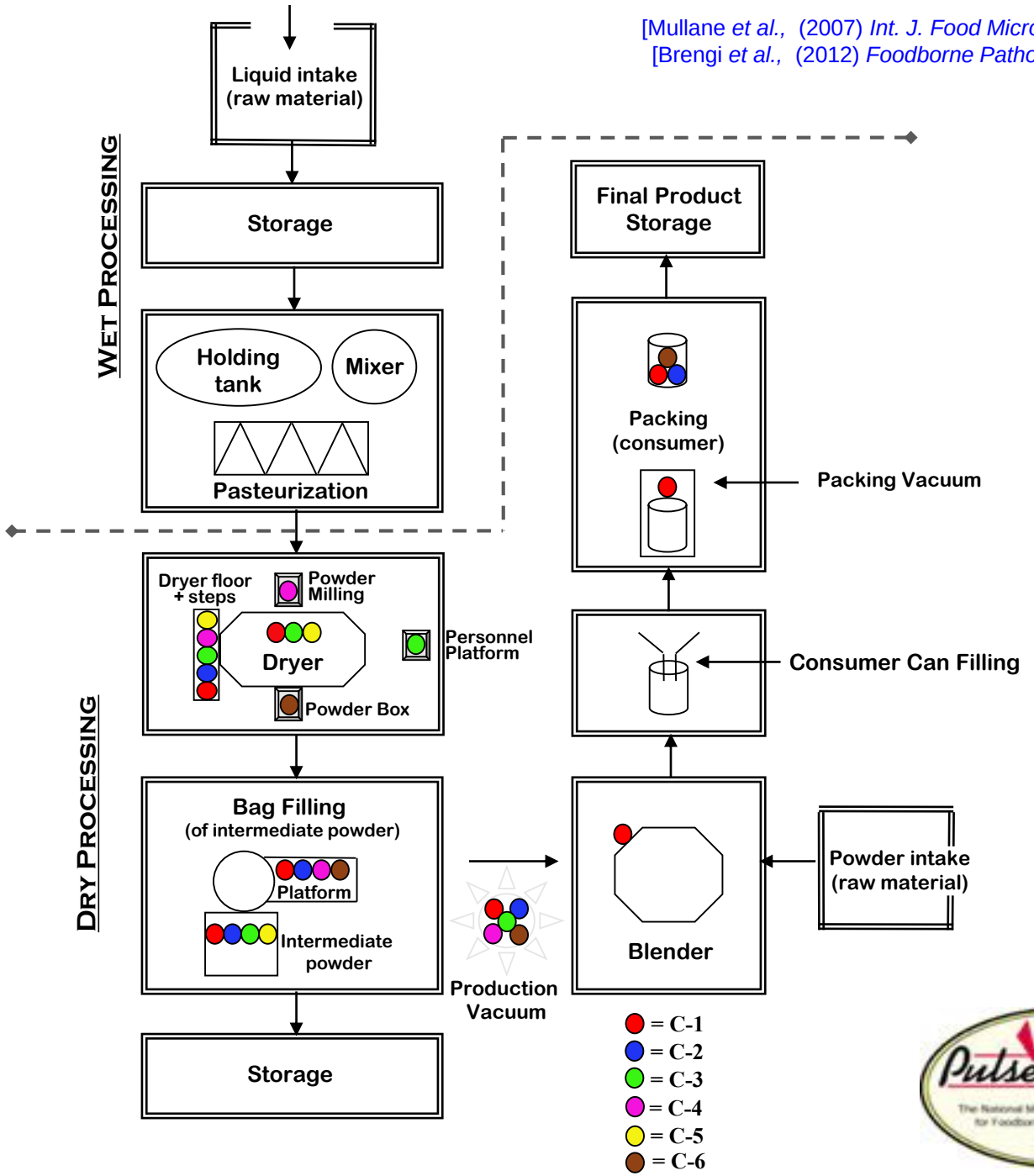


***patho-adaptation* [?]**

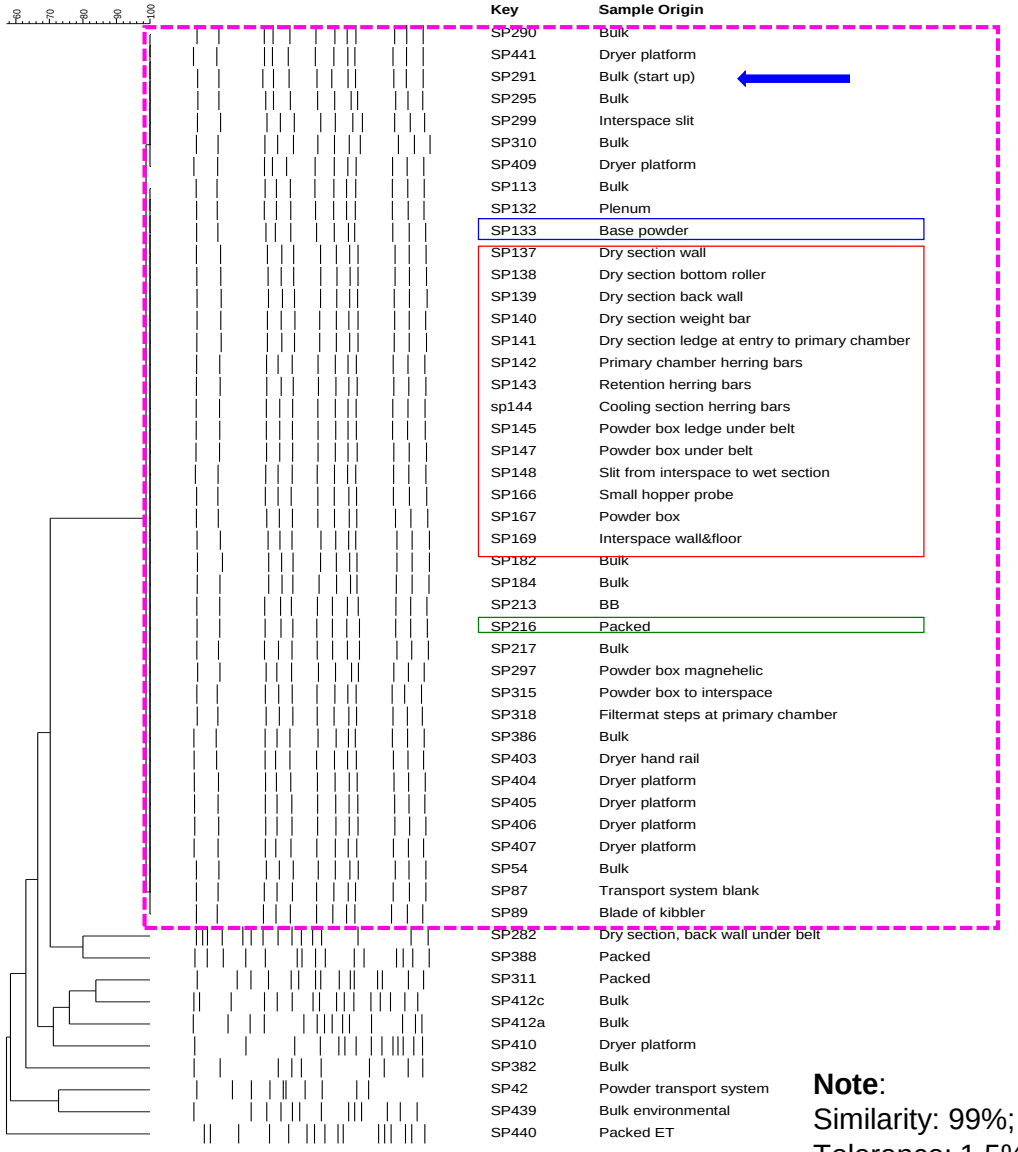
[Yan *et al*., (2015) *Appl. Env. Microbiol*. **81:** 4388-4402]

Pulsed-field gel electrophoresis (PFGE) -

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



PulseNet USA
The National Molecular Subtyping Network for Foodborne Disease Surveillance
CDC

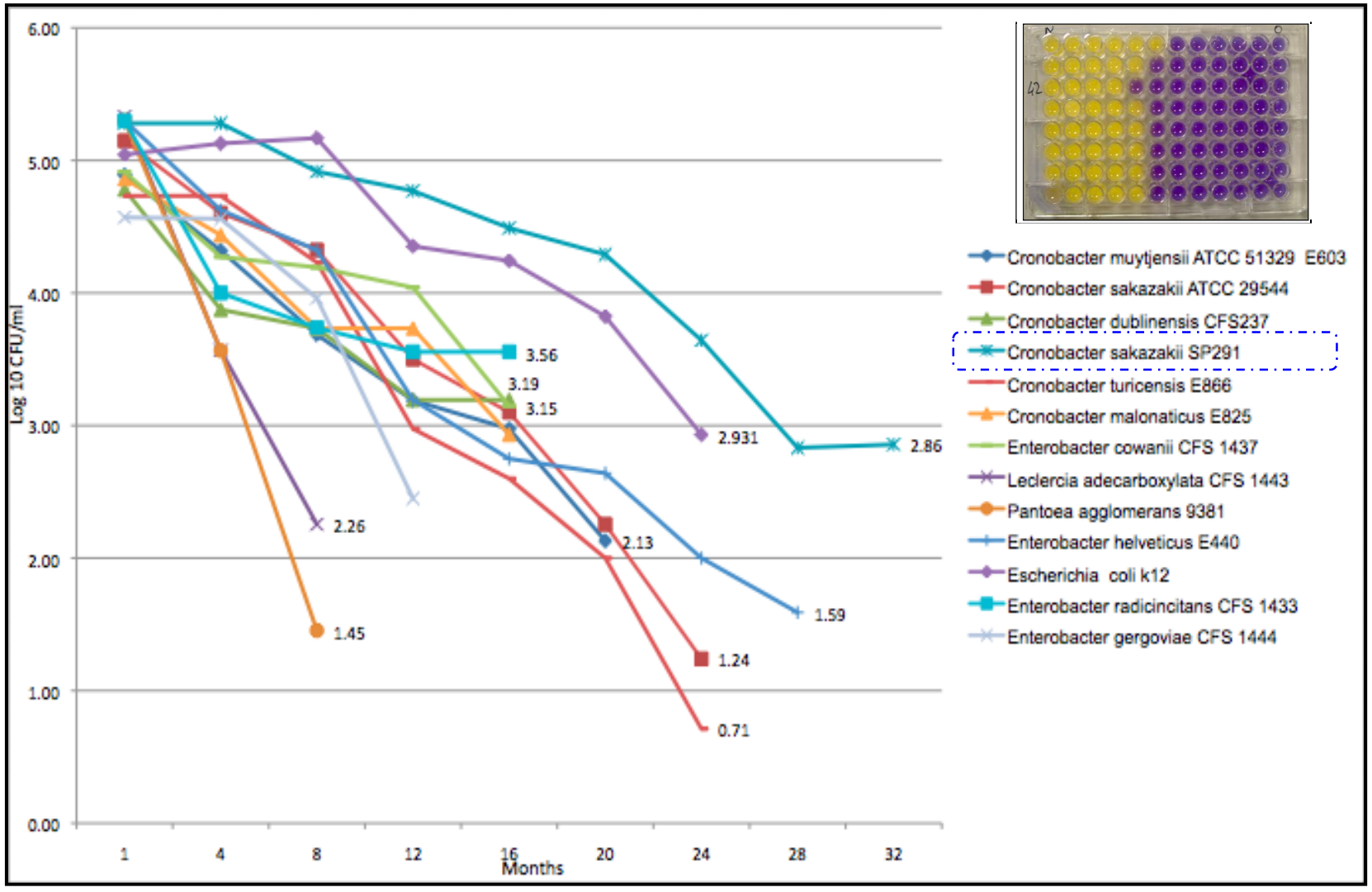


A uniquely adapted *Cronobacter sakazakii* isolate detected in a PIF production environment using PFGE sub-typing

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

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

Tolerance to desiccation with time -



Thermo-tolerant *Cronobacter sakazakii* SP291 -

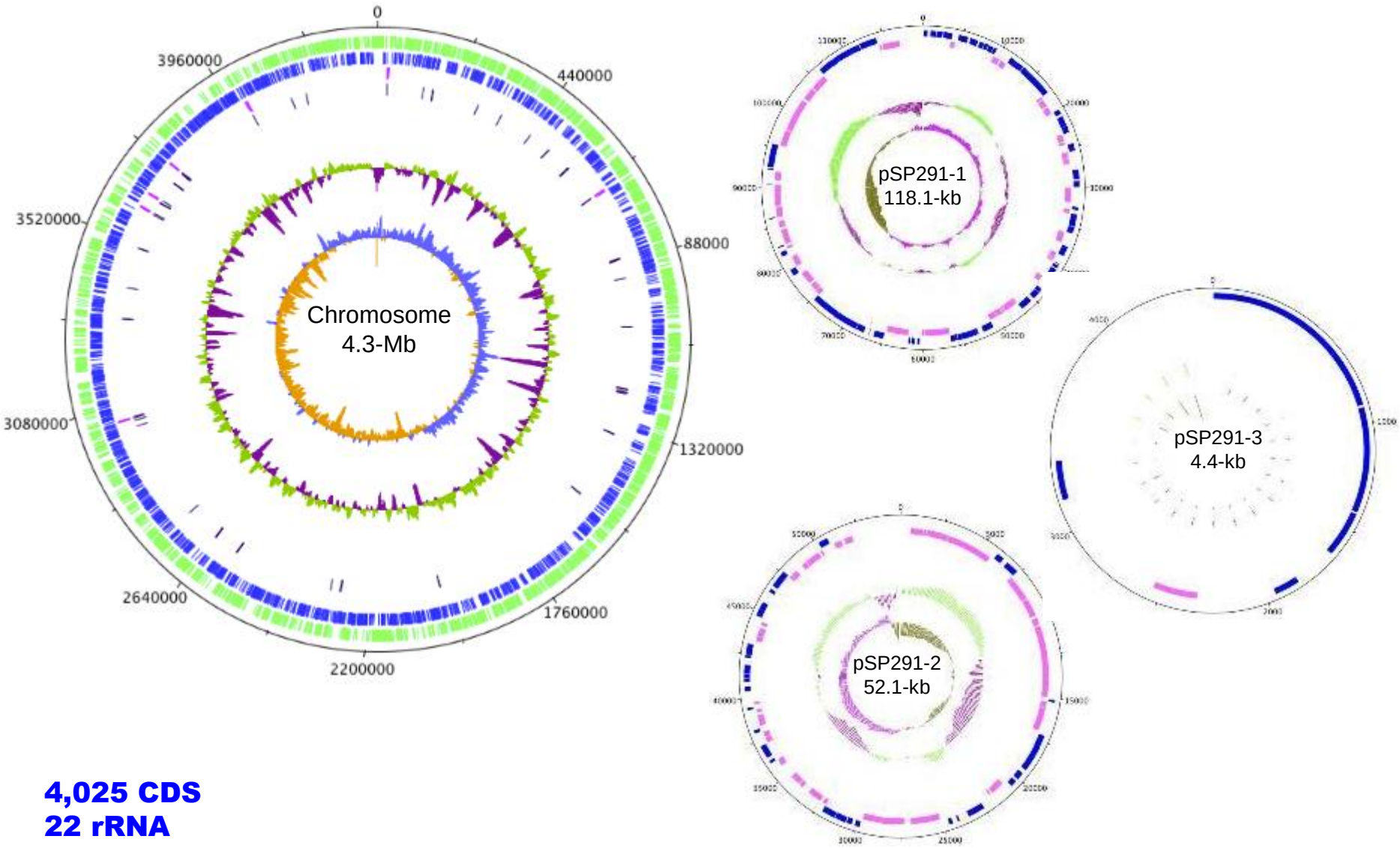
Strain	Species	Origin	D_{60} value (min)	
			Dry heat	Wet heat
ATCC®**12868**	*Cronobacter sakazakii*	Unknown	52.37	0.18
ATCC®**BAA-894**	*Cronobacter sakazakii*	PIF	27.19	0.16
SP291	***Cronobacter sakazakii***	**PIF environment**	**120.28**	**0.15**
S15	*Salmonella* Anatum	PIF	52.37	0.28
S34	*Salmonella* Ealing	Clinical	66.4	0.16
ATCC®**13076**	*Salmonella* Enteritidis	Unknown	90.54	0.18

What are the bacterial characteristics that contribute to survival of *C. sakazakii* SP291 at low-moisture in the PIF environment?

RNA-seq-
to explore the transcriptome

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

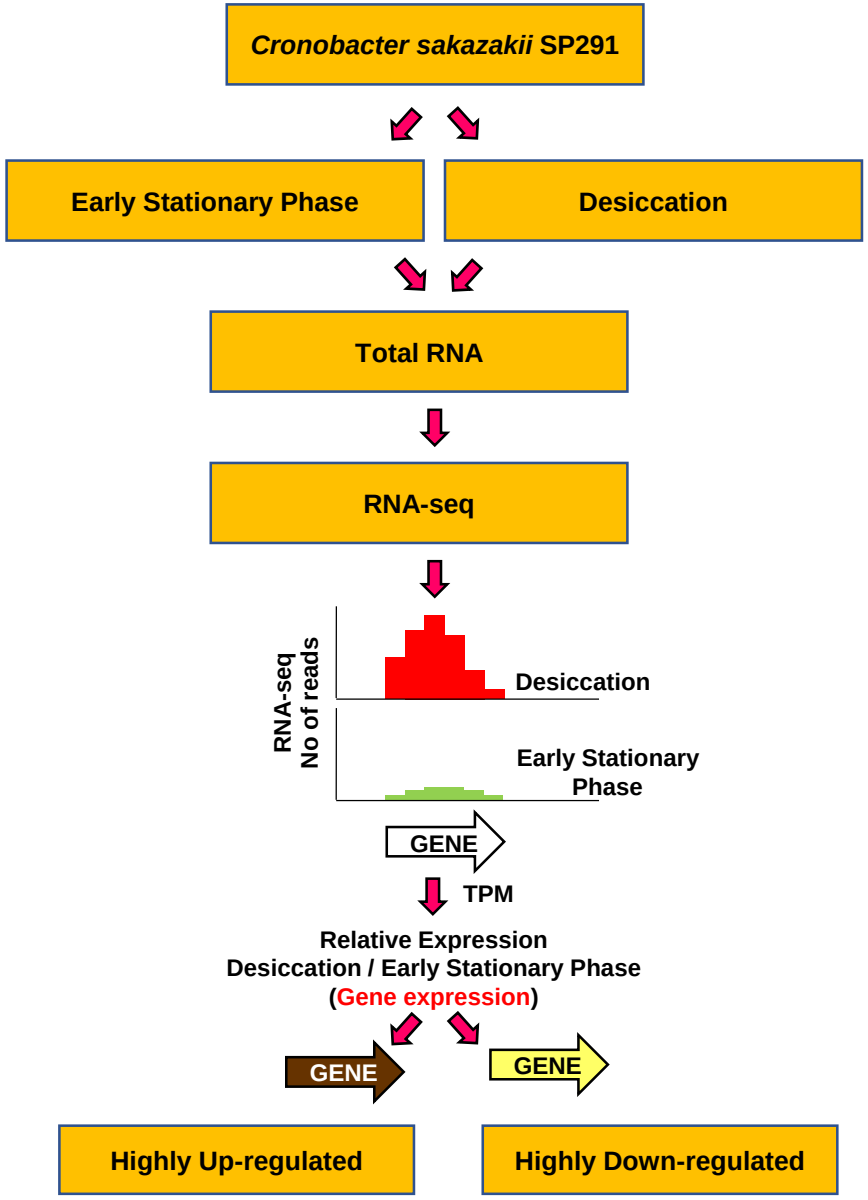
Genome of *Cronobacter sakazakii* SP291 -

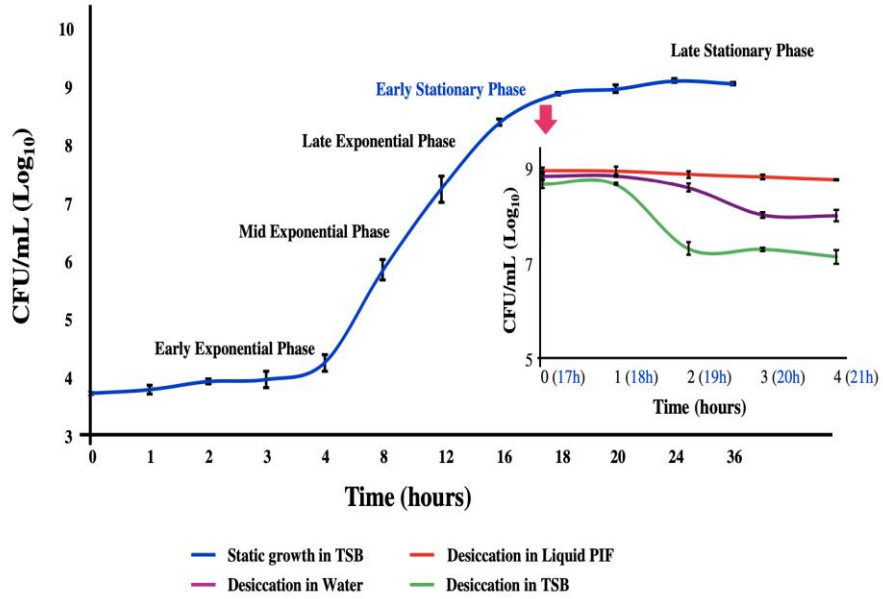


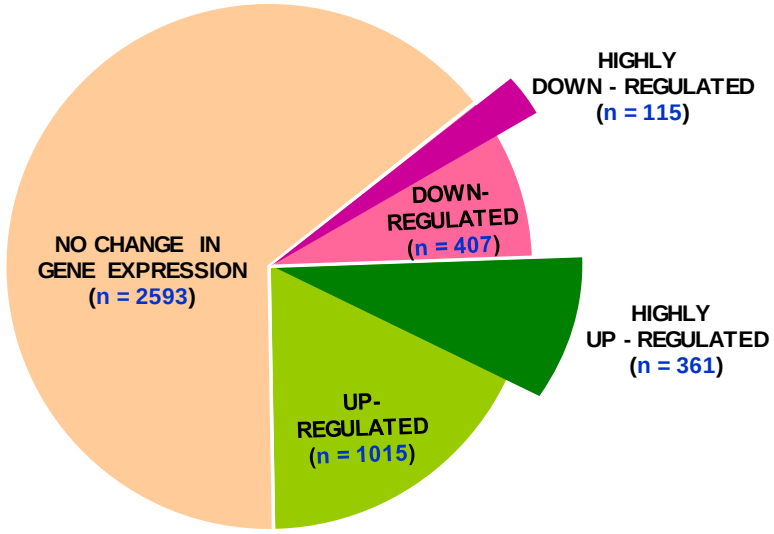
4,025 CDS
22 rRNA
82 tRNA

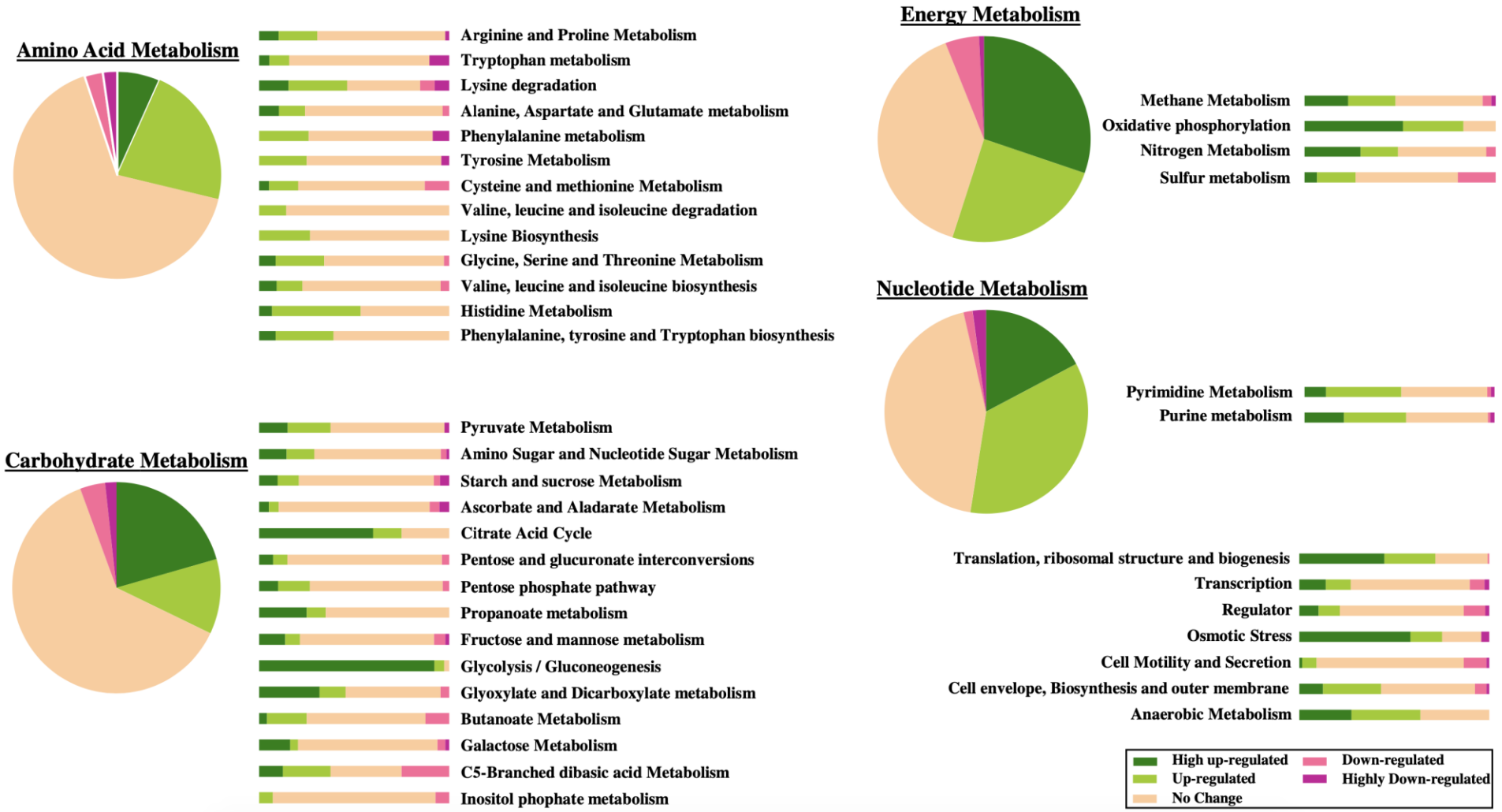
[Yan *et al*. (2013). *Front. in Microbiol.* **4:** 256]

RNA-seq work plan -









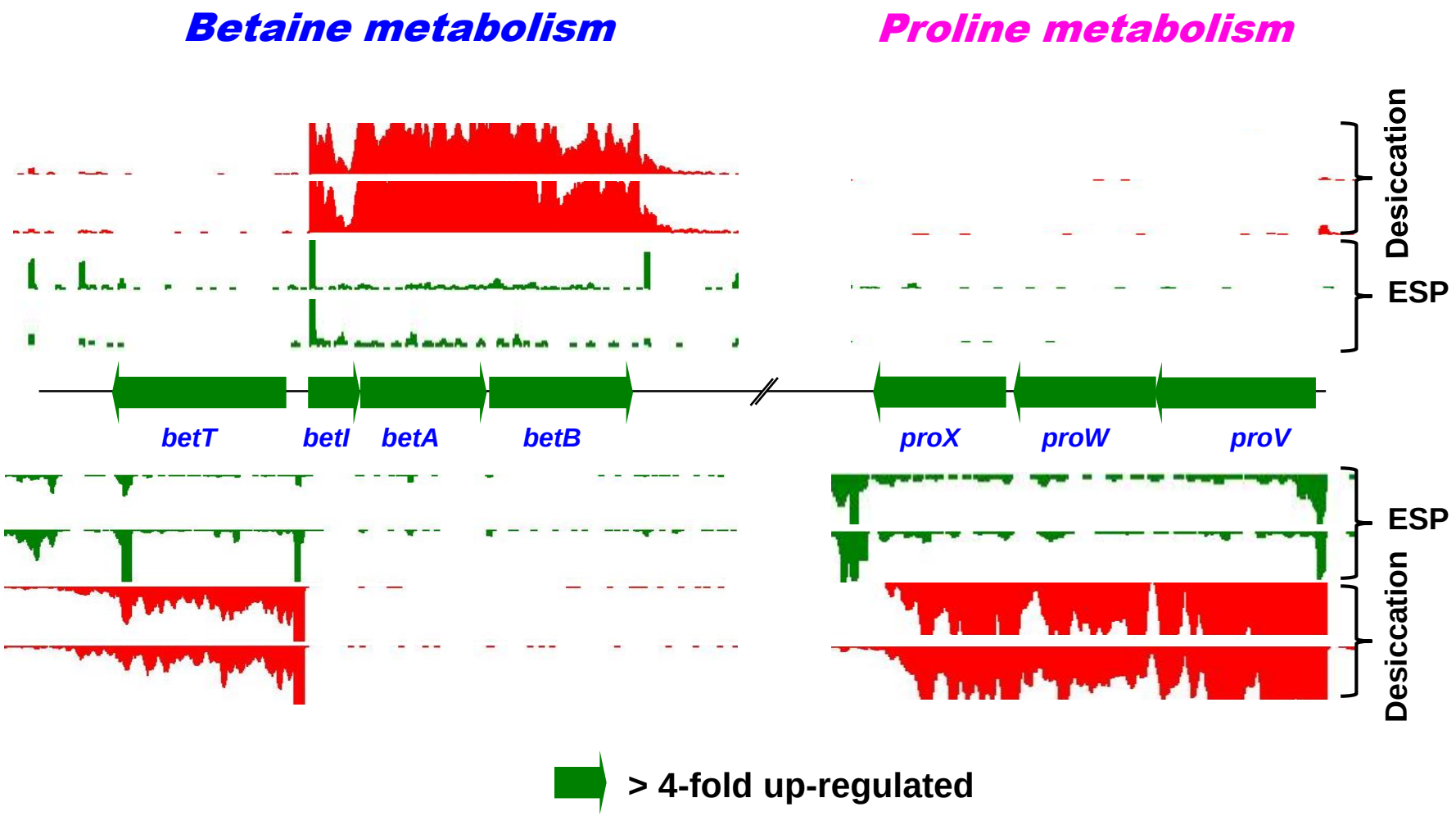
[Srikumar *et al*., (2019) *Appl. Env. Microbiol*. **85:** e019932]

Stress response genes identified in *C. sakazakii* SP291 -

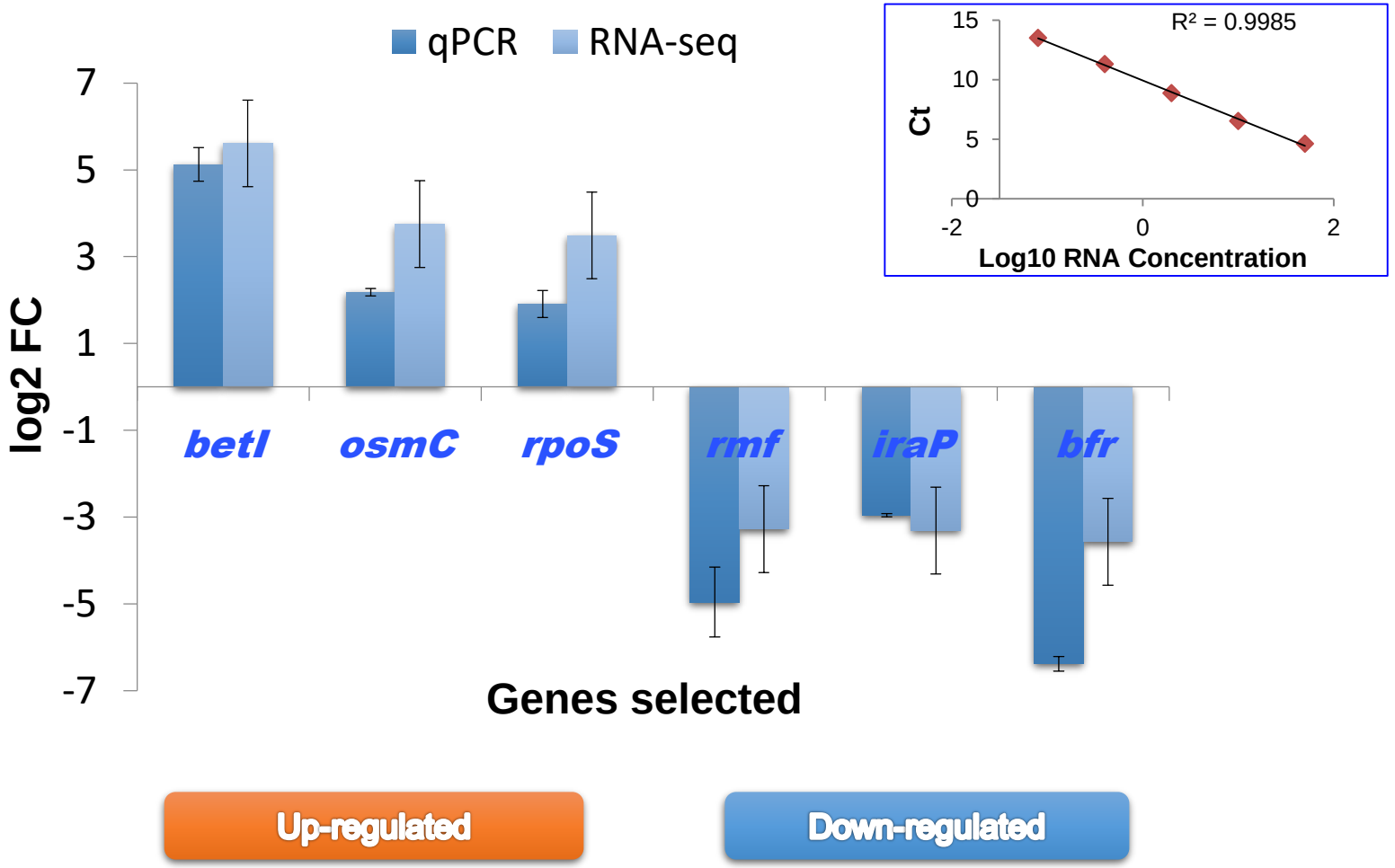
Category	Gene Number
Osmotic stress	**37**
Cold & heat shock	25
Dessication stress	10
Detoxinfication	11
Oxidative stress	65
Periplasmic stress	7
Others	29
Total number	**184**

No.	Gene	Size (bp)	Function
1	*betB*	1472	Betaine aldehyde dehydrogenase
2	*betA*	1679	Choline dehydrogenase
3	*betI*	608	Helix-turn-helix (HTH)-type transcriptional regulator BetI
4	*betT*	2030	High-affinity choline uptake protein BetT
5	*opuCA*	1145	Glycine betaine/carnitine/choline transport, ATP-binding protein OpuCA
6	*opuCB*	647	Glycine betaine/carnitine/choline transport, ATP-binding protein OpuCB
7	*opuCC*	905	Glycine betaine/carnitine/choline transport, ATP-binding protein OpuCC
8	*opuCD*	713	Glycine betaine/carnitine/choline transport, ATP-binding protein OpuCD
9	*proP*	1506	L-Proline/Glycine betaine transporter ProP
10	*proV*	1202	L-Proline/glycine betaine ABC transport system permease protein ProV
11	*proW*	1070	L-Proline glycine betaine ABC transport system permease protein ProW
12	*proX*	995	L-Proline glycine betaine binding ABC transporter protein ProX
13	*yehX*	941	Osmoprotectant ABC transporter ATP-binding subunit YehX
14	*yehZ*	908	Osmoprotectant ABC transporter binding protein YehZ
15	*yehW*	731	Osmoprotectant ABC transporter inner membrane protein YehW
16	*yehY*	1,133	Osmoprotectant ABC transporter permease protein YehY

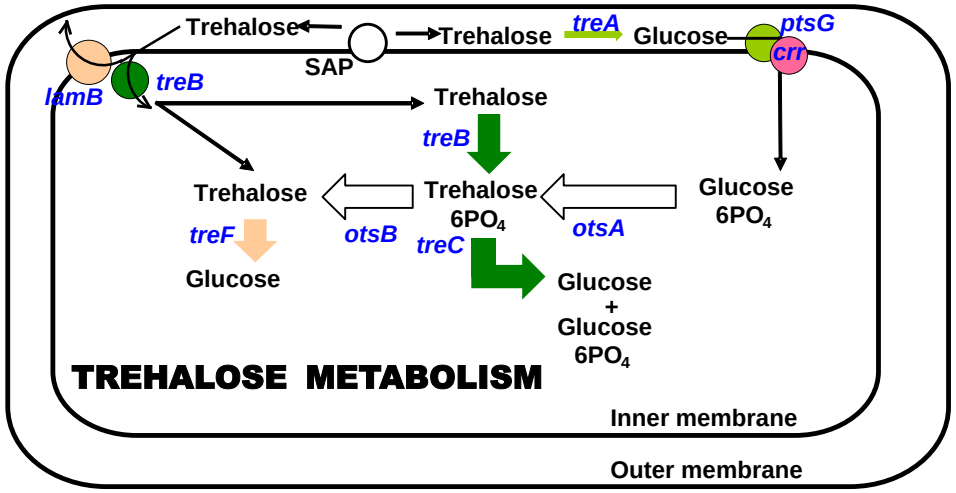
The expression of stress response genes during desiccation -



Confirmation of expression by qPCR -

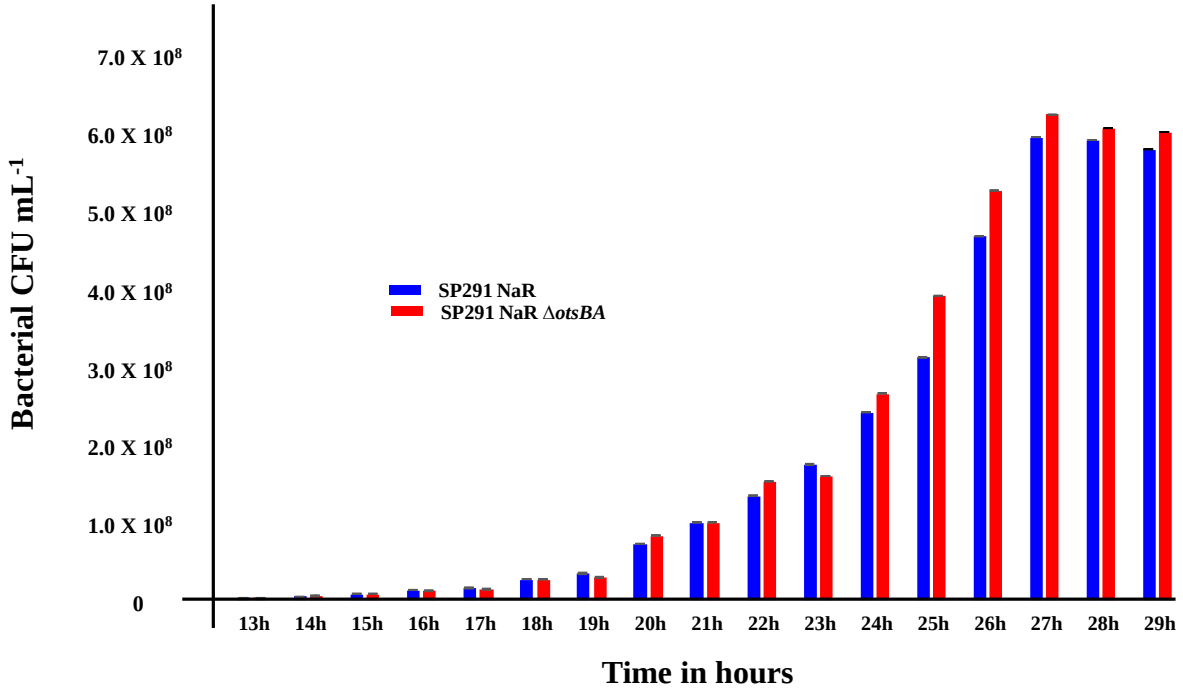


Physiological role of trehalose in desiccation



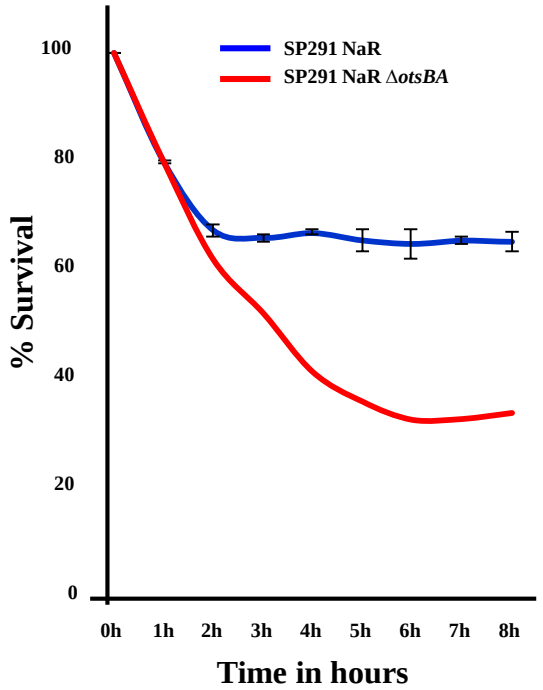
A

Growth in rich media



B

Desiccation Survival



Comparison between the desiccation curves of-

C. sakazakii ATCC™29544^T (*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)

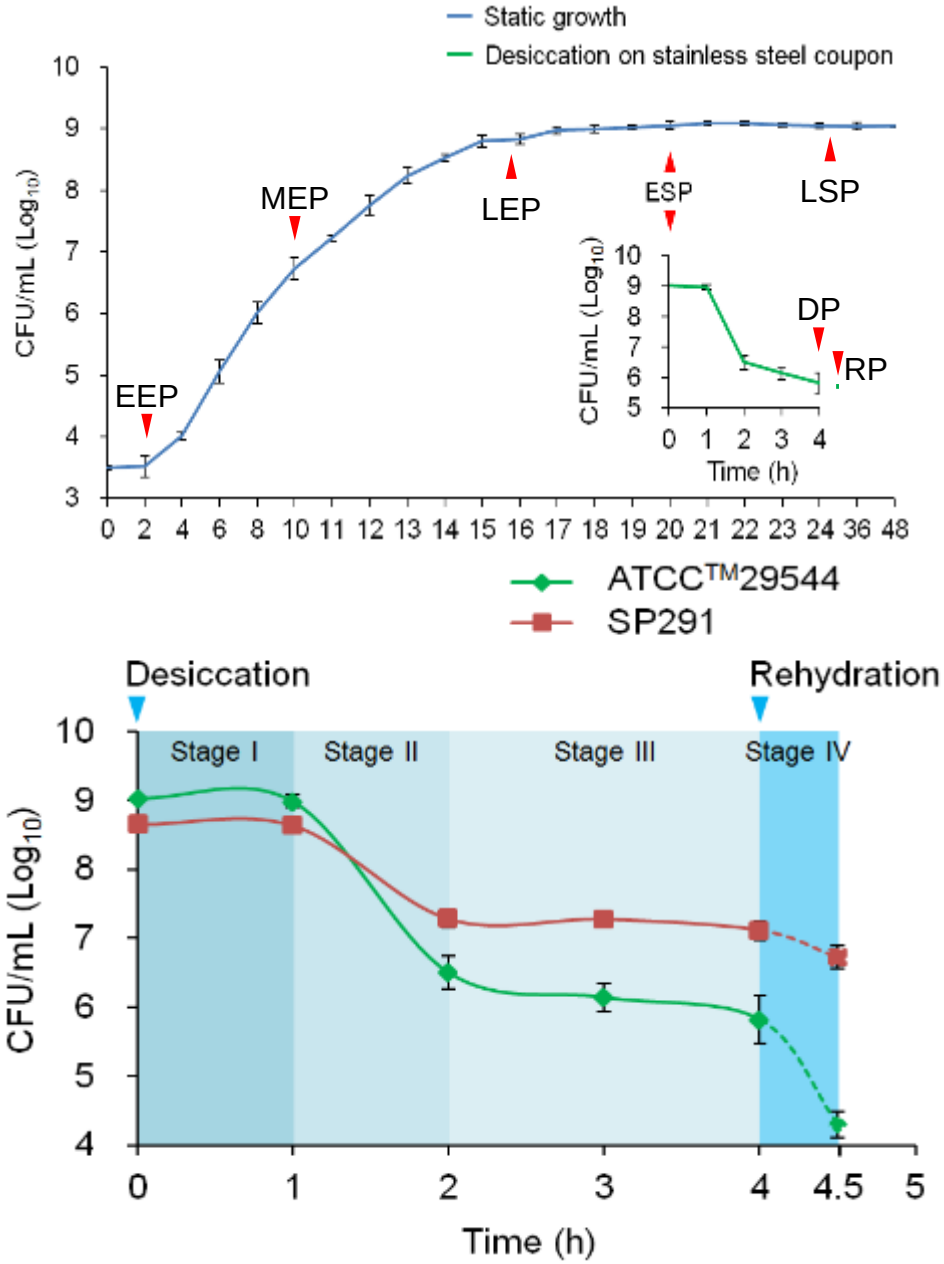
- liquid on the coupon evaporated completely
- a sharp reduction in the viable cell count (~2.5 $\log_{10}$ reduction in 1 h for ATCC™29544)

- Stage III (Desiccation 2 to 4 h)

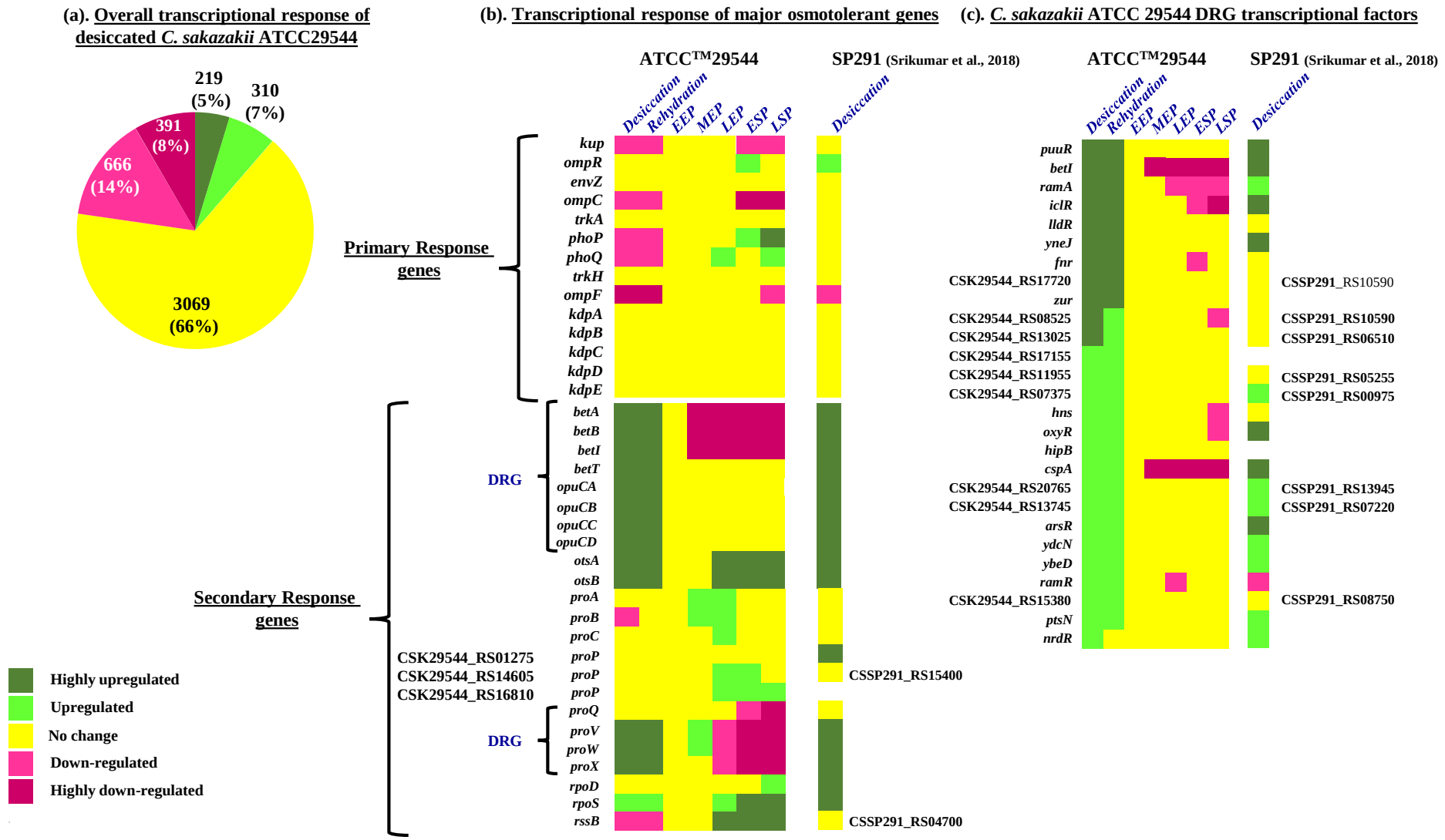
- bacteria were continuously desiccated on the coupon
- decrease in viable cell counts at a much slower rate (~1 $\log_{10}$ reduction in 2 h for ATCC™29544)

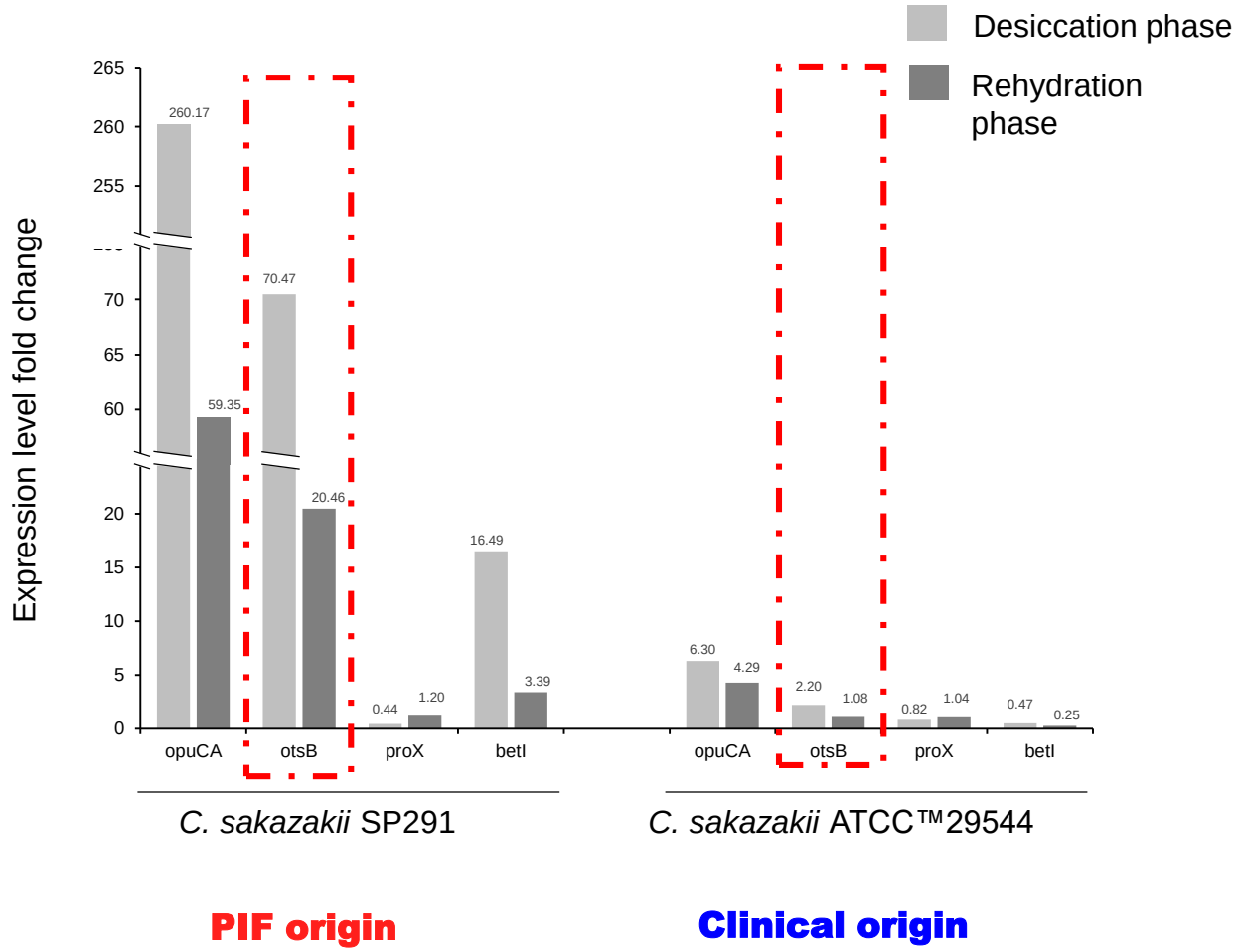
- Stage IV (Rehydration 0-30 min)

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

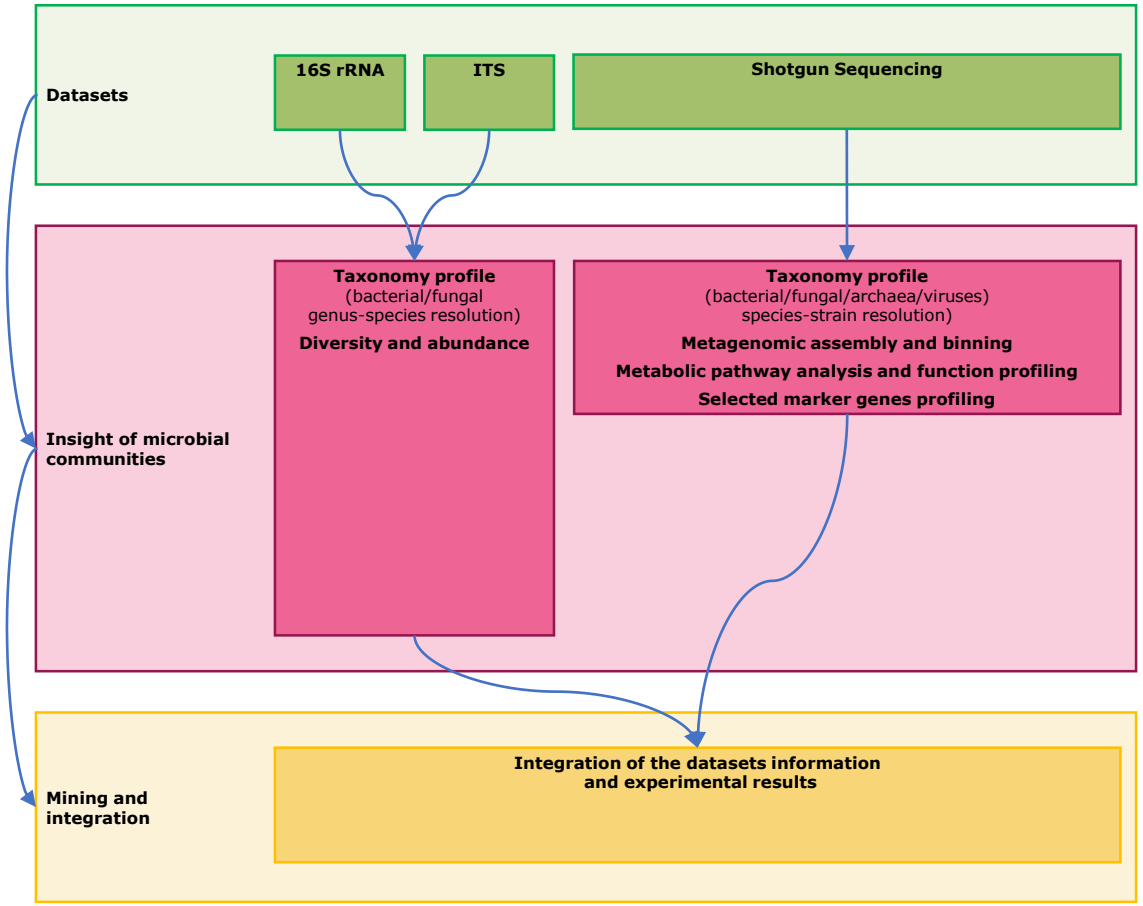


[Cao, *et al. Appl. Env. Microbiol*. (2021) 10.1128/AEM.00820-21]
[Srikumar, *et al. Appl. Env. Microbiol*. (2019) **85**: e01993-18]


(a). Overall transcriptional response of desiccated C. sakazakii ATCC29544
219 (5%)
310 (7%)
391 (8%)
666 (14%)
3069 (66%)
Highly upregulated
Upregulated
No change
Down-regulated
Highly down-regulated
(b). Transcriptional response of major osmotolerant genes
ATCC™29544
SP291 (Srikumar et al., 2018)
Desiccation
Rehydration
EEP
MEP
LEP
ESP
LSP
Primary Response genes
kup
ompR
envZ
ompC
trkA
phoP
phoQ
trkH
ompF
kdpA
kdpB
kdpC
kdpD
kdpE
Secondary Response genes
DRG
betA
betB
betI
betT
opuCA
opuCB
opuCC
opuCD
otsA
otsB
proA
proB
proC
CSK29544_RS01275
CSK29544_RS14605
CSK29544_RS16810
proP
proP
proP
proQ
DRG
proV
proW
proX
rpoD
rpoS
rssB
CSSP291_RS15400
CSSP291_RS04700
(c). C. sakazakii ATCC 29544 DRG transcriptional factors
ATCC™29544
SP291 (Srikumar et al., 2018)
Desiccation
Rehydration
EEP
MEP
LEP
ESP
LSP
puuR
betI
ramA
iclR
lldR
yneJ
fnr
CSK29544_RS17720
zur
CSK29544_RS08525
CSK29544_RS13025
CSK29544_RS17155
CSK29544_RS11955
CSK29544_RS07375
hns
oxyR
hipB
cspA
CSK29544_RS20765
CSK29544_RS13745
arsR
ydcN
ybeD
ramR
CSK29544_RS15380
ptsN
nrdR
CSSP291_RS10590
CSSP291_RS10590
CSSP291_RS06510
CSSP291_RS05255
CSSP291_RS00975
CSSP291_RS13945
CSSP291_RS07220
CSSP291_RS08750

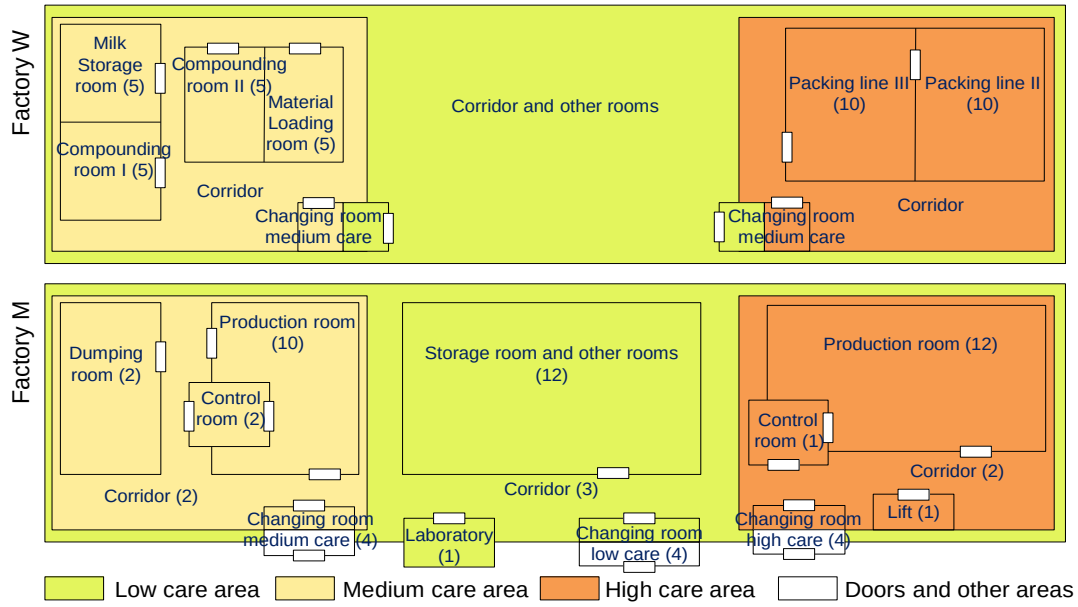

Desiccation phase
Rehydration phase
Expression level fold change
265
260
255
70
65
60
20
15
10
5
0
260.17
59.35
70.47
20.46
0.44
1.20
16.49
3.39
6.30
4.29
2.20
1.08
0.82
1.04
0.47
0.25
opuCA
otsB
proX
betI
opuCA
otsB
proX
betI
C. sakazakii SP291
C. sakazakii ATCC™29544
PIF origin
Clinical origin

16S rRNA sequencing/metagenomics -

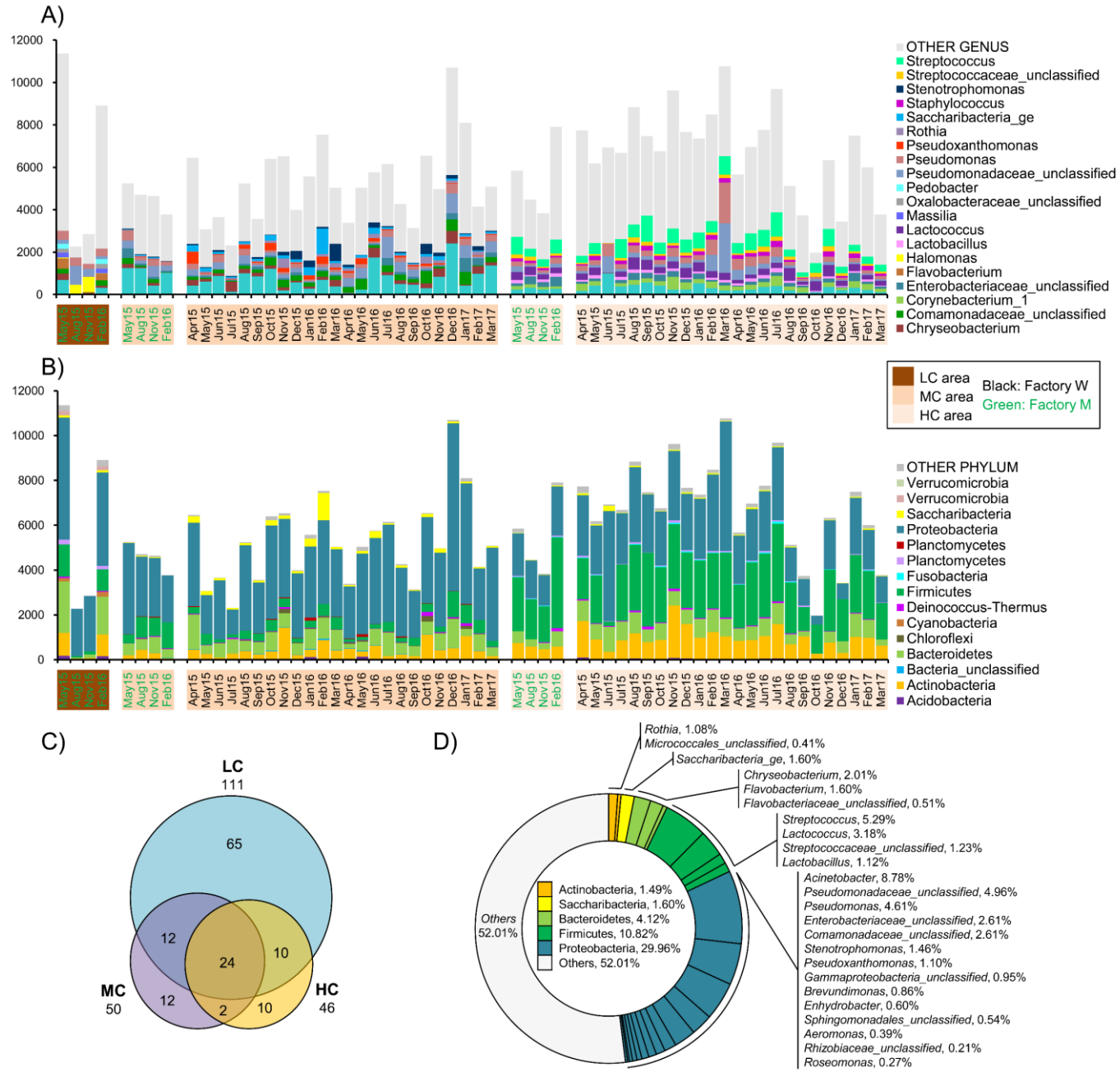


Overall strategy for sequencing a food/environmental sample containing multiple microorganisms

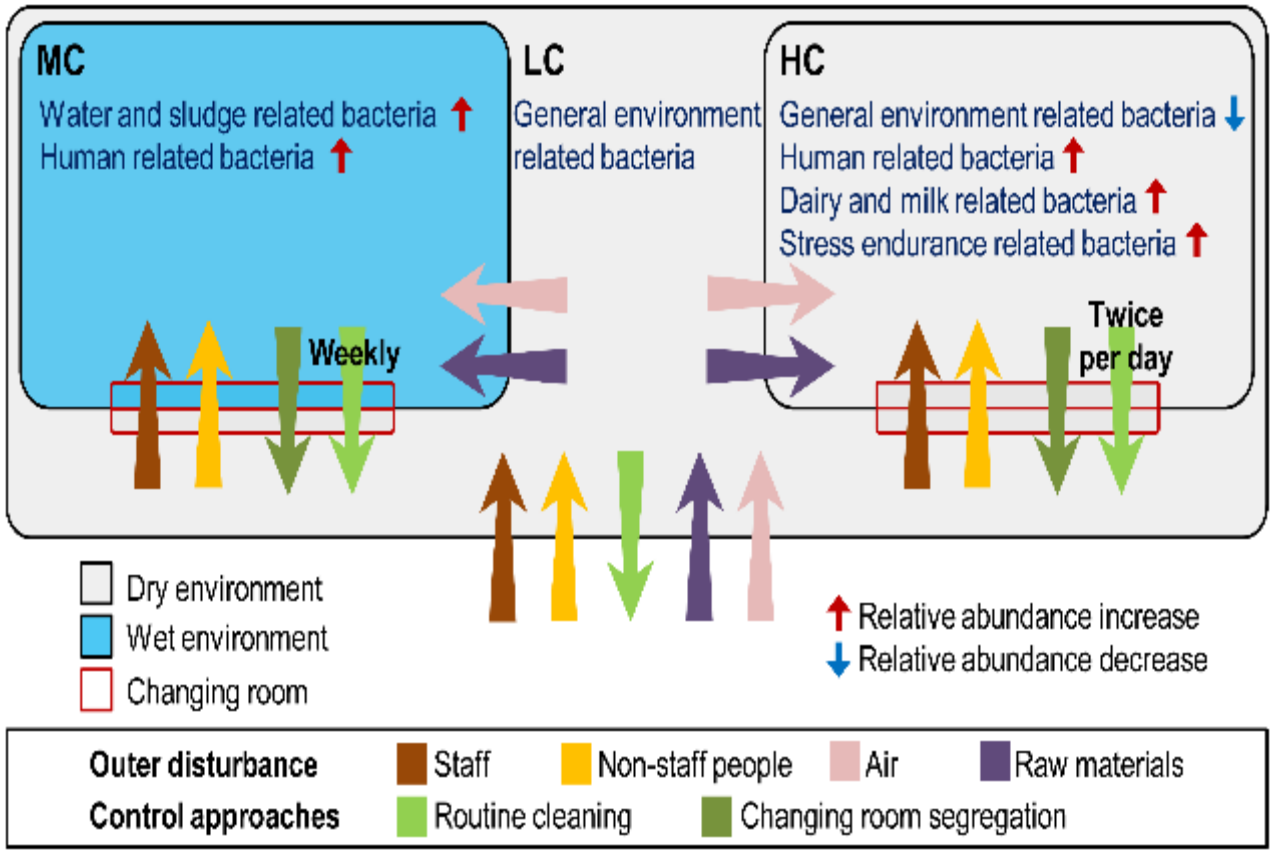
[Bourdichon, *et al.*, (2021) *Int. J. Food Microbiol*. doi: 10.1016/j.ijfoodmicro.2021.109351]



[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, 3.18%
Streptococcaceae_unclassified, 1.23%
Lactobacillus, 1.12%
Acinetobacter, 8.78%
Pseudomonadaceae_unclassified, 4.96%
Pseudomonas, 4.61%
Enterobacteriaceae_unclassified, 2.61%
Comamonadaceae_unclassified, 2.61%
Stenotrophomonas, 1.46%
Pseudoxanthomonas, 1.10%
Gammaproteobacteria_unclassified, 0.95%
Brevundimonas, 0.86%
Enhydrobacter, 0.60%
Sphingomonadales_unclassified, 0.54%
Aeromonas, 0.39%
Rhizobiaceae_unclassified, 0.21%
Roseomonas, 0.27%
Others
52.01%
Actinobacteria, 1.49%
Saccharibacteria, 1.60%
Bacteroidetes, 4.12%
Firmicutes, 10.82%
Proteobacteria, 29.96%
Others, 52.01%

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



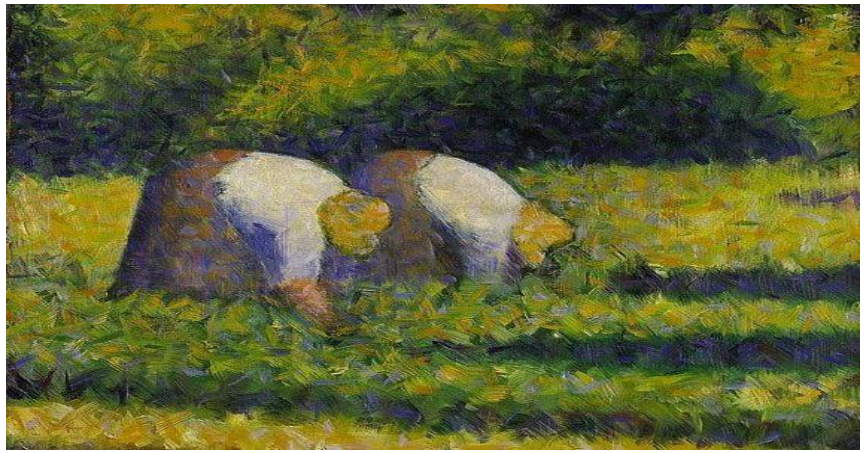
Conclusions -

- understanding the **microbial ecology** of a food production facility is essential

- **differentiating persistent from non-persistent isolates** recovered and based on their phenotype is ***important to support food safety controls***

- modern technological advances, 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 detection and ***subsequent risk reduction***

Thank you



Round table discussion

- Please ask your questions in the question box, not in the common chat – thank you.

Thank you for your attendance!

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