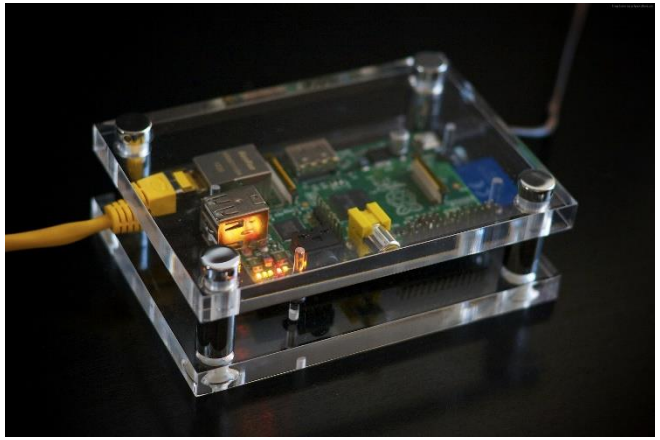
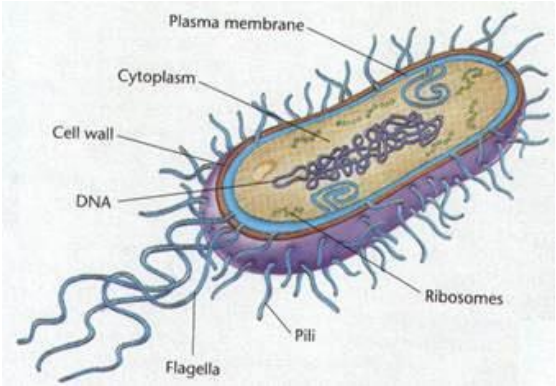
Bacteriocins applications for the control of microbial contaminants

Dr Philippe Gabant
Co-founder & CSO
pgabant@syngulon.com

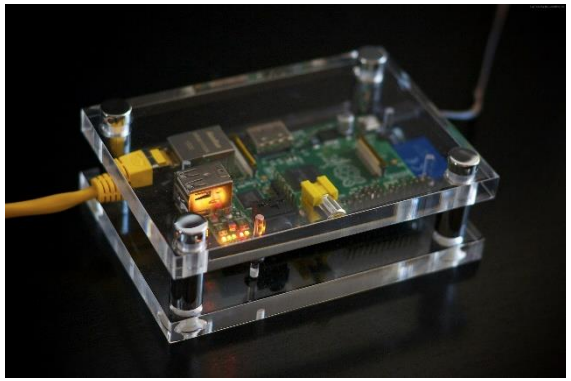
Guy Hélin
Co-founder & CEO
ghelin@syngulon.com

Synthetic Biology: industrialization concept “IT versus genes”

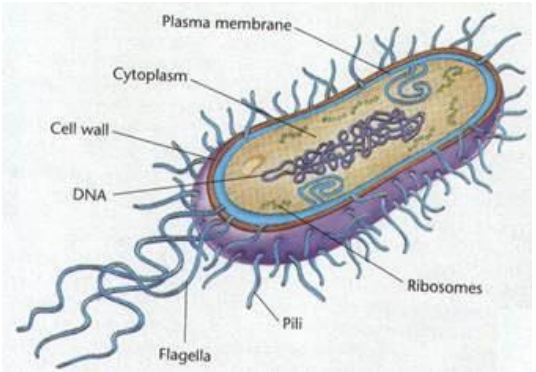




Synthetic Biology: Industrialization concept “IT versus genes”



“Blank” chassis
Constructed by modules (parts)
Behavior code based
Non self replicative
Possible contamination by external code



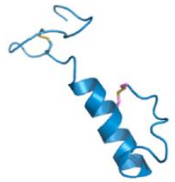
“Evolutionary” based chassis
Constructed by modules (parts)
Behavior code based
Self coding and self replicative
Possible contamination by external code

Similarities with IT exists but fundamental differences

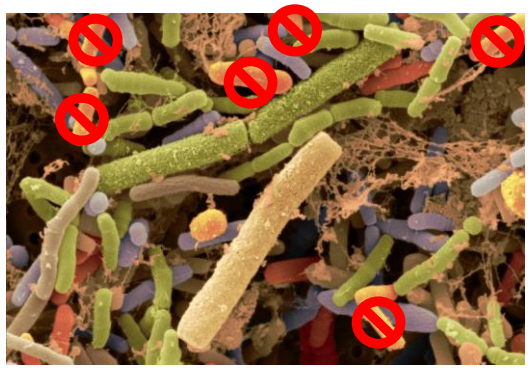
What are bacteriocins? Why use them?

- Discovered in 1925 by Belgian scientist: "**André Gratia** (1893–1950): Forgotten Pioneer of Research into Antimicrobial Agents"
- Heterogenous group of **antimicrobial peptides** produced **ribosomally** by **bacteria**
- Used to **kill related species** to **reduce competition** for resources and space
- **Not toxic**
- Small peptides that in many cases do not undergo post-translational modification to be active = **Ideal for gene-based peptide engineering**[1]
- Active against **antibiotic-resistant bacteria** (VRE, MRSA…)[2]
- Antimicrobial activity at the **nanomolar range scale** (more active than most antibiotics)[2]
- Naturally produced by **probiotic** strains
- **Nisin** is a bacteriocin widely used in the **food industry**
- Availability of broad and **narrow spectrum** of activity

Pediocin PA-1 3



André Gratia



Scimat/Science Photo Library

[1]Cotter et al., Nat. Rev. Microbiol., 2013
[2]Mathur et al., *Front. Microbiol.*, 2017
[3]Oppegård et al., *Biochemistry*, 2015

Increasing Interest in Bacteriocins

The **potential** for **bacteriocins** has been known for many years

Genetic technologies have not yet allowed their widespread use

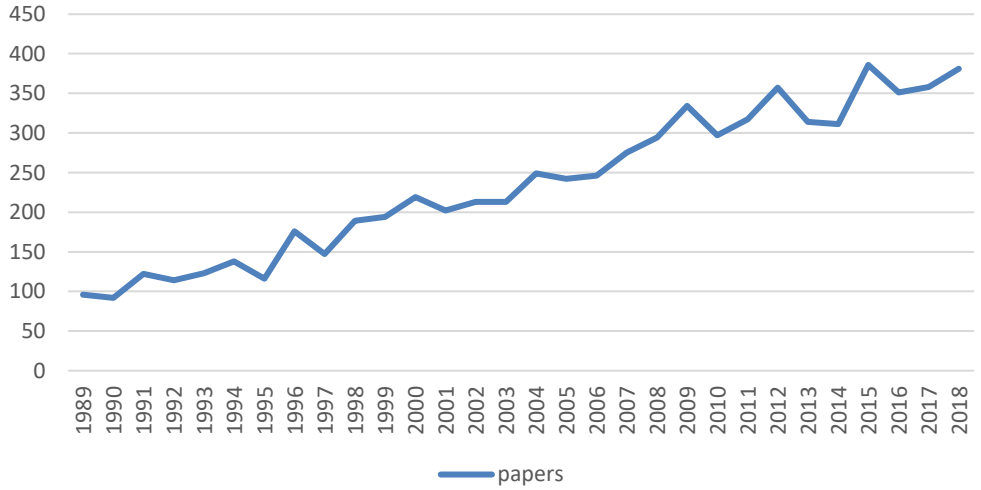
> **800 bacteriocin loci** have been identified

Improvements in **technology** allows their **increased study** and **application**

We use **synthetic biology** to **organise**, **analyse**, and **develop tools** for these purposes

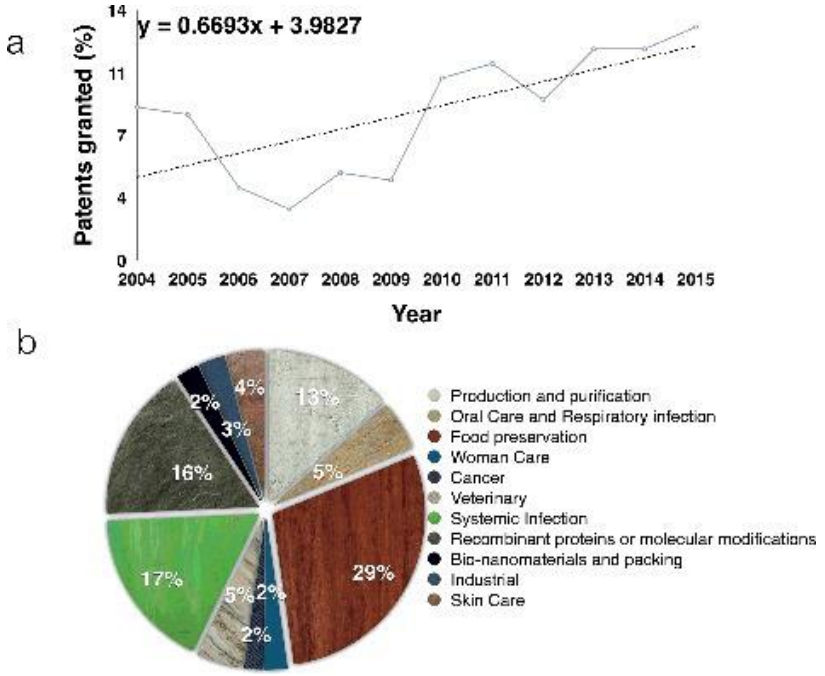
Syngulon has **5 patent application families** related to **bacteriocins**

Bacteriocin papers in the last 30 years (Pubmed)



9379 Papers Total
> 1/day in 2018

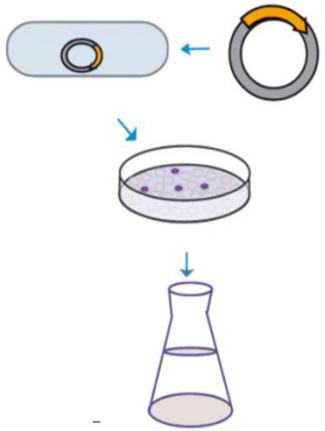
Bacteriocin-related patents issued 2004 - 2015



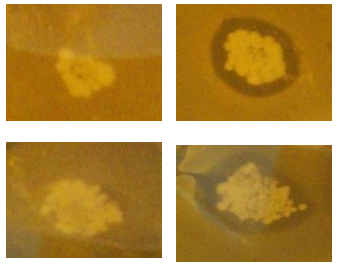
Lopez-Cuellar et al., *Biotech and Biotech Equip.*, 2016

How can bacteriocins be produced?

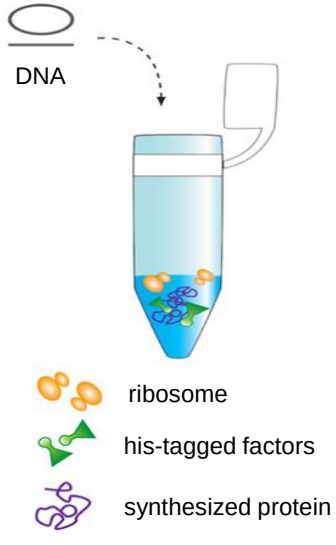
***In vivo* synthesis**



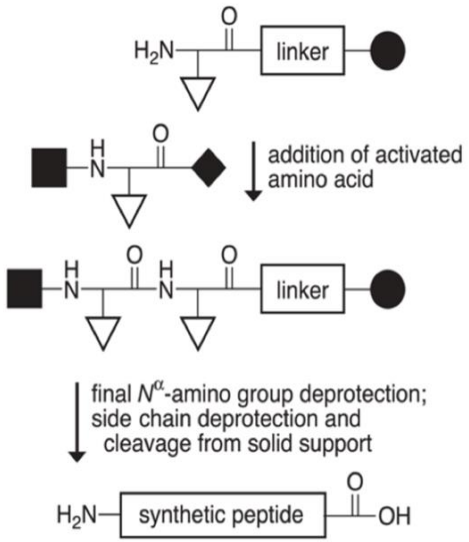
Heterologous production of bacteriocins by bacteria and yeast[4]

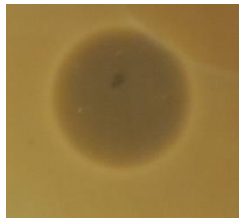


***In vitro* synthesis**



Chemical synthesis





MUCH FASTER AND LESS LABOUR INTENSIVE

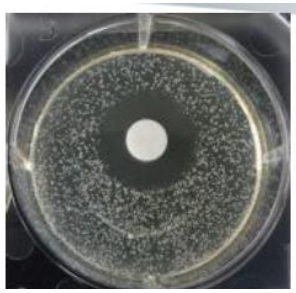
NO ACCESORY OR IMMUNITY GENES NEEDED - ALLOWING THE PRODUCTION OF PEPTIDES TOXIC FOR *IN VIVO* SYNTHESIS

CHEAPER AND MORE VERSATILE THAN CHEMICAL SYNTHESIS ALLOWING THE PRODUCTION OF DIFFERENT BACTERIOCINS IN ONE DAY

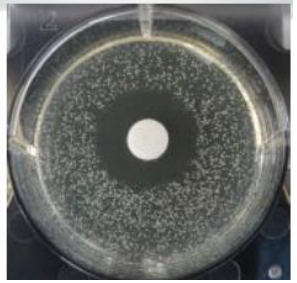
Borrero et al.: *Appl. Microbiol. Biotechnol.*, 2011, 2012 and 2017 / *J. Biotechnol.*, 2011 / *ACS. Synth. Biol.*, 2013 and 2015

Hols et al.: *Trends in Microbiology.*, April 2019

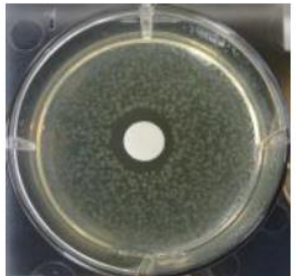
Bacteriocin csEntL50A efficiency



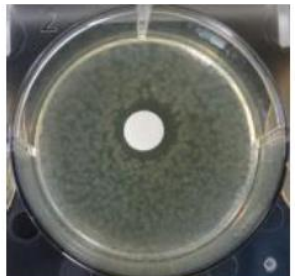
P.acnes ATCC 10390



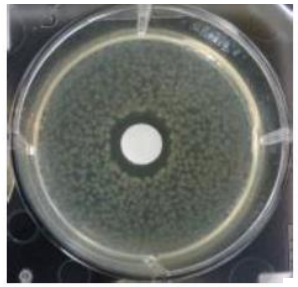
P.acnes ADO36



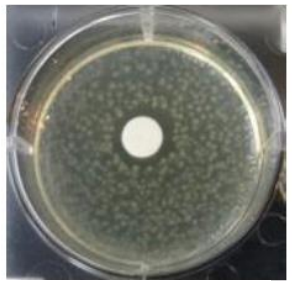
C. difficile DSMZ 1296



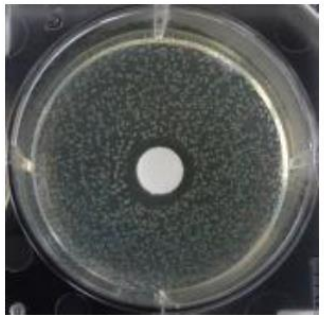
C. difficile 13.22



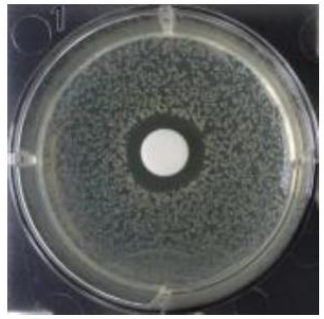
C. perfringens ATCC 13124



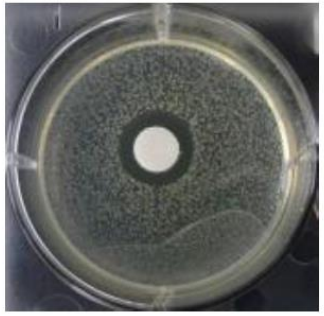
C. perfringens Clinical strain



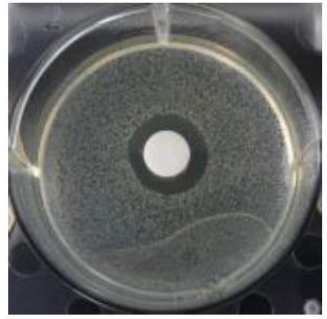
E. faecium ATCC33667



E. faecium 87.71

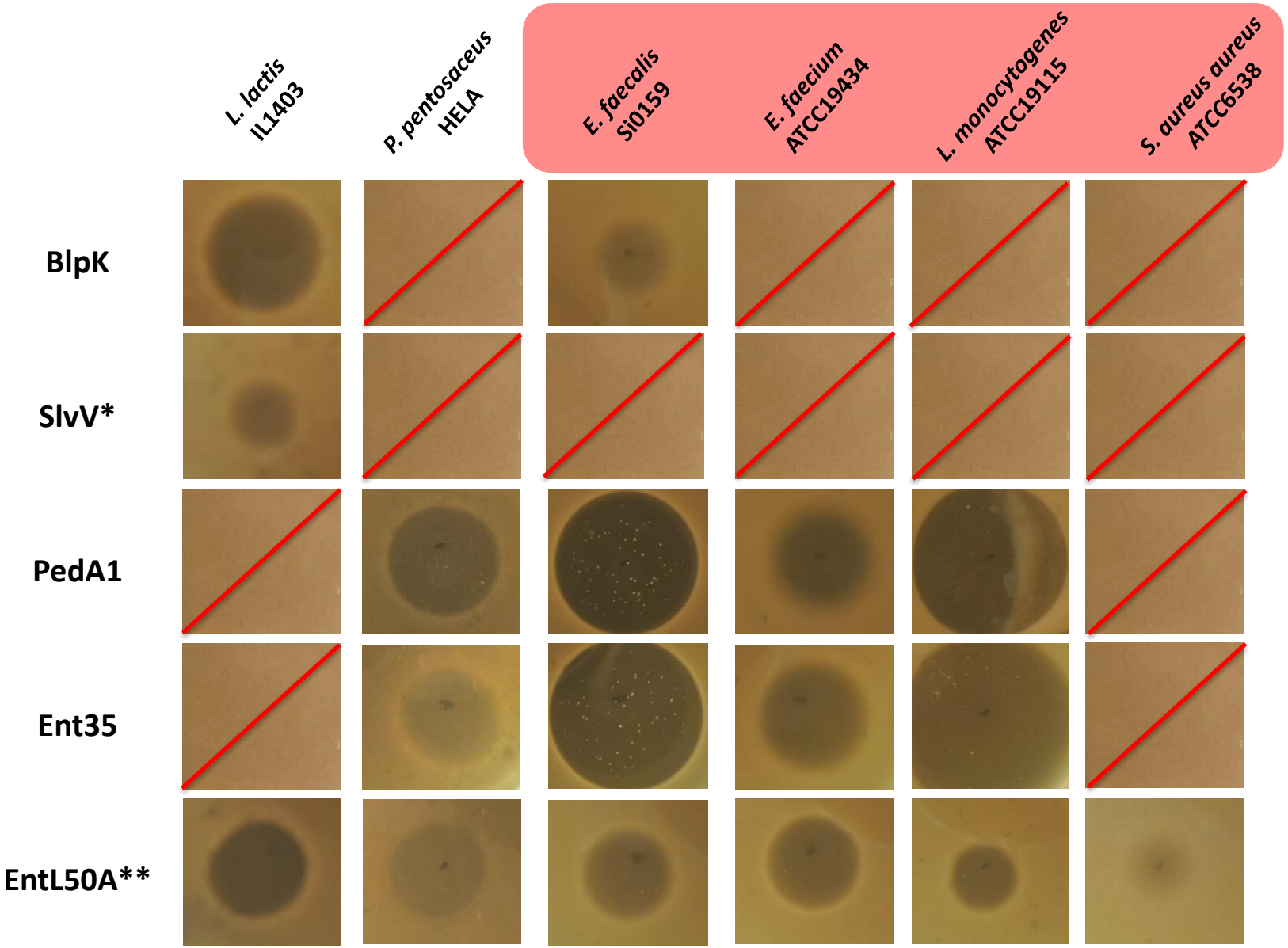


L. monocytogenes BAA679



L. monocytogenes 084PAB

Large diversity of bacteriocin targets and specificity



PARAGEN 1.0 collection

frontiers in Bioengineering and Biotechnology

BRIEF RESEARCH REPORT
published: 06 September 2019
doi: 10.3389/fbioe.2019.00213

PARAGEN 1.0: A Standardized Synthetic Gene Library for Fast Cell-Free Bacteriocin Synthesis

Philippe Gabant and Juan Borrero†*

Syngulon, Seraing, Belgium

OPEN ACCESS

Edited by:
Jean Marie François, UMS3582 Toulouse White Biotechnology (TWB), France

Reviewed by:
Dzung B. Diep, Norwegian University of Life Sciences, Norway
Dae-Hee Lee, Korea Research Institute of Bioscience and Biotechnology (KRIBB), South Korea

****Correspondence:***
Philippe Gabant
pgabant@syngulon.com

†Present address:
Juan Borrero, Sección Departamental de Nutrición y Ciencia de los Alimentos (Facultad de Veterinaria), Universidad Complutense de Madrid, Madrid, Spain

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The continuous emergence of microbial resistance to our antibiotic arsenal is widely becoming recognized as an imminent threat to global human health. Bacteriocins are antimicrobial peptides currently under consideration as real alternatives or complements to common antibiotics. These peptides have been much studied, novel bacteriocins are regularly reported and several genomic databases on these peptides are currently updated. Despite this, to our knowledge, a physical collection of bacteriocins that would allow testing and comparing them for different applications does not exist. Rapid advances in synthetic biology in combination with cell-free protein synthesis technologies offer great potential for fast protein production. Based on the amino acid sequences of the mature peptide available in different databases, we have built a bacteriocin gene library, called PARAGEN 1.0, containing all the genetic elements required for *in vitro* cell-free peptide synthesis. Using PARAGEN 1.0 and a commercial kit for cell-free protein synthesis we have produced 164 different bacteriocins. Of the bacteriocins synthesized, 54% have shown antimicrobial activity against at least one of the indicator strains tested, including Gram-positive and Gram-negative bacteria representing commonly used lab strains, industrially relevant microorganisms, and known pathogens. This bacteriocin collection represents a streamlined pipeline for selection, production, and screening of bacteriocins as well as a reservoir of ready-to-use antimicrobials against virtually any class of relevant bacteria.

Keywords: antimicrobial peptides, bacteriocins, cell-free extracts, *in vitro* synthesis, synthetic biology

Improving industrial fermentation: An R&D case study

Brussels, November 7, 2019

World Ethanol & Biofuels

Elsa Parmentier
R&D project manager
Tereos
elsa.parmentier@tereos.com

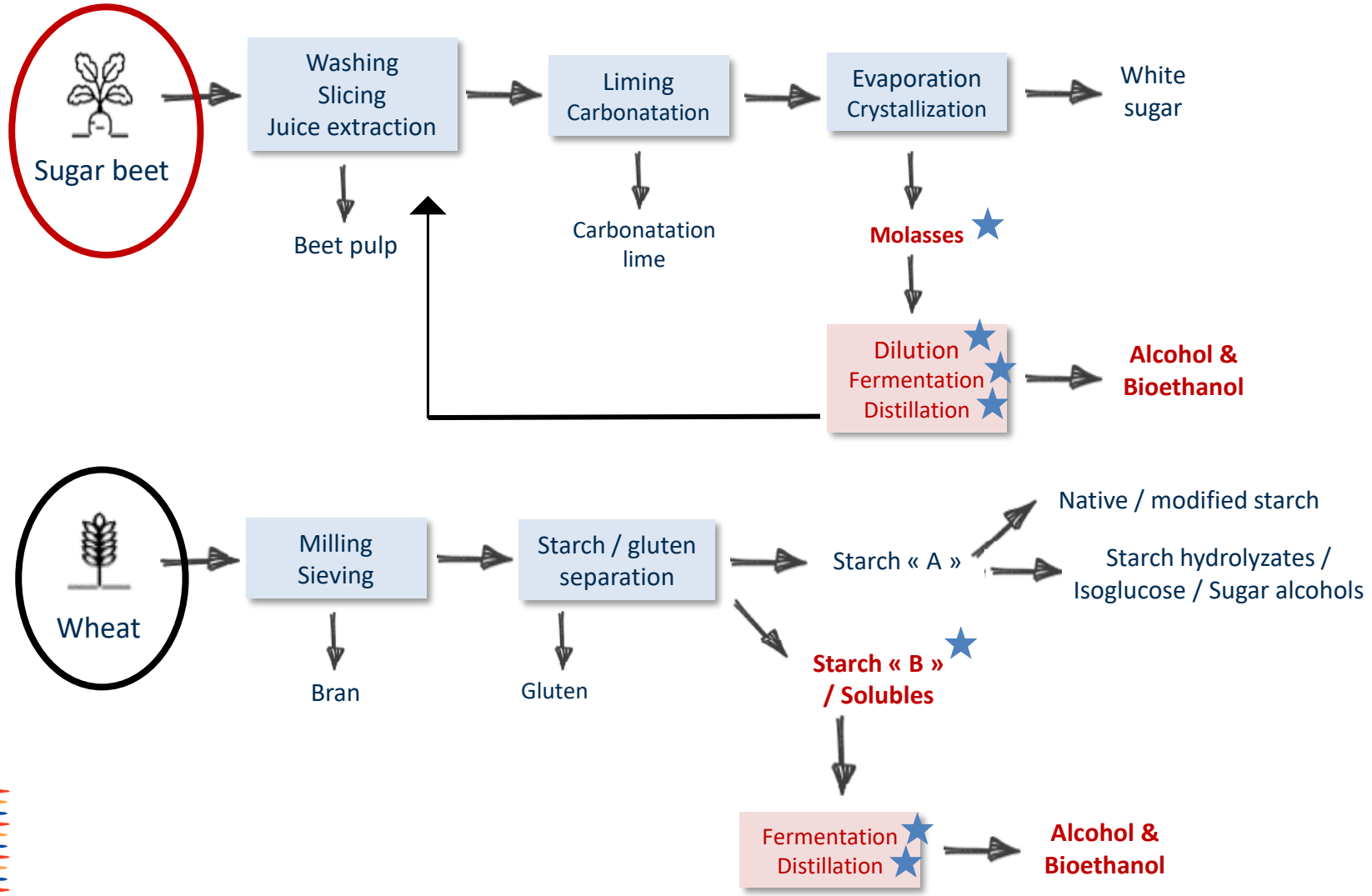
Guy Hélin
Co-founder & CEO
Syngulon
ghelin@syngulon.com

R&D partnership to develop genetic control for the improvement of industrial fermentation

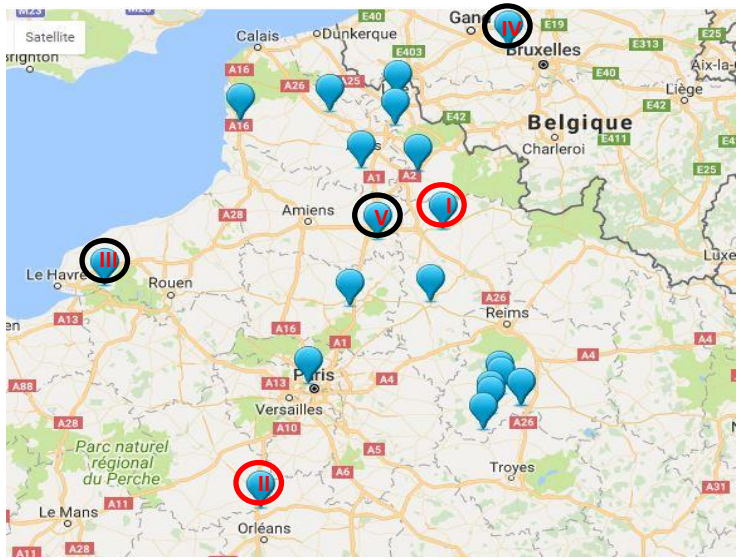
- 3 year R&D project with support of Walloon region of Belgium
- **Purpose:** development of a firewall against contaminants in ethanol production
- Research based on metagenomic analysis of industrial samples from ethanol production based on sugar beet and starch co-products
- Syngulon is working with Tereos, first largest ethanol producer in EU

- (1) Tereos provides samples from **5 different industrial sites** over a one year period
- (2) Syngulon performs **metagenomics analysis**
- (3) Syngulon develops **bacteriocin cocktails** to fight contaminants
- (4) Tereos/Syngulon: test of solutions in R&D lab
- (5) Tereos: test of solutions approved by R&D and regulatory in industrial sites

Ethanol production based on sugar beet and starch co-products



5 industrial plants chosen for the metagenomics analysis in EU



Samples taken weekly over a period of 1 year:

- Substrates
- Yeast propagation step
- Ethanol fermentation: middle + end
- Distillation still bottoms

Microbial contamination basics

Bacterial growth: 4-6 times faster than Saccharmoyces cerevisae

	Growth rate	Propagation time	Generation	Multiplication
Yeast	2 hours	8h	4	16
Bacteria	0.5 hour	8h	16	65.536

X 4.000

- Substrate Competition-Redirection: less sugar & nutrients for selected yeast
- Organic Acids Production: loss in EtOH yield & stress for the yeast

Common rules:

- Lactic acid inhibition > 0.2% w/w
- Acetic acid inhibition > 0.05% w/w

Effect of microbial contamination on yield:

from less than 1% to more than 5%

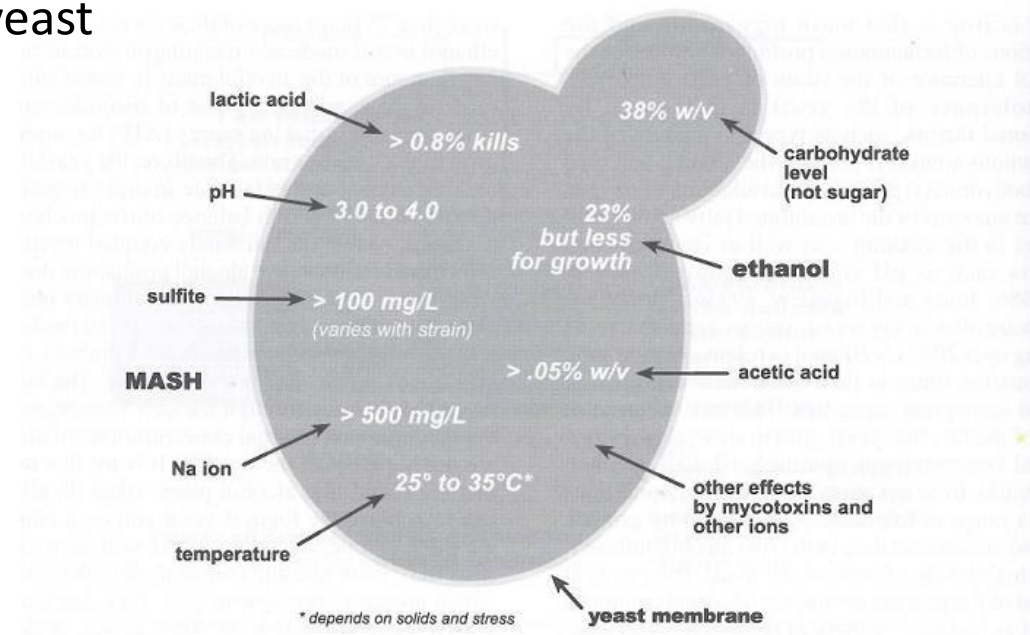
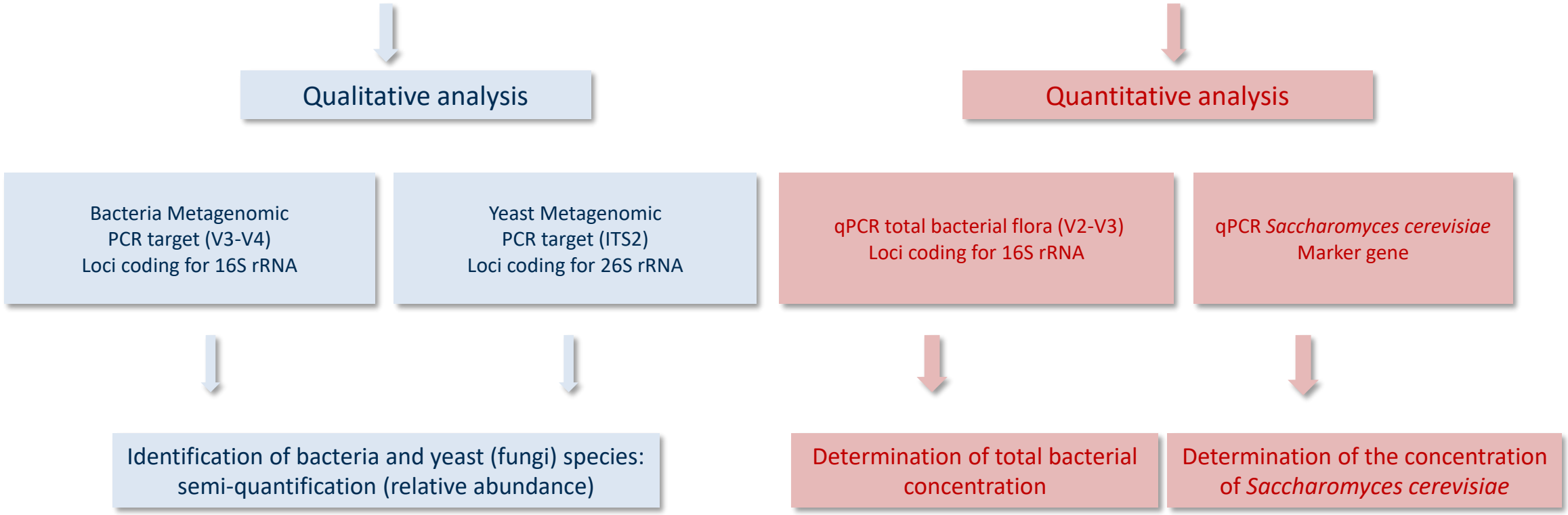


Figure 3. Alarm levels of inhibitory chemicals that affect metabolism in yeast-catalysed fuel alcohol fermentations (Ingledew, 1999).

Metagenomics analyses

Sample DNA Extraction



Analyses performed

Biologicals analysis

Analysis of metabolites:

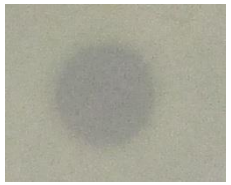
* Determination of the pool of amino acids and other metabolites (carbohydrates, ethanol, glycerol...) present in the medium

Yeast counting:

* Determination of the concentration and activity of S. cerevisiae (cell counting)

Bacteriocidal analysis :

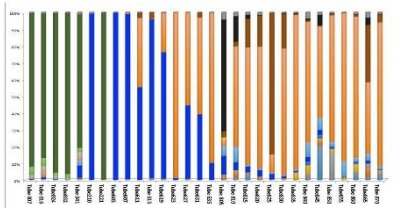
* Test the possible presence of bacteriocins in the fermentation medium



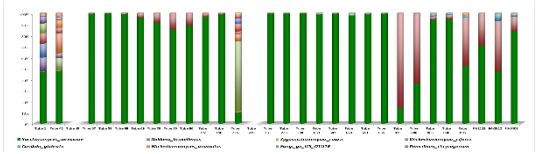
* Test the effect of bacteriocins on the fermentation strain

Metagenomics analysis

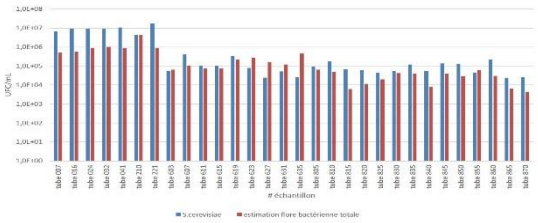
Proportion of bacterial species (%) in the different samples



Proportion of yeast (Saccharomyces cerevisiae / other yeast)



Proportion of yeast / total bacterial flora

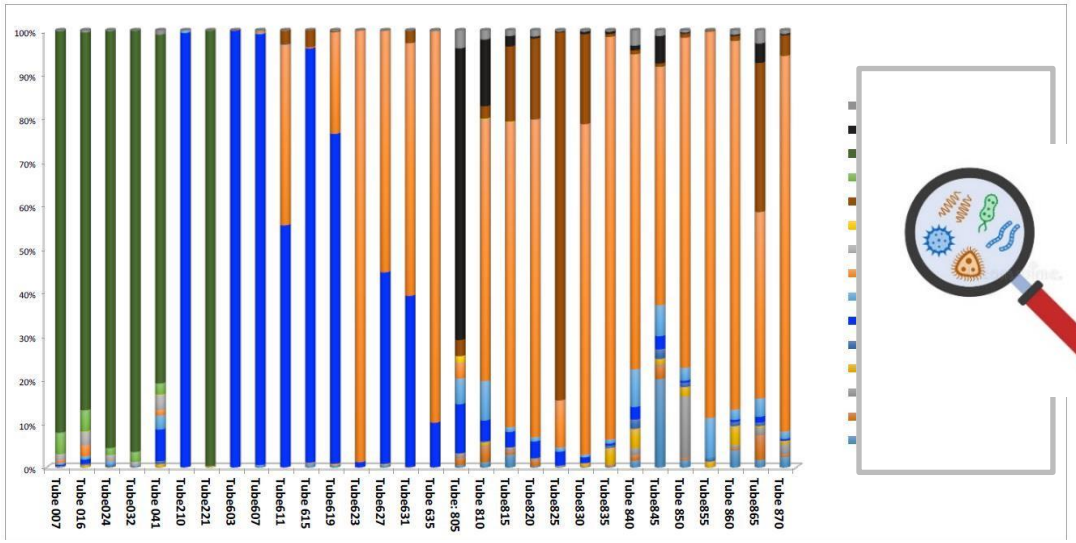


Genome sequencing

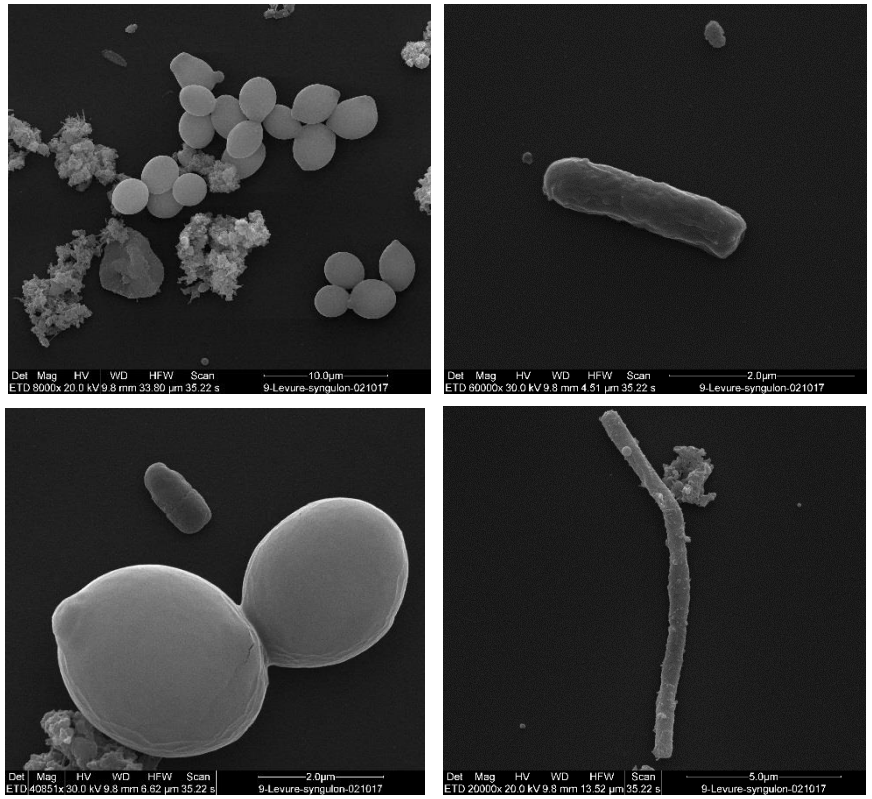
Qualitative analysis

Quantitative analysis

Results to date



Industrial ethanol fermentation samples – SEM analysis



Conclusions:

Simple microbial flora in fermentation whatever the location and the fermentation substrate (sugarbeet or starch based)

Main contaminant **lactic acid bacteria (LAB)** as described in literature for corn and sugarcane based substrate

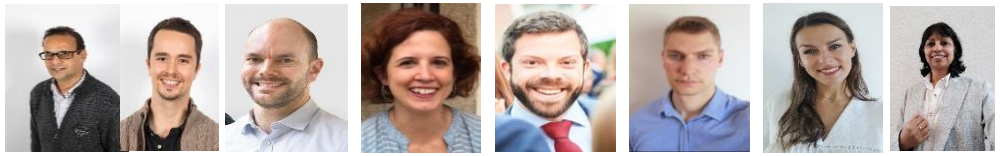
Contaminants causing issues for the storage of the molasses are **not** the same that develop in ethanol fermentation

Test of Bacteriocin solutions on the identified **contaminants that impact the fermentation performances** ongoing at lab scale

Team / SAB / R&D Partners

SYNGULON Team

Guy Hélin, Co-founder, CEO
Dr. Philippe Gabant, Co-Founder, CSO



Dr. Mohamed El Bakkoury, CTO Yeast
Thomas Gosset, Ir, R&D Engineer
Dr. Jason Bland, R&D Project Manager
Dr. Luz Perez, R&D Project Manager
Dr. Baptiste Dumont, R&D Project Manager
Félix Jaumaux, PhD Student
Anaïs Pagès, R&D Scientist
Dr. Anandi Martin, Senior Project Manager - Infectious Disease

Collaboration with:
Universidad Complutense Madrid (UCM)
Dr. Juan Borrero

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Pr Ruddy Wattiez, UMons, Mons (BE)

IN MEMORIAM
Dr Régis Sodoyer, ex-Sanofi Pasteur, Lyon (FR)

R&D Partners

Q & A

Dr Philippe Gabant
Co-founder & CSO
pgabant@syngulon.com

Guy Hélin
Co-founder & CEO
ghelin@syngulon.com