Metabolic Remodelling during Biofilm Development of Pathogenic and Intermediate leptospires

K. Vinod Kumar[1], Prajakta Prashant Bokade[1], Archana Pal[1], Anwesh Maile[2], G. Arun Govind[3], S. Chandan[4], D. Chandan[4], P. Vijayachari[5], B. R. Shome[1], **V. Balamurugan[1]***

[1]Indian Council of Agricultural Research - National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Yelahanka, Bengaluru 560 064, Karnataka, India, [2]DBT-Centre for Microbial Informatics, (DBT-CMI), University of Hyderabad (HCU), Gachibowli, Hyderabad 500046, Telangana, India., [3]CBST, Vellore Institute of Technology (VIT), Vellore- 632014 Tamil Nadu. [4]JSS University, Sri Shivarathreeshwara Nagara, Bannimantap, Mysuru 570004, Karnataka, India., [5]ICMR-Regional Medical Research Centre (ICMR-RMRC), WHO Collaborating Centre for Diagnosis, Reference, Research and Training in Leptospirosis, Port Blair 744101, Andaman and Nicobar Islands, India.

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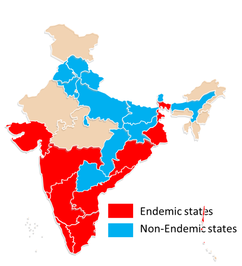
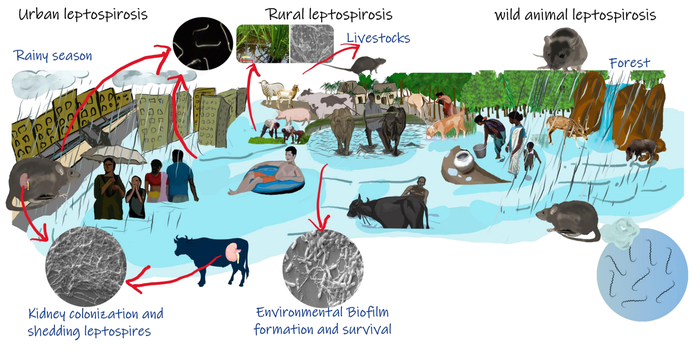
Leptospirosis

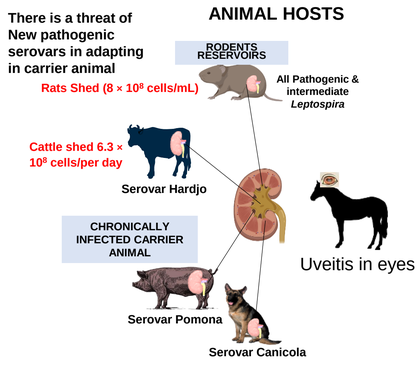
Leptospirosis is a World leading Zoonoses affecting more than one million people worldwide and 58,900 deaths each year (Costa *et al.*, 2015; Torgerson *et al.*, 2015).

- Most common, underdiagnosed neglected zoonosis
- A zoonotic disease of animals and humans caused by infection with the spirochete *Leptospira*
- India - cases are reported from Kerala, Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra, Gujarat & Andamans, etc.,
- Source - Animals (rodents and domestic animals)



CHALLENGES





OBJECTIVES

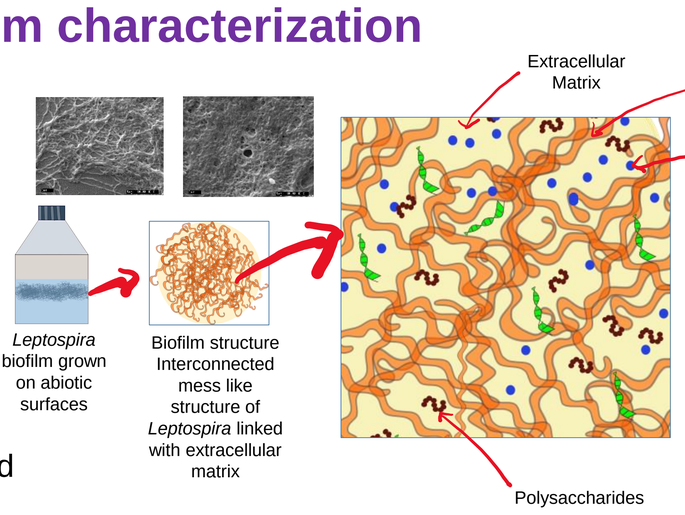
Objective 1: To characterize biofilm formation by intermediate and pathogenic *Leptospira* and study the transcriptomics and metabolomic factors during biofilm formation

Objective 2: To express the biomarkers in heterologous system and to assess their potential use for diagnosis in chronic animal leptospirosis

METHODOLOGY

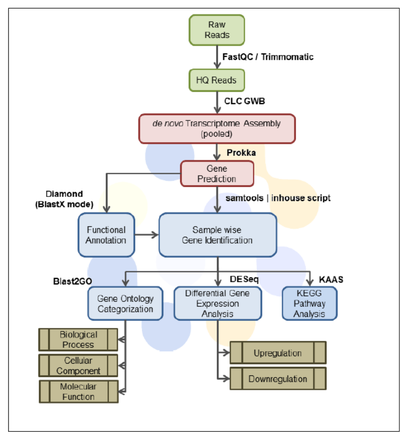
Leptospira biofilm characterization

- Crystal violet assay
- Scanning Electron Microscope (SEM)
- Atomic Force microscopy
- Fluorescence microscopy eDNA staining
- Spicer and meyer staining
- Calcium Staining Method

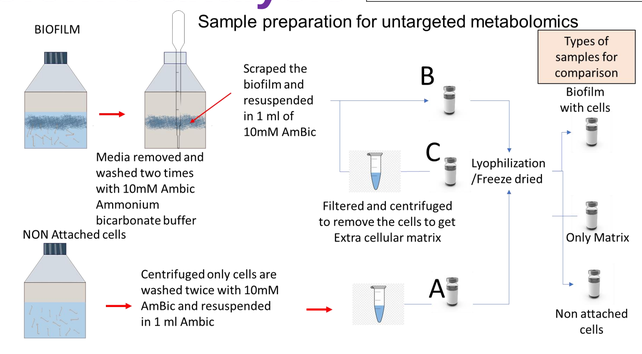


Transcriptomics (Whole genome RNAseq)

- Total RNA Direct-Zol RNA Prep kit (ZYMO Research)
- QC passed RNA samples
- PE libraries were prepared from total RNA using a MICROBExpress kit and TruSeq stranded mRNA Library Prep Kit
- Libraries was sequenced on NovaSeq6000 using 2 x 150 bp chemistry
- Bioinformatic analysis



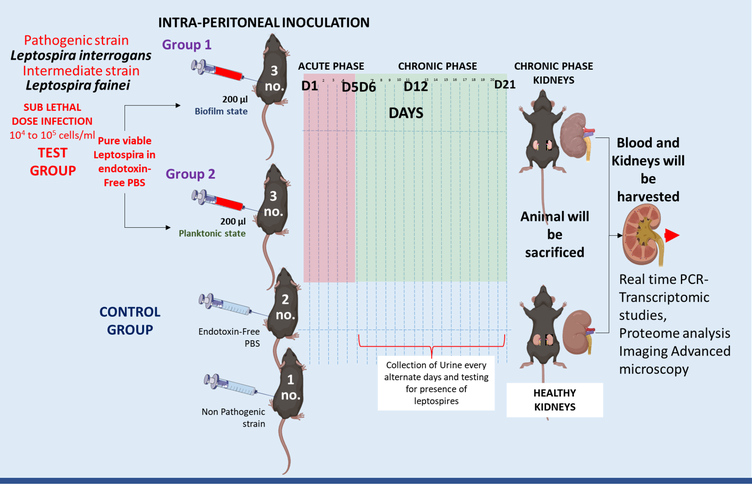
Untargeted Metabolomic analysis



Sample Prep for LC-MS

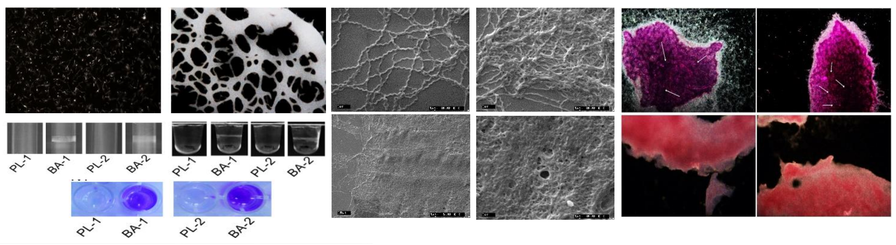
- To the samples 750 uL of extraction buffer [4:4:2 (Methanol: Acetonitrile: water)] was added
- Samples were vortexed, sonicated for 2 mins, and centrifuged for (25mins/14800rpm/4deg)
- Supernatants were transferred to fresh tubes
- To the pellets 250 uL of extraction buffer was added
- Samples were again vortexed, sonicated for 2 mins, and centrifuged (25mins/14800rpm/4deg) and supernatants were pooled
- 200 uL each from supernatant was pooled together for quality control (QC) QC and supernatants of samples were dried under vacuum
- Dried samples and QC were each reconstituted in 60 ul of 50% Acetonitrile and to this 20 uL each of reserpine and taurocholate-D8 were added
- 10ul of each sample and QC were injected for analysis

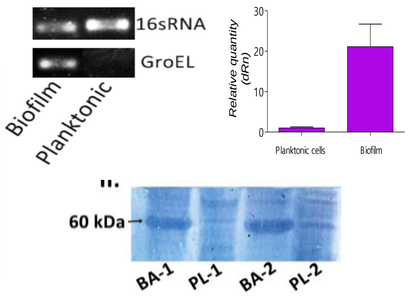
ANIMAL EXPERIMENTS FOR *LEPTOSPIRA* BIOFILM

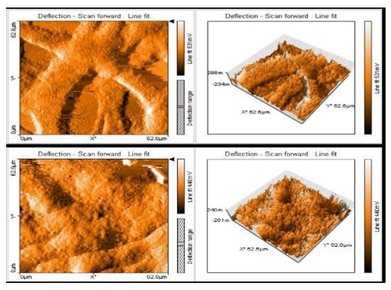


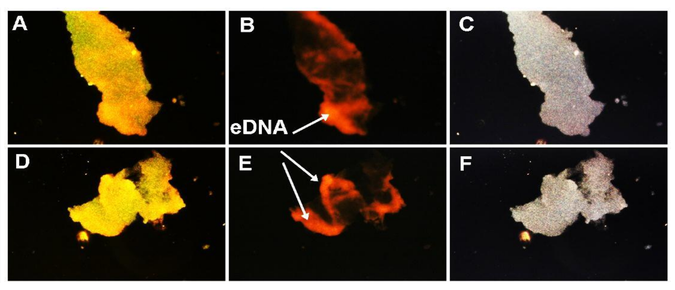
RESULTS

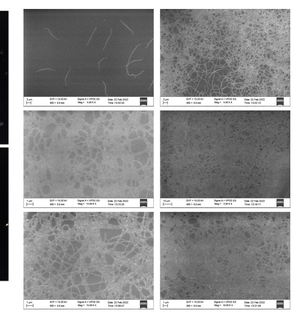
Leptospira biofilm characterization





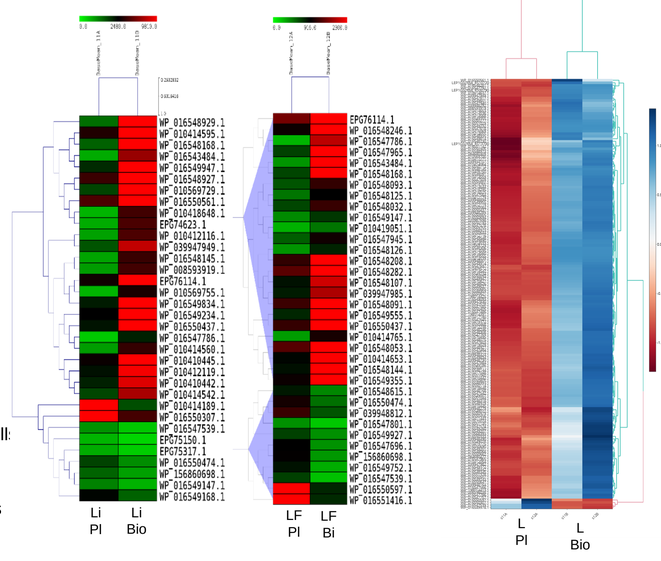






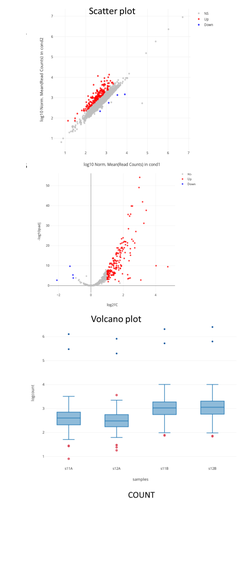
Transcriptomics (Whole genome RNAseq)

Differential expression heat m

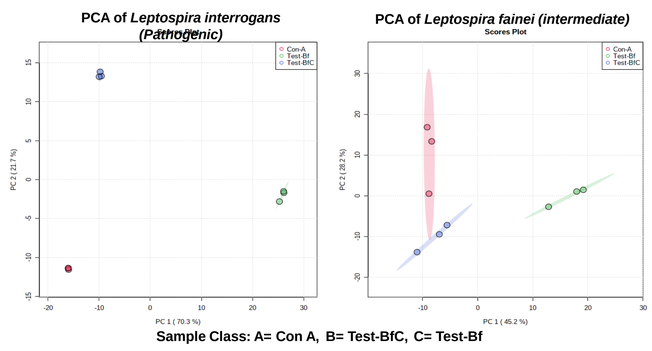


	Gene	Symbol	pvalue	foldChange
Replication and repair				
	WP_039947949.1	ssb	3.3E-39	5.48
	WP_016551145.1	xerD	6.1E-09	2.70
Transcription				
	WP_016551044.1	rpoA	1.7E-15	2.77
	WP_010414653.1	NusG	3.8E-10	2.29
Translation				
	WP_039947830.1	raiA	1.3E-13	3.00
	WP_016550507.1	NA	4.8E-08	2.05
	WP_016548993.1	EF-Ts	3.5E-14	2.68
	WP_010414595.1	cysN	1.9E-58	8.08
	WP_010412119.1	infC	1.2E-21	3.21
Cell motility				
	WP_016547484.1	FlaA	6.1E-18	3.53
	WP_010569729.1	FlgL	2.0E-43	5.65
Outer Membrane Protein				
	WP_039948279.1	NA	3.7E-06	2.01
	WP_016550561.1	OmpA	4.8E-17	3.48
	WP_016549947.1	OmpL1	4.4E-53	7.84
	WP_016550561.1	lrpA-like	5.0E-04	3.30
	WP_010416525.1	LipL21	5.7E-25	4.33
	WP_016548168.1	LipL32	3.4E-41	10.67
	WP_016549834.1	LipL41	2.3E-25	6.11
	WP_016548068.1	LipL45	4.6E-09	2.29
	WP_016548318.1	LipL46	7.3E-19	3.18
	WP_016547542.1	LipL71	8.9E-19	2.94
	WP_016548345.1	NA	7.3E-11	2.78
Carbohydrate metabolism				
	WP_039948001.1	gapA/GAPDH	1.4E-13	2.26
	WP_016550068.1	NA	1.8E-12	2.61
	WP_016550405.1	NA	1.1E-08	2.08
	WP_016549232.1	fbaB	4.3E-10	2.10
	WP_016548306.1	mdh	1.4E-13	2.29
	WP_010412179.1	atpE	2.2E-24	4.78

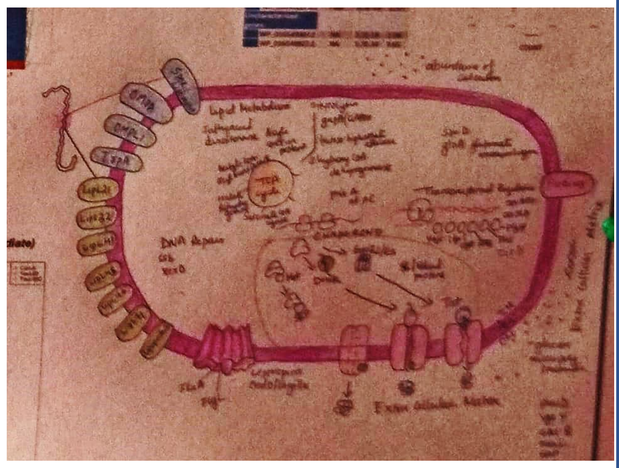
	Gene	Symbol	pvalue	foldChange
Chaperone				
	WP_016551328.1	Hsp20/lbpA	3.6E-16	4.16
	WP_016550690.1	DnaK	9.9E-08	2.12
	WP_016548230.1	TatE/TatA	3.4E-05	2.17
	WP_016548075.1	GroEL	5.1E-22	3.77
	WP_010418648.1	GroES	2.7E-30	6.85
	WP_010411443.1	Hfq	5.4E-11	3.53
Lipid metabolism				
	WP_016549637.1	NA	1.0E-21	3.06
	WP_016549234.1	NA	5.3E-39	4.91
	WP_010412054.1	acpP	6.1E-15	5.12
Amino Acid Metabolism				
	WP_039948894.1	speD	4.0E-19	3.46
	WP_016548959.1	glnA	1.1E-11	2.16
	WP_016548091.1	metK	1.4E-13	2.40
Energy metabolism				
	WP_016550694.1	FixA	4.0E-27	3.96
	WP_016547757.1	atpC	5.2E-06	2.04
Regulators				
	WP_016548935.1	YajC	3.1E-07	2.11
	WP_016548572.1	ClpP	1.1E-13	2.84
	WP_016547940.1	KhpA	2.4E-07	2.54
	WP_016547645.1	NA	1.6E-11	2.95
	WP_016548144.1	FliS	2.0E-08	2.08
	WP_010418295.1	FlgM	7.8E-15	2.96
	WP_010417048.1	CarD	1.7E-13	2.71
	WP_010570495.1	NA	4.1E-11	2.99
	WP_010570024.1	NA	2.8E-12	3.70
	WP_010409572.1	NA	4.0E-13	2.46
	WP_010414655.1	SecE	6.0E-06	2.06
Poorly characterised				
	WP_198014425.1	PuuD	7.9E-10	2.03
	WP_016547817.1	YqeY	1.0E-09	2.17
Unclassified; metabolism				
	WP_016548442.1	NA	3.0E-08	2.23
	WP_010412271.1	PaaI	8.3E-09	2.57
Uncharacterised genes				
	WP_016549007.1	NA	2.2E-18	2.98
	WP_016548927.1	NA	1.7E-34	9.65



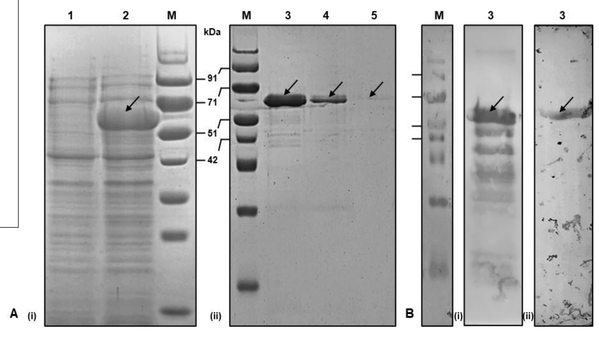
Untargeted Metabolomic analysis



Sample Class: A= Con A, B= Test-BfC, C= Test-Bf



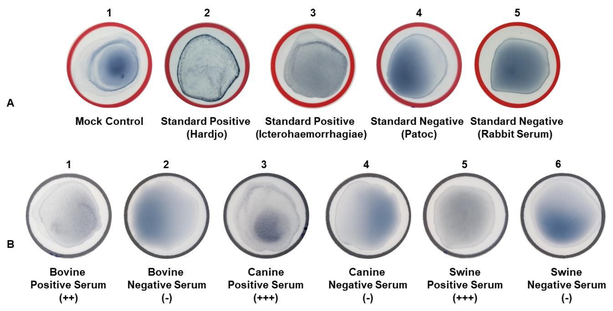
Expression of Recombinant proteins



A. SDS-PAGE analysis of expressed rGroEL.

B. Immunoblot analysis of expressed rGroEL

Latex agglutination test (LAT



CONCLUSIONS

Analysis of *in-vitro Leptospira* biofilm formation by metabolomics and transcriptomics indicates the remodelling of metabolism during biofilm development through an extensive change in gene expression. The dynamic remodelling of metabolism involves bio-synthetic pathways and secondary metabolites associated with biofilm formation.

Transcriptomics in the present study and our earlier protein study revealed potential immunogenic proteins shared between the other pathogenic species/serovars, which can be used as a diagnostic or vaccine candidate.

Further, analyses are ongoing to understand the role of hypothetic genes and their function in the colonization of kidneys in the animal model.

The developed rGroEL LAT is an extremely simple and rapid test that can be used as a diagnostic tool in resource-limited diagnostic laboratories, especially at the field level and it will complement existing serodiagnosis tests.

Reference

Vinod Kumar, K., Lall, C., Vimal Raj, R., Vedhagiri, K. and Vijayachari, P. 2015. Coexistence and survival of pathogenic leptospires by formation of biofilm with *Azospirillum. FEMS Microbiology Ecology 91(6), fiv051.*

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