In vitro assessment of P. freudenreichii adaptation to caecal environment

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IN VITRO ASSESSMENT OF *PROPIONIBACTERIUM FREUDENREICHII* ADAPTATION TO CAECAL ENVIRONMENT

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CONTEXT

P. freudenreichii

PF

A 2-in-1 Bacterium

Flavor compounds ← Cheese starter

Probiotic → Immunomodulatory proteins (Slps); Short chain fatty acids; Bifidogenic factors

Delivery vehicle

Tolerance towards digestive stresses	Slps proteolysis	Adaptation to colon environment
✓	✓	?

- The tolerance response to digestive stresses depends on the delivery vehicle
- Slps digestive proteolysis depends on the delivery vehicle

Does the delivery vehicle affect *Pf* adaptation ability to caecal environment ?

STRATEGY

Yeast extract-lactate (YEL)	Milk ultrafiltrate (UF)	Single-strain cheese (Cheese)
Pf 129 *Pf* 129 KO SlpB	*Pf* 129	*Pf* 129

Bacterial pellets extraction and surface proteins "shaving"

± Trypsin

Surface protein proteolysis =shaving: 1 hour-37°C- 300 U of trypsin-Agitation at 180 rpm

Incorporation of bacterial pellets in caecal medium

Cecum content → 10 000 g, 15 min → Supernatant Recovery → Autoclaving 115°C-15 min → 20 000 g, 30 min → Sterilization by filtration → Caecal medium

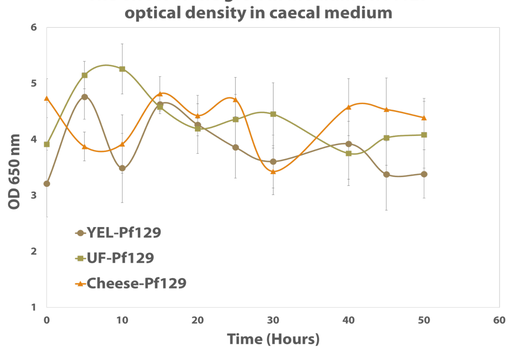
Caecal cultures incubation

50 hours — 37°C — Anaerobiosis

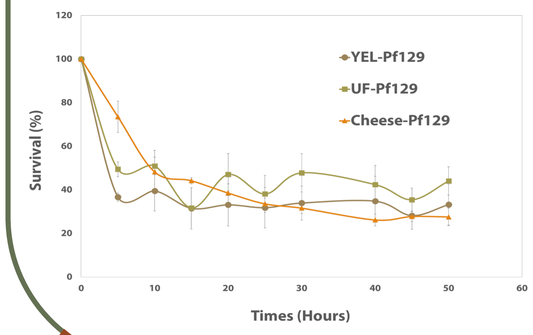
- Survival counting
- Optical density monitoring
- live/dead fluorescence labeling

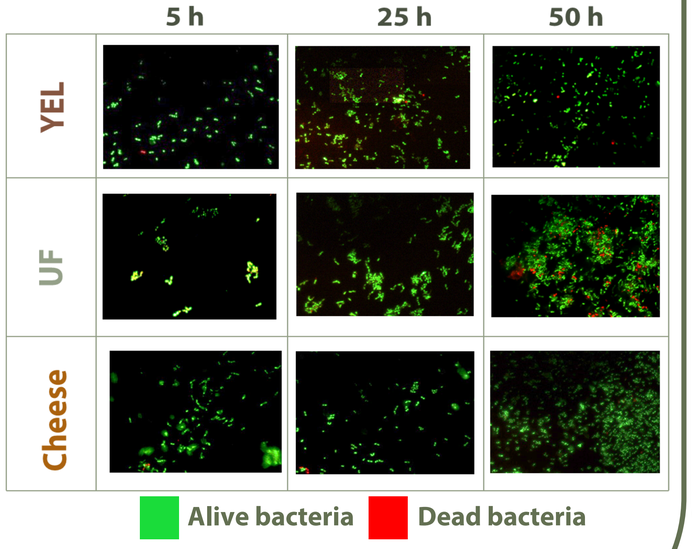
Pf 129 uses a viable but nonculturable state to adapt to caecal environment , whatever the growth medium of *Pf 129*

The effect of the growth medium on *Pf129* optical density in caecal medium



The effect of the growth medium on *Pf129* survival in caecal medium

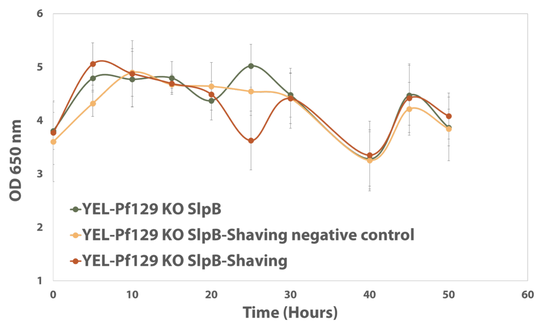




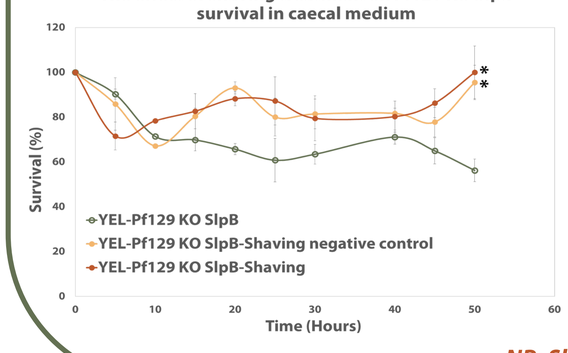
Means ± Standard deviation, P <0.05

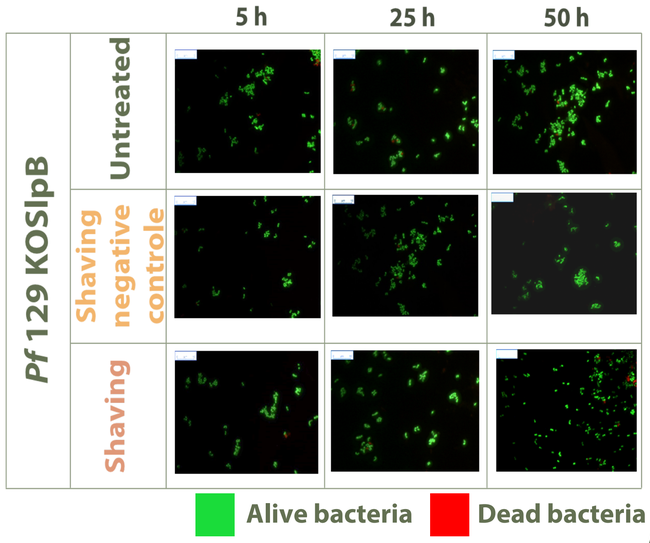
The mutation of SlpB does not change *Pf 129* culturable state but shaving treatment enhances also *Pf* KO SlpB culturable state

The effect of shaving on *Pf129* KO SlpB optical density in caecal medium



The effect of shaving treatment on *Pf129* KO SlpB survival in caecal medium



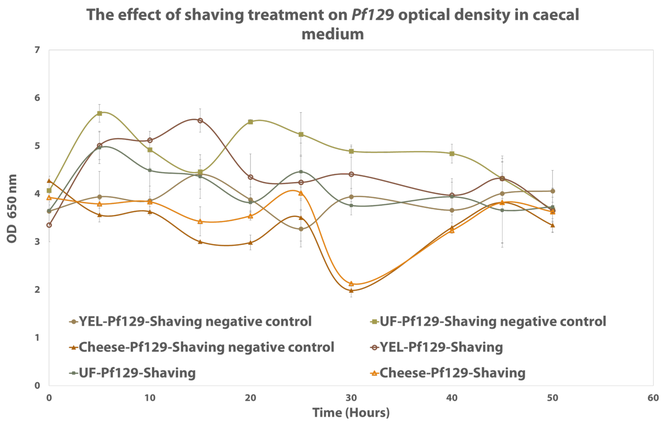


Means ± Standard deviation, * P <0.05

NB: SlpB is the major S-layer protein (Slps) expressed by Pf 129

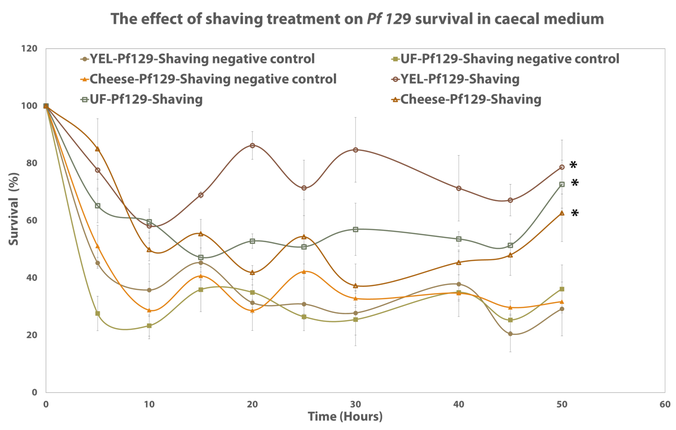
Shaving treatment enhances *Pf* 129 culturable state in caecal medium, whatever the growth medium of *Pf 129*

The effect of shaving treatment on *Pf129* optical density in caecal medium

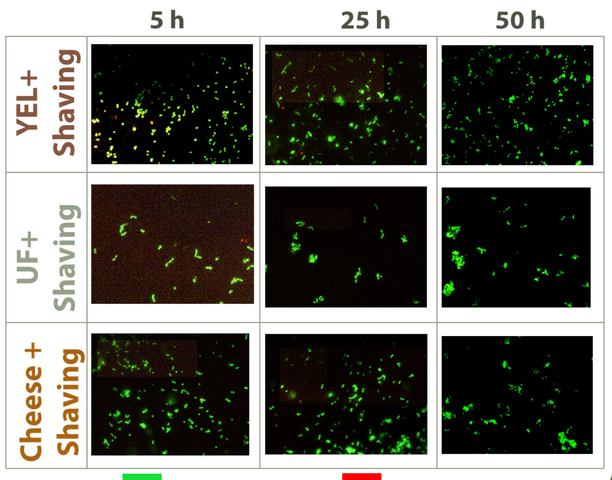


Means ± Standard deviation, P <0.05

The effect of shaving treatment on *Pf 129* survival in caecal medium



Means ± Standard deviation, * P <0.05



NB: the shaving negative control consists in a treatment of 1 hour at 37°C without trypsin

The surface proteins proteolysis enhances *Pf 129* culturable state and so may be its metabolic activity in caecal medium.
The Slps proteins seem to be not involved directly in *Pf 129* adaptation to caecal environment.
=> A comparative proteomic analysis will address the molecular mechanisms involved in an enhanced culturable state of shaved bacteria.

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