Role of biofilm formation in developing synbiotic food for promoting well-being and heath

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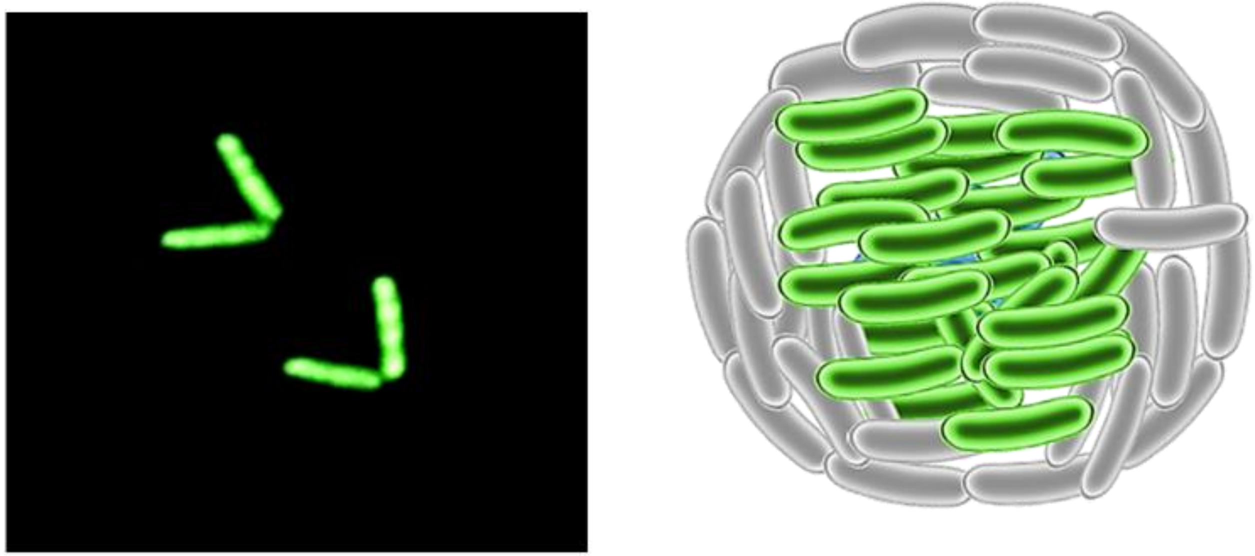
Cone-shaped story

Figure 1. Cone-shaped colonies formed by a lactic acid bacteria



V-shaped cells | Consolidated bundles

Figure 3. V-shaped cells and consolidated bundles consisting of live (green) and dead cells (grey)

Spatiotemporal bio-shielding of bacteria through consolidated geometrical structuring

Rajasekhharan and Shemesh, npj biofilms and microbiomes 2022

What are probiotics?????

Live bacteria (not –pathogenic) that proved beneficial for humans when take as supplements

Probiotics form beneficial biofilms , that can remove pathogen biofilms

Probiotics can be used to treat pathogens

What are prebiotics ?????

Chickpea

→ Rich in fibre → **prebiotics**

What are Synbiotics ?????

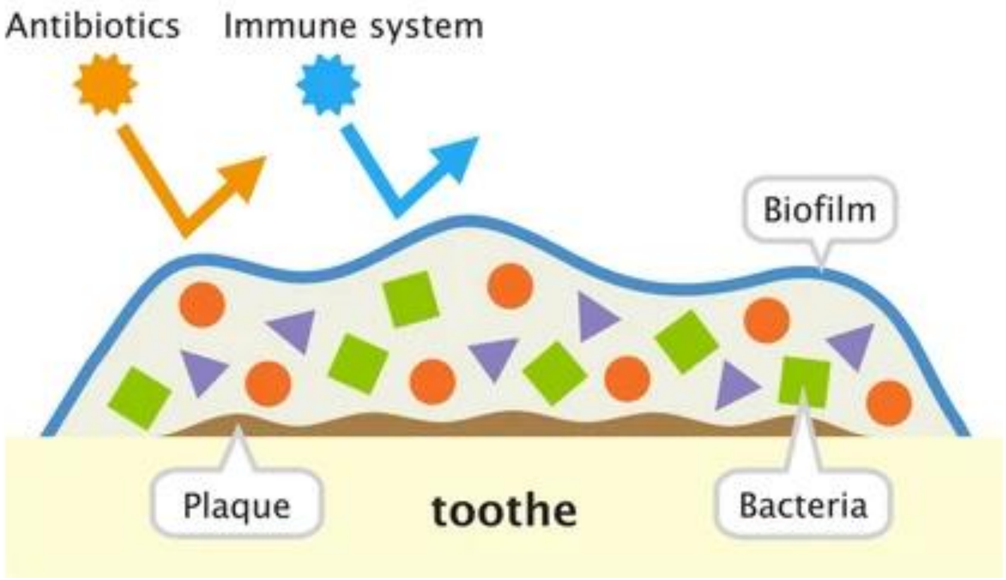
Probiotic – prebiotics complex

Beneficial bacteria – fibre complex

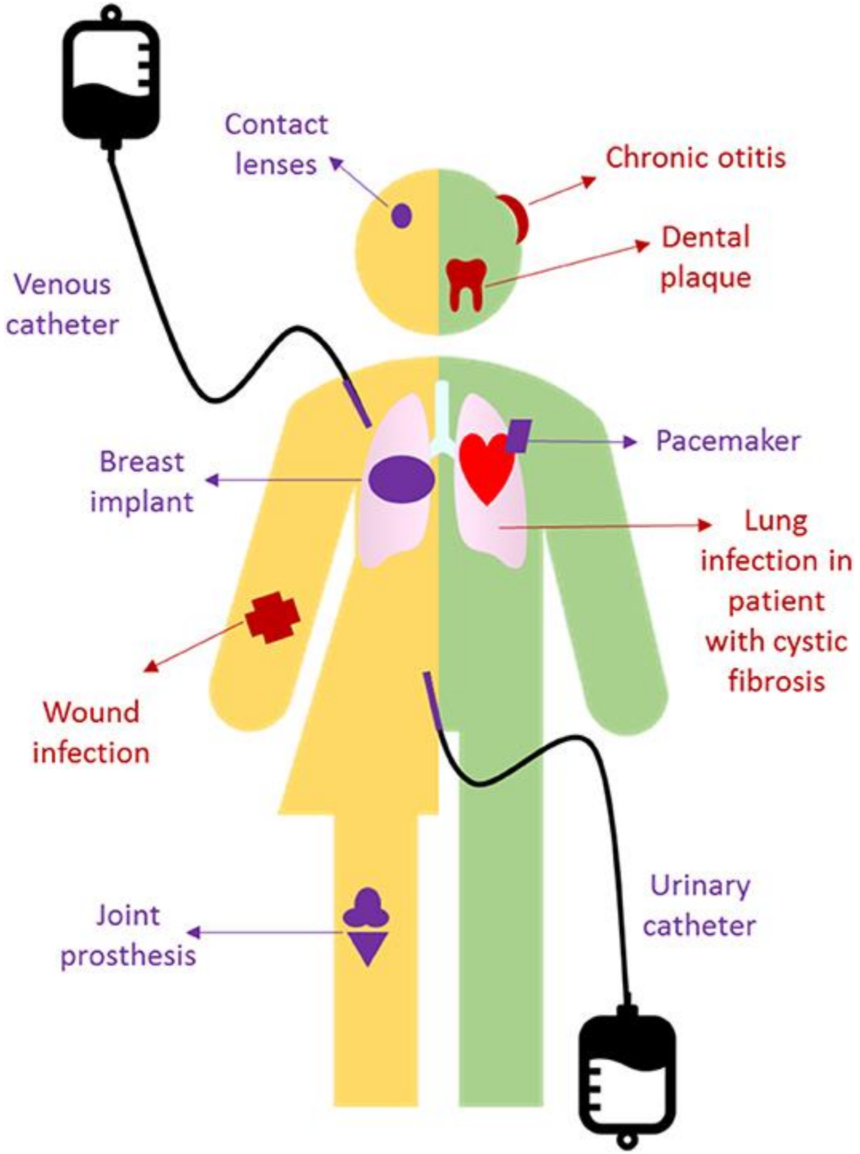
What are biofilms???????

A sessile community formed by bacteria that attaches to biotic and abiotic surfaces

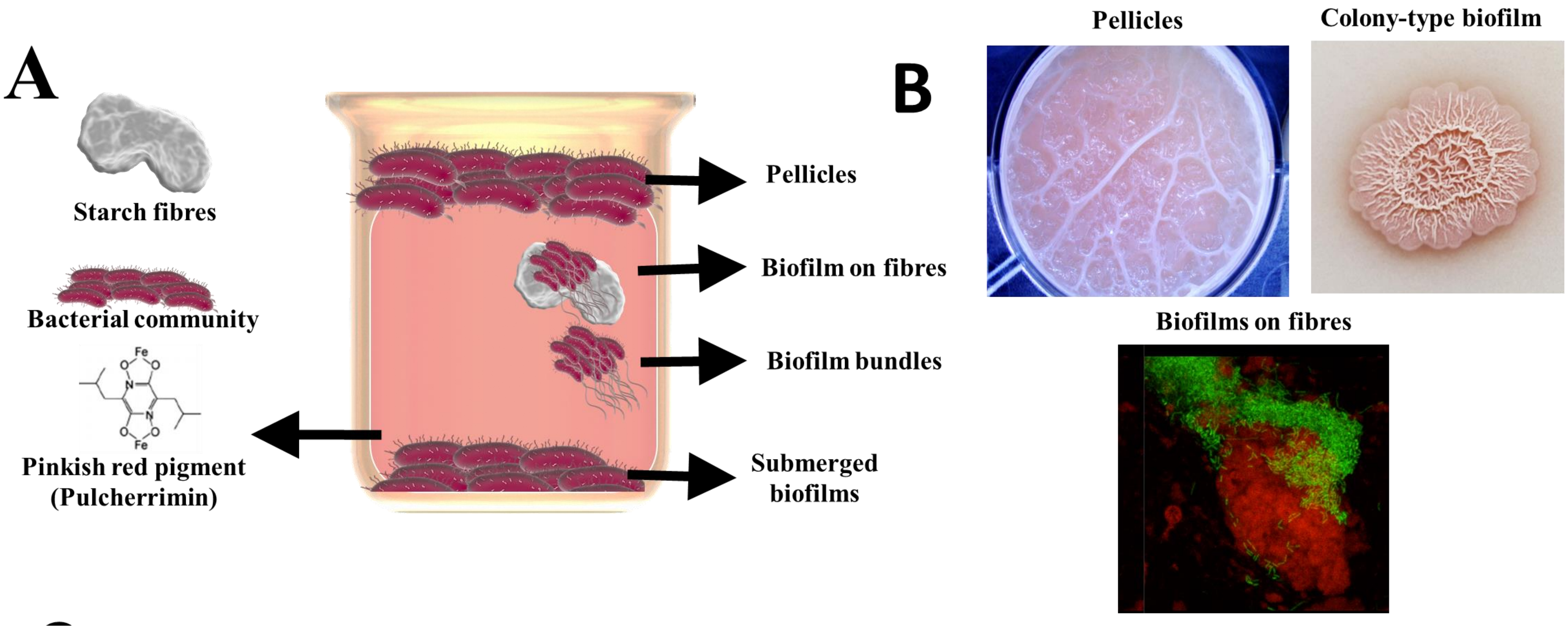
Can be harmful (if formed by pathogens), and beneficial (if formed by probiotics)



Sites for biofilm formation in human by pathogens



Chickpea milk– a prebiotic model


A
Starch fibres
Bacterial community
Fe
N
O
Pinkish red pigment
(Pulcherrimin)
Pellicles
Biofilm on fibres
Biofilm bundles
Submerged
biofilms
B
Pellicles
Colony-type biofilm
Biofilms on fibres

Benefits of probiotic biofilms on food matrices

- Prebiotics (food matrices) serve as a scaffold for probiotic colonization (biofilm formation)

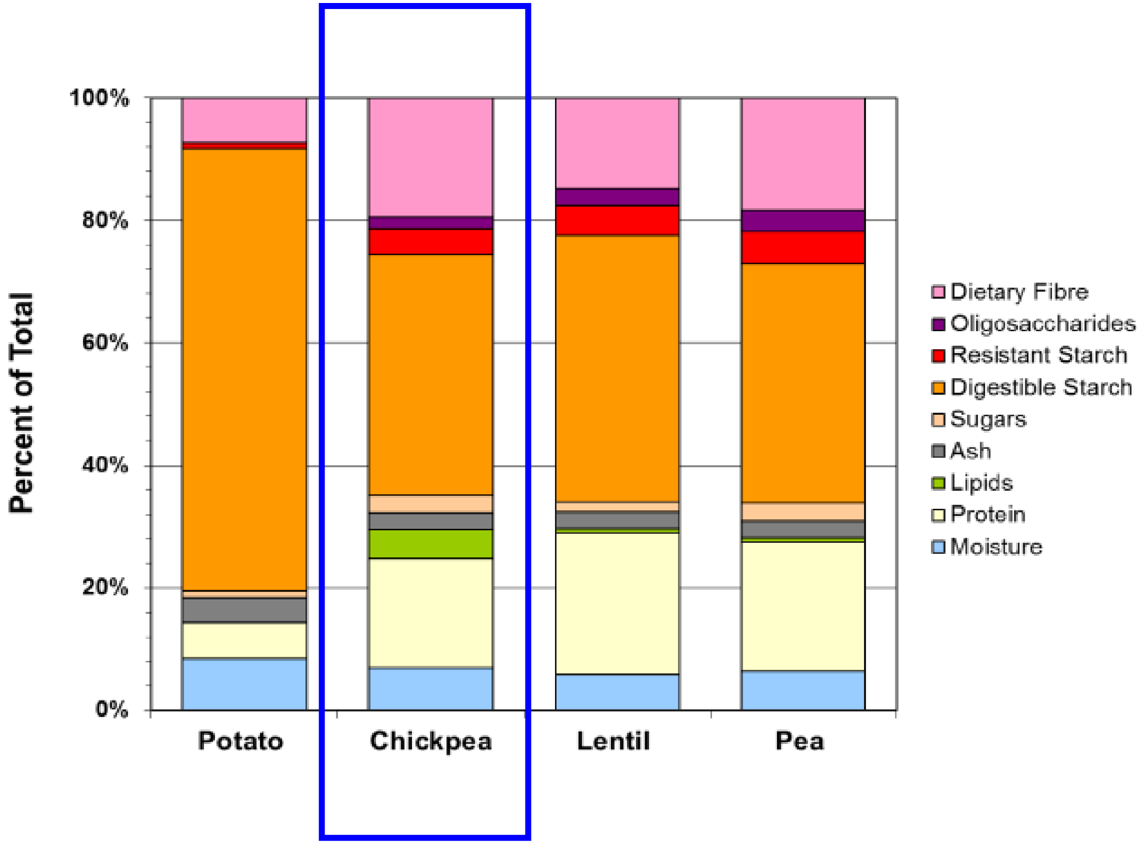
- Probiotic biofilms on these scaffold prevent pathogen
colonization – probiotic blanketing

- Allows safe and fast passage through the GIT
to gut ,where probiotics can detach and colonize.

Allows probiotic adaptation to hostile conditions.

Designing chickpea-based synbiotic food

Brief data on chickpea (Cicer Arietinum L.)

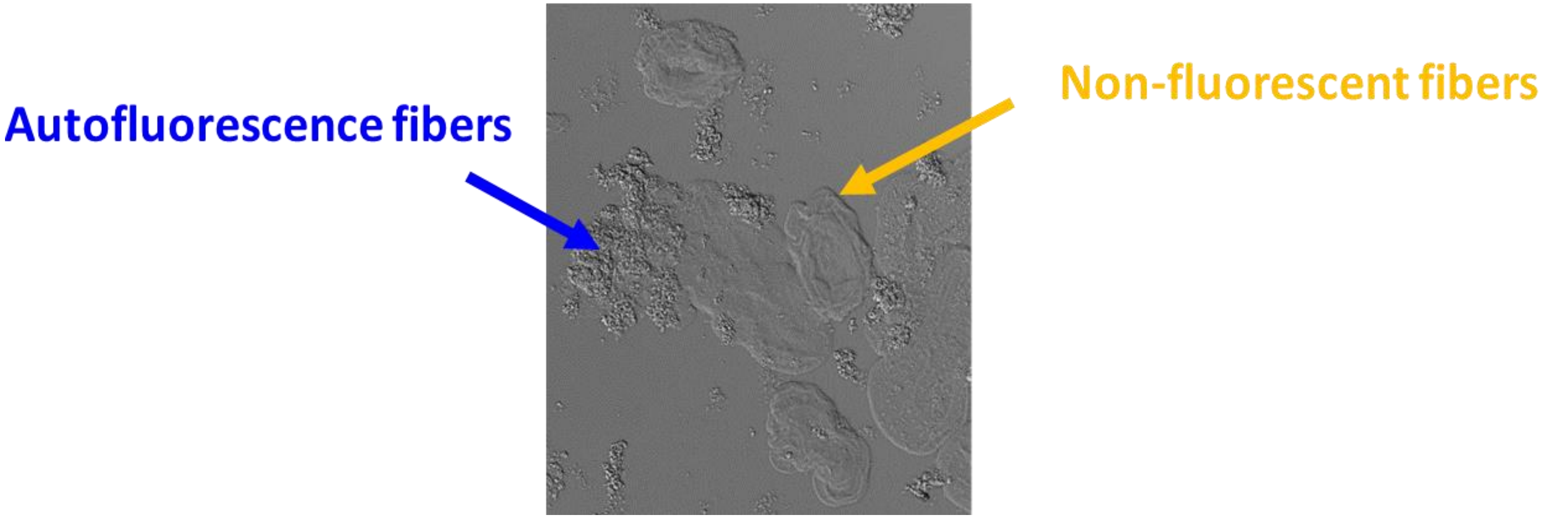


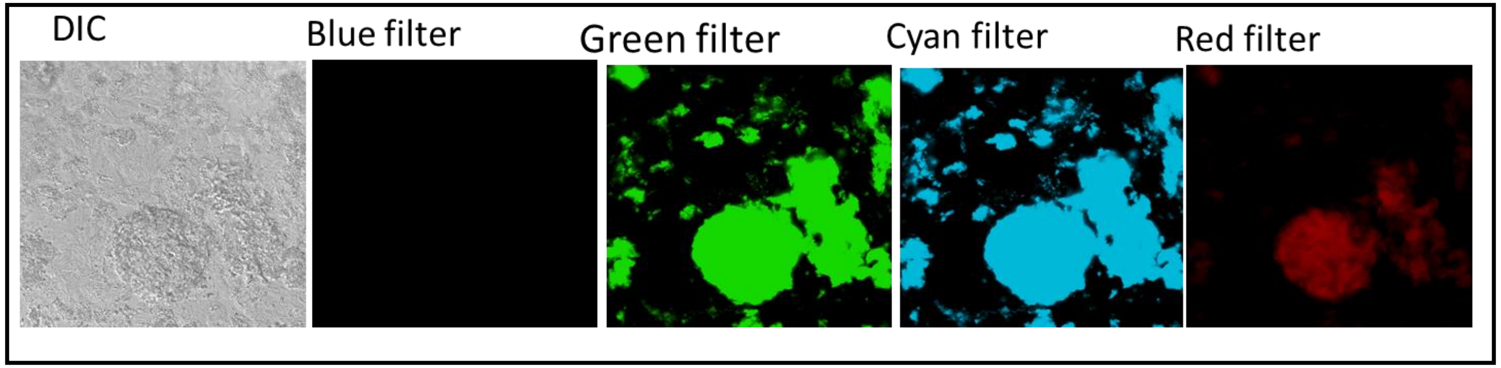
(Susan et al., 2013, Foods)

Compounds	Chickpea grains
Monosaccharides	0.32-0.97
ribose	0.03-0.19
fructose	0.23-0.28
glucose	0-0.065
Disaccharides:	
sucrose	1.09-2.28
maltose	0.16-0.68
Oligosaccharides:	3.87-6.98
raffinose	0.62-1.45
ciceritol	2.51-2.78
stachyose	0.74-2.56
verbascose	0-0.19

Source: Sánchez-Mata et al., 1998; Alajaji et al., 2006

Chickpea milk components that favours colonization

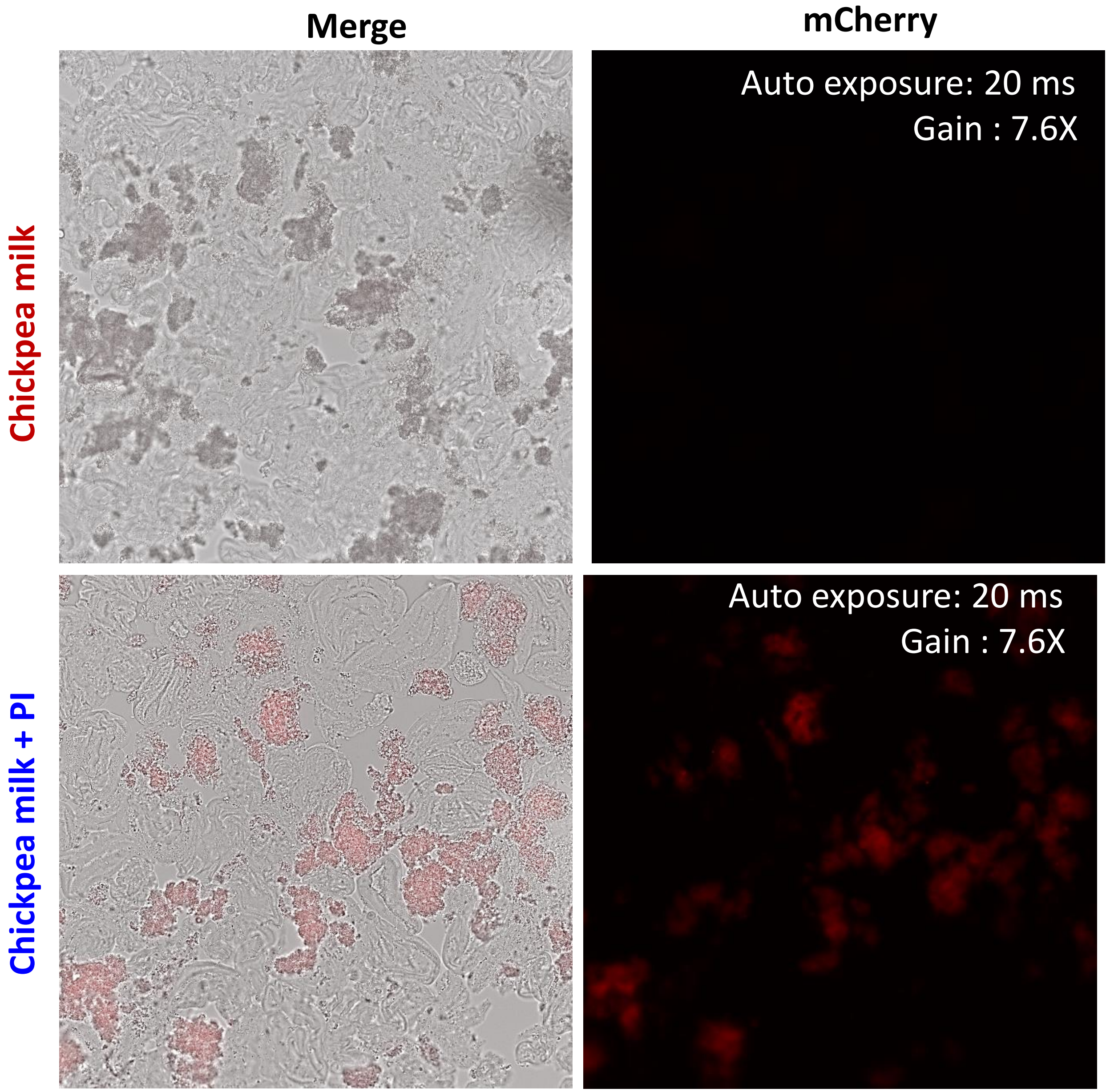




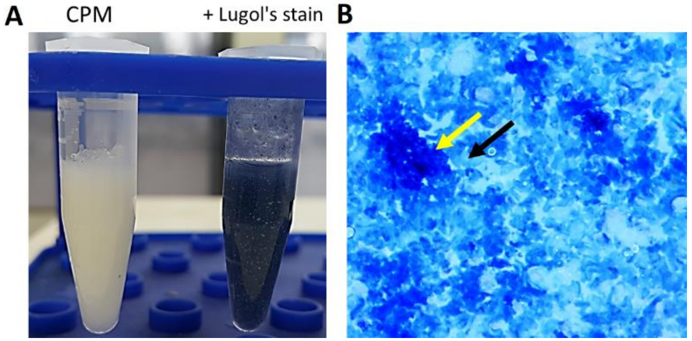
Dietary Fibers in chickpea

1. *Lignin (detected by green autoflouresence)*
2. *Cellulose*
3. *Hemicellulose*
4. *Resistant starch*

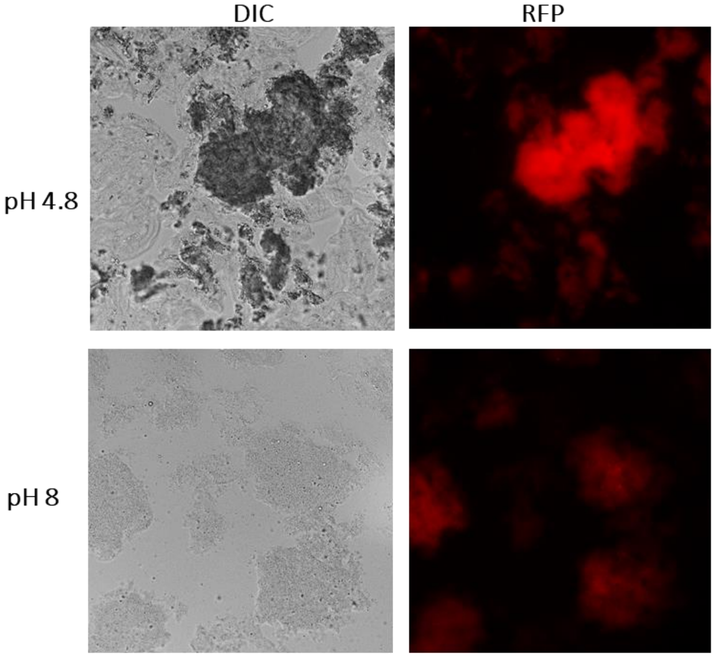
PI stains the autoflouresent components



CPM contains predominantly insoluble starch



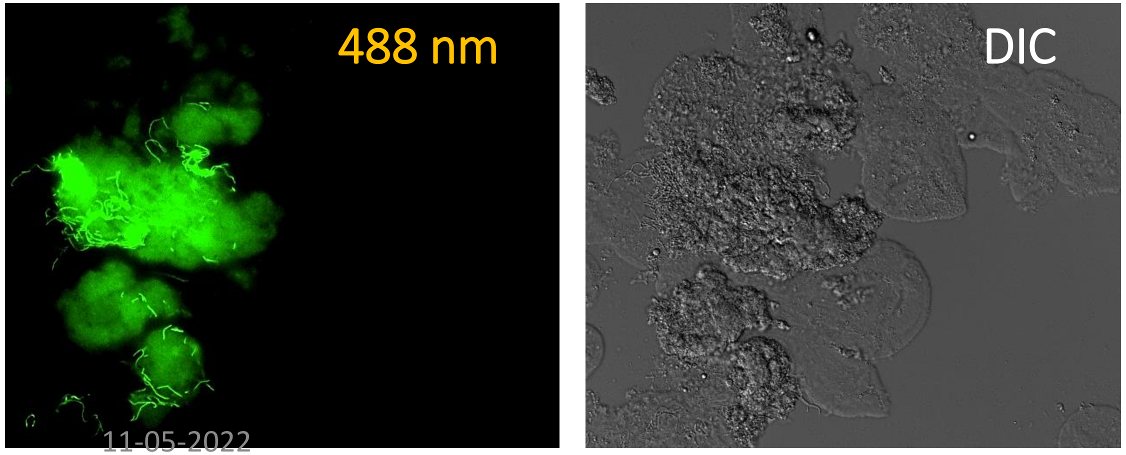
KOH treatment enhances solubility suggesting them to be 'Resistant starch fibres'



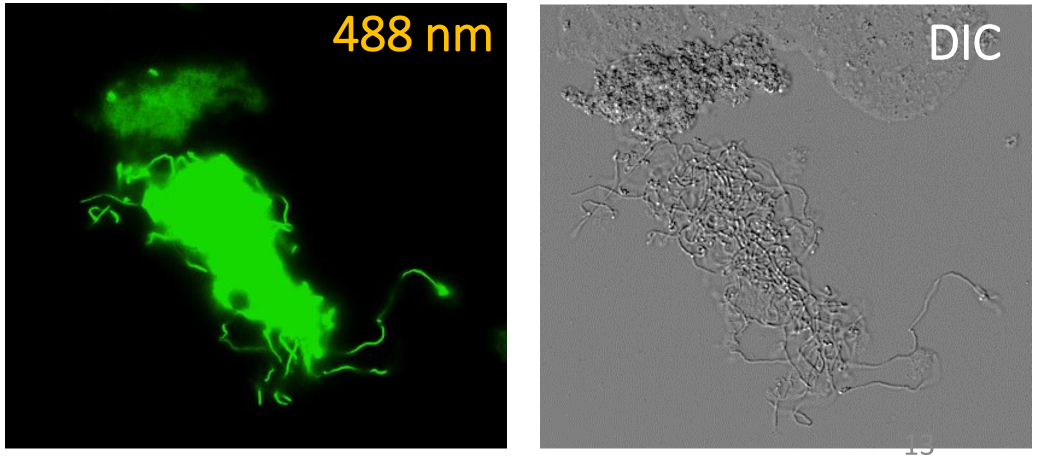
The fibers are Resistant starch

Subsets of sessile B. subtilis cells in chickpea milk

Flocs on fibers (16- 24 h)



Flocs in suspension (16-24 h)



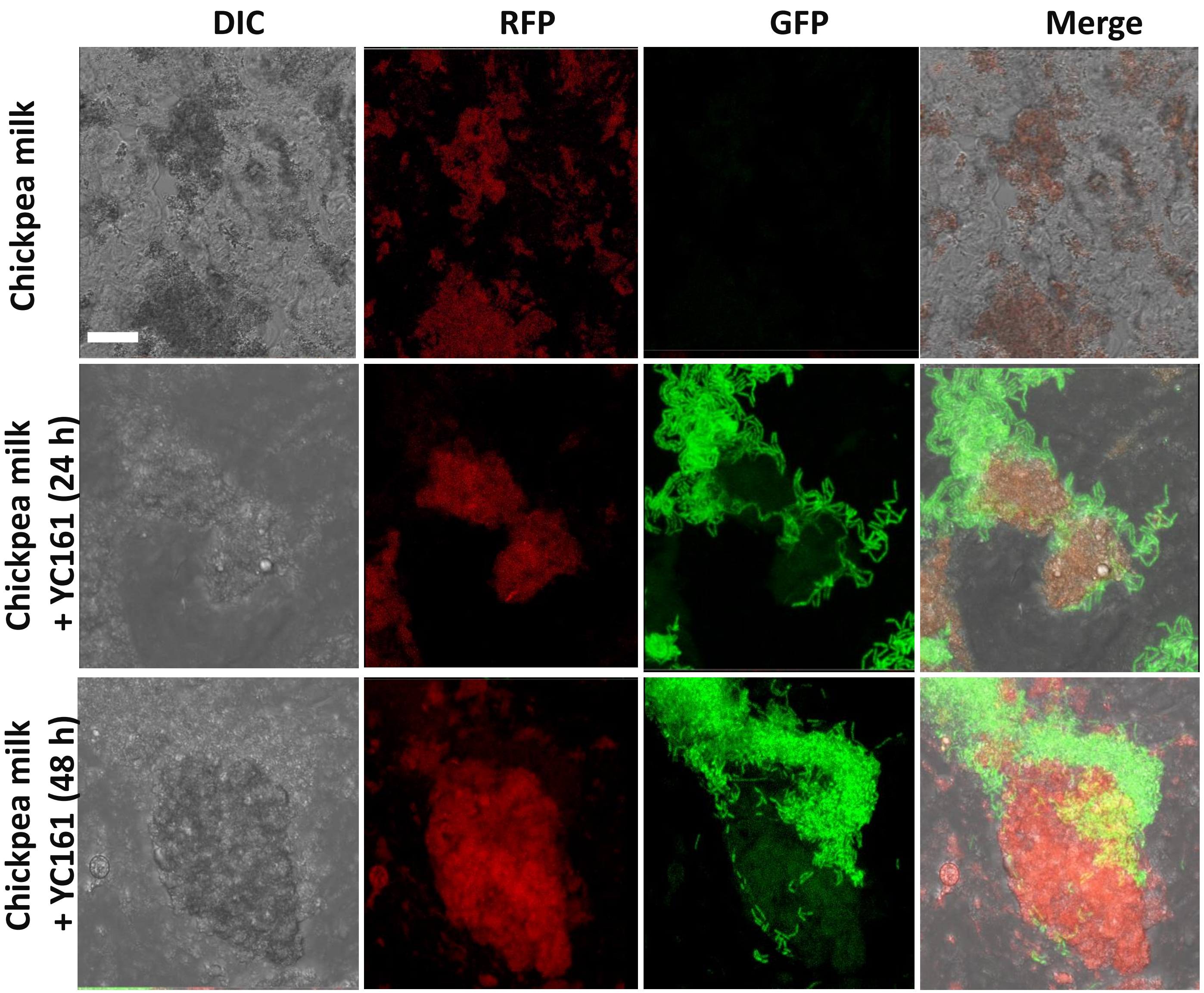
In chickpea milk, 3 subsets of cells exist.

1. Cells that form pellicle
2. Cells that form “attached flocs/biofilm” particularly on “autofluoresent chickpea fibres.
3. Cells that are in suspension and that are loosely positioned over “non-fluorescent fibers.

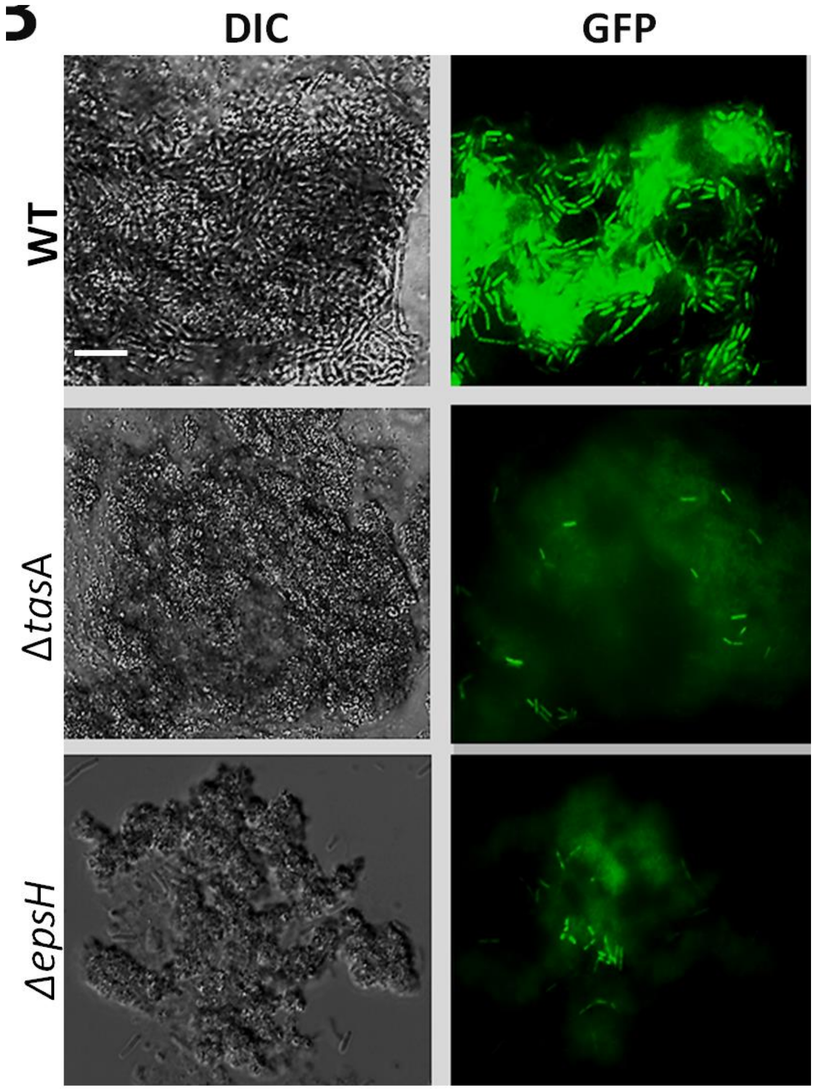
Pellicles with reddish-pink pigment (72 h)

Pigment production is an ‘adaptive response’

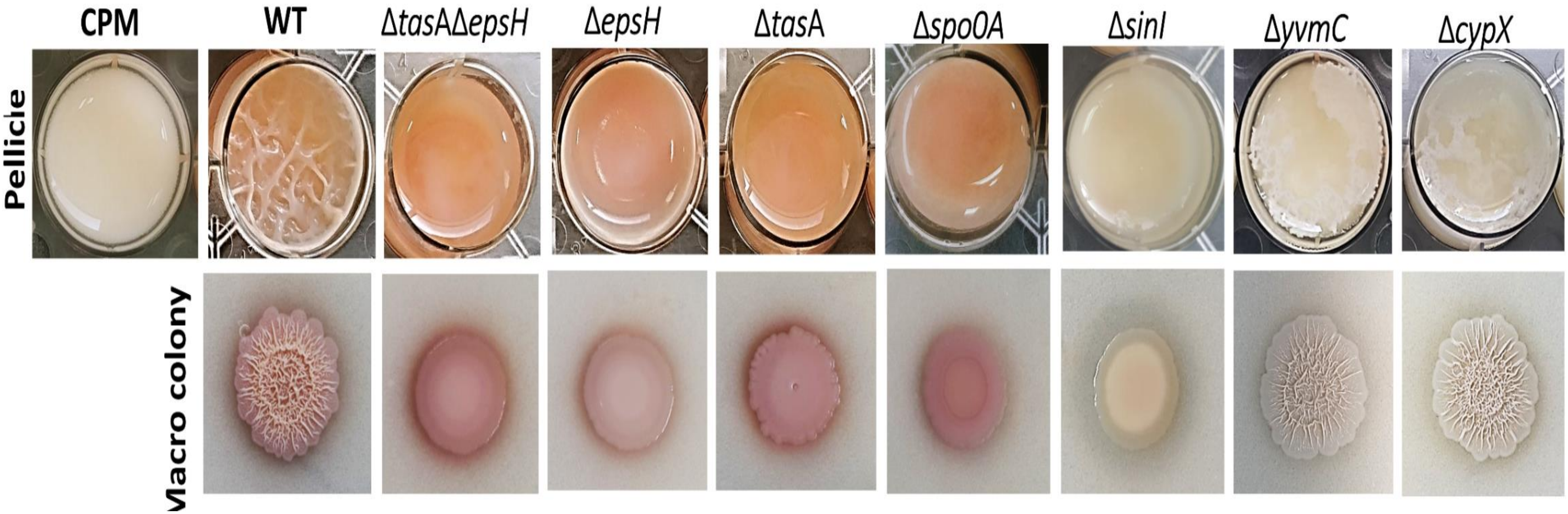
Z-section confirms bacterial attachment to auto-fluorescent fibres

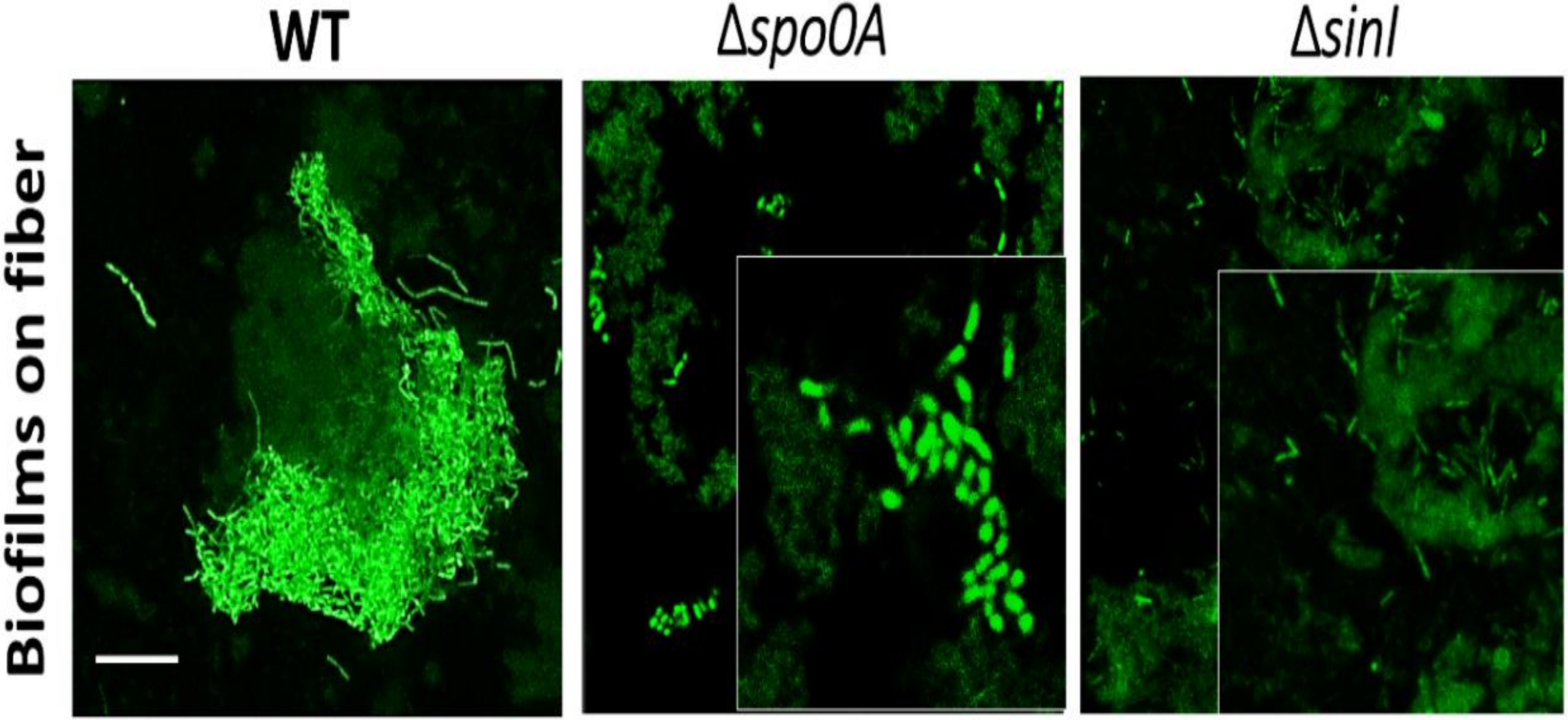


Matrix mutants did not attach to the fibres



Macroscopic phenotypes in CPM





WT
Δspo0A
ΔsinI
Biofilms on fiber

Pigment Production

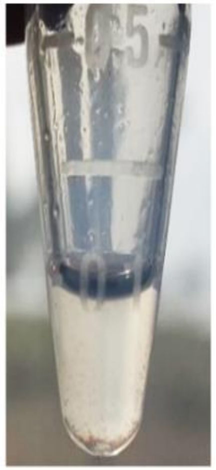
Pigmentation under shaking condition – 24 h



Wild-type 3610 *ΔtasA* *ΔepsH*

WT *ΔsinI*

pulcherrimin

Pigment extraction- exhibits biochemical traits of pulcherrimin

48 h *B. subtilis* in chickpea milk

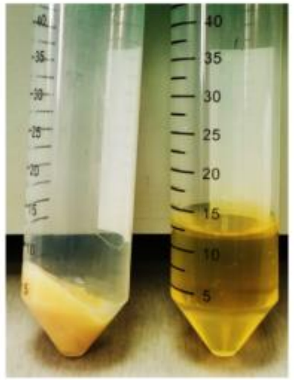
9000 rpm, 10 min, 4°C →

Pellets with pigment, cells and chickpea debris

Methanol treatment + NaOH/KOH (2mM) →

Red pigment turns yellow

9000 rpm, 10 min, 4°C →



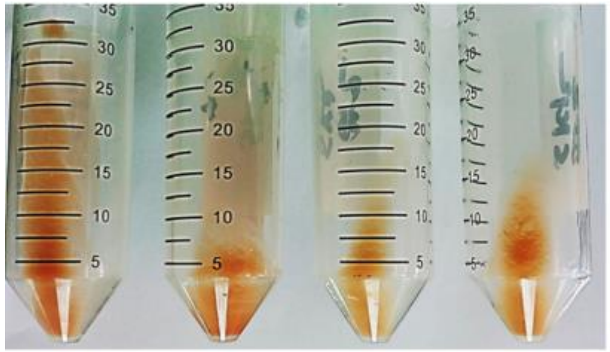
Debris, cells separated from pigment

Supernatant adjusted to pH 1.0 ↓ + incubate 100°C, 30 min (water bath)

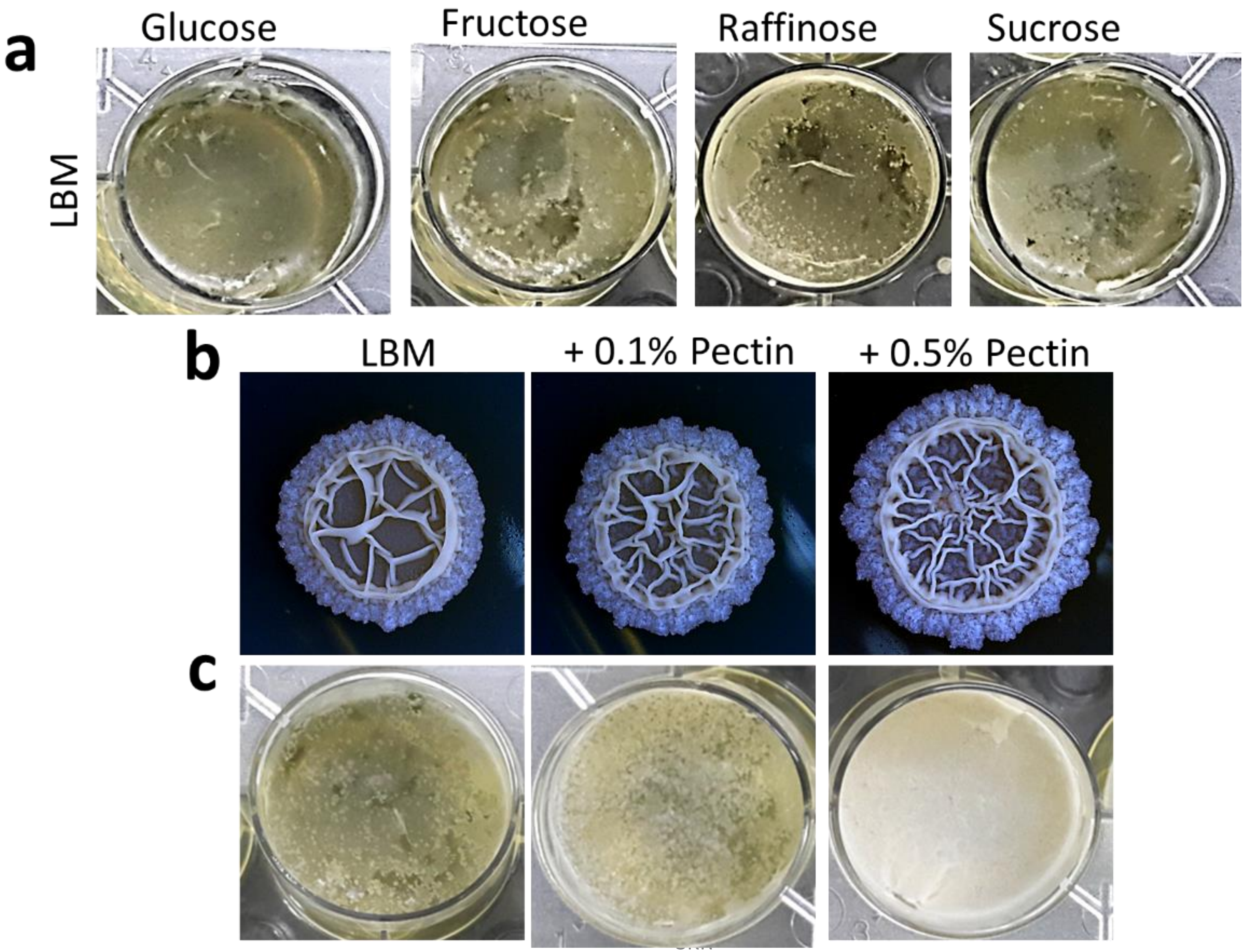
Red precipitate reappears

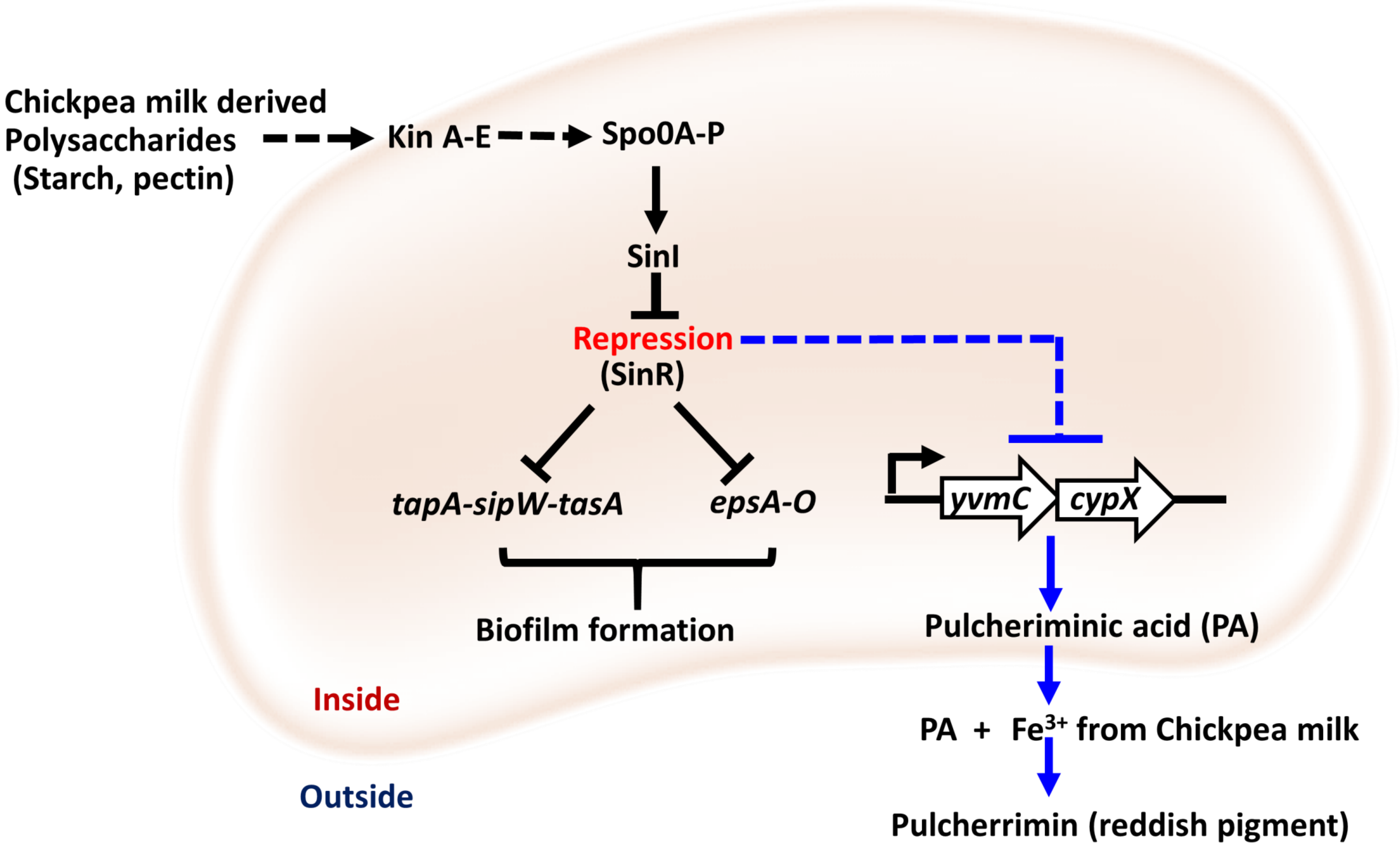
← 2000 rpm, 10 min, 4°C

← Remove supernatant, Drying

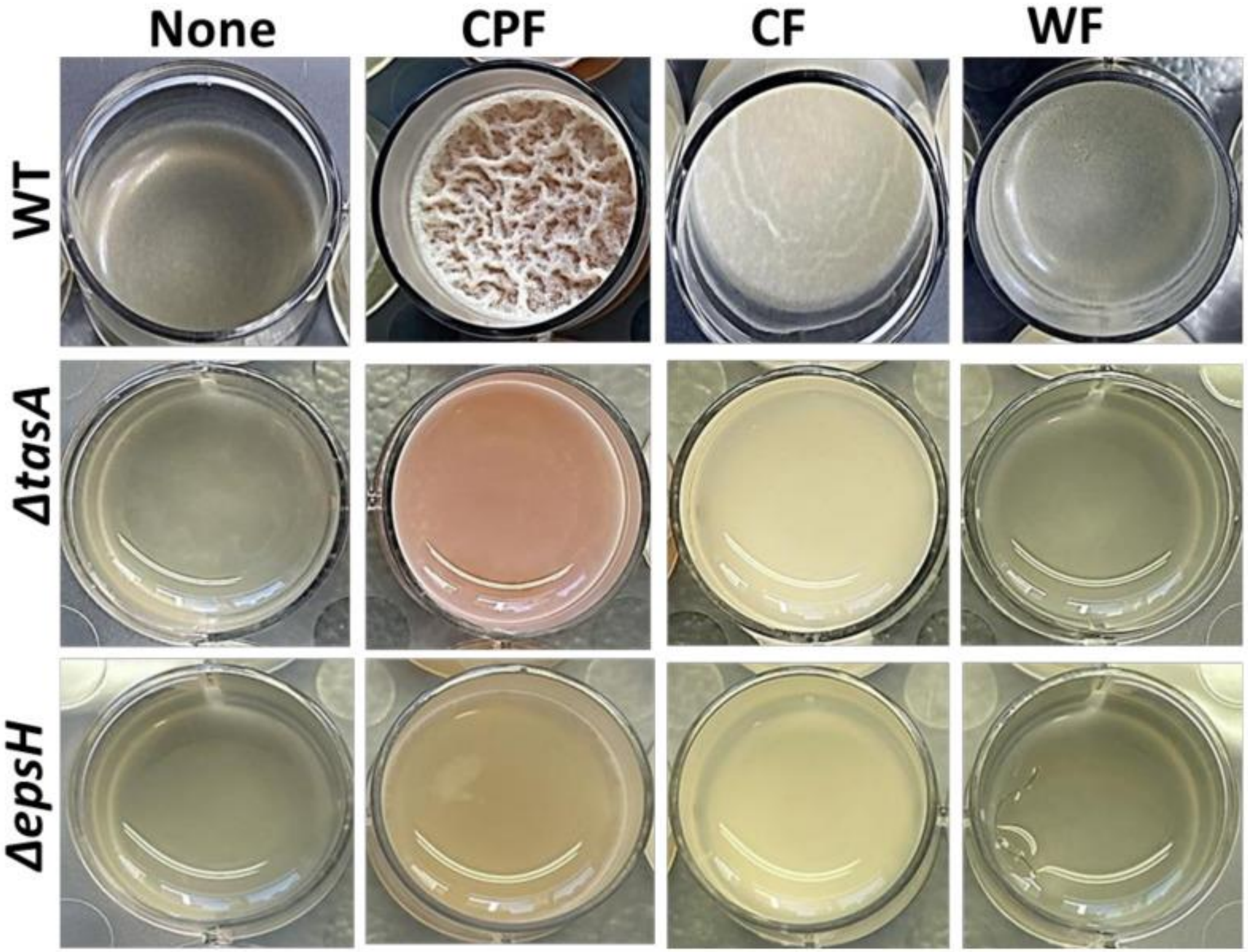


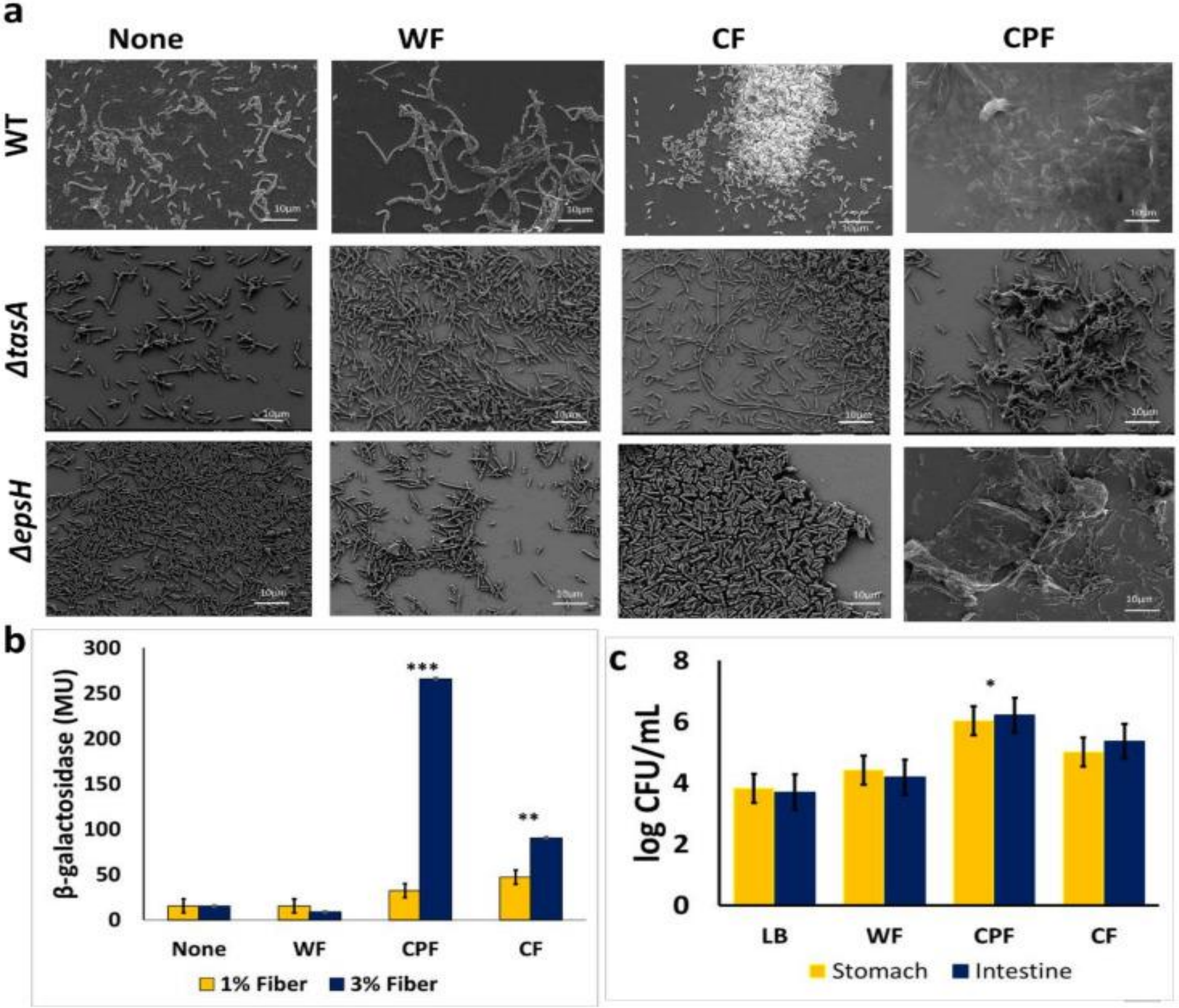
Soluble Fiber (Pectin) might Act as an Environmental Cue



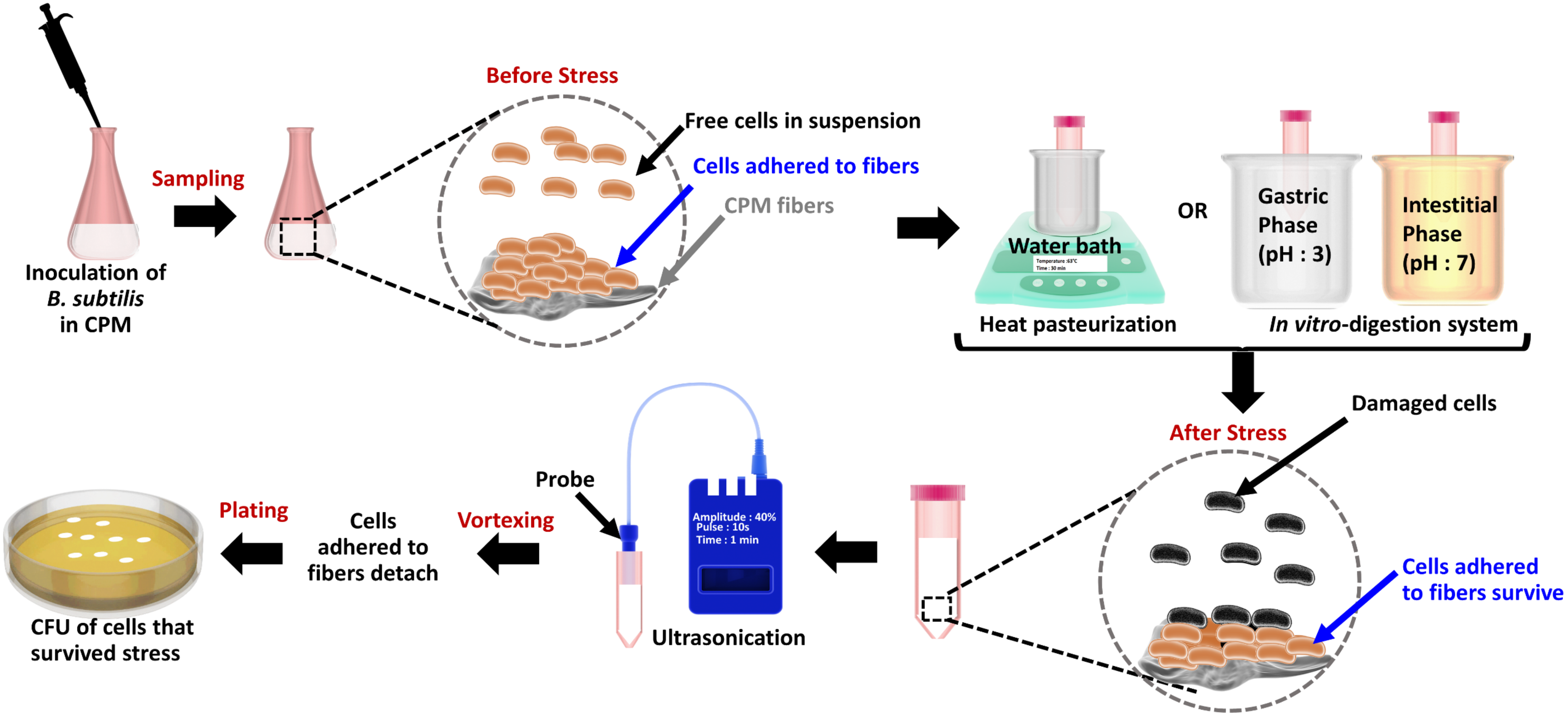

Chickpea milk derived
Polysaccharides
(Starch, pectin)
Kin A-E
Spo0A-P
SinI
Repression
(SinR)
tapA-sipW-tasA
epsA-O
yvmC
cypX
Biofilm formation
Pulcheriminic acid (PA)
PA + Fe^{3+} from Chickpea milk
Pulcherrimin (reddish pigment)
Inside
Outside

Chickpea-Derived Prebiotic Substances Trigger Biofilm Formation by *Bacillus subtilis*

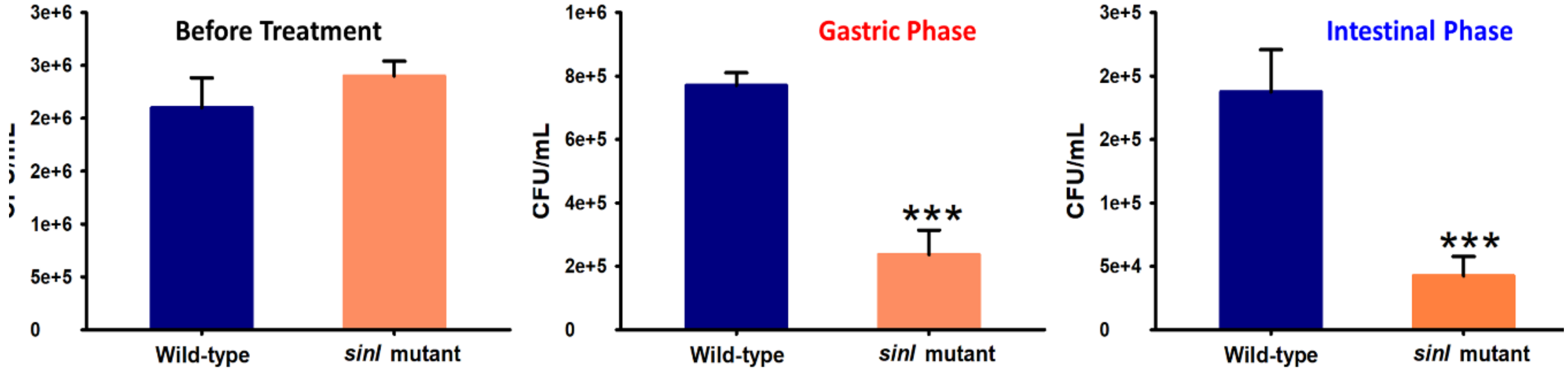



a
None
WF
CF
CPF
WT
ΔtasA
ΔepsH
10µm
b
β-galactosidase (MU)
300
250
200
150
100
50
0
***
**
None
WF
CPF
CF
1% Fiber
3% Fiber
c
log CFU/mL
8
6
4
2
0
*
LB
WF
CPF
CF
Stomach
Intestine

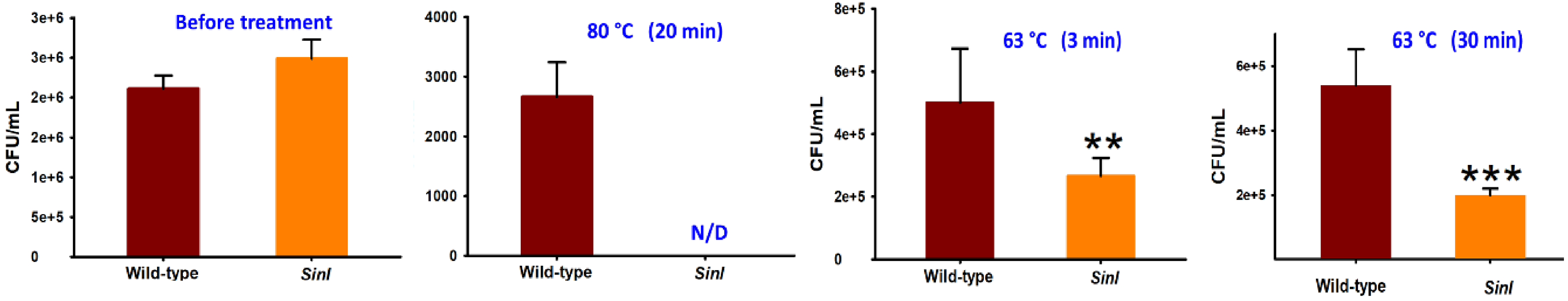
Probiotic blanketing, adaptation, and protection in chickpea milk



Survivability following *In vitro* gastro-intestinal digestion.



Survivability following pasteurization



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- **I thank Dr. Moshe Shemesh and members of the Shemesh lab**

- ***Thank you***