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In vitro studies of the impact of pectins on adhesion of *Lactobacillus* spp. to human epithelial cells and intestinal barrier integrity

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Introduction and objectives

Pectins are complex polysaccharides extracted from plants and used in food industry as stabilizers and gelling agents. Pectins are commonly referred as emerging prebiotics which beneficial effects in the gut need to be confirmed.

The aim of this study: To investigate the effect of pectins on adhesion of probiotic *Lactobacillus* species to human epithelial cells and integrity of intestinal cell monolayers.

Materials and Methods

Pectins: P1 (Harsh extracted from orange), P2 (Mild extracted from lemon), P3 (Harsh extracted chemically deesterified from lime) and P4 (Harsh extracted pectin from lemon) (provided by CP Kelco ApS, Denmark).

Bacterial strains: *Lactobacillus fermentum* PCC and *Lactobacillus reuteri* RC-14 (provided by Chr. Hansen, Denmark).

Cell culture: Human colon adenocarcinoma Caco-2 cell line.

Assays conditions:

- Caco-2 cells were maintained in DMEM, 37ºC, and 5% CO_2 for 14 days to reach differentiation.
- Bacterial strains PCC and RC-14 (overnight culture): 10^8 CFU per well
- Pectins P1 – P4: final concentration 0.2% (w/v) in test solution (PBS + DMEM, pH 7.3).

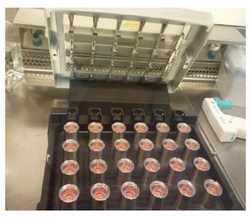
➢ Adhesion assay

12 well-plate

1h → Counts of bacterial cells bound to Caco-2 cells on MRS agar

➢ TEER assay

Transwell filter insert (0.4 µm pore size)



CellZscope2 (Nano Anatytics)

➢ Gene expression analysis

12 well-plate

Incubation 1, 4 and 10h → Extract RNA from Caco-2 cells → qRT-PCR (Fluidigm/SYBR Green)

Conclusion

- Pectins have a potential to improve bacterial adhesion to intestinal cells and further enhance strengthening of epithelial barriers by probiotic *Lactobacillus* spp. in the gut.
- The beneficial effects of bacterial-pectin combinations in this study were strain-specific and differed between the pectins, indicating involvement of specific structural factors in bacterial-pectin interactions.
- The mechanisms behind these interactions and the interplay between the structural properties of pectins and their beneficial effects need to be further elucidated.

Results

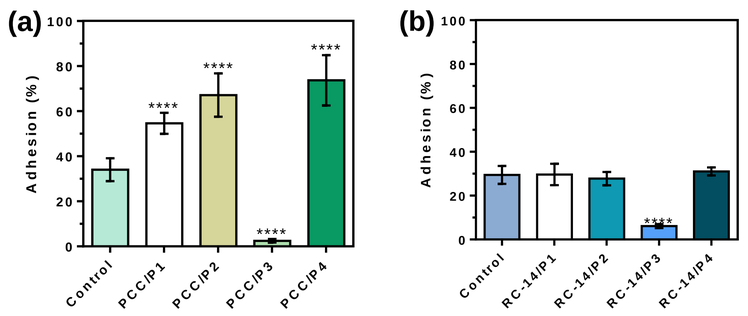


Figure 1. Adhesion of *Lactobacillus spp.* to Caco-2 cell monolayers as affected by pectins after 1h incubation ((a) PCC and (b) RC-14).

❖ ***Adhesion assay*:** PCC and RC-14 increased adhesion to Caco-2 cells of 30% and 35%, respectively.

- ❖ Pectins P1, P2, and P4 improved adhesion of PCC (2-fold); their effect on RC-14 adhesion was insignificant
- ❖ Pectin P3 reduced the binding of both strains to Caco-2 cells.

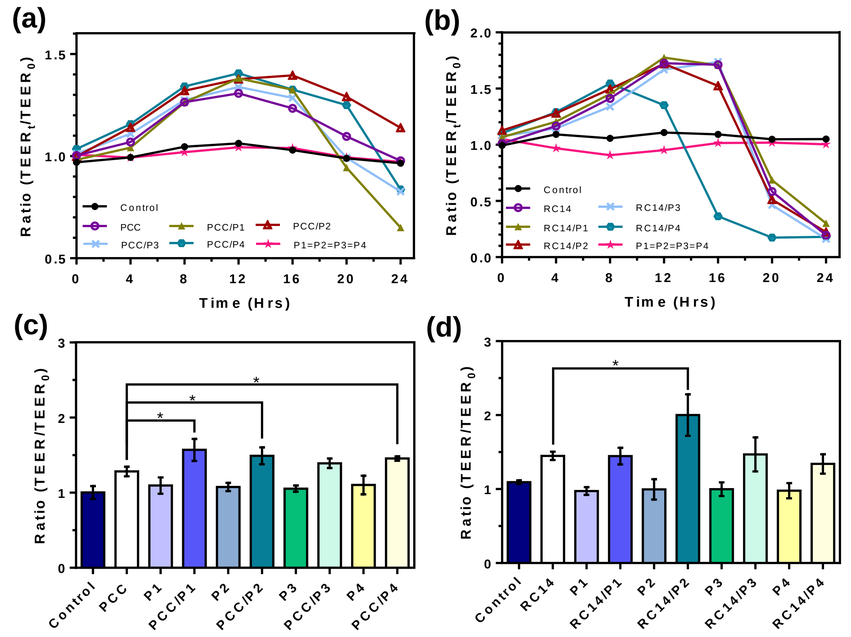


Figure 2. Transepithelial electrical resistance (TEER) across Caco-2 cell monolayers treated with PCC and RC-14 alone and in combination with pectins P1, P2, P3 and P4. (a) and (b) TEER curves during 24 h treatment; (c) and (d) differences between treatments at maximum TEER (after 12h incubation).

❖ ***Measurement of transepithelial electrical resistance (TEER)*:**

- ❖ TEER was increased up to 30% by treatments with PCC and RC-14.
- ❖ PCC in combination with P1, P2 and P4 increased TEER up to 16% compared to PCC alone.
- ❖ RC-14 in combination with P2 increased TEER up to 37%.
- ❖ Other pectin combinations with PCC (P3) and RC-14 (P1, P3 and P4) did not have significant effect on TEER.

	1 Hours						
	Gene	Control	P2	P3	PCC	PCC/P2	PCC/P3
Adhesion	CLDN1	1.00	1.11	0.42	0.50	0.56	0.26
	CLDN2	1.00	0.77	0.92	0.91	1.08	0.42
	CLDN4	1.00	1.03	0.56	1.10	1.18	0.32
	TJP1	1.00	0.94	0.57	0.86	1.02	0.49

	4 Hours						
	Gene	Control	P2	P3	PCC	PCC/P2	PCC/P3
Immune reponse	CCL20	1.00	0.86	5.59	11.73	16.41	10.08
	CXCL1	1.00	0.72	26.17	79.75	64.61	43.68
	CXCL2	1.00	0.95	0.99	14.47	13.65	10.54
	CXCL10	1.00	2.86	9.68	13.97	18.40	11.48
	IL8	1.00	1.28	9.21	28.97	30.37	14.34
	TNF	1.00	1.15	2.54	101.06	77.37	81.58
Adhesion	CLDN1	1.00	0.25	0.49	1.29	0.77	0.38
	CLDN2	1.00	4.09	1.13	0.43	0.34	0.19
	CLDN4	1.00	0.96	0.90	2.20	2.66	2.88
	TJP1	1.00	1.24	0.55	0.84	0.76	0.30

	10 Hours						
	Gene	Control	P2	P3	PCC	PCC/P2	PCC/P3
Adhesion	CLDN1	1.00	1.13	1.02	0.89	1.03	1.02
	CLDN2	1.00	1.00	3.22	0.16	0.17	0.31
	CLDN4	1.00	0.86	0.48	2.79	2.65	9.67
	TJP1	1.00	0.85	1.09	0.62	0.52	1.36

Figure 3. Expression of genes encoding adhesion and immune response proteins in Caco-2 cells treated with PCC and pectins P2 and P3.

- Responses in adhesion genes were determined after 1, 4, and 10 hours treatment (SYBR Green assay) and immune genes - after 4 h treatment (TaqMan assay, Fluidigm).

❖ ***PCC in combination with pectins P2 and P3 altered mRNA expression in Caco-2 cells*:**

- ❖ Expression of genes CCL20, CXCL1, CXLC2, CXCL10, IL8 and TNF∝ involved in immune responses were increased after 4h in all treatments.
  - ➢ Highest by 70-100 fold in TNF∝ after treatment with PCC alone and combined with P2 and P3.
- ❖ CLDN4 was induced by both P2 and P3 combined with PCC after 4h.
- ❖ CLDN1, CLDN2 and TJP1 were decreased (0.2-0.8 fold) after 1h in all treatments.
- ❖ TJP1 was slightly increased (1.36-fold) after 10h incubation (PCC with P3).

Acknowledgements

Bioactive components from by-products of food processing used in a synbiotic approach for improving human health and well-being.

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