Department of Food Quality and Safety
Institute of Postharvest and Food Sciences
The Agricultural Research Organization The Volcani Center

Bacterial communities in food production – friend or foe?

Moshe Shemesh

ARO, 4.11.20

Outline

- Kinds of microorganisms in food
- Concept of food safety and quality
- Biofilm as a bacterial community
- Biofilm problem in dairy industry
- Signaling mechanism for biofilm formation
- Novel means to mitigate biofilm formation
- Using biofilm concept to protect on beneficial bacteria

History of civilization and food preservation

Pre-Leeuwenhook period

Food preservation as

an art

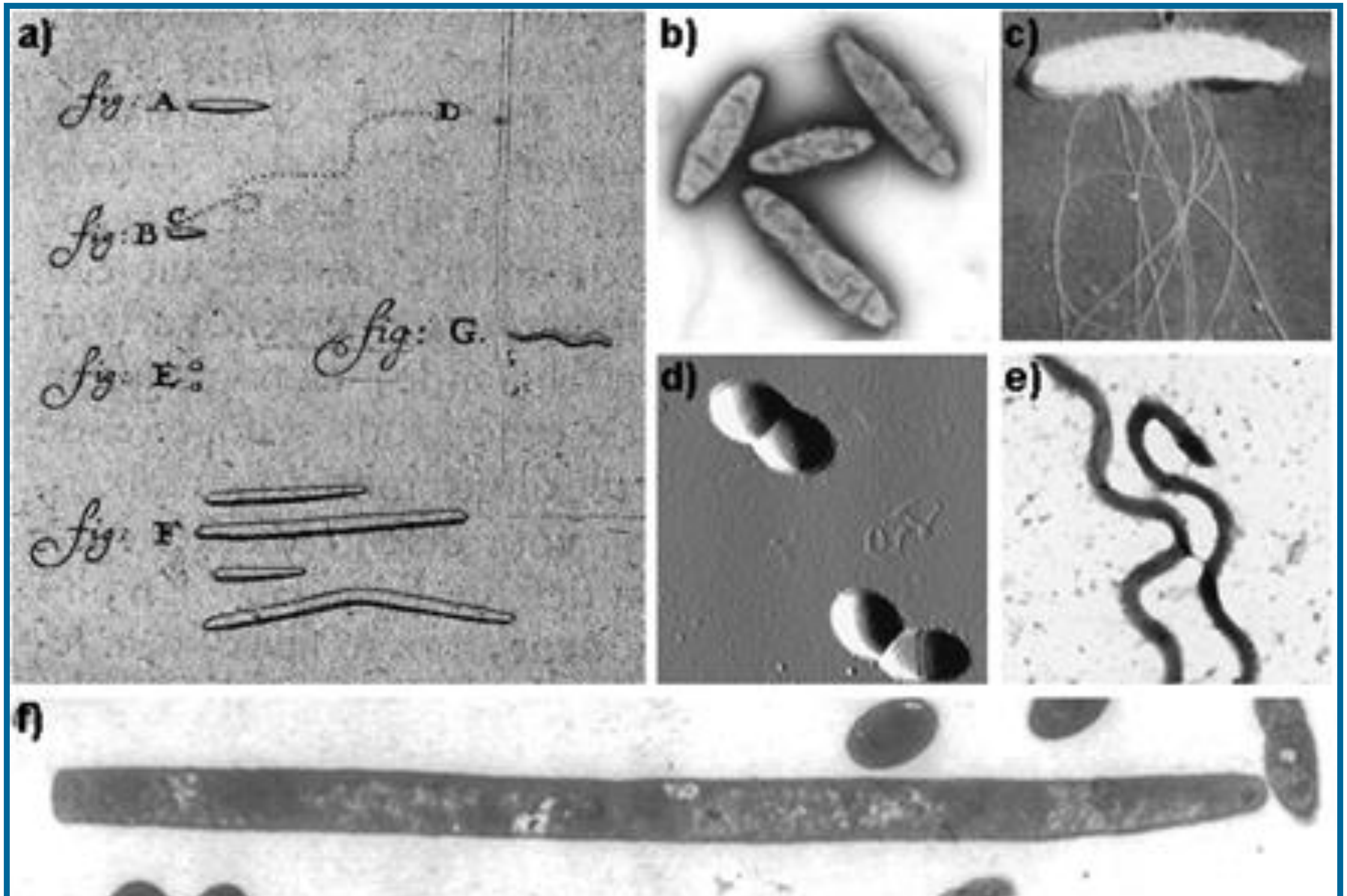
Post-Leeuwenhook period

Food microbiology as

a science

The science of food microbiology allows food to be processed, distributed and marketed with a high degree of confidence in product's quality and safety

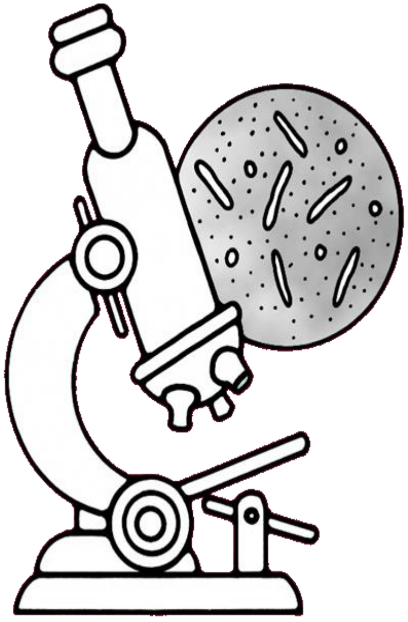
Dental plaque bacteria observed by Antoni van Leeuwenhoek and their contemporary equivalents



Kuramitsu *et al*. 2007

Food microbiology

- Microorganisms are small, living unicellular or multicellular.
- They include bacteria, viruses, yeasts, molds, and parasites.
- They can be....
  - —The good
  - —The bad, and
  - —The ugly!



The good (helpful) microorganisms

- Add them to foods or they are there naturally.
- They ferment foods to preserve them and/or create unique flavors and textures.
- Examples: cheese, yogurt, sour cream, bread, sauerkraut and pickles.

The bad (spoilage) microorganisms

– Change foods and cause them to “go bad” or spoil.

– Examples: Discolored, mushy, or fuzzy vegetables; sour milk; and slimy, putrid meat.

The ugly microorganisms (pathogenic)

- Illness can range from mild to life-threatening.
- Examples include foods contaminated with *Salmonella* or *Bacillus cereus*. Common signs and symptoms include nausea, vomiting, and diarrhea.

Microorganisms that cause:

Food spoilage -

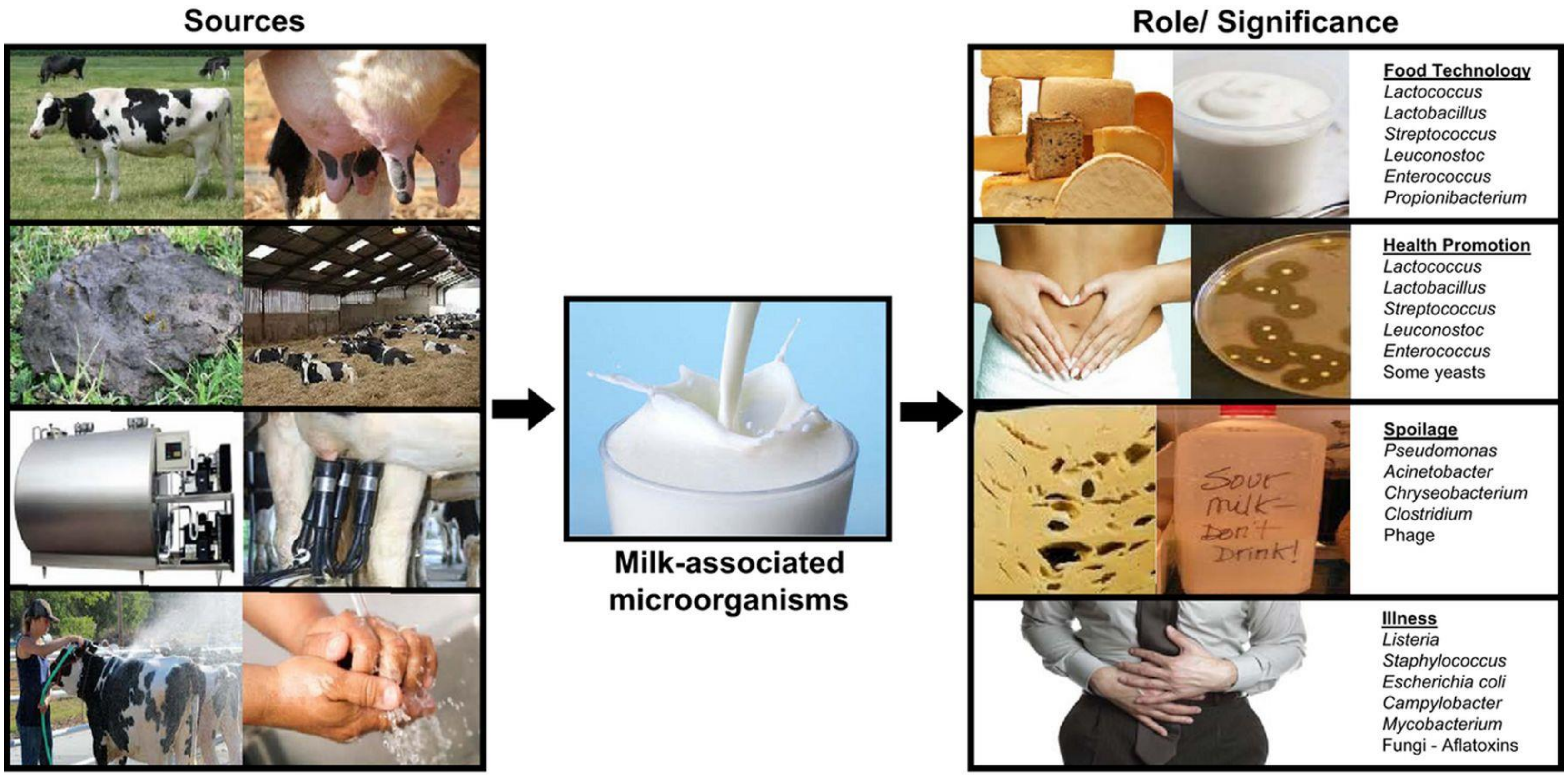
- **Affect aroma, texture and/or appearance of food**

Foodborne illness -

- **May or may not affect sensory characteristics of the food**

- **Only laboratory testing can tell if harmful microorganisms or toxins are present – some are difficult to detect or cannot be detected**
- **Bacteria are involved in greatest number of foodborne illnesses**

Potential sources of microorganisms that are present in raw milk and their effect on dairy products



Microorganisms can enter milk from contact with the animal including teat, hides, faeces; also from the housing, bedding, feed, air and water. Contact with farm equipment and milking equipment as well as insufficient farm or personnel hygiene may influence the microbial content of milk.

Once in the milk these microorganisms can play an important role in dairy product manufacture; they may contribute to promoting human health or enhancing food safety. On the other hand these microorganisms can lead to spoilage of milk and dairy products or they may contribute to disease and illness in humans.

Lisa Quigley et al. FEMS Microbiol Rev 2013;37:664-698

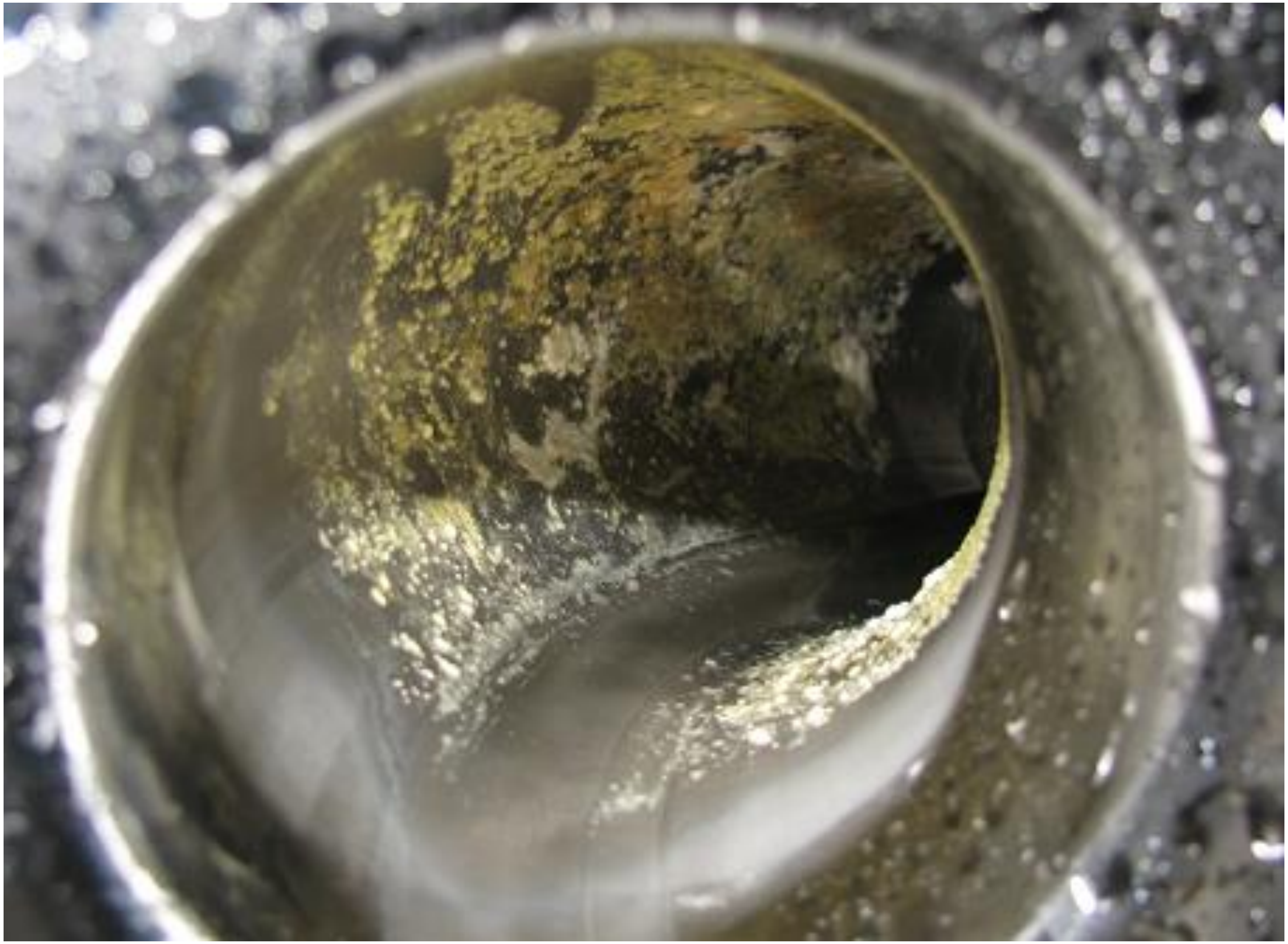
FEMS MICROBIOLOGY REVIEWS

Milking parlor at modern dairy farm

Milking machine

Dairy tank

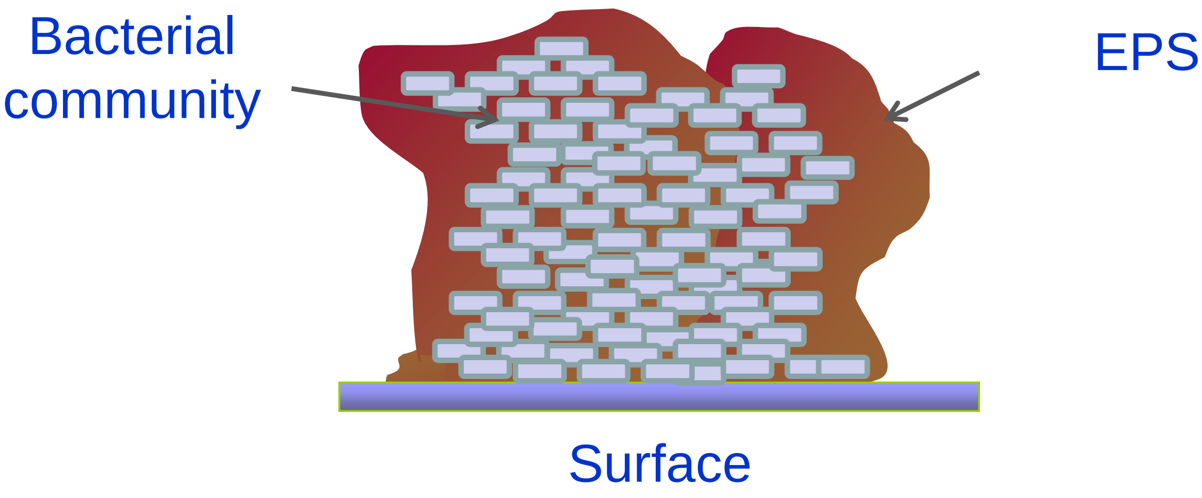
Contaminated surfaces in milking system



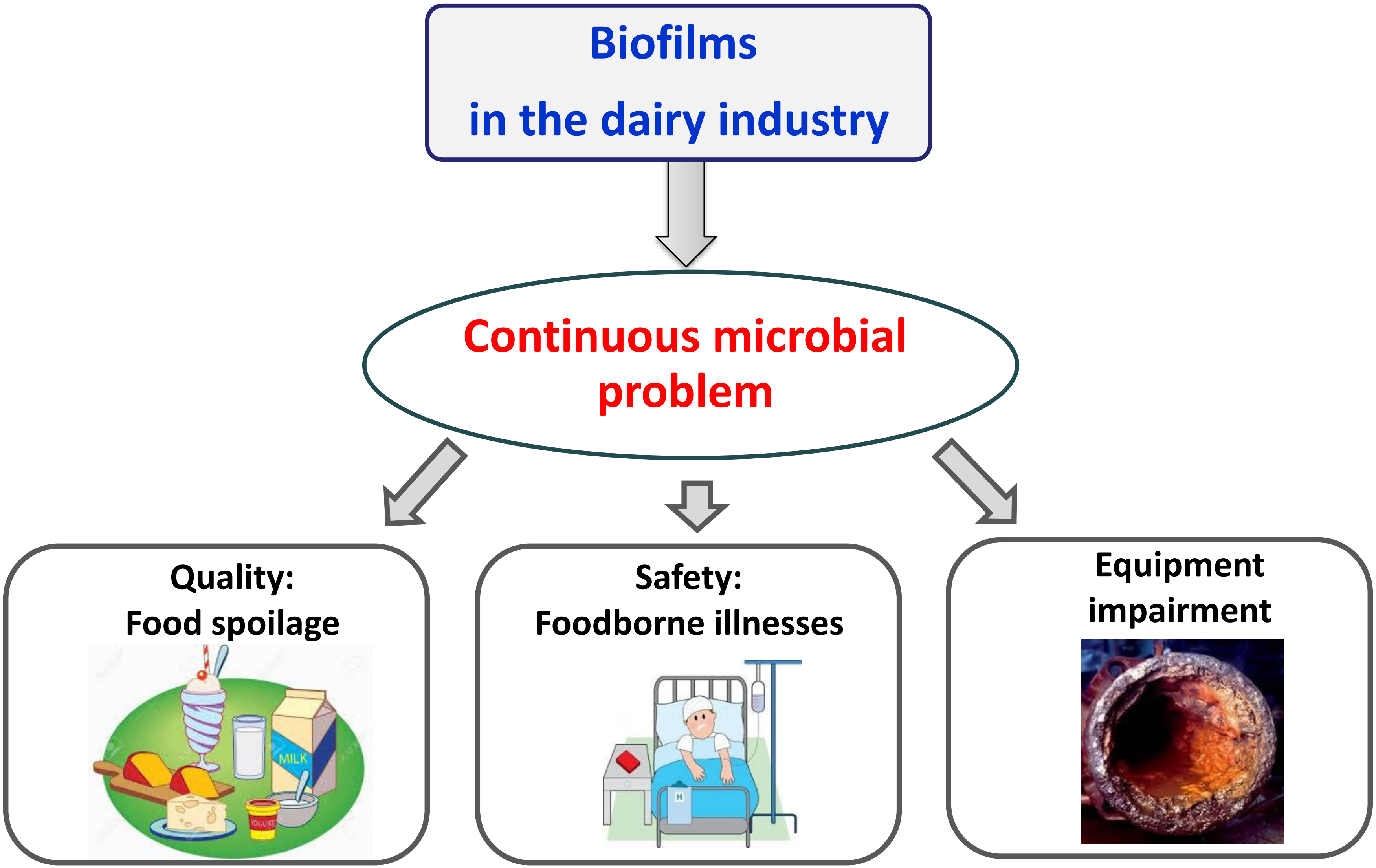
Contributed by Avraham Harel, Israel Dairy Board

In general biofilm can be defined as:

multicellular community encased within exopolymeric substances formed by bacteria

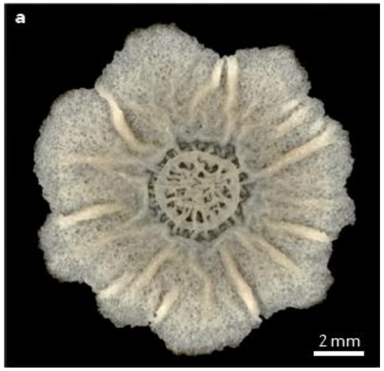


The EPS may provide extraordinary protection on biofilm bacteria against adverse environmental conditions


Biofilms
in the dairy industry
Continuous microbial problem
Quality:
Food spoilage
MILK
Safety:
Foodborne illnesses
Equipment impairment

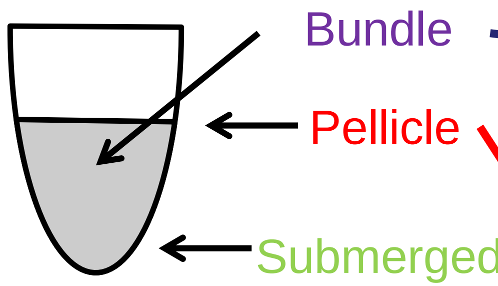
Types of biofilms

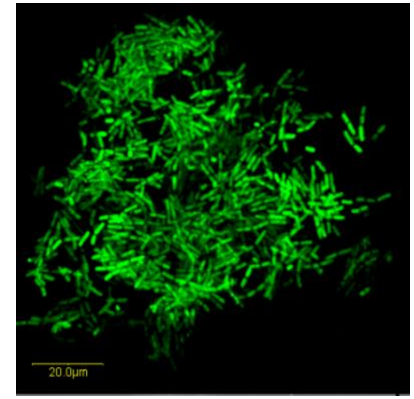
Solid



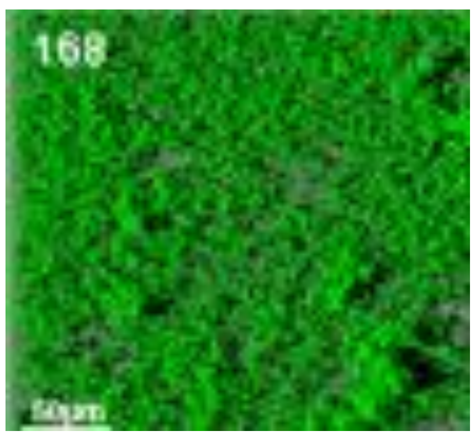
Vlamakis et al., Nat Rev Microbiol, 2013

Liquid

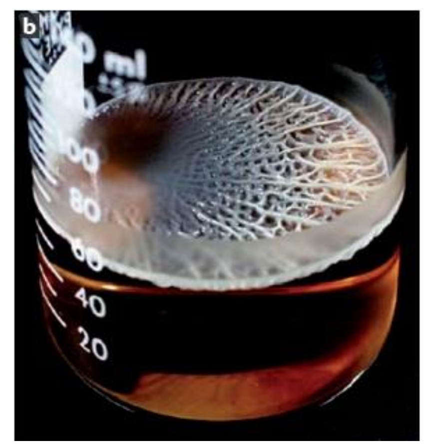




Pasvolsky et al., IJFM 2014

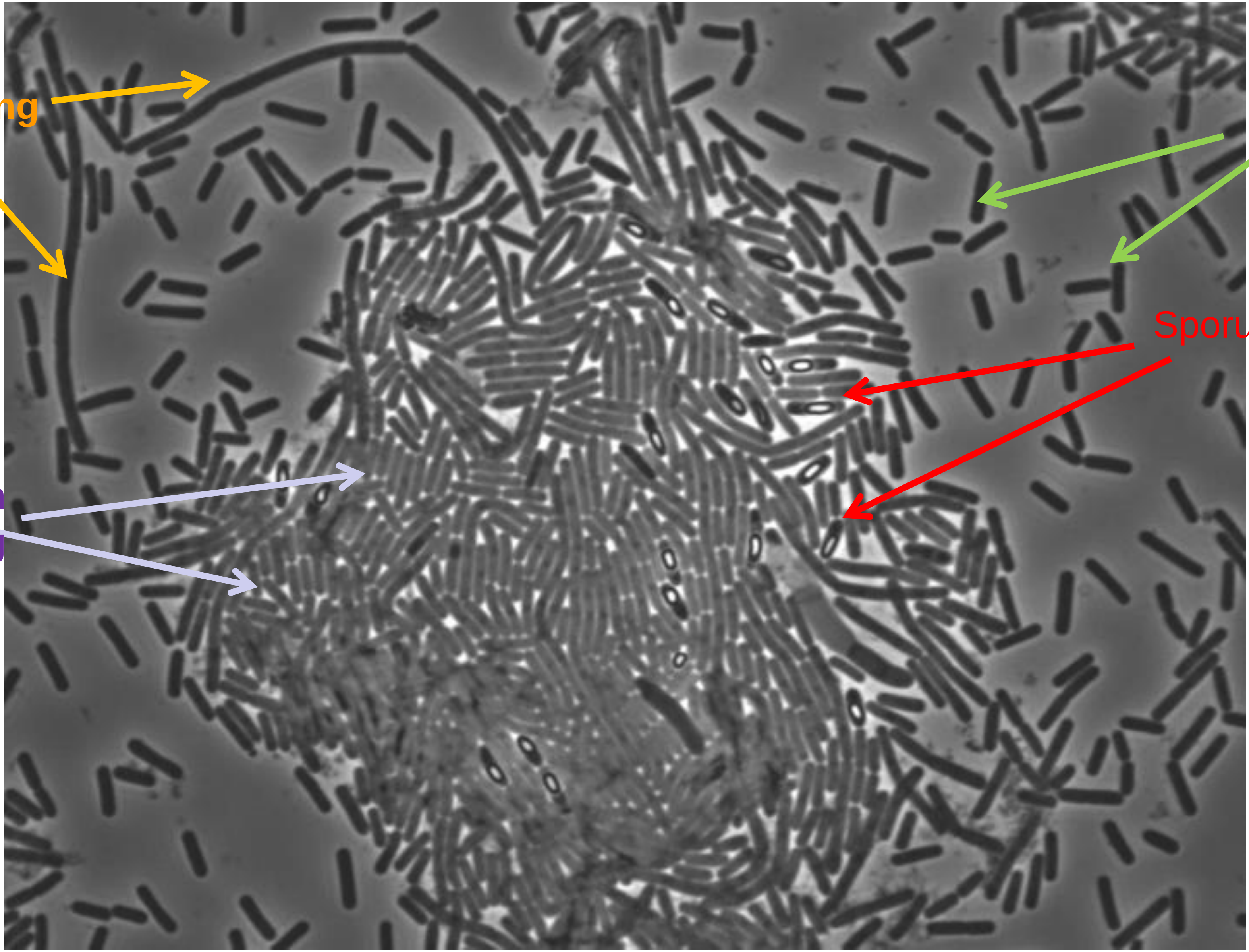


Bridier et al., Plos One, 2011



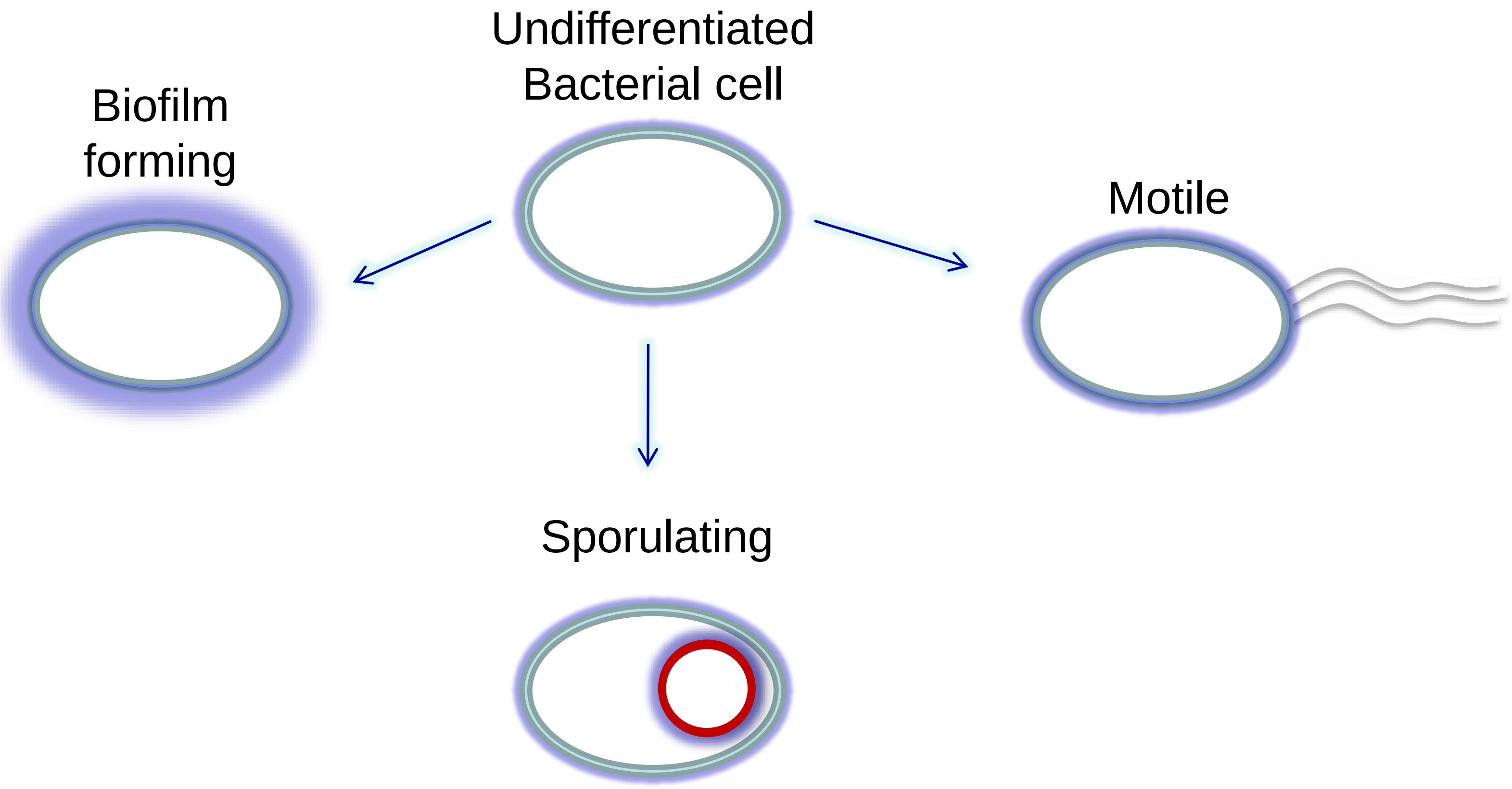
Branda et al., PNAS, 2001

Heterogeneous population during biofilm formation



Shemesh *et al.* 2015

Differentiation to biofilm forming bacteria

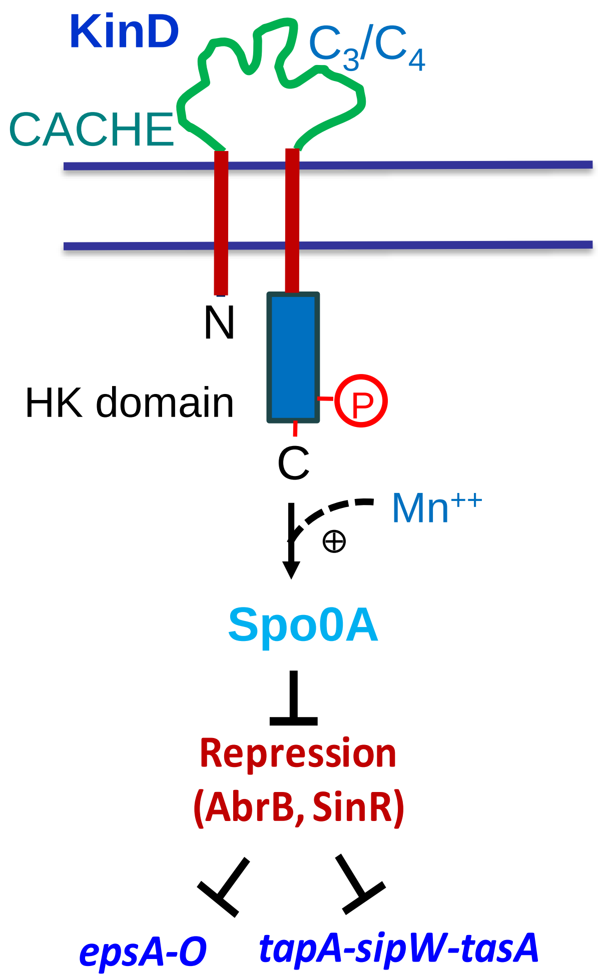


Journal of Bacteriology

A Combination of Glycerol and Manganese Promotes Biofilm Formation in Bacillus subtilis via Histidine Kinase KinD Signaling

Moshe Shemesh and Yunrong Chai
J. Bacteriol. 2013, 195(12):2747. DOI: 10.1128/JB.00028-13.
Published Ahead of Print 5 April 2013.

KinD is a principal kinase for sensing C3/C4 molecules as a biofilm signal



Shemesh and Chai, 2013

International Journal of Food Microbiology 181 (2014) 19–27

Contents lists available at ScienceDirect

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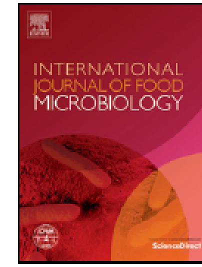
journal homepage: www.elsevier.com/locate/ijfoodmicro



Butyric acid released during milk lipolysis triggers biofilm formation of *Bacillus* species

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Department of Food Quality and Safety, Agricultural Research Organization (ARO), Bet-Dagan, Israel

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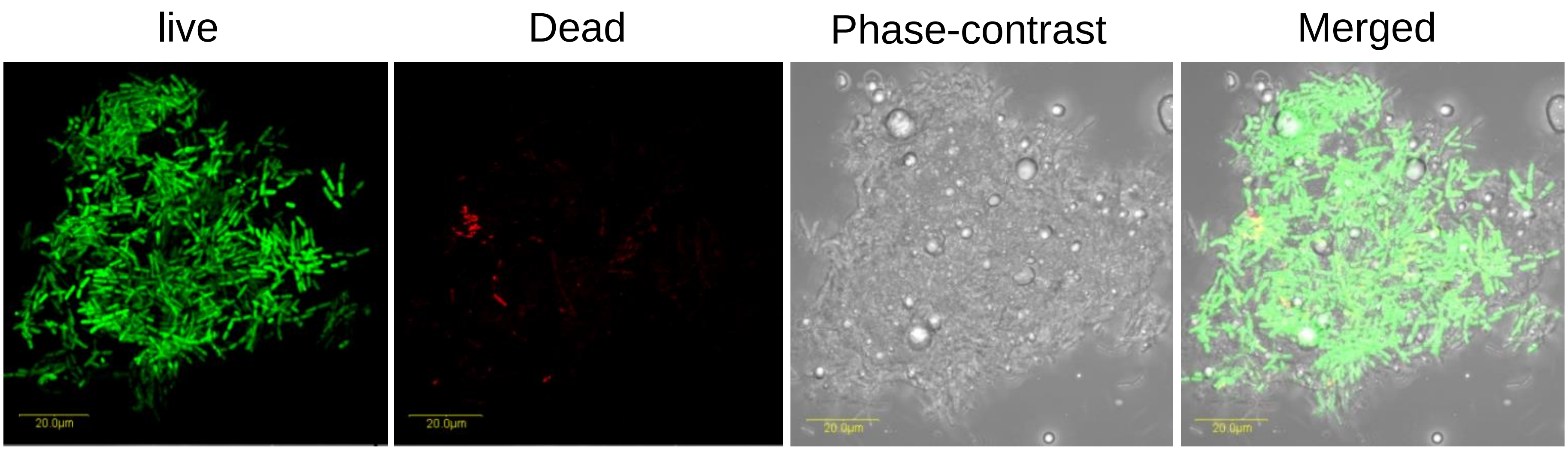
ABSTRACT

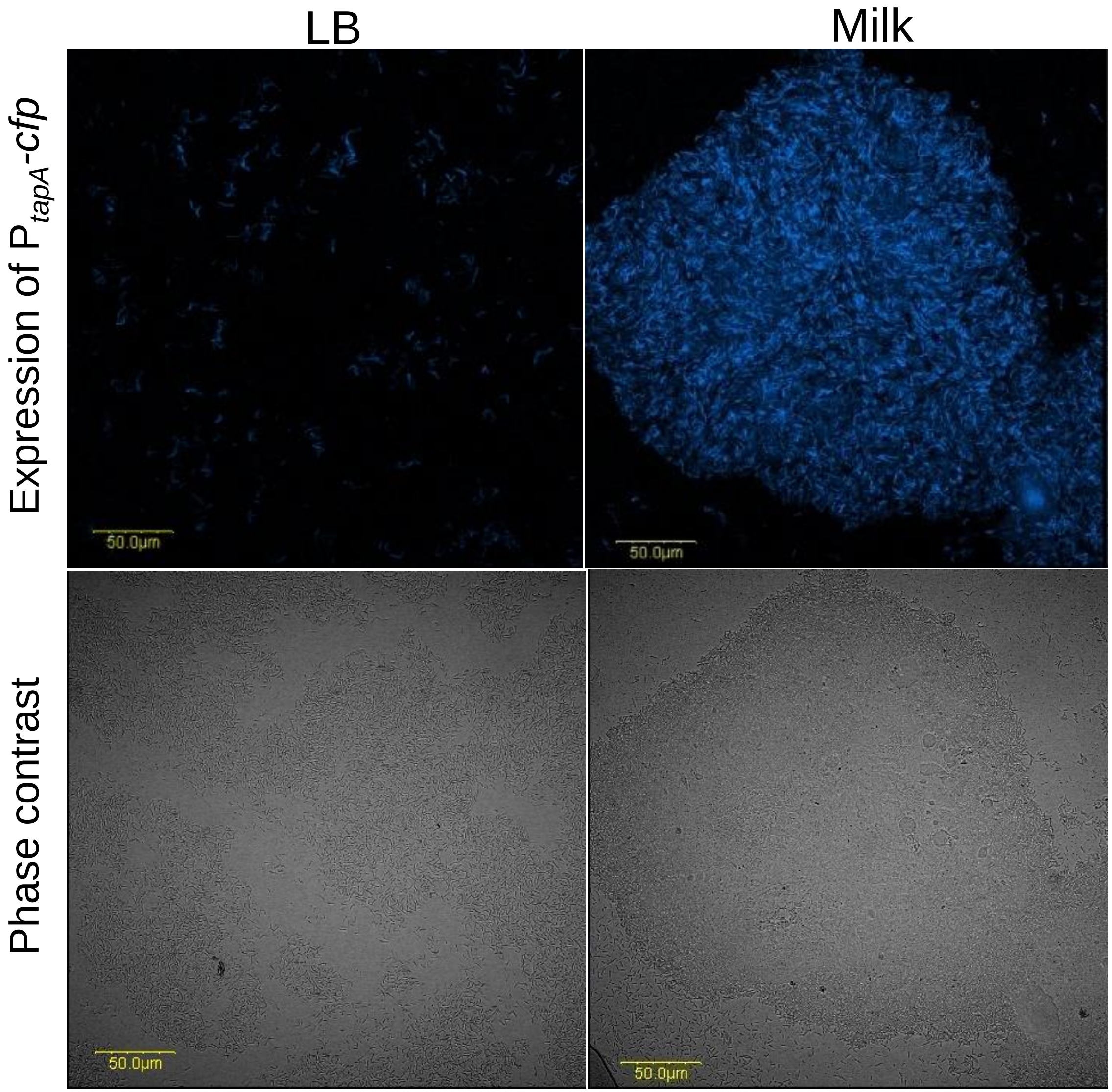
Bacillus species form biofilms within milking pipelines and on surfaces of equipment in the dairy industry which represent a continuous hygiene problem and can lead to serious economic losses due to food spoilage and equipment impairment. Although much is known about the mechanism by which the model organism *Bacillus subtilis* forms biofilms in laboratory mediums in vitro, little is known of how these biofilms are formed in natural environments such as milk. Besides, little is known of the signaling pathways leading to biofilm formation in other *Bacillus* species, such as *Bacillus cereus* and *Bacillus licheniformis*, both of which are known to contaminate milk. In this study, we report that milk triggers the formation of biofilm-related structures, termed bundles. We show this to be a conserved phenomenon among all *Bacillus* members tested. Moreover, we demonstrate that the *tasA* gene

The milk components trigger the biofilm bundles formation



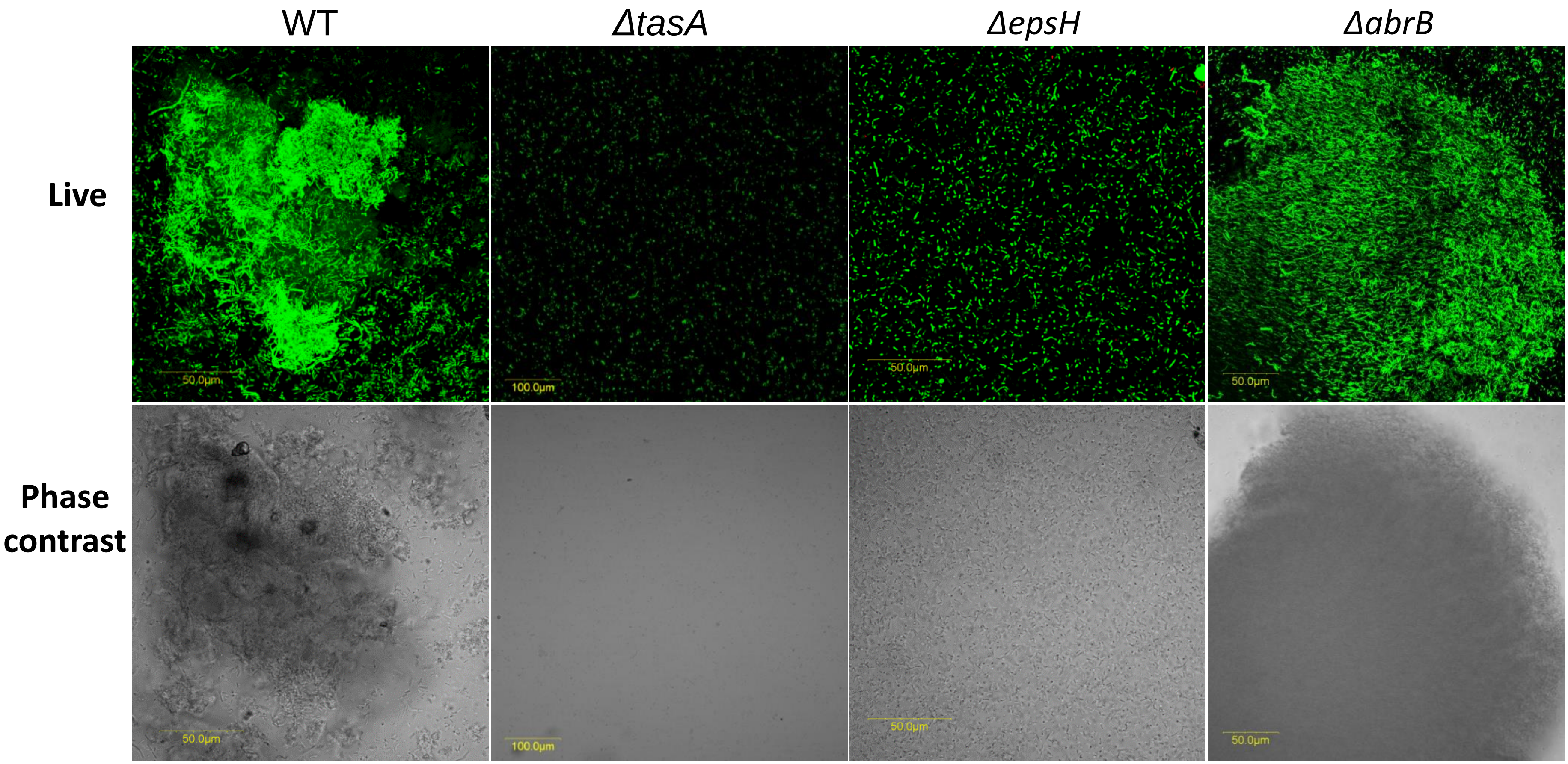
Pasvolsky et al., 2014

The milk components induce transcription of *tapA* operon responsible for the matrix production



Pasvolsky et al., 2014

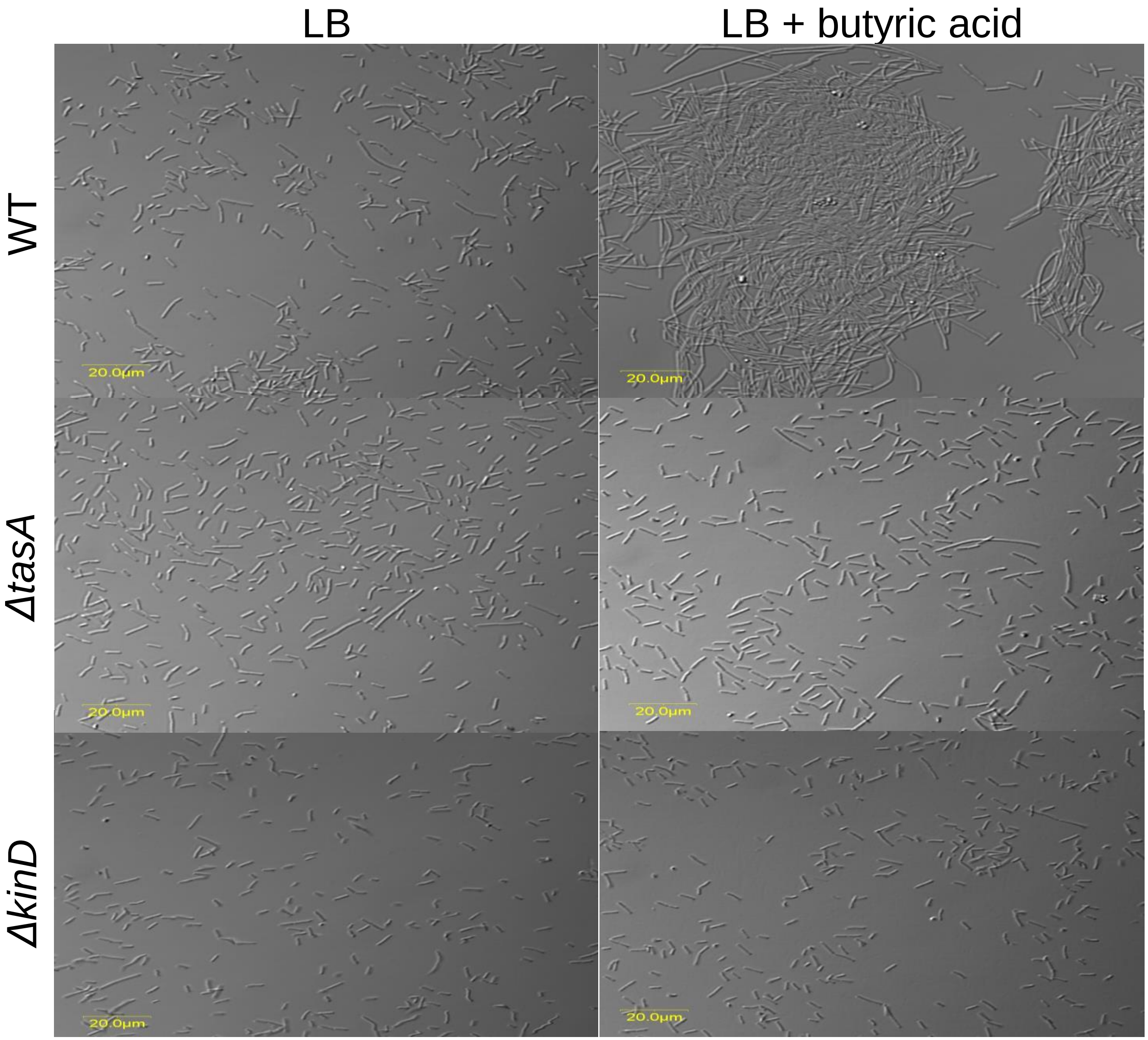
The *tasA* and *epsH* are required for biofilm formation in milk, while AbrB represses the biofilm formation



Pasvolsky et al., 2014

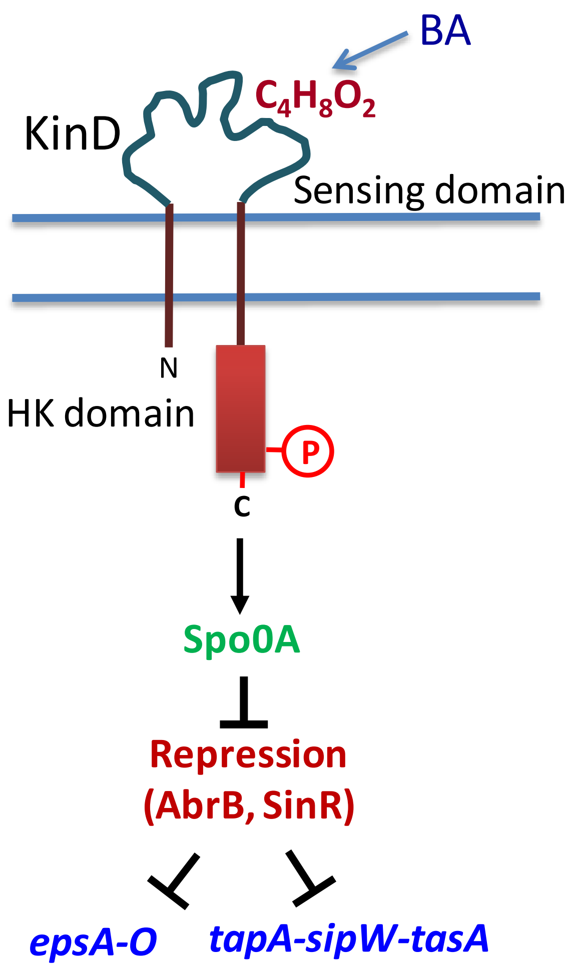
What are the milk components triggering biofilm bundles formation?

Free fatty acids, derived from milk breakdown, trigger biofilm formation



Pasvolsky et al., 2014

Signaling pathway leading for biofilm formation



Pasvolsky et al., 2014

How we use the knowledge about the signal transduction pathway for biofilm formation?

Preventing undesirable (negative) biofilm formation

Inducing desirable (positive) biofilm formation

npj | Biofilms and Microbiomes

www.nature.com/npjbiofilms

BRIEF COMMUNICATION OPEN

Enrichment of milk with magnesium provides healthier and safer dairy products

Noa Ben-Ishay[1,2], Hilla Oknin[1,3], Doron Steinberg[3], Zipi Berkovich[2], Ram Reifen[2] and Moshe Shemesh[1]

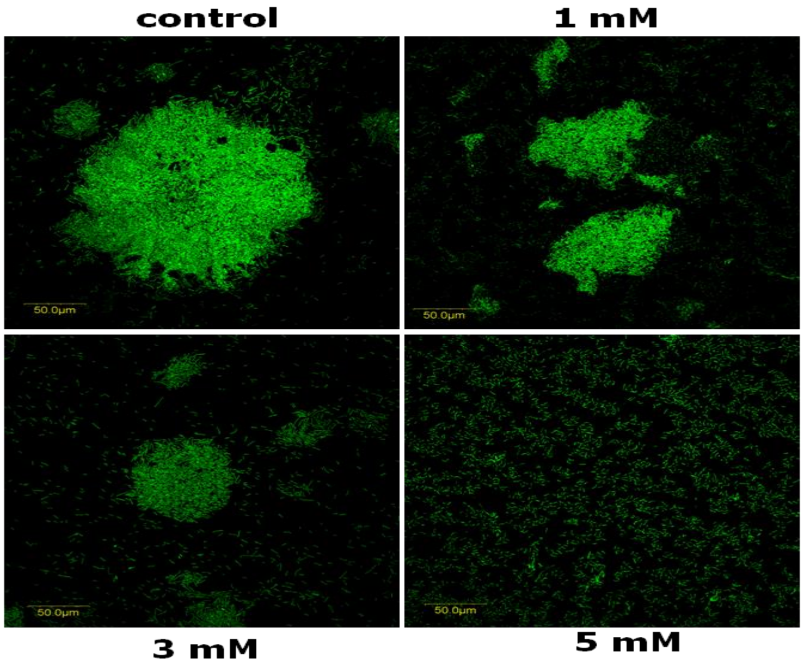
Biofilms on the surfaces of milk-processing equipment are often a major source of contamination of dairy products. Members of the genus *Bacillus* appear to be among the most commonly found bacteria in dairy farms and processing plants. *Bacillus* species may thrive in dairy farm equipment and in dairy products since they can form robust biofilms during growth within milk. We found that fortification of milk with magnesium mitigated biofilm formation by *Bacillus* species, and thus could notably reduce dairy product spoilage. We also show that the mode of action of Mg^{2+} ions is specific to inhibition of transcription of genes involved in biofilm formation. Our further findings indicate that in the presence of Mg^{2+} bacterial cells are hypersensitive to the heat pasteurization applied during milk processing. Additionally, we demonstrated that enrichment of milk with magnesium improved technological properties of milk products such as soft cheeses. Finally, we report that there is a notable increase in the intestinal bioavailability potential of magnesium from supplemented milk compared with that from non-supplemented milk.

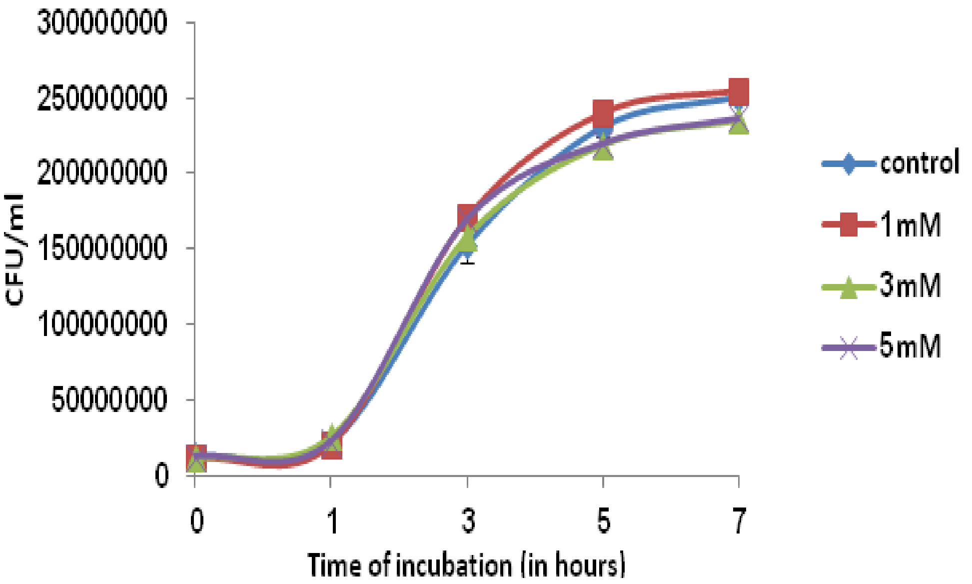
npj Biofilms and Microbiomes (2017)3:24 ; doi:10.1038/s41522-017-0032-3

INTRODUCTION

Bacterial contamination can adversely affect the quality, functionality, and safety of milk and its derivatives. It appears that the are able to survive pasteurization procedures, and psychrotrophic bacteria thrive at the low temperatures at which milk is stored.[3] Moreover, bacterial spores can survive treatment with reagents

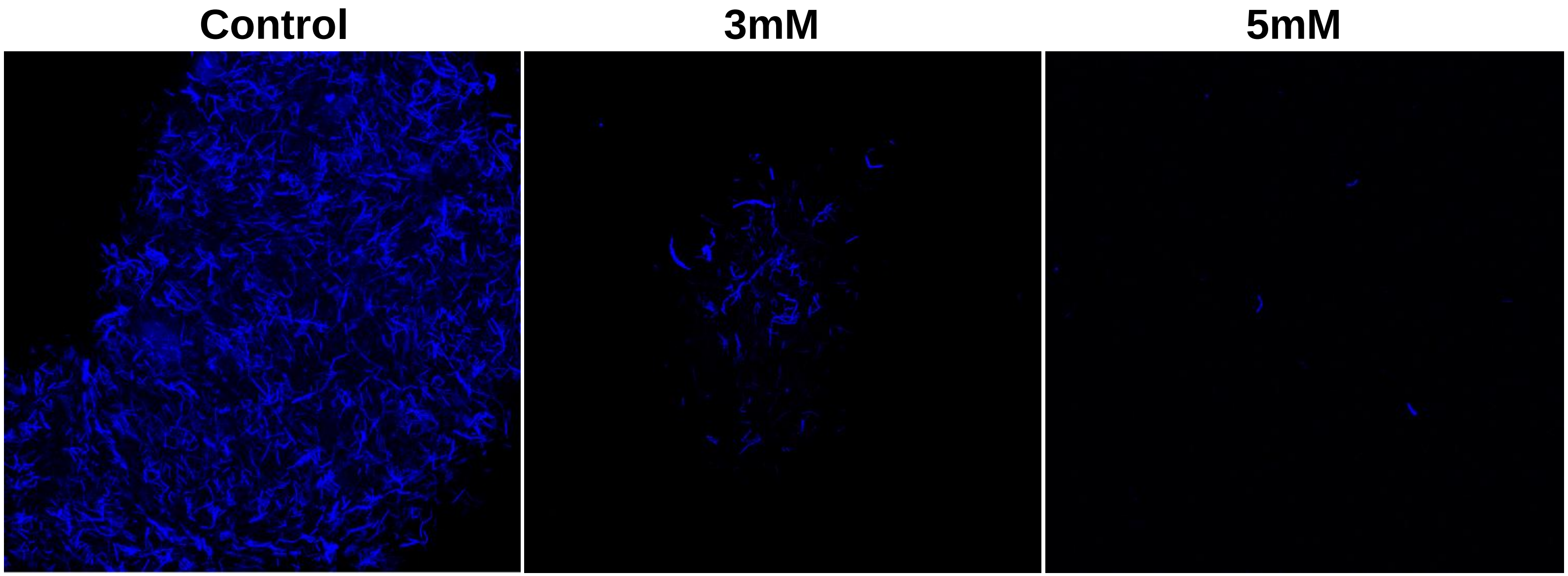
The effect of Mg^{2+} ions on *Bacillus subtilis* during growth within milk

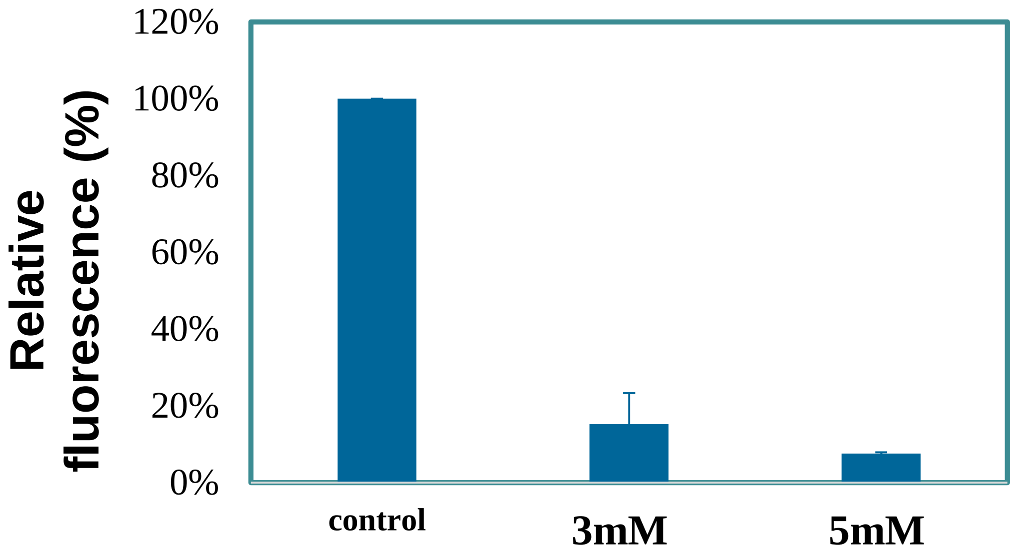




Ben-Ishay et al, 2017

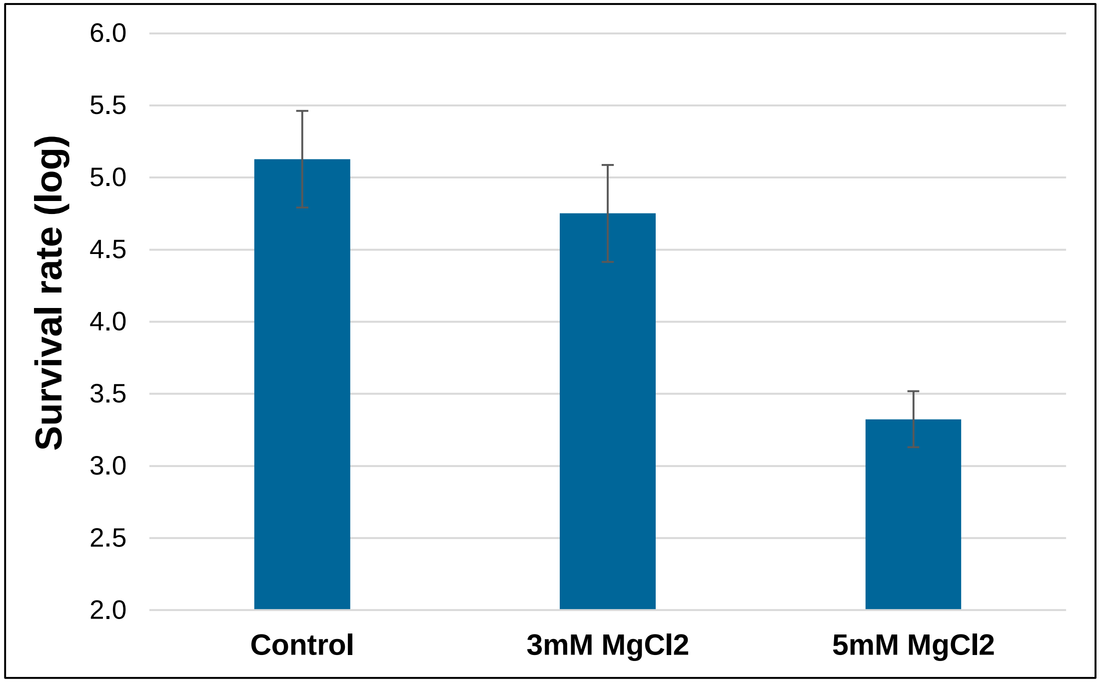
Transcription of *tapA* operon is down-regulated in response to Mg^{2+} ions





Ben-Ishay et al, 2017

Bacteria are more sensitive to heat treatment in the presence of Mg^{2+} ions within milk



Ben-Ishay et al, 2017

Scaling up

5ml milk

Initial experiments in the lab

20L milk

300L milk

Pilot experiments

40,000L milk

Industrial scale experiments

Product

COPIA Ag & Food
Funding Sustainable Technologies

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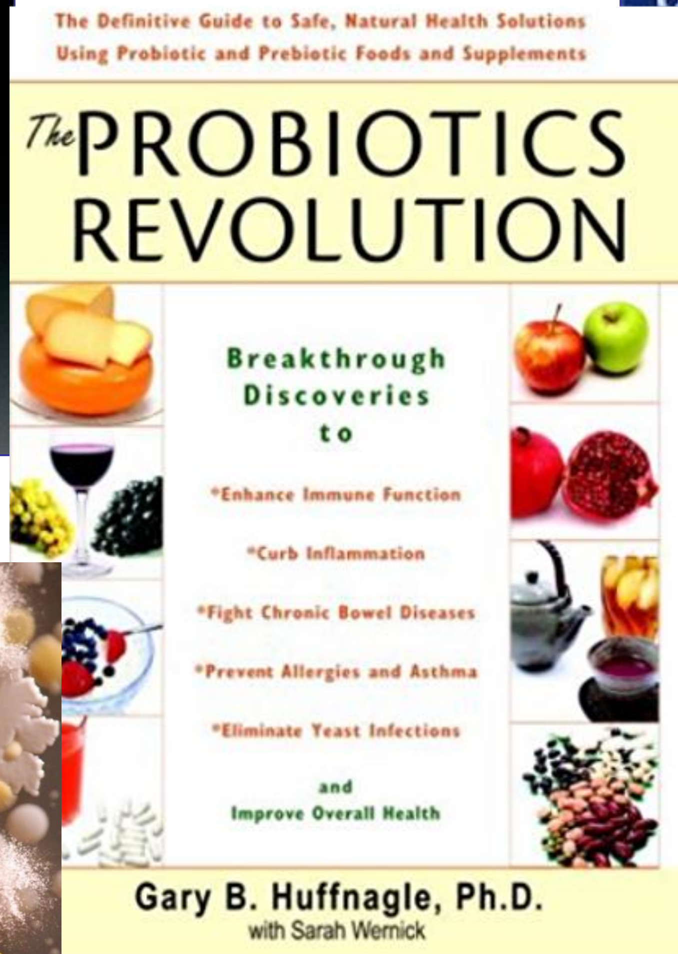
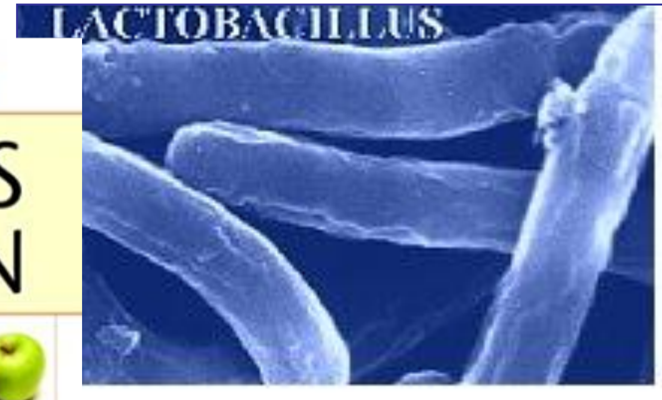
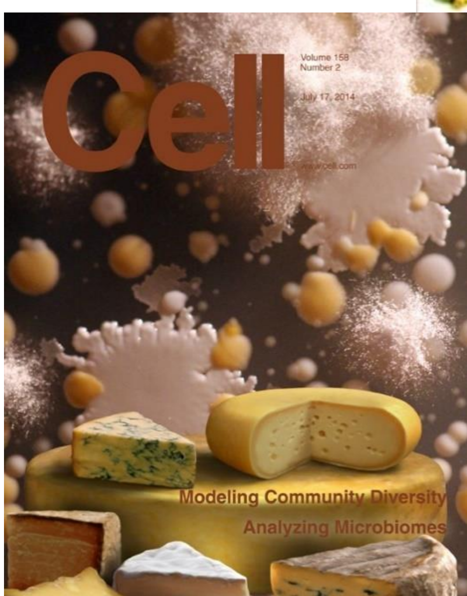
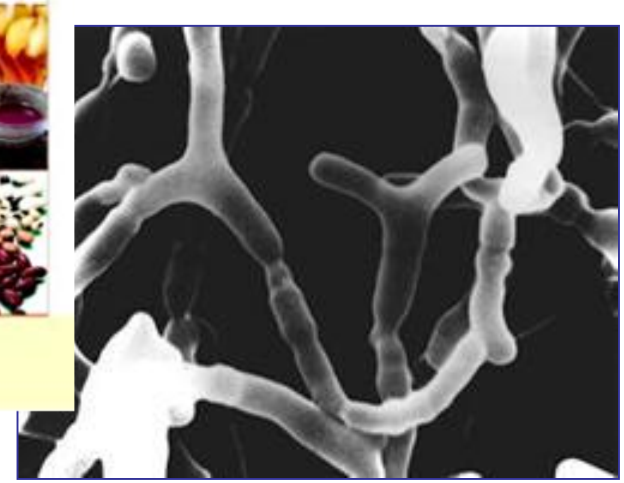
Food and Probiotics

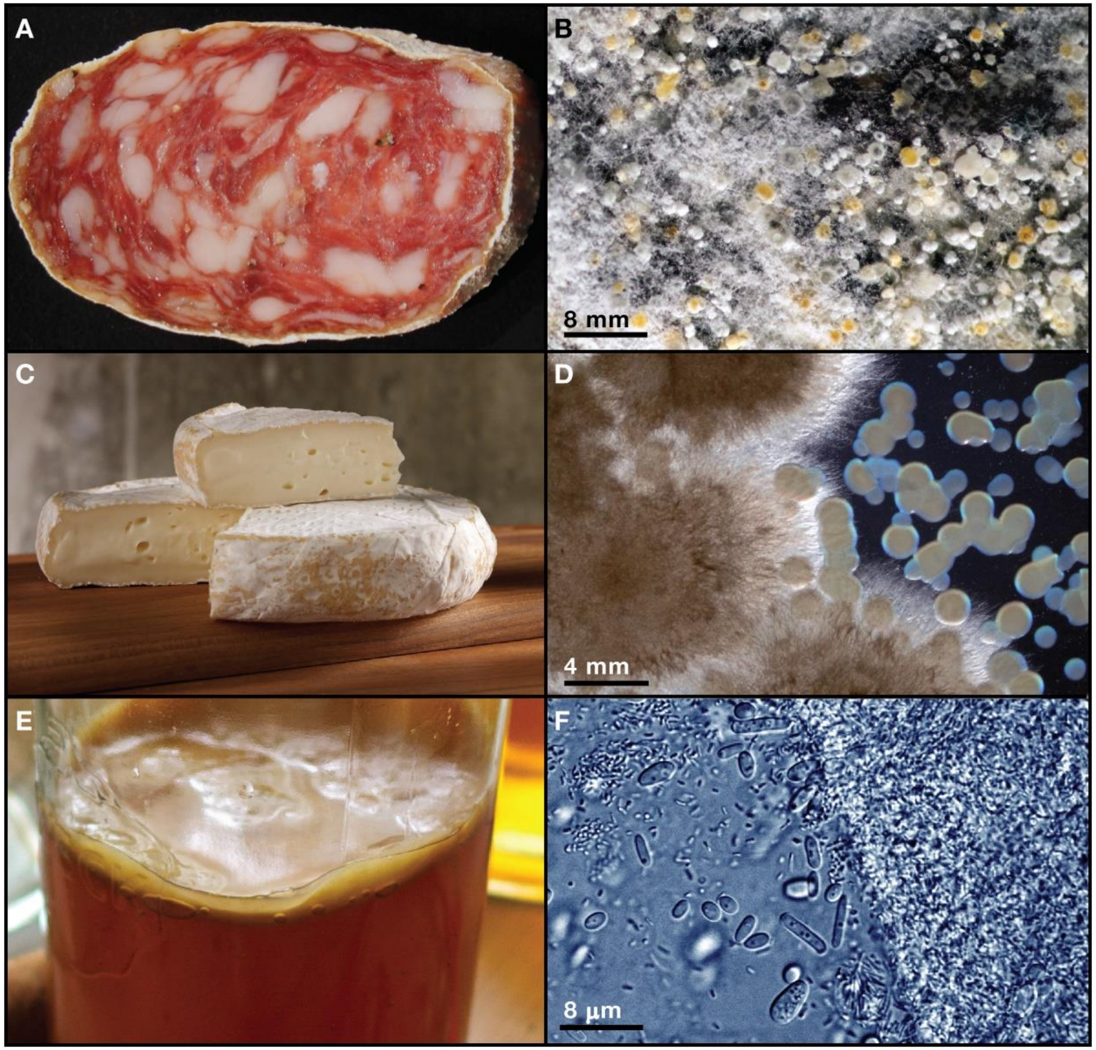








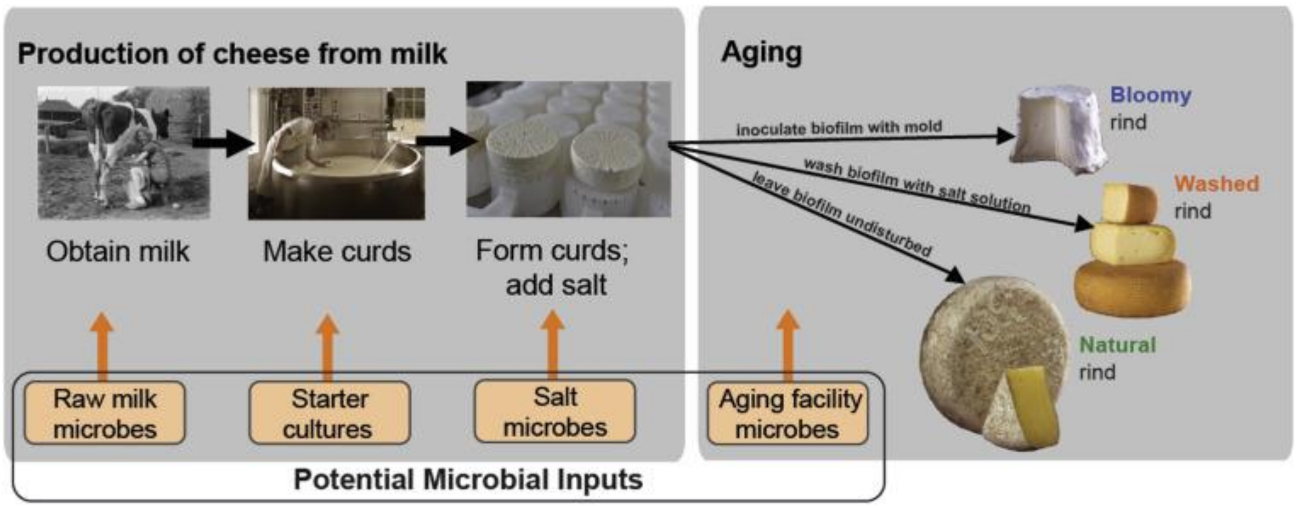
Multi-species Microbial Communities Form during the Production of Fermented Foods

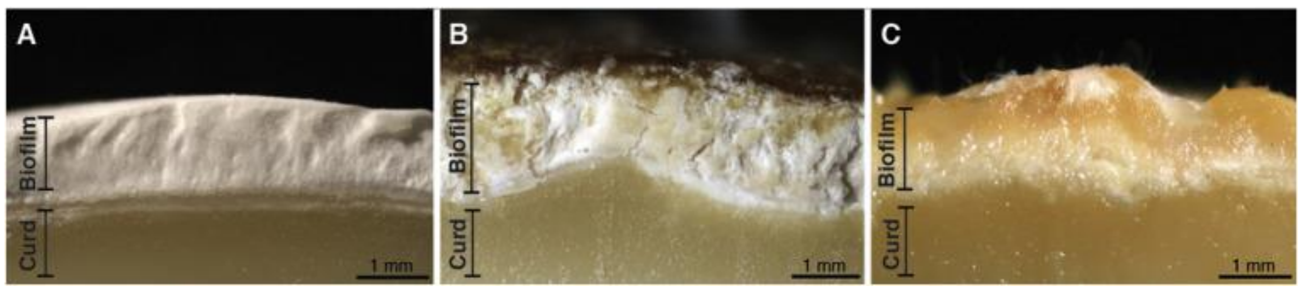


Wolfe and Dutton, 2015

https://www.cell.com/cell/fulltext/S0092-8674(15)00200-7

Microbial communities in the dairy food

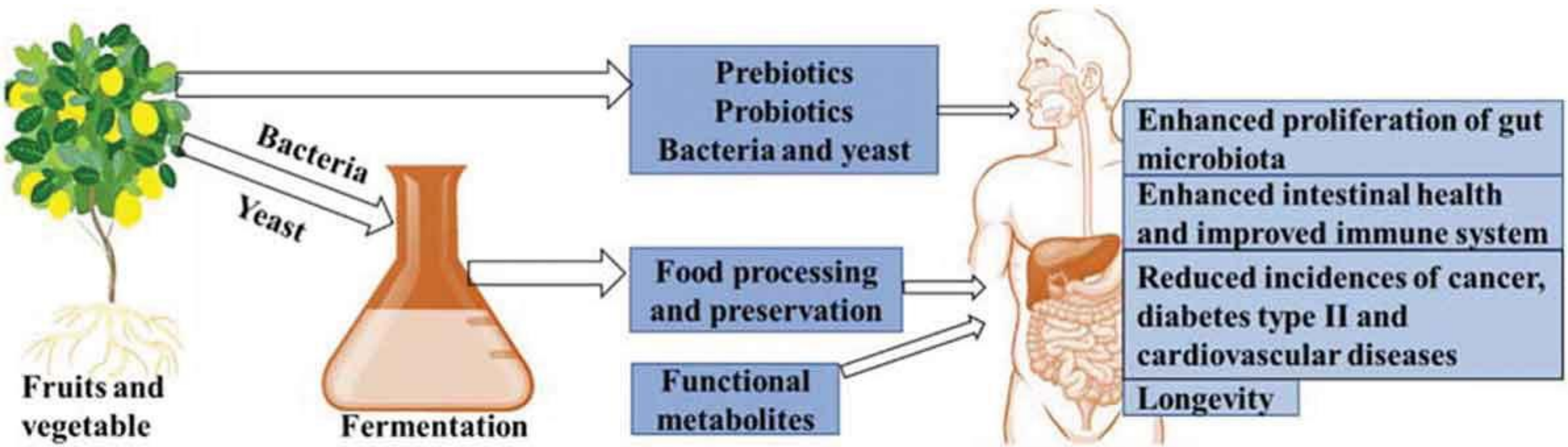




https://www.sciencedirect.com/science/article/pii/S00928674%3aiv?14007454Dihub#fig1

Wolfe et al, 2014

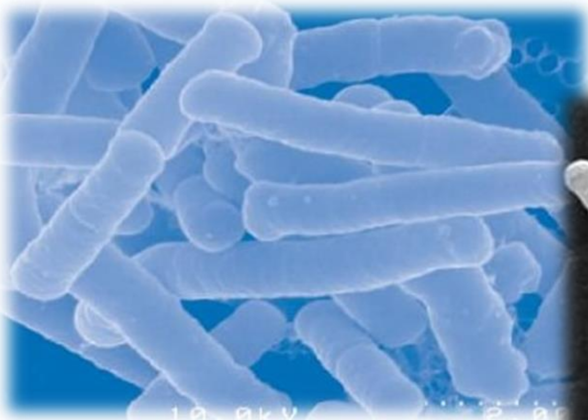
Prebiotics, probiotics and gut microbiota relationship in maintaining human health



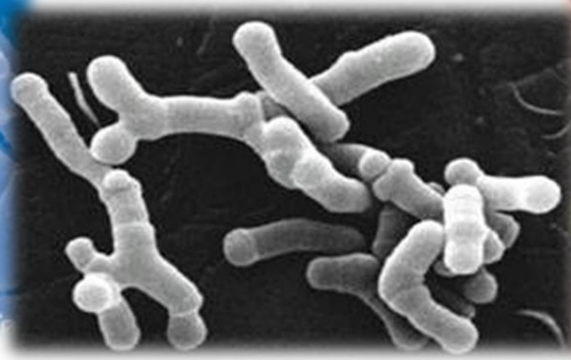
James & Wang, 2019

Armachius James & Yousheng Wang (2019) Characterization, health benefits and applications of fruits and vegetable probiotics, CyTA - Journal of Food, 17:1, 770-780, DOI: 10.1080/19476337.2019.1652693

Most probiotic products contain lactic-acid-producing (LAB) bacteria



Lactobacillus acidophilus



Bifidobacterium longum

Lactococcus lactis

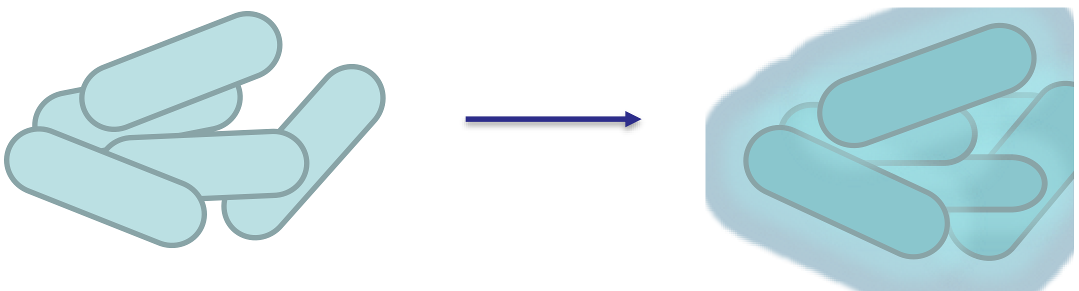
- The problem: there is considerable loss in viability of probiotic bacteria in the acidic conditions of the stomach and the high bile concentration in the small intestine.

How to protect on probiotic species?

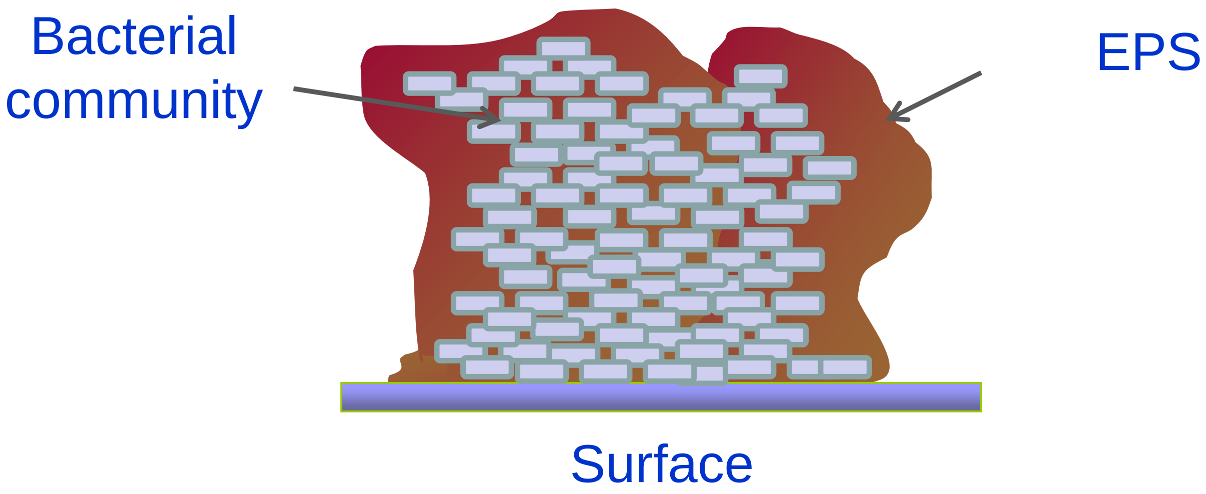
- Chemical encapsulation using various polymers to create physical barrier for protection.

- Encapsulation using natural food matrices for instance milk fat globules.

- Encapsulation using extracellular polymeric substances (EPS) produced by probiotic species themselves

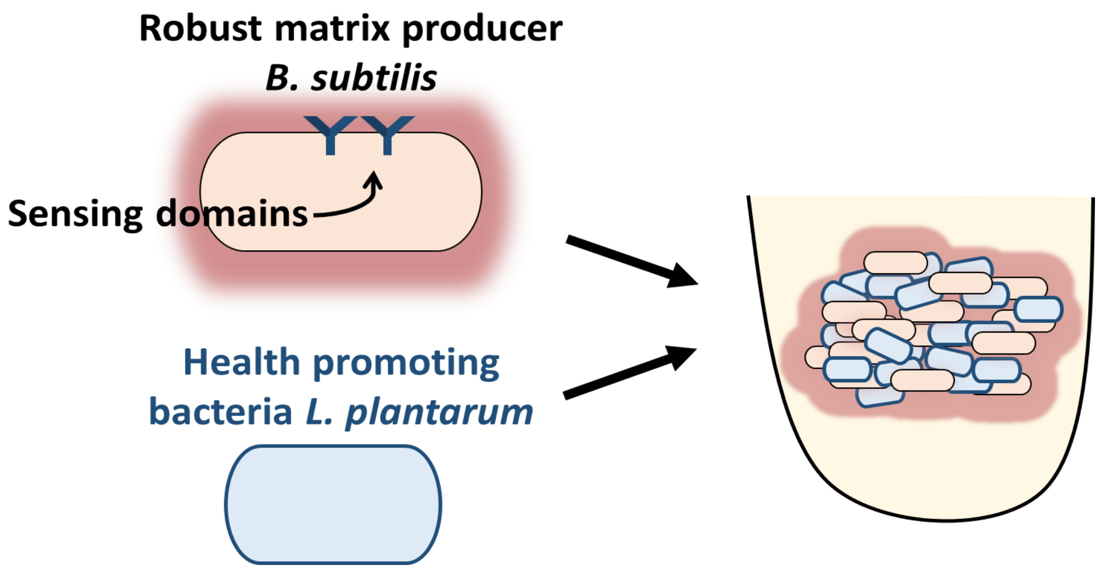


Biofilm formation as a natural way of encapsulation



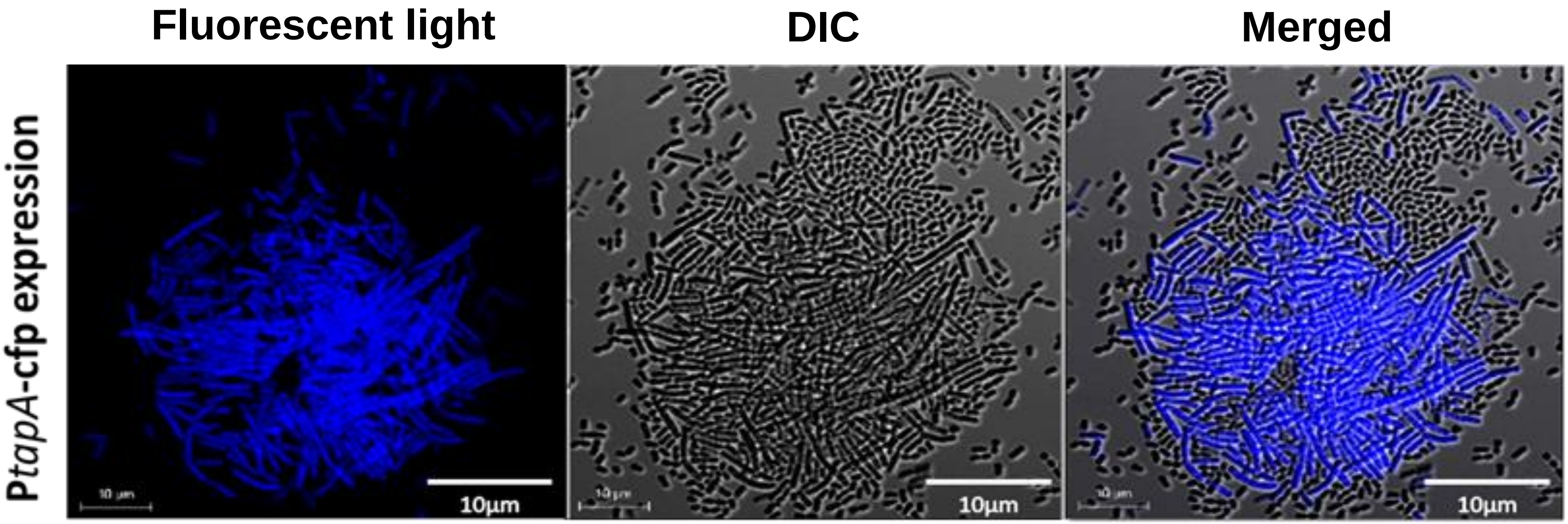
- EPS is naturally produced by bacteria during formation of multicellular community called biofilm
- EPS can provide extraordinary protection on bacteria embedded into biofilm
- The problem: not all the probiotic species produce the EPS.

Hypothesis



EPS, produced by robust biofilm former probiotic bacterium *B. subtilis*, would provide increased protection to other probiotic bacteria during their growth in co-culture biofilm system

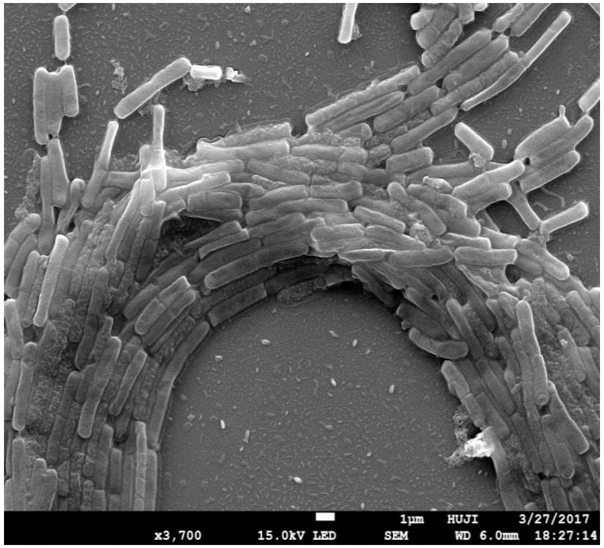
B. subtilis produces EPS during formation of a dual-species biofilm with *L. plantarum*



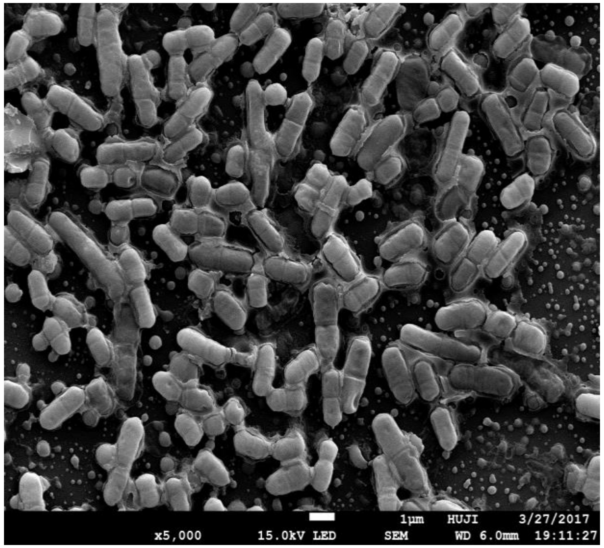
Yahav et al., 2018

SEM images of dual species biofilm composed of *B. subtilis* and *L. plantarum*

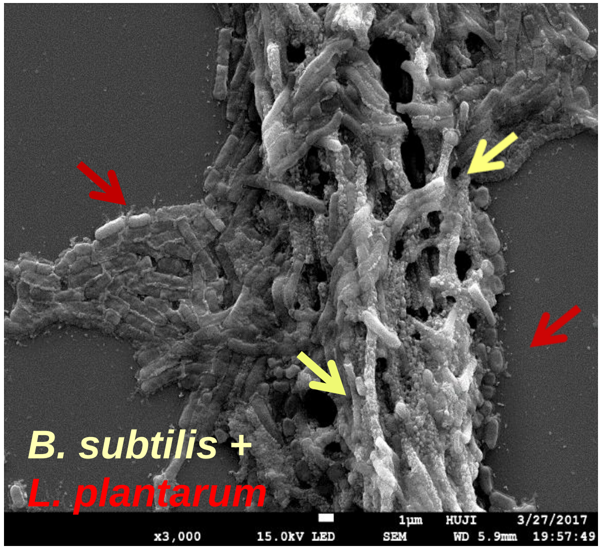
B. subtilis



L. plantarum

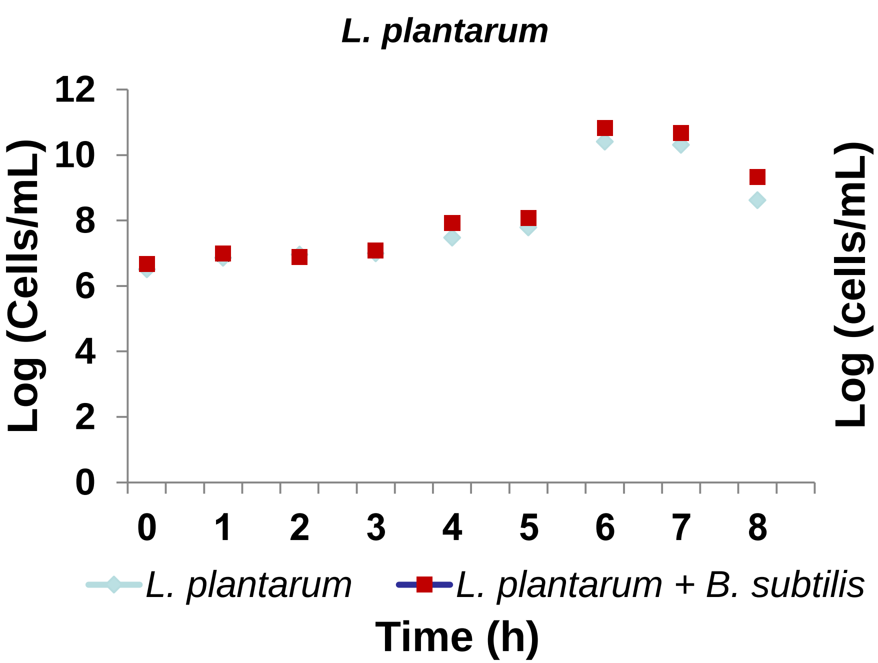


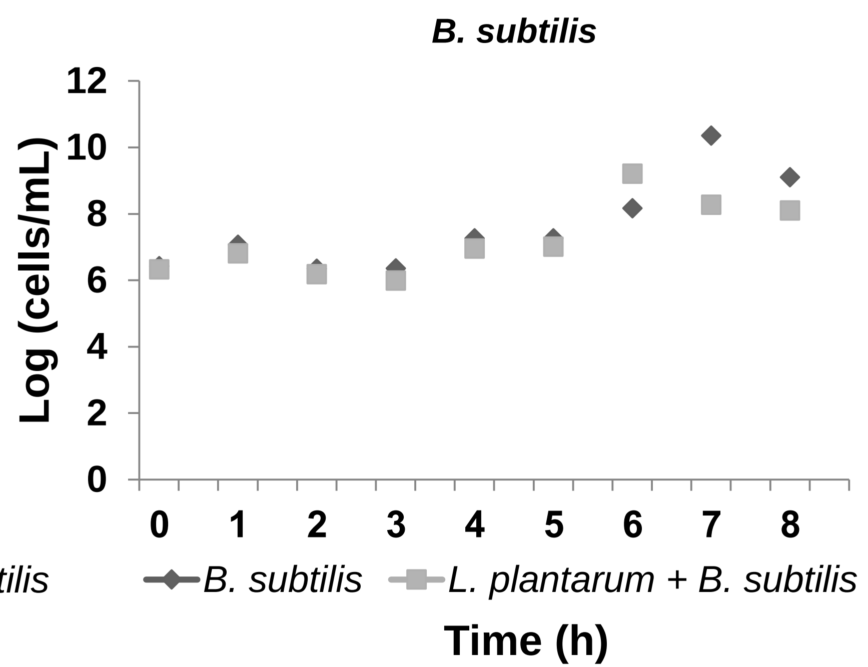
B. subtilis + L. plantarum



Yahav et al., 2018

B. subtilis and *L. plantarum* do not inhibit each other while grown in co-culture

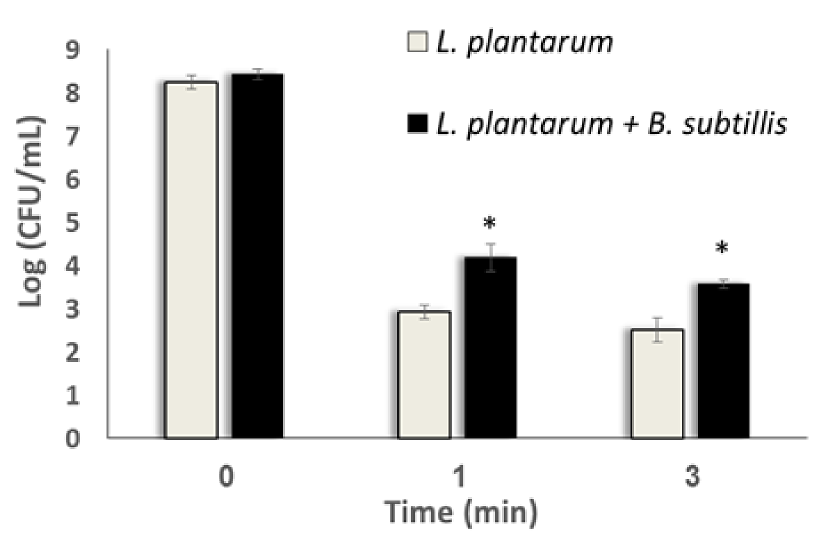




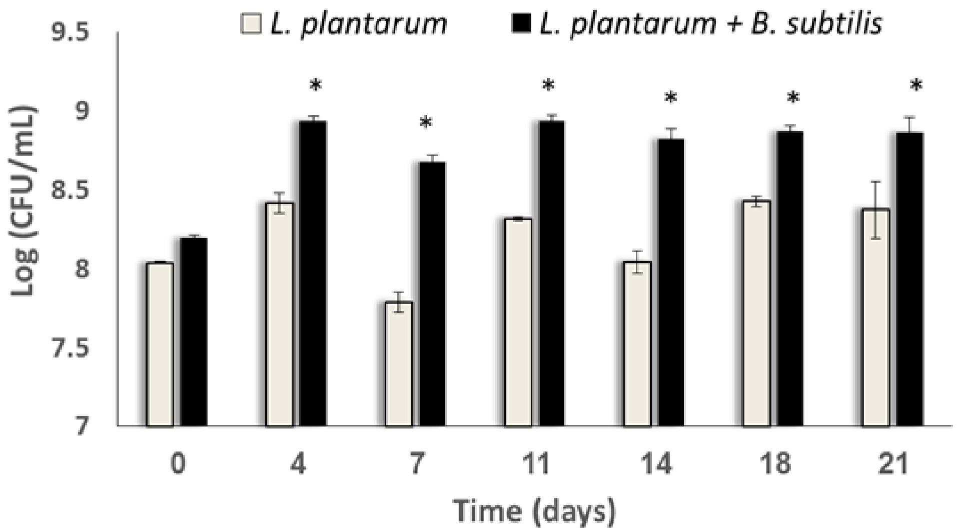
Yahav et al., 2018

B. subtilis biofilm facilitates survival of *L. plantarum* during stressful conditions

Heat treatment

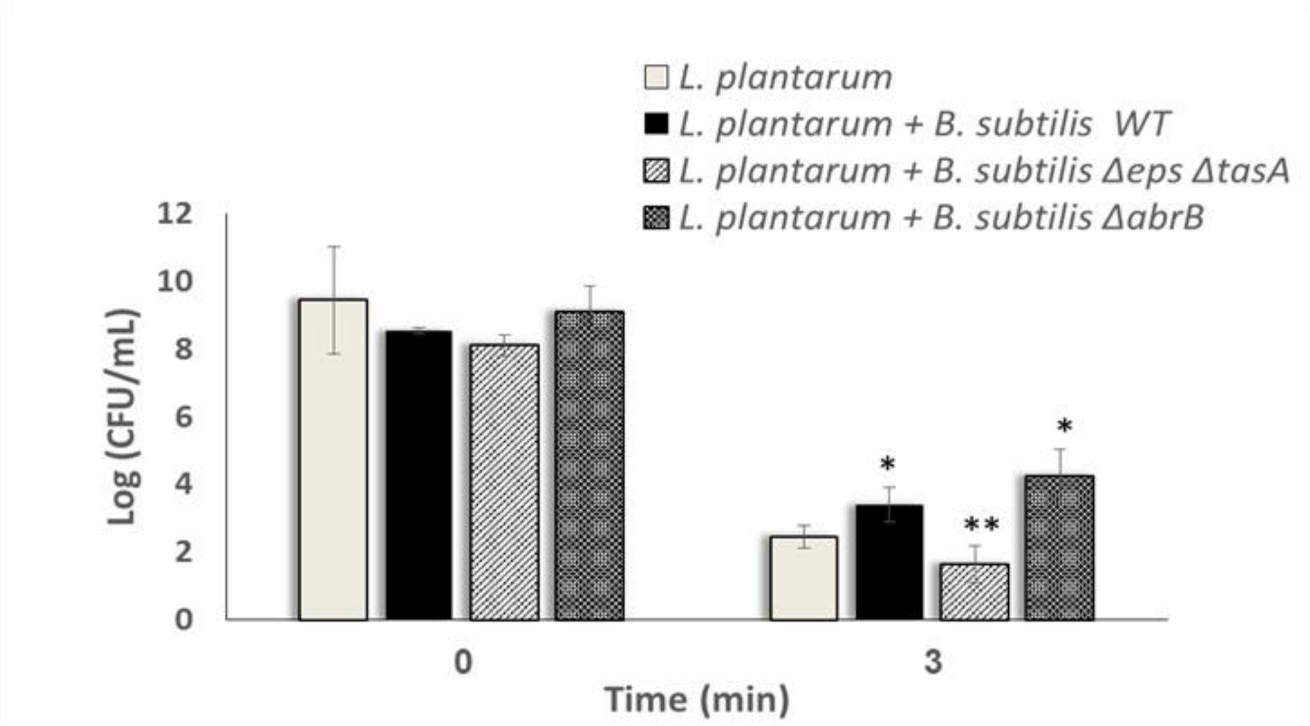


Low temperature treatment



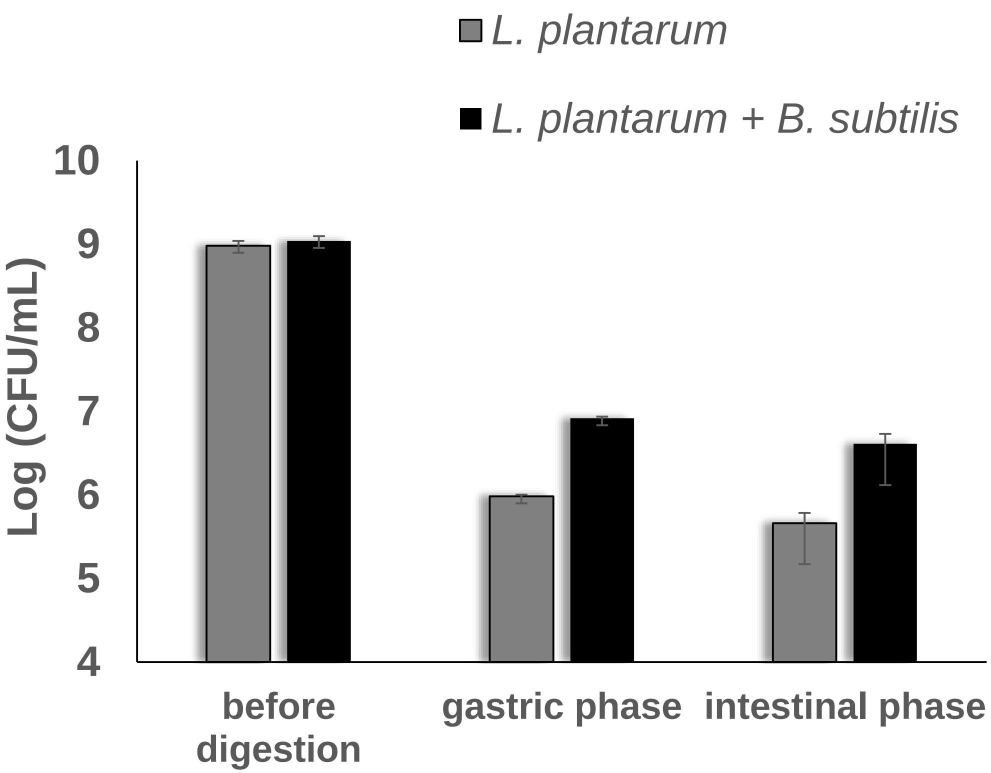
Yahav et al., 2018

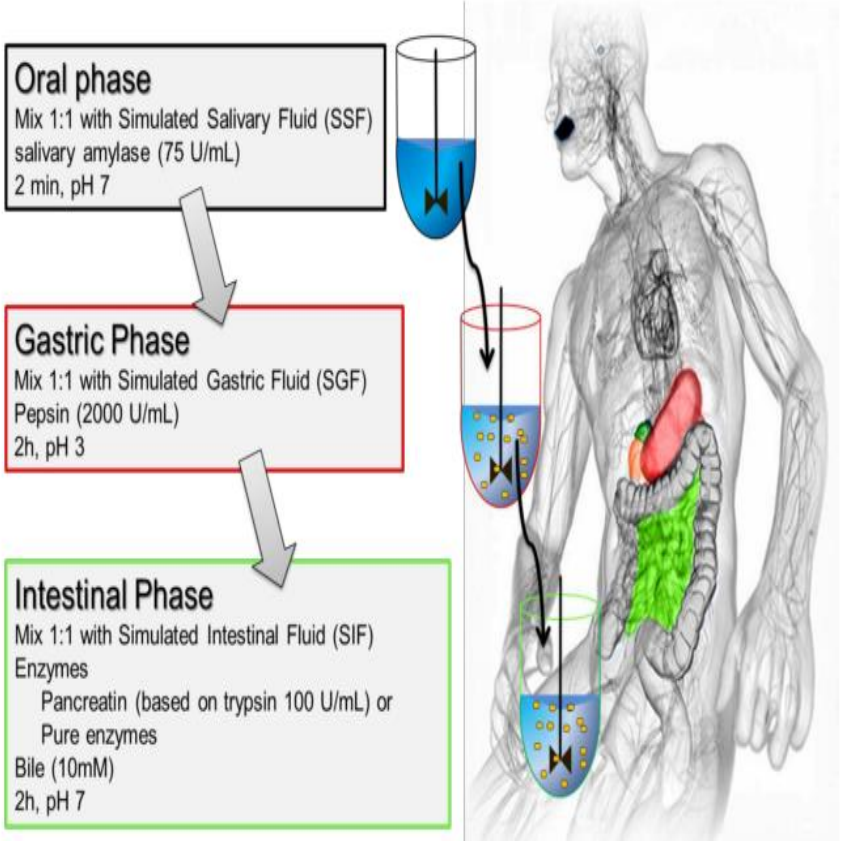
EPS of *B. subtilis* is responsible for increased survival of *L. plantarum* during heat treatment



Yahav et al., 2018

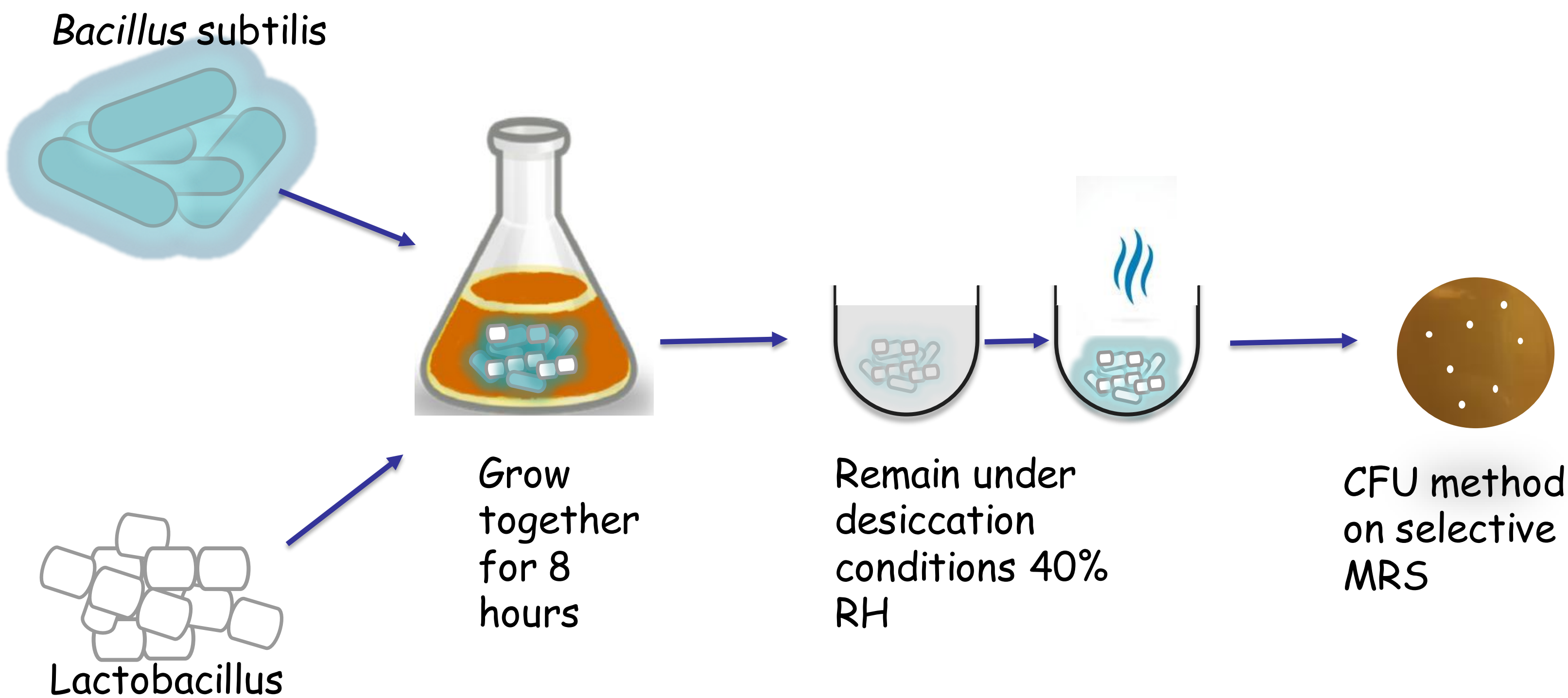
B. subtilis biofilm facilitates survival of *L. plantarum* during gastric and intestinal digestion *in vitro*





Yahav et al., 2018

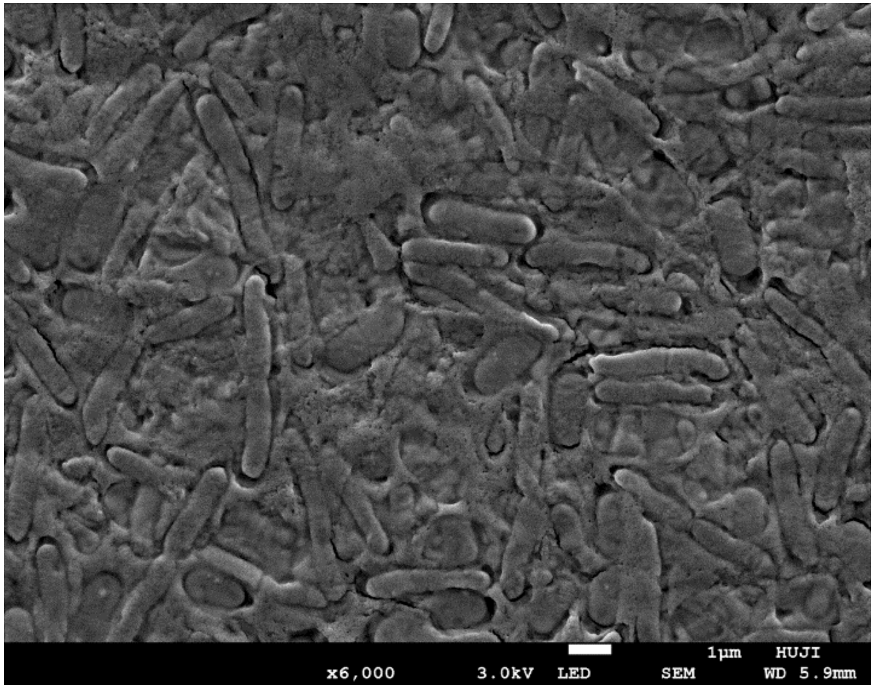
Does EPS protect *L. plantarum* during desiccation?

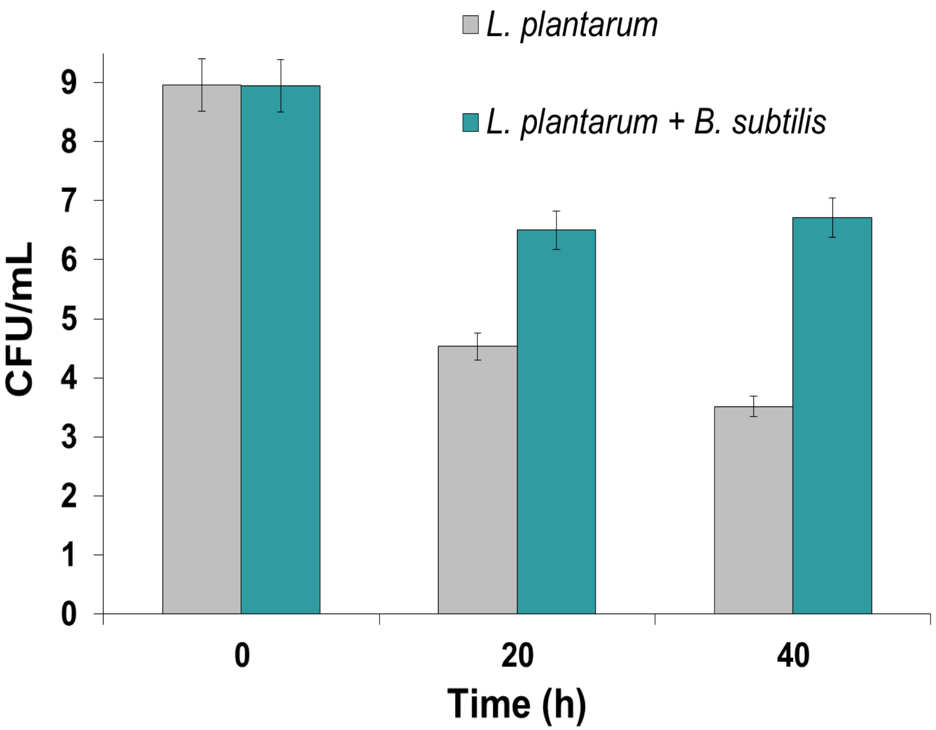


Kimelman et al. 2019

Survival of *L. plantarum* during desiccation

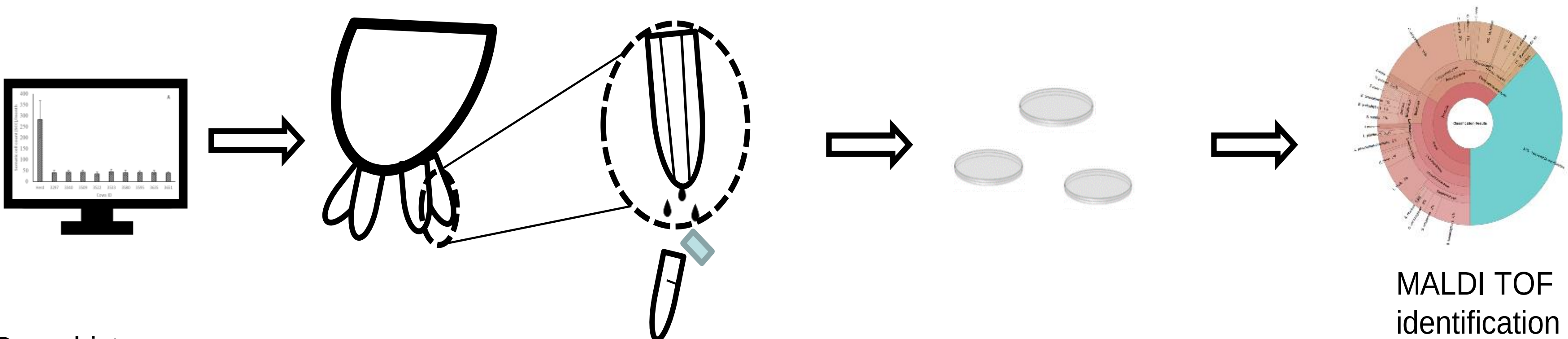
SEM imaging of the dual-species biofilm following 20 h dehydration





Kimelman et al. 2019

Schematics of sampling system for screening for new probiotic bacteria from milk



Cows history checking for no instance of mastitis

Sterile teat cistern and streak canal milk sampling

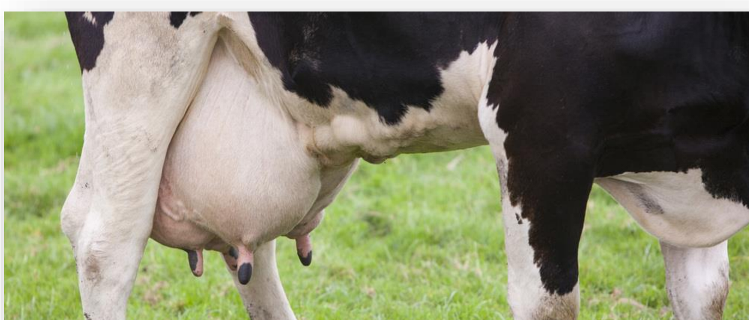
Initial bacteria isolation

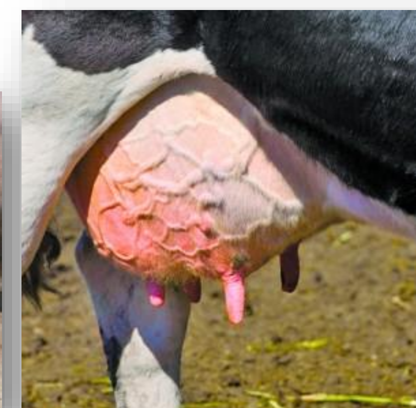
MALDI TOF identification and physiological characterization of the isolates

Mastitis - serious problem in the dairy farm

causing more than $0.99/cow/day economic losses for farmers

highly expensive to treat





Current treatment for mastitis

Mostly – Antibiotic therapy

Drawbacks – Some of the serious problems associated with this therapy include the low cure rate, the bacterial resistance and the presence of antimicrobial residues in milk

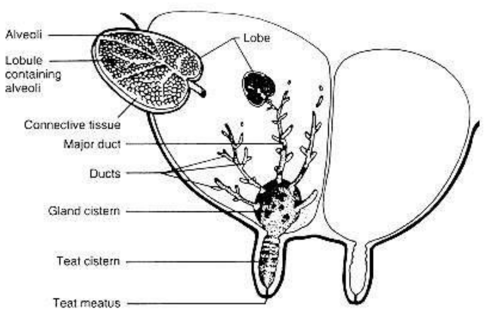
The need – development and discovery of new anti-mastitis treatment for clinical practice

How can we help to improve udder health?

develop probiotic anti-mastitis treatment

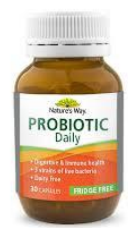
1

Isolating probiotic bacteria from the udder of healthy cows



2

development of probiotic anti-mastitis treatment



3

experiments in the cows - efficiency check

4

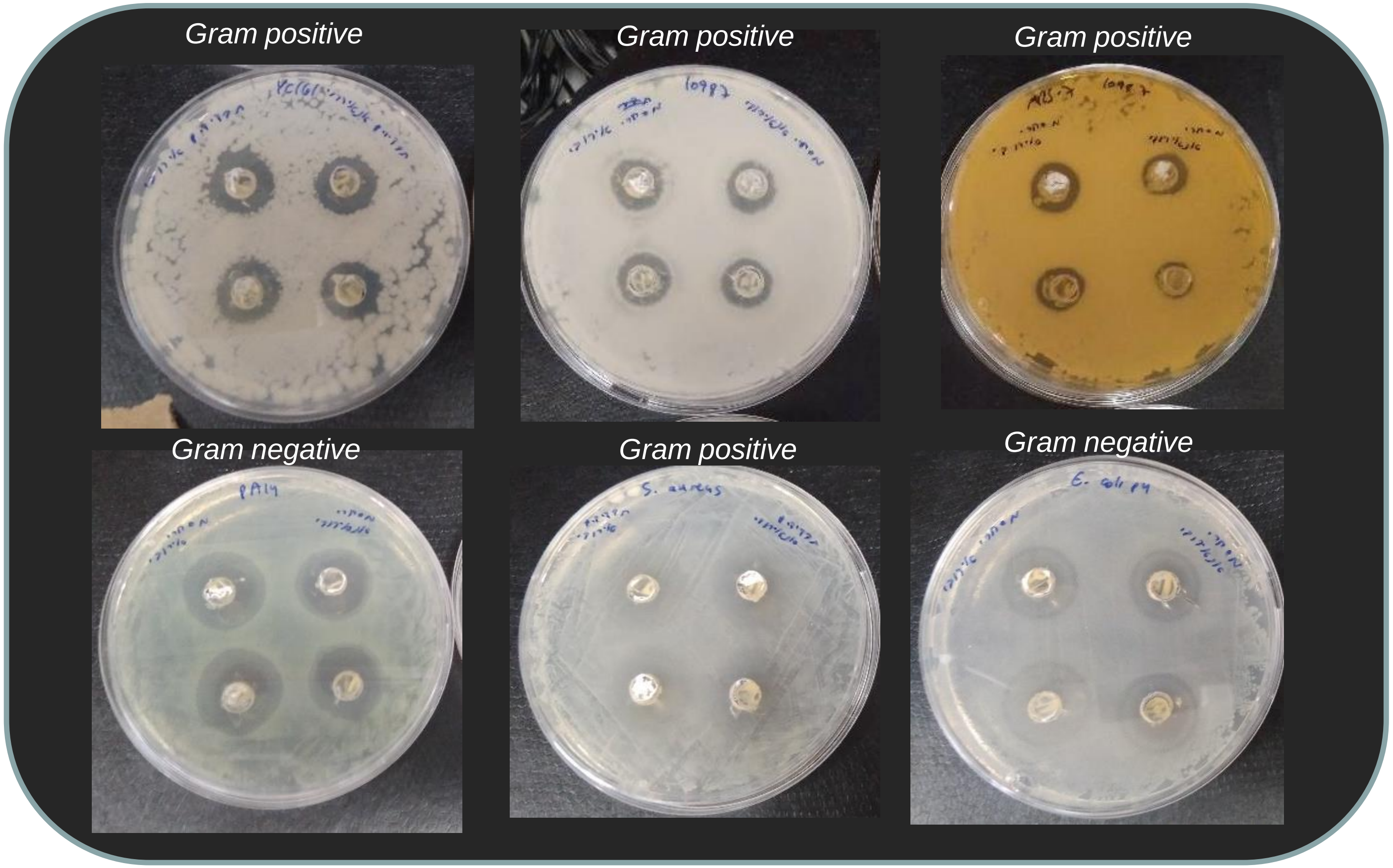
Formulation and licencing

5

Healthier cows,
less mastitis cases
- less money waste

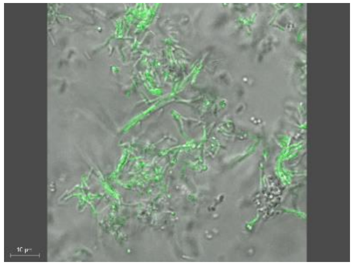
Isolated probiotic bacteria inhibit the growth of pathogenic species

Liquid (agar) diffusion



Cohen et al, unpublished

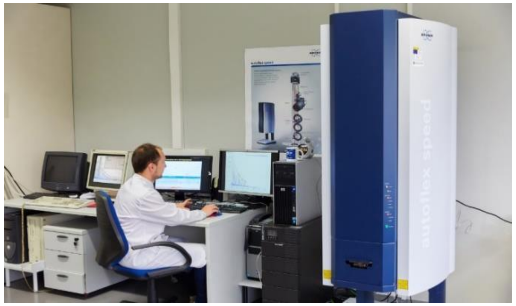
Experimental approach



Screening for selection of potential isolates for the anti-mastitis treatment

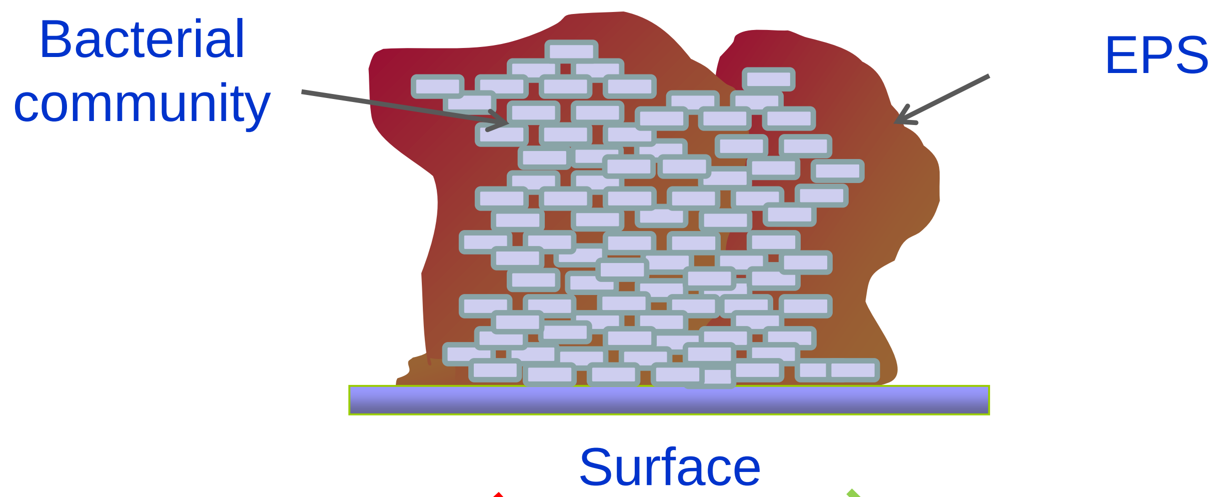
Molecular and physiological characterization of selected isolates

Developing combinatorial approach through identifying symbiotic interactions for better probiotic formulations



In vitro, *ex vivo* and *in vivo* testing of the formulations for their effectiveness as an anti-mastitis treatment

Understanding a role of bacterial communities during food production



Developing novel means to inhibit undesirable bacterial proliferation

Developing novel systems to protect beneficial bacteria

Questions?