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► To cite this version:

Sarah Barrau, C. Gaiani, Claire Retourney, C. Mangavel, Yves Le Roux, et al.. Crucial role of milk proteins on adhesion of intestinal microbes. *Biomolecules: Research & Development, Markets and Acceptability*, Oct 2019, Palma de Majorque, Spain. hal-02504336

HAL Id: hal-02504336

<https://hal.univ-lorraine.fr/hal-02504336>

Submitted on 10 Mar 2020

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Crucial role of milk proteins on adhesion of intestinal microbes

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Introduction

Milk

Main source of nutrition and immunological protection for the neonate
Contain glycoproteins (GPs)

Potentially bind bacteria' pili to adhesion site of model intestinal cells

Use of probiotic bacteria

Beneficial for health and intestinal diseases

"Generally Recognized as Safe" (GRAS)

Glycoproteins

What are the different existing interactions ?

Aim of the study

- ❖ Identification SpaCBA pili role in interaction with intestinal cells and GPs
- ❖ Establishment of interactions and benefits of GPs on bacteria adhesion
- ❖ Improved understanding of the role of milk in the adhesion of bacteria to epithelial intestinal cells

Use of differentiated cell monolayer obtained after 22 culture days

Materials and Methods

Materials used

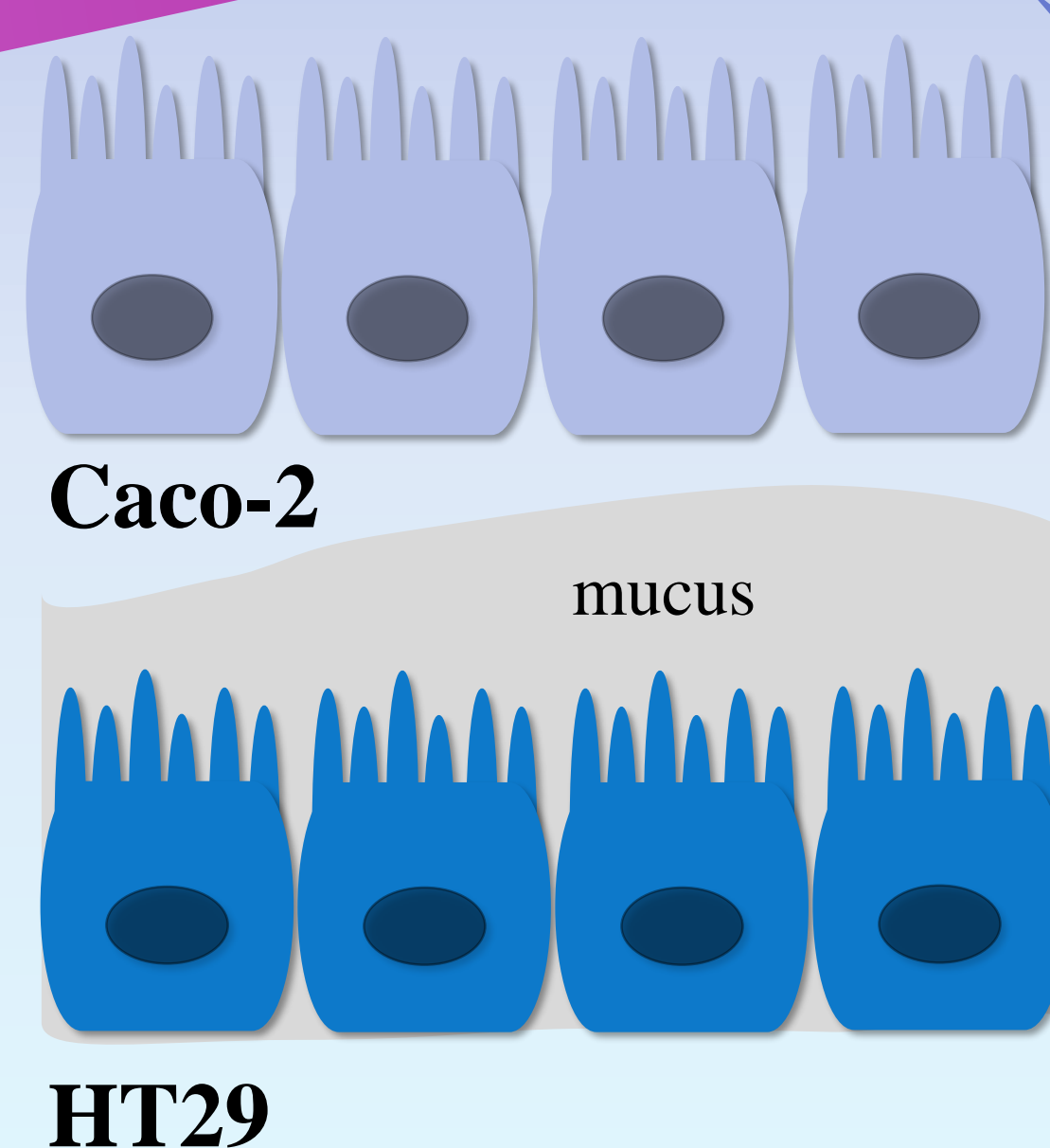
Milk GPs
Osteopontin
Lactoferrin
OPN **Lf**

Intestinal epithelial cells
Caco-2 TC7 cells
HT29-CL16E cells (producing mucus)

Probiotic bacteria
L. rhamnosus GG (LGG) WT
LGG pili depleted (spaCBA)

LGG WT

LGG spaCBA



Mucus layer: potentially impact the bacterial adhesion

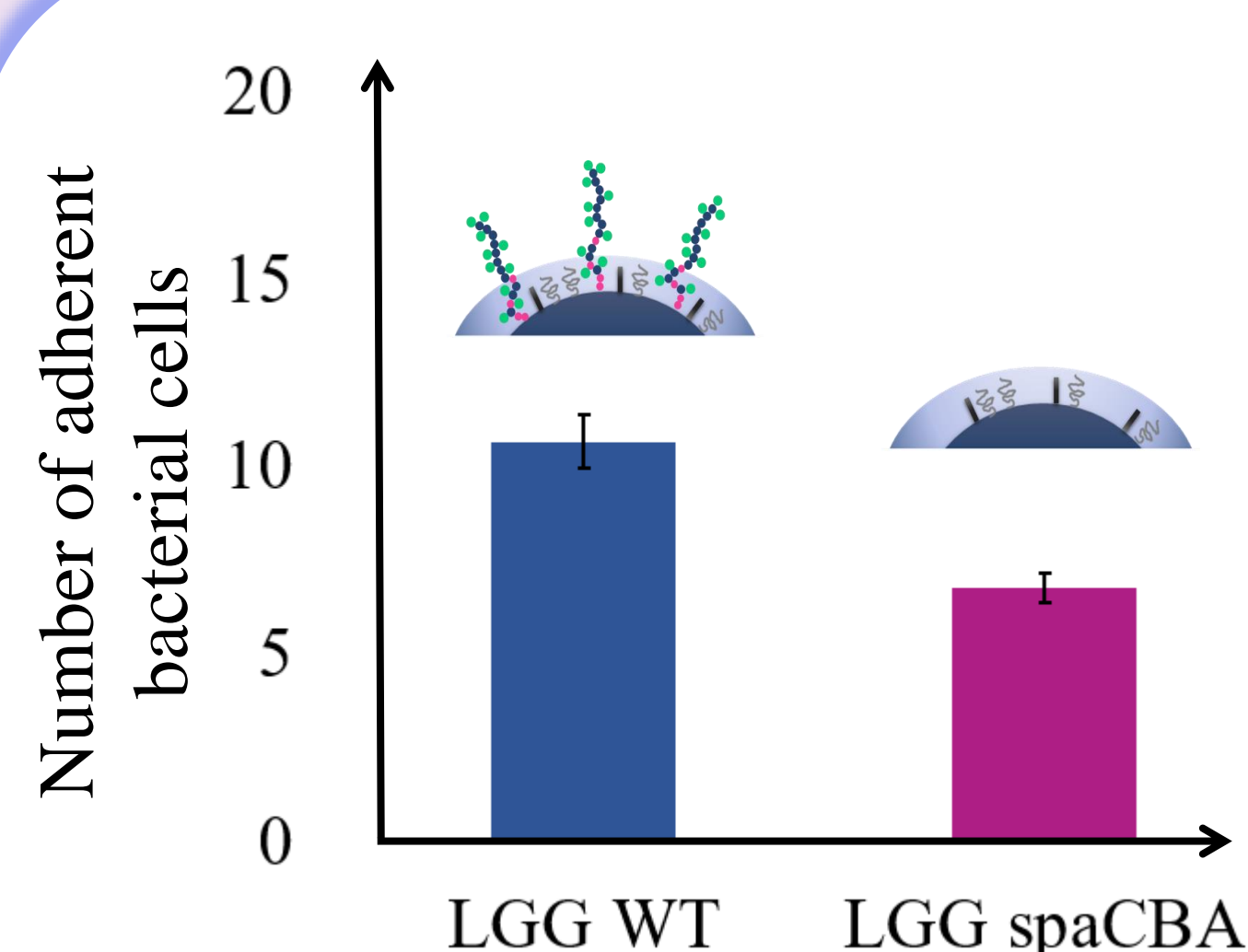
Cell adhesion assay

Cell plate with Caco-2 TC7 or HT29-CL16E

- ① Bacterial culture on fresh MRS broth: end of exponential phase (12h)
- ② Epithelial monolayer incubated for 2h with a) Bacteria suspension or b) Bacteria + GPs
- ③ Sequential washing of intestinal cells
- ④ Counting of bacteria attached to epithelial cells

Results and Discussion

Caco-2 TC7 cells



* Percentage increase in cell adhesion compared to results obtained without GPs

Addition of GPs

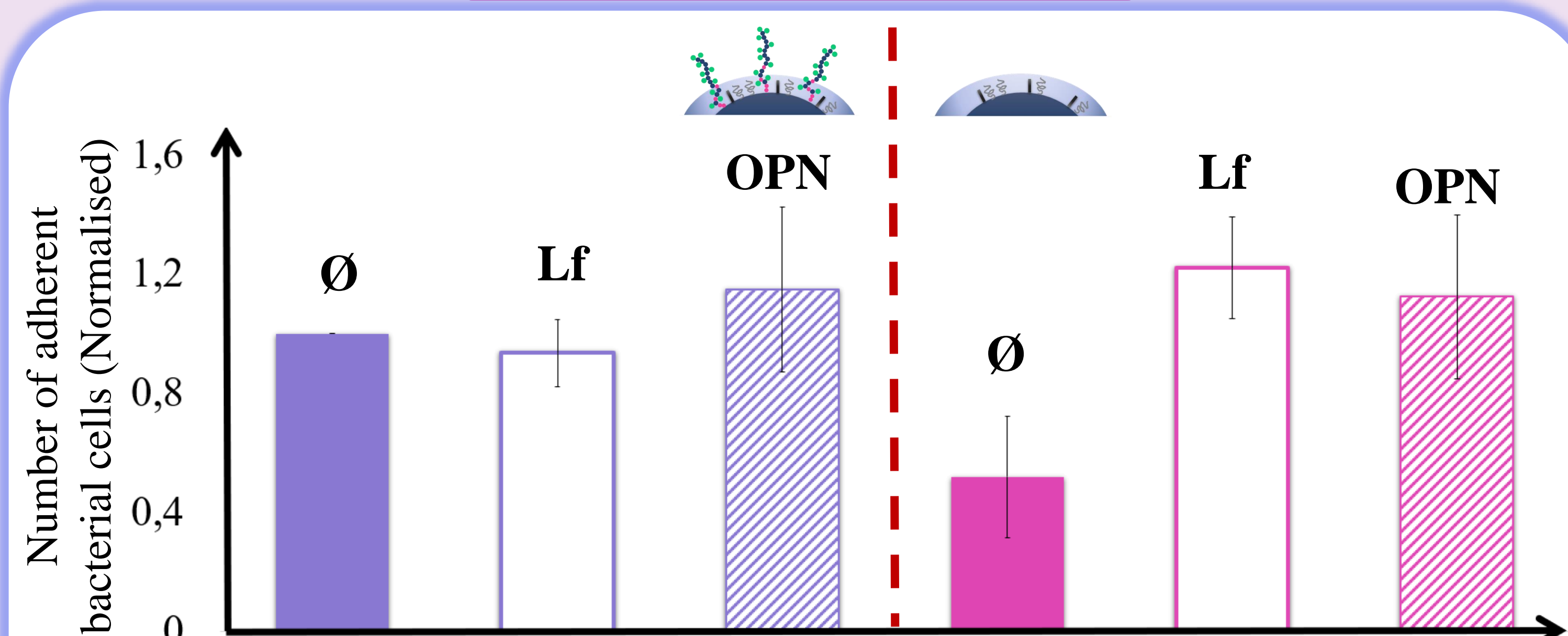
Lf + 23.4 %*
OPN + 51.4 %*

Lf + 14.7 %*
OPN + 70.6 %*

Key role of SpaCBA pilus in adhesion of LGG to Caco-2 cells

- ❖ GPs improve LGG adhesion
- ❖ OPN: major impact on bacterial adhesion

HT29-CL16E cells

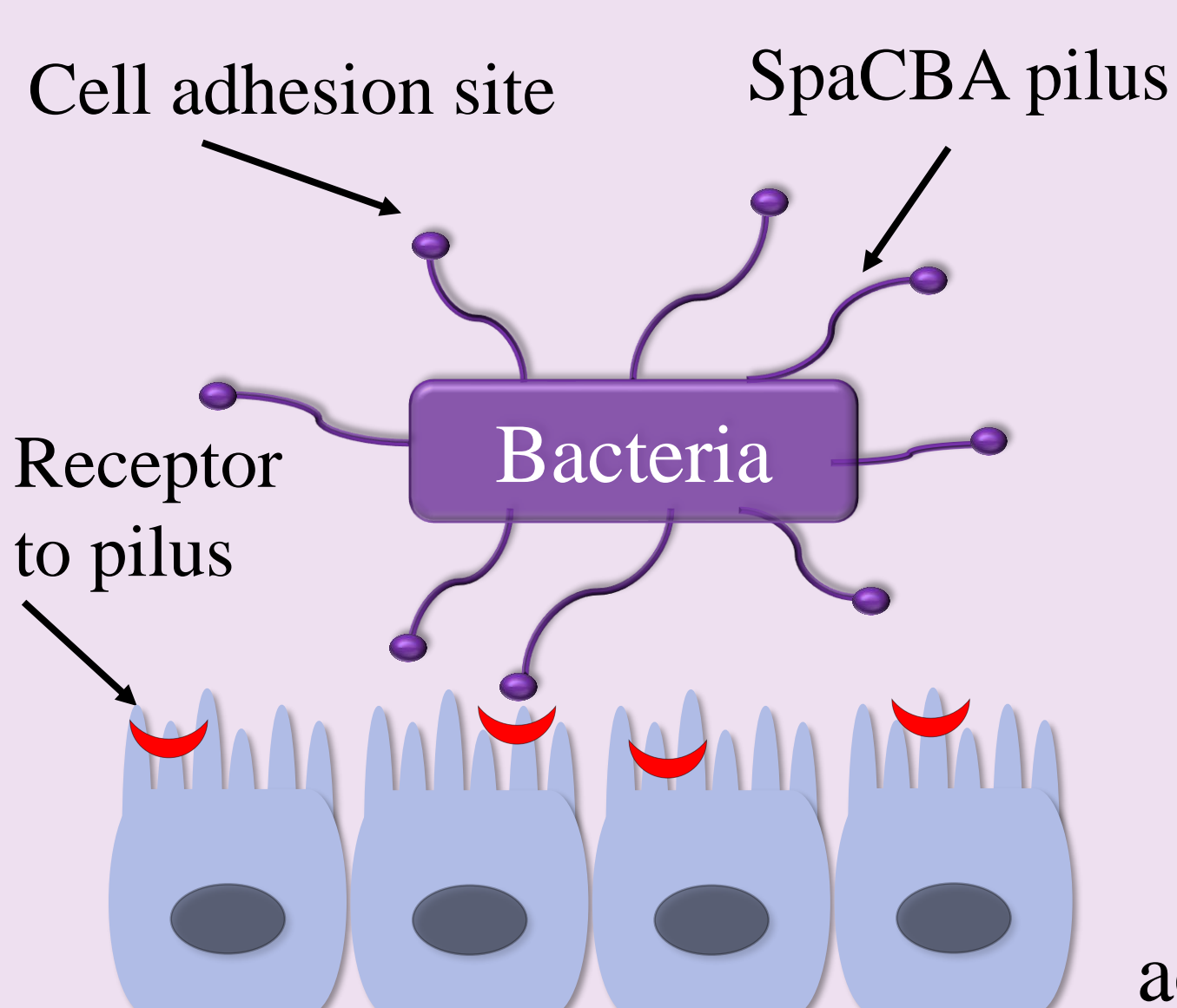


- ❖ Impact of mucus on bacterial adhesion (compared to Caco-2 cells)
- ❖ SpaCBA and WT: almost similar adhesion with GPs
- ❖ OPN: best adhesion results of both cell types in all present cases

Conclusion / Perspectives

- ❖ Identification of all interactions

(1) Without GPs: bacteria adhesion with epithelial cells

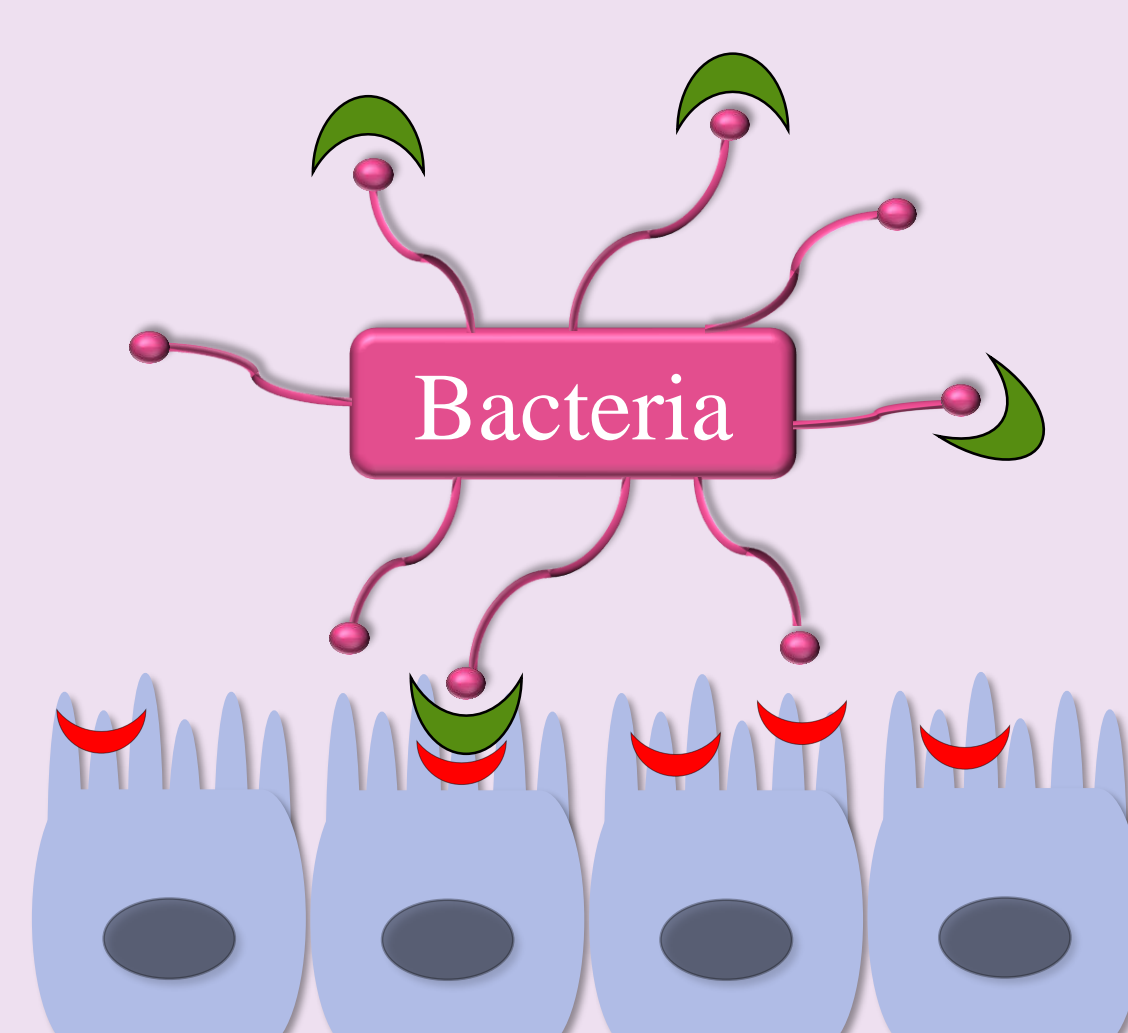


GPs

Bacteria

(2) Bacterial adhesion with GPs

(3) With GPs: increase of bacteria adhesion to epithelial cells



Future works: Identification of molecules engaged in adhesion like Peptins (adhesins, GPs, surface molecules...)

Acknowledgments

Thanks to:

- ❖ Arla Foods and Glanbia for providing milk GPs
- ❖ LUE Impact Biomolécules for funding COMPET project.

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