

## Antiviral Effects of Lactococcus Lactic Strain Plasma (LC-Plasma) Against Upper Respiratory Tract Infectious Viruses.

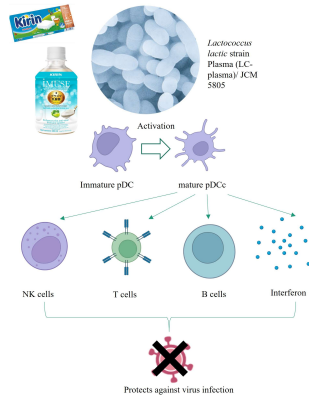
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### General understanding of immune system



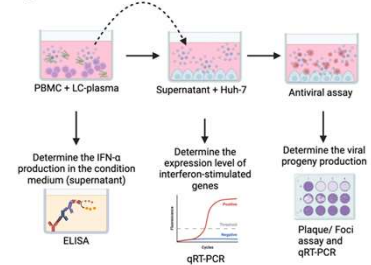
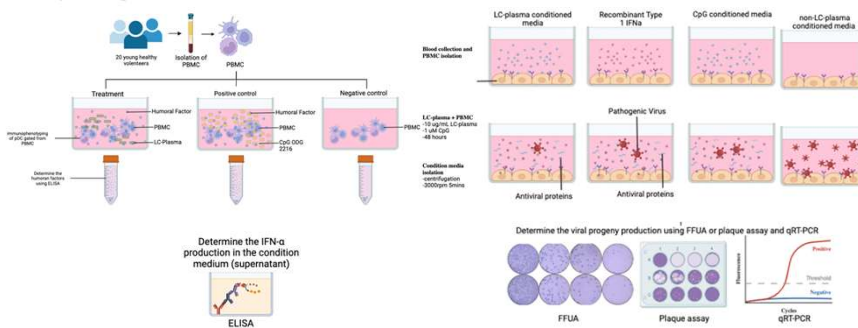
### Abstract

Viruses, like influenza and SARS-CoV-2, remain major causes of upper respiratory tract infections worldwide, with symptoms ranging from asymptomatic to lethal outcomes. While antivirals and vaccines have helped ameliorate disease morbidity and mortality, these infections still pose significant challenges. Probiotics, including *Lactococcus lactis* strain Plasma (LC-Plasma), have recently shown antiviral effects by activating plasmacytoid dendritic cells (pDCs), though their detailed mechanism remains unclear. In this study, we stimulated peripheral blood mononuclear cells (PBMCs) collected from healthy participants with LC-Plasma and conducted immunological analyses to investigate the immunomodulatory mechanisms of LC-Plasma. The supernatant derived from LC-Plasma-stimulated PBMCs (LCP-Sup) exhibited dose-dependent inhibition of H1N1 and SARS-CoV-2 replication. LCP-Sup significantly reduced SARS-CoV-2 viral load in Huh-7 cells. However, in the H1N1 antiviral assay using A549 cells, LCP-Sup was required at a higher concentration against H1N1 in A549 cells compared to SARS-CoV-2 in Huh-7 cells. Treatment with LCP-Sup significantly upregulated interferon-stimulated genes (ISGs) expression, particularly MxA, in A549 cells. While MxA showed the most notable increase, other ISGs also exhibited elevated expression levels compared to negative control. Other cytokines, chemokines, and growth factors were also induced by LC-Plasma and CpG-DNA stimulation, and the effects of LC-Plasma were much higher than those of CpG-DNA. These results provide *in vitro* evidence of the antiviral mechanisms of LC-Plasma via upregulation of IFN- $\alpha$  and related ISGs for host defense against respiratory viruses.

### Study summary (Aim & Endpoint)

- To evaluate the immunostimulatory effect of LC-plasma on PBMC isolated from young, healthy Malaysian volunteers
- To evaluate the antiviral effects of LC-plasma against influenza virus, and SARS-CoV-2 by *ex vivo*.
- To evaluate the mechanism of LC-plasma in inhibiting viral infections.
- To evaluate the interferon stimulative gene (ISG) expression in cells upon LC-plasma supernatant treatment via qRT-PCR

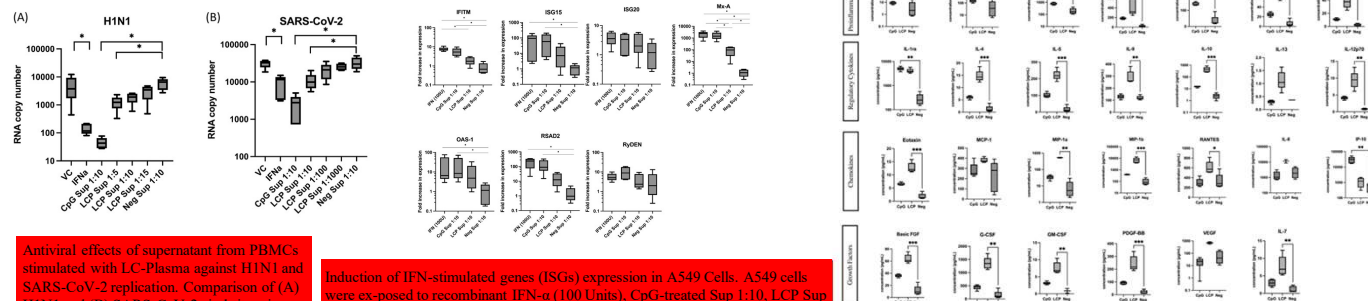
### Study design



Fold-increase in ISGs of A549 cells following treatments with recombinant IFN- $\alpha$  (100 Units), CpG Sup 1:10, and LCP Sup 1:10

ISGs	Fold-increase in ISGs in A549			
	IFN- $\alpha$ (100 U)	CpG 1:10	LCP 1:10	Neg 1:10
IFITM-1	7.06 $\pm$ 1.91	5.30 $\pm$ 2.64	1.75 $\pm$ 0.88	0.67 $\pm$ 0.53
ISG15	94.50 $\pm$ 78.80	57.67 $\pm$ 85.45	7.16 $\pm$ 0.74	1.16 $\pm$ 0.74
ISG20	3.55 $\pm$ 1.92	3.35 $\pm$ 2.11	2.00 $\pm$ 2.20	1.19 $\pm$ 1.28
Mx-A	2442.00 $\pm$ 1243.00	1441.00 $\pm$ 1253.00	96.04 $\pm$ 70.30*	1.12 $\pm$ 0.77
OAS-1	6.57 $\pm$ 31.02	8.42 $\pm$ 28.68	4.82 $\pm$ 13.35	1.15 $\pm$ 1.08
RSAD2	239.60 $\pm$ 136.90	89.41 $\pm$ 122.10	12.52 $\pm$ 15.44	0.96 $\pm$ 1.77
RYDEN	5.09 $\pm$ 2.60	8.89 $\pm$ 6.85	2.37 $\pm$ 3.50	2.00 $\pm$ 5.37

### Results



Antiviral effects of supernatant from PBMCs stimulated with LC-Plasma against H1N1 and SARS-CoV-2 replication. Comparison of (A) H1N1 and (B) SARS-CoV-2 viral titers in treated A549 and Huh-7 cells, respectively.

Induction of IFN-stimulated genes (ISGs) expression in A549 Cells. A549 cells were exposed to recombinant IFN- $\alpha$  (100 Units), CpG-treated Sup 1:10, LCP Sup 1:10, or Neg Sup 1:10 for 24 hours, and the expression of ISGs, RyDEN, IFITM-1, OAS-1, ISG15, ISG20, RSAD2 and MxA were determined.

Cytokines, chemokines growth factors analysis of PBMC supernatant analyzed using the multiplex BioPlex Pro Human Cytokine 27-plex assay kit.

### Conclusion

The finding suggest that enhanced IFN- $\alpha$  production, including other humoral factors stimulation leading to upregulation of ISGs (MxA) expression, is a possible mechanism contributing to LC-Plasma's antiviral activity against H1N1 and SARS-CoV-2.