Recombinant surface layer protein of *Lactobacillus helveticus* inhibits the binding of enterotoxigenic *E. coli* to human intestinal cell line

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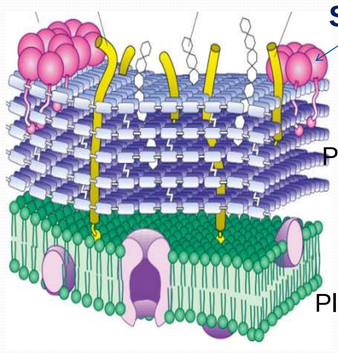
INTRODUCTION

The surface layer protein (Slp) form a crystalline bi-dimensional porous outermost layer of several archaea and bacteria including probiotics such as lactic acid bacteria, providing a protective coat against harsh environmental conditions. Some probiotic properties of whole bacteria, such as adhesion in the gastrointestinal tract, aggregation, pathogens inhibition as well as eliciting host's immune response are partly mediated by surface layer protein found in lactobacilli species.



Functions of Surface layer protein

Pathogen Exclusion	**Adhesion to Intestinal cell line and ECM**	Protective Coat
Cell shape Maintainanace	Molecular Sieve	Antimicrobial Substance Production

Hypothesis: Surface proteins should interact with specific gut cell receptors and exclude pathogens like probiotic organisms. It could be a better proposition to use specific surface proteins to achieve the targeted function of probiotics instead of using whole cell for exclusion of pathogens.

We have cloned, expressed and purified the surface layer protein of *L. helveticus* and studied the interaction of this protein with human gut cell line, Caco-2 and inhibition of enterotoxigenic *E. coli*.

METHODS

- ***slp*A (1.2kb)** gene of *L. helveticus* was cloned and expressed in *E. coli* using pMAL-c2x expression vector.
- Purification of over-expressed protein (approximately of 88 kDa fusion protein along with MBP purification tag) has been done by affinity and ion-exchange chromatography.
- Adhesion assays were performed with human intestinal cell line, Caco-2 using immunostaining. Anti-MBP primary antibody and phycoerythrin labelled secondary antibody were used for immunostaining.
- Pathogen exclusion and competitive inhibition assays were performed.

RESULTS

Cloning of *slp* gene in *E.coli* using pMAL-c2X Expression Vector

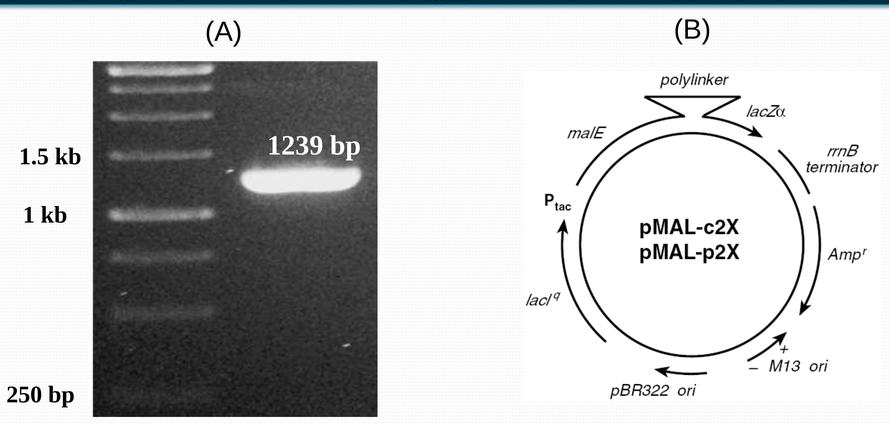


Fig 1.. Panel (A) shows cloning confirmation of *slp* gene (1239 bp) *L. helveticus* in *E. coli* TOP10 by PCR and Panel (B) shows pMAL-c2X vector map.

Expression and Purification of Slp Protein of *L. helveticus*

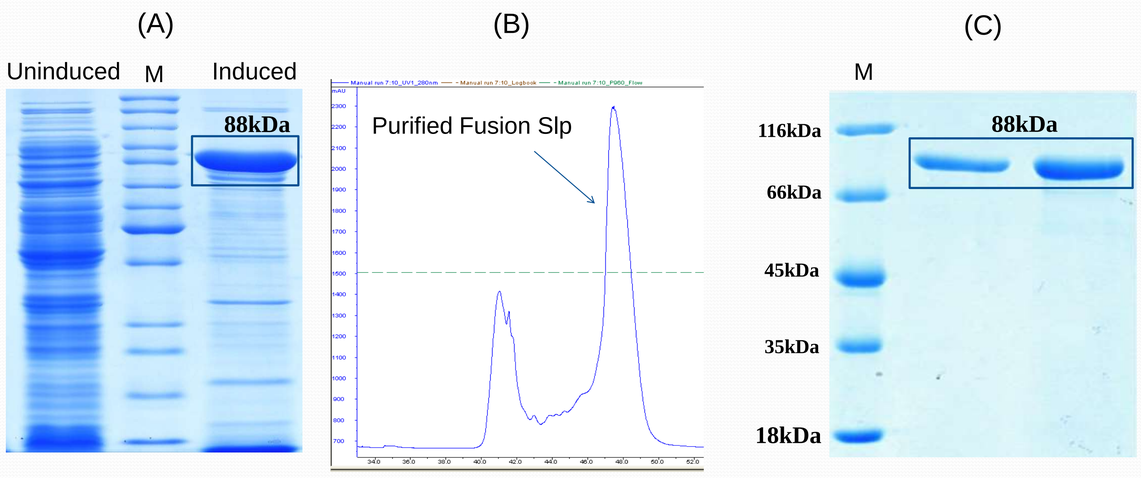


Fig 3.. Panel (A). Lane 1 – Uninduced, Lane 2 –Marker, Lane 3 – Induced with 0.3mM IPTG at 12°C in Shuffle *E.coli* cells
Panel (B) Chromatogram shows peak of purified Slp
Panel (C) shows purified Fusion SlpA (88kDa, 45kDa SlpA with 42.5kDa MBP vector tag).

Recombinant Slp is able to bind with human cell line, Caco-2

Binding of Slps to human intestinal cells, detected by immunofluorescence

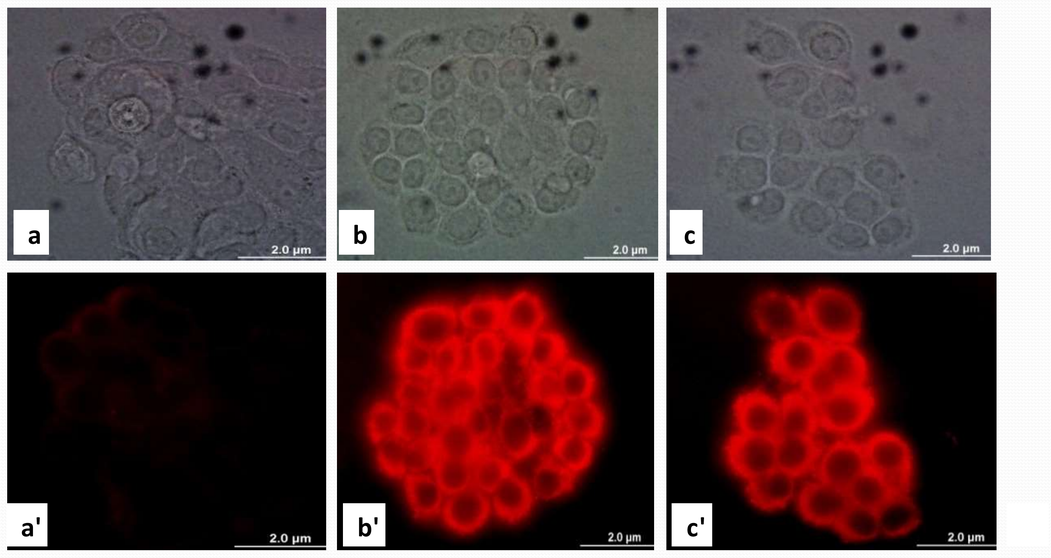


Fig 4. Images a, b, c shows bright fields and a', b', c' shows respective fluorescent fields.
(a) and (a') - control or 150µg/ml MBP(Tag) protein, (b) and (b') - 150µg/ml Slp protein
(c) and (c') - 300µg/ml Slp

Slp is able to inhibit pathogen binding in exclusion assay

S. No.	Pre-incubation of Slp (4 hrs)	ETEC incubation (2 hrs)	ETEC Adhesion (in %)	Decrease in Adhesion (in %)
1	Caco-2 +PBS	ETEC (10^9 cfu)	100	0± 2.673
2	Caco-2 + MBP ((150µg/ml)	ETEC (10^9 cfu)	100	0± 1.735
3	Caco-2 + **Slp (150µg/ml)**	ETEC (10^9 cfu)	30.78	**69.22** ± 2.673
4	Caco-2 + **Slp (300µg/ml)**	ETEC (10^9 cfu)	28.91	**71.09** ± 2.673

Slp is able to inhibit pathogen binding in competition assay

S. No.	Co-incubation of Slp + ETEC to Caco-2 for 2hrs)	ETEC Adhesion (in %)	Decrease in Adhesion (in %)
1	Caco-2 + PBS + ETEC (10^9 cfu)	100	0± 1.989
2	Caco-2 + MBP ((150µg/ml) + ETEC (10^9 cfu)	100	0± 2.214
3	Caco-2 + **Slp (150µg/ml) +** ETEC (10^9 cfu)	25.87	**74.13** ± 2.009
4	Caco-2 + **Slp (300µg/ml) +** ETEC (10^9 cfu)	21.81	**78.19** ± 2.791

SUMMARY & CONCLUSION

- ***slp* gene (1.2kb) of *L. helveticus* was cloned and expressed successfully in *E.coli* and purified**
- **Slp of *L. helveticus* has potential to interact with human intestinal cell line, Caco-29 and is able to inhibit enteric pathogen adhesion to human intestinal cells**
- **Practical Application: This recombinant proteins may find application in the prophylaxis of gastrointestinal tract infections.**

ACKNOWLEDGEMENTS

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