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Latarcins as membrane active peptides for therapeutic applications

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ABSTRACT

Venoms, a rich source of bioactive peptides present promising avenues for drug discovery and development. Latarcins, a class of seven linear membrane active peptides (MAPs) isolated from the ant spider *L.tarabaevi*, exhibit notable biological activities including antimicrobial and anticancer effects. Studying the membrane-targeting ability of Latarcins is crucial for understanding their molecular mechanisms. Our ongoing research focuses assessment of antimicrobial efficacy of Latarcins, revealing a broad spectrum of activity. Propidium Iodide (PI) uptake assay confirms the membrane-disrupting activity of Latarcins on MRSA cells. Time kill kinetics assays demonstrated rapid microbial killing effect (Mentioned data is not published yet). Cellular translocation and cargo delivery efficiency of modified Latarcin peptide sequences fused with a nucleus- targeting sequence (LDP-NLS) was also investigated. Also, *in-vitro* anticancer activity is currently being investigated in different cancer cell lines. Collectively, this multidimensional exploration combined with *in-silico* Molecular Dynamics (MD) studies contribute to our understanding of molecular interaction of these MAPs with biological membranes and holds potential for the development of efficacious therapeutics.

APPROACHES

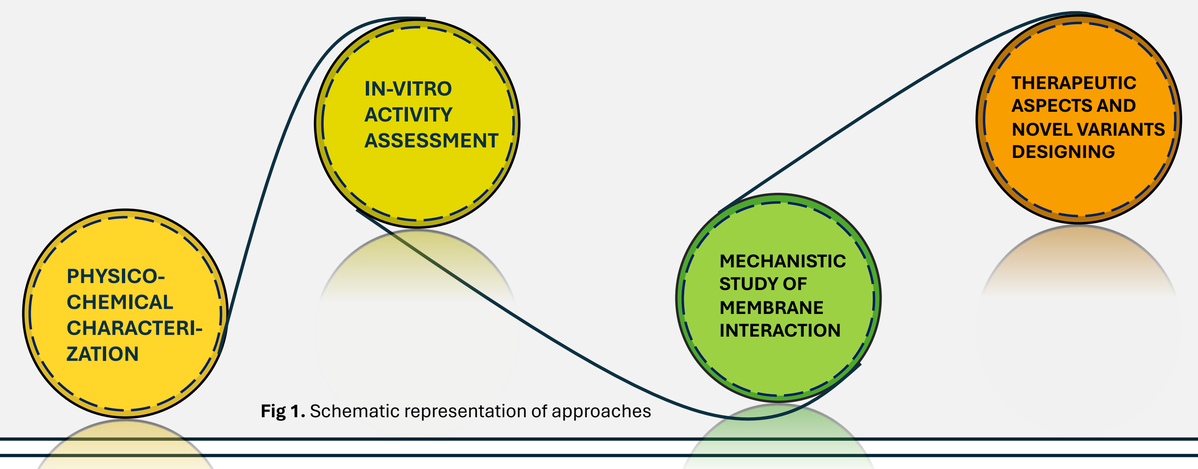


Fig 1. Schematic representation of approaches

RESULTS

MEMBRANE TARTGETING ANTIBACTERIAL ACTIVITIES (Data unpublished)

MIC AND MBC DETERMINATION

Strain	Ltc1	Ltc2a	Ltc3	Ltc4	Ltc5	Ltc6	Ltc7
Methicillin resistant *Staphylococcus aureus*	16, 32	4, 8	8, 16	4, 8	8, 16	>128	>128
Escherichia coli	128,128	8,128	128,128	128,128	128,128	>128	>128
Pseudomonas aeruginosa	16,16	8,8	128,128	>128	32,32	>128	>128
Vibrio cholerae	128,128	32,32	64,64	64,64	128,128	>128	>128
Rhizopus zygospores	>128	32,32	>128	>128	>128	>128	>128
Mucor indicus	>128	32,32	>128	>128	>128	>128	>128
Fusarium dimerum	16	4,8	32,64	32,64	32,64	>128	128, >128

Table 1. MIC and MBC values of Ltcs

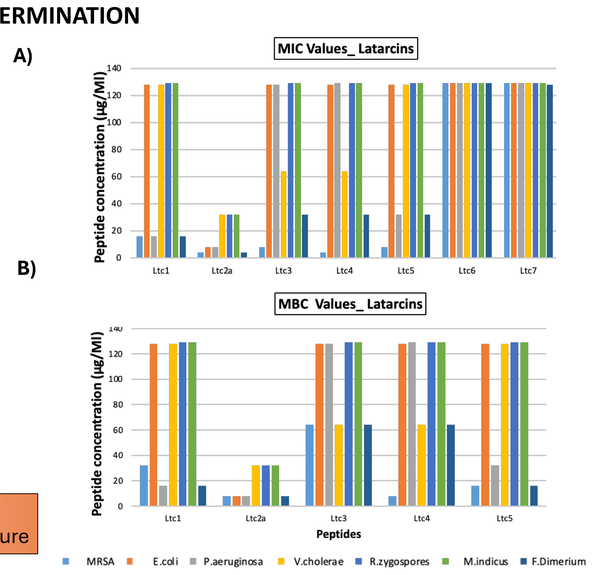


Fig 2. A) Bar graph representation of MIC values of Ltcs B) Bar graph representations of MBC values of Ltcs against different pathogens

Ltc2a exhibited broad-spectrum antimicrobial activity at lower concentrations;
The close correspondence between the MIC and MBC values highlights their bactericidal nature

PROPIDIUM IODIDE (PI) UPTAKE ASSAY

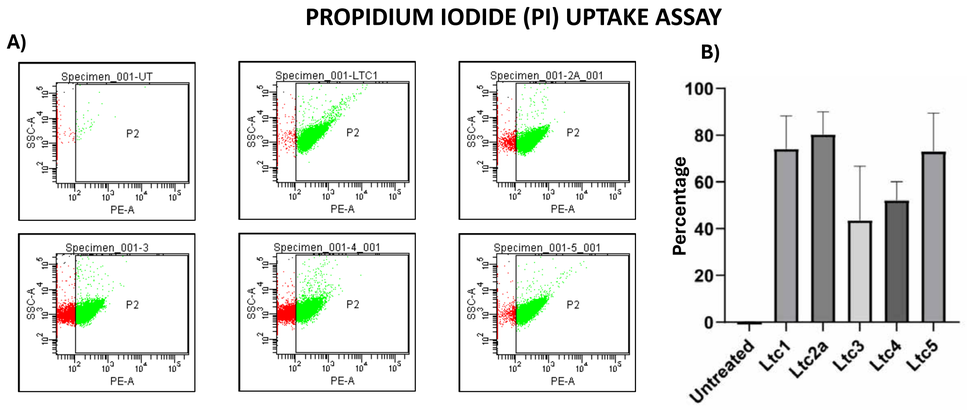


Fig 3. Flow cytometry analysis of PI uptake A) i) Untreated ii) Ltc1 iii) Ltc2a iv) Ltc3 v) Ltc4 vi) Ltc5 B) Population cell death percentage in treated and untreated cells.

After 1h treatment, ~50 or more percent of cells were stained with PI in Ltcs treated populations

TIME KILL KINETICS

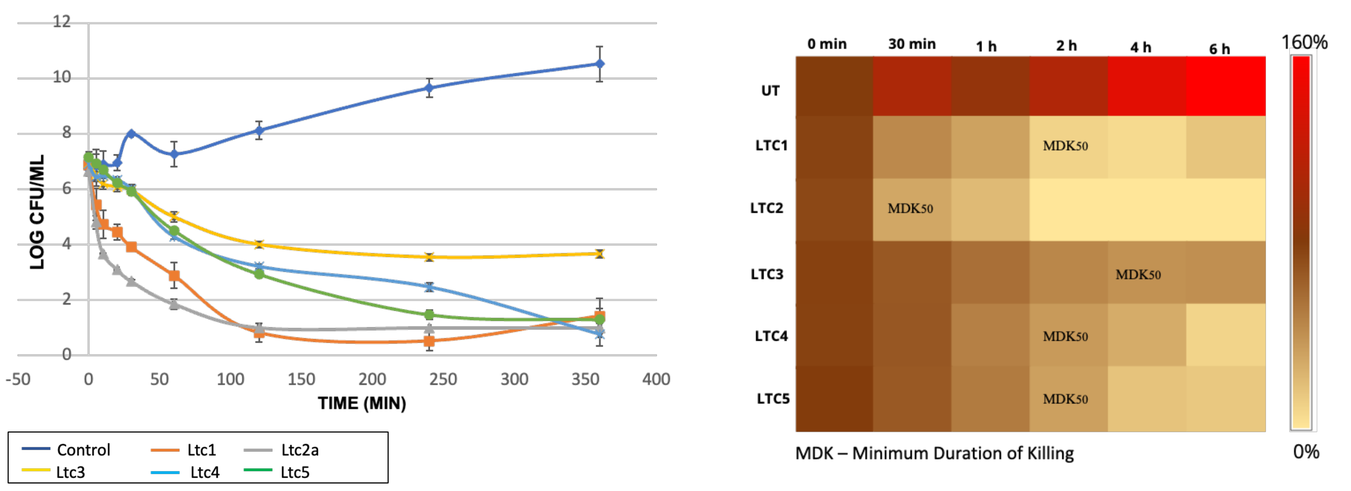


Fig 4. A) Time kill curve for Ltc treated cells. B) Heat map representation of population viability over time in ltc treated populations

At 6h post treatment the population size declined nearly to Log1 (~100 %) (Except Ltc3) ; Ltc2a treated cells showed ~log5 reduction after 2h treatment

MEMBRANE INTERACTION AND MEMBRANE DAMAGE EFFECTS

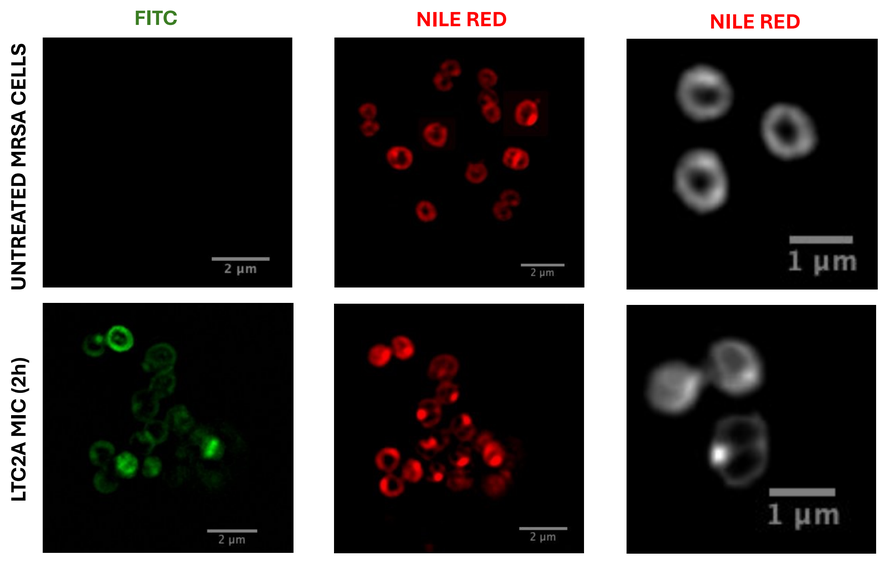
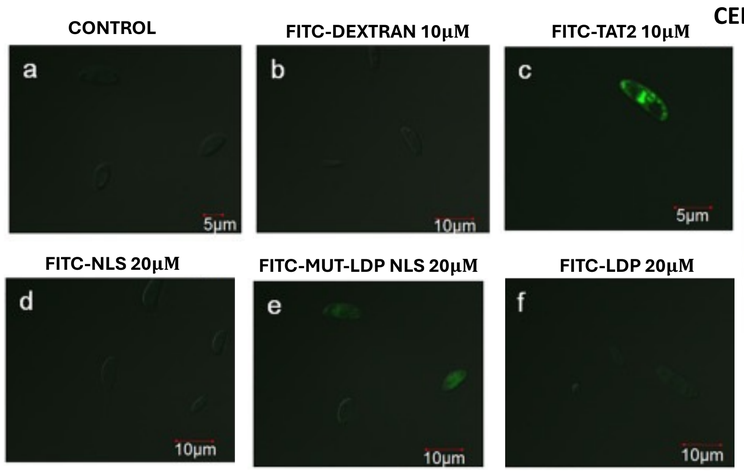


Fig 5. SIM images of FITC-Ltc2a treated cells stained with Nile Red.

Nile red dye accumulation and diffusion confirms the membrane damage in Ltc2a cells

CELL PENETRATING ACTION INSIDE FUNGAL SPORES AND ANTIFUNGAL EFFECTS (Budagavi et al., 2018)

CELL PENETRATION ACTION OF LTC VARIANTS



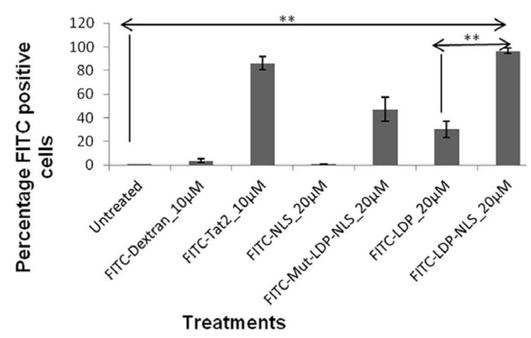


Fig 6. Epi fluorescence microscopy images of FITC-Latarcin variants treated cells.

Fig 7. Flow cytometry analysis of FITC-Latarcin variants treated cells.

Fluorescence microscopy and flow cytometry analyses confirm the penetration of FITC-LDP-NLS inside fungal spores

ANTIFUNGAL ACTIVITY OF VARIANTS AGAINST *Fusarium solani*

S.No.	Peptide name	Sequence[a]	MIC of peptides against *F. solani* (µM)	
			Spores	Hyphae
1.	LDP-NLS	KWRRKLKKLRPKKKRKV	2.5–5	20–30
2.	LDP	KWRRKLKKLR	5–10	30–40
3.	Mut-LDP-NLS	AWRRKLKALAPAKKAKV	20–25	> 50
4.	NLS	PKKKRKV	No effect till 50 µM	No effect till 50 µM
5.	Tat2	RKKRRQRRRRKKRRQRRR	5–10	15–30

Table 2. MIC determination of Latarcin derived variants against *F.solani* spores and hyphae

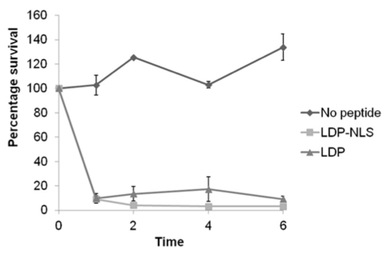


Fig 8. Killing kinetics of variants

LDP-NLS showed effective anti-fungal activity against *F.solani* spores and hyphae

DISCUSSION

Currently, mechanistic aspects of membrane damage induced bactericidal action of Ltcs are being investigated using microscopic and biophysical approaches. Beyond their antimicrobial and cell-penetrating capabilities, these MAPs also exhibit promising anticancer properties (data is not shown). Ongoing research is focused on the *in-vitro* validation of their anticancer efficacy and elucidating the mechanistic pathways involved in their cytotoxic effects. By offering a strong foundation for investigating the various biological functions of these MAPs, these integrated, multidisciplinary approaches demonstrate their potential as viable therapeutic candidates in the future.

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