Establishing the Taxa and Functional profile of Microbiota implicated in West Nile Fever

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Abstract: West Nile fever causing microbiome is taken in this work. *Culex nigripalpus* mosquito is the causative factor for West Nile Virus. Using Metatranscriptomic sequencing, identified the taxa and functional profile of the microbiome is identified.

Keywords: West Nile fever, microbiome, Metatranscriptomics, taxonomy, functional profile, gene receptors, docking.

1. INTRODUCTION

West Nile fever is an infection caused by the West Nile virus, which is spread by mosquitoe, *Culex nigripalpus*. *Culex nigripalpus* is a primary vector for causing West Nile fever; they proliferate in the nutrient rich media and colonize in freshwater aquatic habitats. The microbiota of mosquitoes provides nutrition for the development and transmission of the pathogen [1, 2]. The virus replicates affecting the brain causing neuro-virulence, it turns to be neuroinvasive when it gains access to the central nervous system. The viral serine protease, NS2B-NS3 plays a crucial role in viral replication. The distribution of the virus is seen throughout Africa, the Middle East, southern Europe, western Russia, southwestern Asia, and Australia [3].

The Src family kinase c-Yes was recently reported to be important for maturation of West Nile virus particles. Individuals with West nile fever recover within days to months In acute flaccid paralysis individuals develop symptoms headache, fever, malaise, gastrointestinal upset, skin rash, and some patients have neck rigidity and changes in mental status [4].

2. MATERIALS AND METHODS

Metatranscriptomic analysis

Next-generation sequencing (NGS) is an advanced version of non-Sanger-based sequencing technology that offers ultra-high throughput, scalability, and speed. Galaxy is an open source, web-based platform for next generation computational biomedical research [5]. Metatranscriptomics analysis enables understanding of how the microbiome responds to the environment by studying the functional analysis of genes expressed by the microbiome [6, 7].

Structure based drug designing technique is used here to build, display, simulate and analyze the molecular structure. Here we have used SWISS-MODEL tool [8] for modelling the proteins (gene receptors) responsible for West Nile fever CCR5, CLEC4M, IFITM2, IRF3. Selected models from homology modelling [9] output are docked with selected phytocompound from medicinal herbs.

Selection of phytocompounds was done using Lipinski rule for drug which is based on the ADME properties. Molecular docking [10] was done using Patchdock tool and best interacting phytocompounds with the gene receptors can be selected as ligands.

West Nile viruses' fastq sequences SRR10017187.1.1 and SRR10017187.1.2 were retrieved from SRA database.

Sequences' quality was checked using FASTQC [11]. MultiQC [12] was done to aggregate results from FASTQC analyses into a single report.

Sequences were trimmed using cutadapt.

FASTQC followed by MultiQC was re-run using the results of cutadapt.

Next, using SortMeRNA tool [13-17] any reads identified as rRNA in dataset was removed.

Next, using FASTQ INTERLACE tool [18] paired end FASTQ reads from two separate files were joined.

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MetaPhlAn tool [19] was used for profiling the composition of microbial communities (Bacteria, Archaea and Eukaryotes) from our microbiota.
Krona tool [20, 21] was used to visualize the results of a metagenomic profiling as a zoomable pie chart and GraPhlAn tool [22] for visualizing high-quality circular representations of taxonomic and phylogenetic trees.
Further, HUMAnN [23] pipeline was used for efficiently and accurately profiling the presence/absence and abundance of microbial pathways in our microbiota.

3. RESULTS AND DISCUSSION

Metagenome, having accession number SRR10017187, for West Nile virus was downloaded from SRA database.
As, per **Per base sequence quality** results of FASTQC and MultiQC, the sequence quality is not good hence we go ahead with trimming the sequence.
CUTADAPT tool [24] is used for trimming. It finds and removes adapter sequences, primers, poly-A tails, and other types of unwanted sequence from our data. It searches for the adapter in all reads and removes it when it finds it. Further, sequence quality of the cutadapt output is checked using FASTQC and MultiQC and it is found within the range.
SortMeRNA tool removes any reads identified as rRNA from our dataset. Fastq Interlace tool joins paired end FASTQ reads from two separate files. Taxonomic profiling [25] was done using MetaPhlAn tool.
The output is visualized using Krona and Graphlan.

Fig. 1: Generation, personalization and annotation of tree: Tree in PhyloXML

Table 1: MetaPhlAn: Predicted taxon relative abundances at each taxonomic levels

kingdom	kingdom_id	phylum	class	order	family	family_id	genus	genus_id	species	species_id	strains	strains_id
Viruses	10239					100.0						
Viruses	10239	Viruses unclassified					100.0					
Viruses	10239	Viruses unclassified	Viruses unclassified					100.0				
Viruses	10239	Viruses unclassified	Viruses unclassified	Viruses unclassified					100.0			
Viruses	10239	Viruses unclassified	Viruses unclassified	Viruses unclassified	Flaviviridae	11050				100.0		
Viruses	10239	Viruses unclassified	Viruses unclassified	Viruses unclassified	Flaviviridae	11050	Flavivirus	11051			100.0	
Viruses	10239	Viruses unclassified	Viruses unclassified	Viruses unclassified	Flaviviridae	11050	Flavivirus	11051	West Nile virus	11082		100.0

After generation of taxonomy, we move to functional information of our microbiome. Functional information of the above microbiome community [28] was done using HUMAnN pipeline.

# Gene Family	humann_Abundance-RELAB
UNMAPPED	0.888769
UniRef90_D9MXB1	0.0073362
UniRef90_D9MXB1|unclassified	0.0073362
UniRef90_UPI0001DD3770	0.00495637
UniRef90_UPI0001DD3770|unclassified	0.00495637
UniRef90_A0A1B1WW31	0.00484378
UniRef90_A0A1B1WW31|unclassified	0.00484378
UniRef90_B9DF73	0.00459488
UniRef90_B9DF73|unclassified	0.00459488
UniRef90_A0A067ZQ74	0.00422115
UniRef90_A0A067ZQ74|unclassified	0.00422115
UniRef90_A0A077EZ14	0.00417222
UniRef90_A0A077EZ14|unclassified	0.00417222
UniRef90_A0A067ZS80	0.00390475
UniRef90_A0A067ZS80|unclassified	0.00390475
UniRef90_UPI0003F054D7	0.00376104
UniRef90_UPI0003F054D7|unclassified	0.00376104
UniRef90_F1BA45	0.0036982
UniRef90_F1BA45|unclassified	0.0036982
UniRef90_A0A1X3CH90	0.00318336
UniRef90_A0A1X3CH90|unclassified	0.00318336
UniRef90_UPI000D0C92C0	0.00302072
UniRef90_UPI000D0C92C0|unclassified	0.00302072
UniRef90_K9L2G3	0.00265002
UniRef90_K9L2G3|unclassified	0.00265002
UniRef90_A0A1B1WWN4	0.00258533
UniRef90_A0A1B1WWN4|unclassified	0.00258533
UniRef90_K7ET80	0.00256502
UniRef90_K7ET80|unclassified	0.00256502

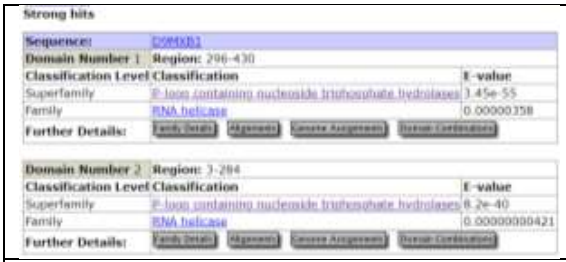
UniRef90_Q91CD9	0.00251161
UniRef90_Q91CD9|unclassified	0.00251161
UniRef90_UPI00018E17CF	0.00240202
UniRef90_UPI00018E17CF|unclassified	0.00240202
UniRef90_G7PXF9	0.00234066
UniRef90_G7PXF9|unclassified	0.00234066
UniRef90_F6Z663	0.00218081
UniRef90_F6Z663|unclassified	0.00218081
UniRef90_G7MFM1	0.00215974
UniRef90_G7MFM1|unclassified	0.00215974
UniRef90_A0A2K6NB76	0.0020891
UniRef90_A0A2K6NB76|unclassified	0.0020891
UniRef90_UPI000181CCFE	0.00203554
UniRef90_UPI000181CCFE|unclassified	0.00203554
UniRef90_C9WPK8	0.00195453
UniRef90_C9WPK8|unclassified	0.00195453
UniRef90_P14335	0.00180304
UniRef90_P14335|unclassified	0.00180304
UniRef90_F7HJ82	0.00180269
UniRef90_F7HJ82|unclassified	0.00180269
UniRef90_A0A0D3MDX0	0.00160595
UniRef90_A0A0D3MDX0|unclassified	0.00160595
UniRef90_UPI0000E69E2F	0.00153697
UniRef90_UPI0000E69E2F|unclassified	0.00153697
UniRef90_I3VPR6	0.00149368
UniRef90_I3VPR6|unclassified	0.00149368
UniRef90_G8E0A9	0.00146336
UniRef90_G8E0A9|unclassified	0.00146336
UniRef90_D3X888	0.0013421
UniRef90_D3X888|unclassified	0.0013421
UniRef90_UPI00003BECBC	0.00130469
UniRef90_UPI00003BECBC|unclassified	0.00130469
UniRef90_UPI0002C39258	0.00127138
UniRef90_UPI0002C39258|unclassified	0.00127138
UniRef90_Q6VXX6	0.00120319
UniRef90_Q6VXX6|unclassified	0.00120319
UniRef90_D3X8F1	0.0011535
UniRef90_D3X8F1|unclassified	0.0011535
UniRef90_A0A1S6WNB4	0.00110115
UniRef90_A0A1S6WNB4|unclassified	0.00110115
UniRef90_D3X8F7	0.00102768
UniRef90_D3X8F7|unclassified	0.00102768
UniRef90_D3X8D8	0.000998791
UniRef90_D3X8D8|unclassified	0.000998791
UniRef90_Q91KP3	0.000989086
UniRef90_Q91KP3|unclassified	0.000989086
UniRef90_D9IFF5	0.000952321
UniRef90_D9IFF5|unclassified	0.000952321
UniRef90_F7EY45	0.000912381
UniRef90_F7EY45|unclassified	0.000912381
UniRef90_D5K1B9	0.00090469
UniRef90_D5K1B9|unclassified	0.00090469
UniRef90_Q67428	0.000861045
UniRef90_Q67428|unclassified	0.000861045
UniRef90_Q91AB0	0.000854561
UniRef90_Q91AB0|unclassified	0.000854561
UniRef90_D3X875	0.000850089
UniRef90_D3X875|unclassified	0.000850089

UniRef90_A0A2K5WQL4	0.000795493
UniRef90_A0A2K5WQL4|unclassified	0.000795493
UniRef90_B1P6F1	0.000689513
UniRef90_B1P6F1|unclassified	0.000689513
UniRef90_P29984	0.000618302
UniRef90_P29984|unclassified	0.000618302
UniRef90_A0A0B5H4C4	0.000582165
UniRef90_A0A0B5H4C4|unclassified	0.000582165
UniRef90_Q6YFU6	0.00051542
UniRef90_Q6YFU6|unclassified	0.00051542
UniRef90_A0A0P0RST2	0.000505187
UniRef90_A0A0P0RST2|unclassified	0.000505187
UniRef90_A0A0D5CWD5	0.000489091
UniRef90_A0A0D5CWD5|unclassified	0.000489091
UniRef90_A0A068AXW2	0.000463204
UniRef90_A0A068AXW2|unclassified	0.000463204
UniRef90_A0A1X3CWX3	0.000451315
UniRef90_A0A1X3CWX3|unclassified	0.000451315
UniRef90_I1TEC3	0.000443267
UniRef90_I1TEC3|unclassified	0.000443267
UniRef90_A0A229QXW6	0.000390111
UniRef90_A0A229QXW6|unclassified	0.000390111
UniRef90_UPI0000485C0B	0.000373856
UniRef90_UPI0000485C0B|unclassified	0.000373856
UniRef90_Q6VXX7	0.000367549
UniRef90_Q6VXX7|unclassified	0.000367549
UniRef90_H2NBX3	0.000356109
UniRef90_H2NBX3|unclassified	0.000356109
UniRef90_UPI000642CDD8	0.000349761
UniRef90_UPI000642CDD8|unclassified	0.000349761
UniRef90_I3N2P9	0.000333919
UniRef90_I3N2P9|unclassified	0.000333919
UniRef90_D9IFG5	0.000299914
UniRef90_D9IFG5|unclassified	0.000299914
UniRef90_D3X874	0.000283626
UniRef90_D3X874|unclassified	0.000283626
UniRef90_Q9DP92	0.000282183
UniRef90_Q9DP92|unclassified	0.000282183
UniRef90_D3X8B2	0.000279068
UniRef90_D3X8B2|unclassified	0.000279068
UniRef90_A0A075FC35	0.000211602
UniRef90_A0A075FC35|unclassified	0.000211602
UniRef90_B7U2U9	0.000200363
UniRef90_B7U2U9|unclassified	0.000200363
UniRef90_C9WPM0	0.000194638
UniRef90_C9WPM0|unclassified	0.000194638
UniRef90_A0A0P7UTC2	0.000191691
UniRef90_A0A0P7UTC2|unclassified	0.000191691
UniRef90_F7FBV8	0.000191691
UniRef90_F7FBV8|unclassified	0.000191691
UniRef90_G3SQL6	0.000191691
UniRef90_G3SQL6|unclassified	0.000191691
UniRef90_H3BYH5	0.000191691
UniRef90_H3BYH5|unclassified	0.000191691
UniRef90_P60866	0.000191691
UniRef90_P60866|unclassified	0.000191691
UniRef90_D3X8C7	0.000186455
UniRef90_D3X8C7|unclassified	0.000186455

UniRef90_A0A2K6EGR5	0.000170037
UniRef90_A0A2K6EGR5|unclassified	0.000170037
UniRef90_A0A0B7JKQ7	0.000166418
UniRef90_A0A0B7JKQ7|unclassified	0.000166418
UniRef90_UPI0005215E94	0.000165638
UniRef90_UPI0005215E94|unclassified	0.000165638
UniRef90_G3I2D3	0.00016095
UniRef90_G3I2D3|unclassified	0.00016095
UniRef90_A0A2I0MVY6	0.000152329
UniRef90_A0A2I0MVY6|unclassified	0.000152329
UniRef90_L9LBB7	0.000143369
UniRef90_L9LBB7|unclassified	0.000143369
UniRef90_UPI0006B0C551	0.000133289
UniRef90_UPI0006B0C551|unclassified	0.000133289
UniRef90_A0A091DCD6	0.000130236
UniRef90_A0A091DCD6|unclassified	0.000130236

UniRef90_UPI000D3231B4	0.00012363
UniRef90_UPI000D3231B4|unclassified	0.00012363
UniRef90_UPI000359548B	0.000119308
UniRef90_UPI000359548B|unclassified	0.000119308
UniRef90_UPI00051F13F9	0.000116856
UniRef90_UPI00051F13F9|unclassified	0.000116856
UniRef90_UPI0005216B30	0.000112244
UniRef90_UPI0005216B30|unclassified	0.000112244
UniRef90_K7P5A3	0.000108977
UniRef90_K7P5A3|unclassified	0.000108977
UniRef90_L5JTI1	8.00319e-05
UniRef90_L5JTI1|unclassified	8.00319e-05
UniRef90_Q6VYH3	7.08503e-05
UniRef90_Q6VYH3|unclassified	7.08503e-05
UniRef90_A0A2U3XJL7	5.7032e-05
UniRef90_A0A2U3XJL7|unclassified	5.7032e-05

Next, from the gene family information, we obtain the functional information of our microbiome using Superfamily server. The Functional information of 1st five families from Normalized gene families as detected by Superfamily (HMM library and genome assignments server) is given below.



UniRef90_D9MXB1| Abundance-0.0073362

Protein sequence

Sequence length	2584
Comment	(tr|A0A1B1WW31|A0A1B1WW31_WNV) Genome polyprotein {ECO:0000256|SAAS:SAAS00368684} OX=11082 OS=West Nile virus (WNV). GN=MZ11_60591gpGP3 OC=Flaviviridae; Flavivirus; Japanese encephalitis virus group. OH=7158,8782,9606,34610,34627,34630,53527,308735,308737

UniRef90_A0A1B1WW31 | Abundance-0.00484378

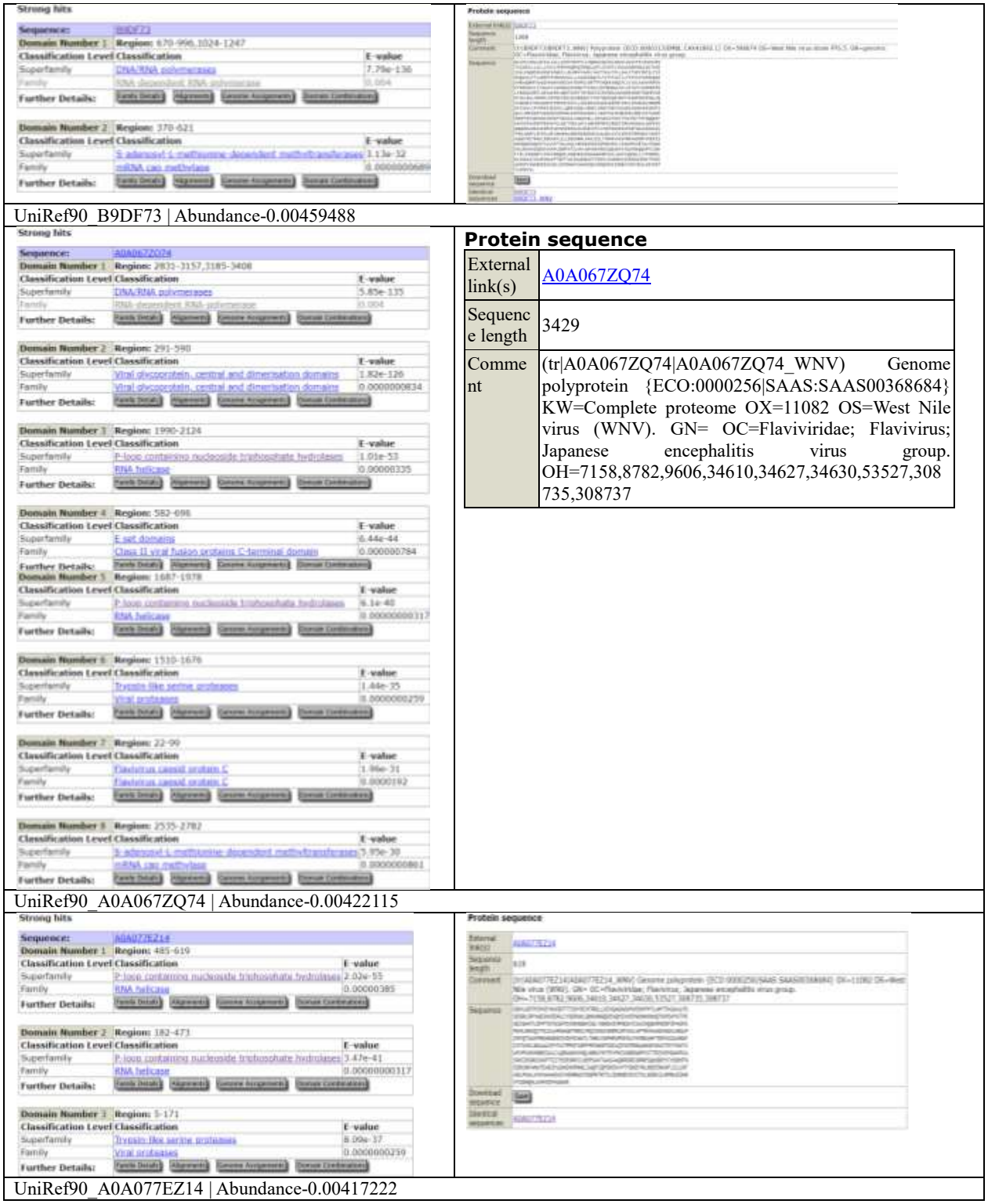


Fig. 2: Functional information of 1st five gene families as detected by Superfamily server [26]

CONCLUSION

The taxonomy information of West Nile fever microbiome are identified. The functional

information of the microbiome was identified using Superfamily database. This family information will be useful for further drug designing studies.

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