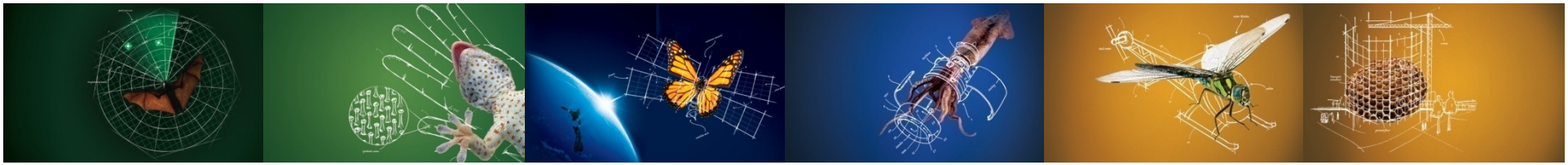
The Probiotic Bacterium *Lactobacillus reuteri* DPC16: Some Properties and Applications.

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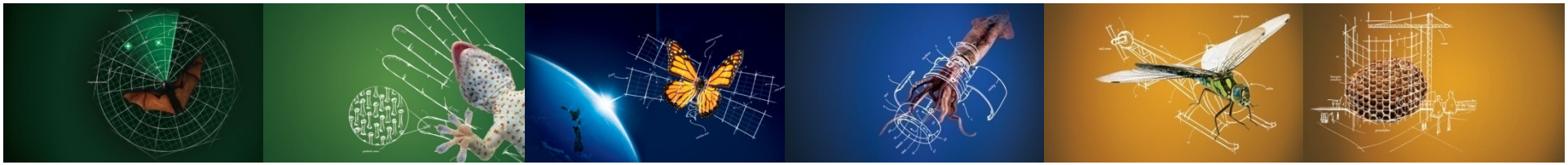


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To include:

1. **metabolic products of DPC16**
2. **antimicrobial studies with some foodborne pathogens**
3. **adhesion studies with intestinal epithelial cells**
4. **other properties**
5. **applications in the food industry**
6. **bibliography**



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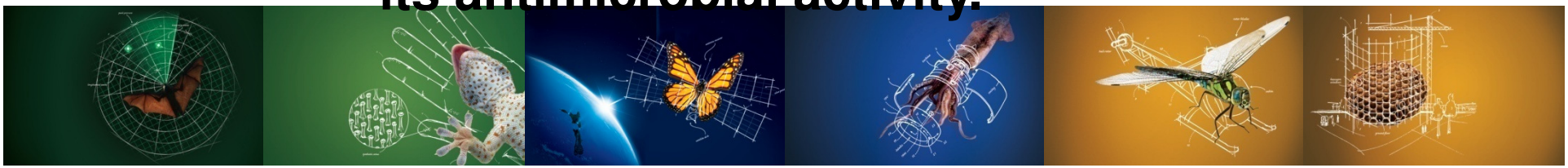
L. reuteri DPC16

--- isolated from a human source

--- protected by patent

--- commercially available as a probiotic bacterium
and in some food products
www.Drapac.co.nz

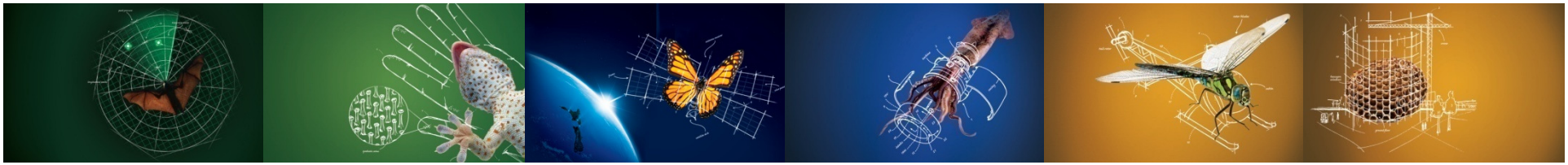
--- selected because of its superiority to other tested strains of lactic acid bacteria, with respect to its antimicrobial activity.



L. reuteri **DPC16 produces short chain fatty acids, scfa, (acetic, 1- 5 g/l, and lactic, 1-7 g/l) and reuterin (3-hydroxypropionaldehyde, upto 60mM).**

At pH 4, antibacterial activity is due to both scfa and reuterin.

At pH 6, antibacterial activity is due mainly to reuterin.



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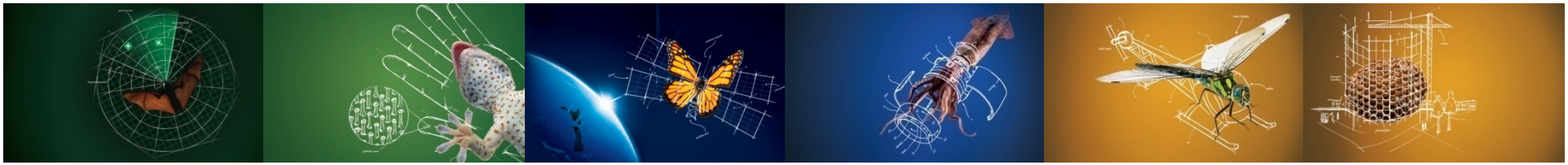
Production of reuterin

--- stationary phase cells, reuterin is produced from glycerol (DPC16 possesses glycerol dehydratase gene).

--- concentrations upto 60mM reuterin.

Target pathogens

Listeria monocytogenes; E. coli* O157:H7*;
Salmonella typhimurium; Staphylococcus aureus;
Vibrio parahaemolyticus



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Antibacterial effect at minimum effective dose of diluted DPC16 culture supernatant ----

At pH 4, (scfa in absence of reuterin)

--- lowers growth rate

--- lowers final number of cells

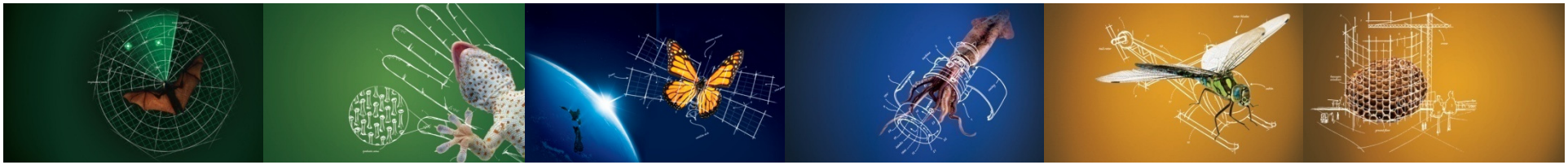
--- no effect on lag phase duration.

At pH 6, (reuterin)

--- no effect on growth rate

--- lowers final number of cells

--- extends duration of lag phase



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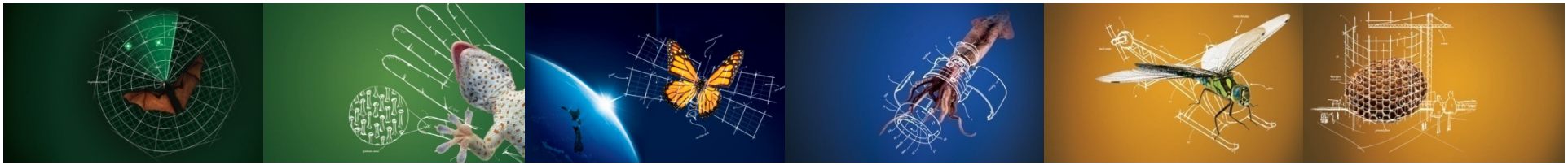
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Reuterin at 60 mM (in culture supernatant)

Bactericidal activity

3-4 log cycles over 3 hours duration for pathogens tested (at 10 Exp 9 per ml). (effective at 10C and 37C).

Much less inhibitory effect on DPC16 itself or on other probiotic lactic acid bacteria.



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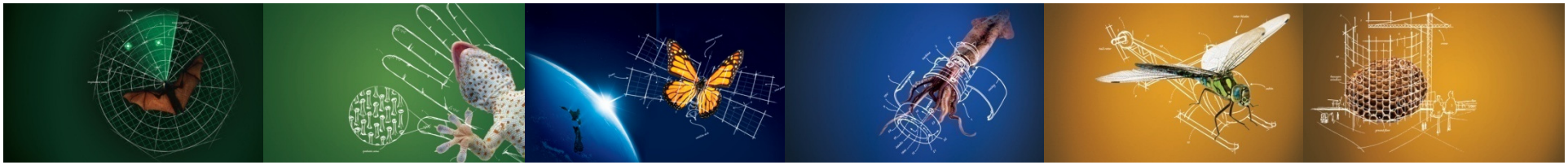
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Adhesion of DPC16 to Caco-2 and HT29 human carcinoma cells

--- slightly less than other probiotic bacteria, but same order of magnitude (due to exopolysaccharides).

Effect of DPC16 on adhesion of *E. coli* to CaCo-2 cells:

Displacement assay	**---**	**positive**
Competition assay	**---**	**positive**
Exclusion assay	**---**	**positive**



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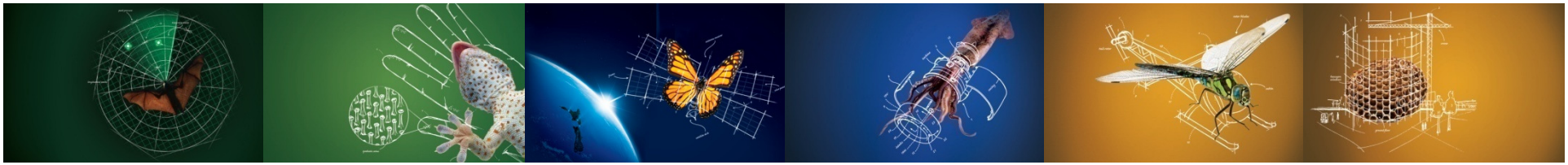
Some other properties of DPC16

--- removes indole from faecal water (probably due to physical adsorption onto cells)

--- the supernatant and heat-killed cells prevent epithelial (HT29) cell DNA damage caused by faecal water.

(Comet assay) (Carcinogenesis model)

(Exopolysaccharides?)



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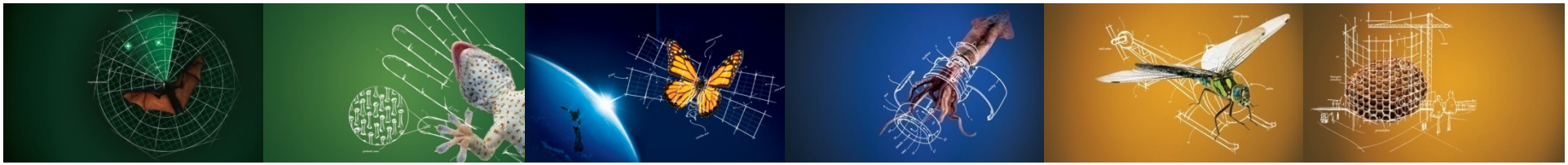
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Some applications

1. **Probiotic (commercially available).**
   Using simulated gastrointestinal tract of gastric juice, intestinal fluid and colonic fluid---

DPC16 cells encapsulated in alginate (3%), Ca chloride and skim milk (8%).

Protection during passage through gastric juice and intestinal fluid, followed by release in colonic fluid.



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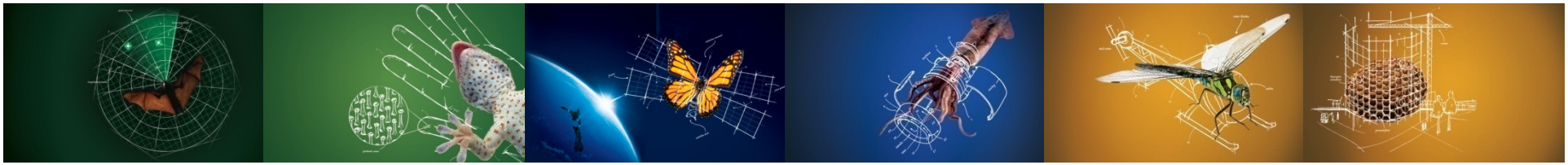
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2. Synergy with bovine lactoferrin

Lactoferrin inhibits growth of pathogens but not of probiotic bacteria.

Possible joint delivery of DPC16 and lactoferrin to take advantage of synergy, i.e. joint encapsulation of DPC16 and lactoferrin in probiotic tablet/food.

--- different modes of action.



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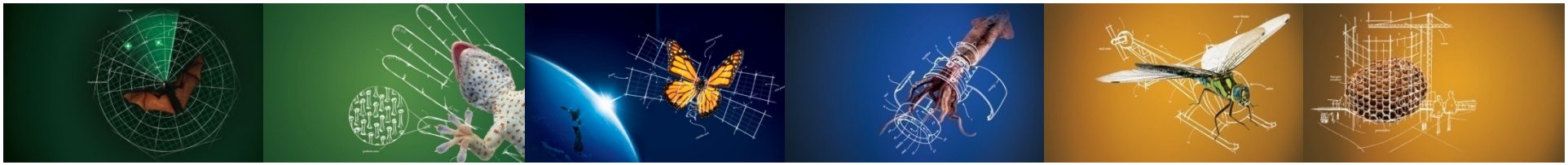
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3. Combination of DPC16 with Controlled Atmosphere Packaging.

CAP (60% CO_2, 40% N_2) inhibits growth of pathogens by reduction of growth rates and extension of lag phase duration.

Combination of CAP and DPC16 supernatant ---- combined effect is additive.

Possible food preservation technique.



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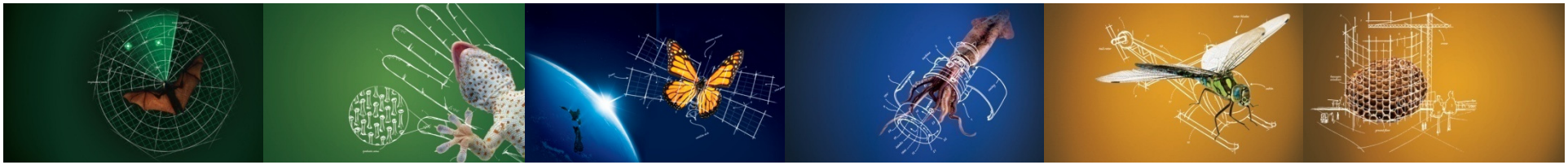
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4. Manuka honey

--- contains methyl glyoxal (2-keto propionaldehyde).
(unique manuka factor)

Note similarity between reuterin and methyl glyoxal structures (3-carbon aldehydes).

Addition of cell-free DPC16 supernatant containing reuterin to non-manuka honey (commercially available).



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