IPD Project Details

Project ID: IPD3284

Project Title: Exploiting Dynamics of Genomic Islands to Edit the Genome of Vibrio cholerae and Identify Novel Interactions Between Core and Acquired Genome

Description: Horizontally acquired genetic elements (HGEs) plays a major for determination of virulence, antimicrobial resistance, adaptation and evolution in pathogenic bacteria. Conserved integrative mobile genetic elements (MGEs) of Vibrio cholerae contribute in the disease development, antimicrobial resistance and metabolic functions. To understand the dynamics of integrative MGEs and cross talk between MGEs and core genome, engineered genome of V. cholerae was monitored in the presence and absence of horizontally acquired genetic elements. Deletion of more than 250 revealed that CTX? contributes to the essentiality of SOS response master regulator LexA in V. cholerae. Also, he core genome encoded RecA helps CTX? to bypass the host immunity and replicate in the host cell in the presence of similar prophage in the host chromosome. Finally, our multiomics data reveal importance of MGEs in modulating the level of cellular proteome and metabolome in V. cholerae. This study for the first time engineered the genome of V. cholerae strains to eliminate all the GIs, ICE and prophages from their genome and revealed new interactions between core and acquired genomes. The engineered strain could be a potential candidate for understanding evolution of cholera pathogen and development of therapeutics.

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Sample Preparation: Overnight grown V. cholerae strains N16961 and BB1 were used for total protein extraction and trypsin digestion. Protein were extracted using lysis buffer (RIPA) and subsequent sonication. Reduced (10 mm DTT) and alkylated (20 mm IAA) protein sample was trypsin digested for 18 hrs at 37 °C followed by C18 desalting step for further LC-MS/MS analysis.

Peptide Separation: Peptides from the cell lysate of both the V. cholerae strains were fractionated using an Agilent 1200 HPLC system on high-pH reverse-phase Zorbax 300 Extend-C18 column (Agilent Technology, Santa Clara, CA, USA) to the final 12 fractions. For identification and spectral library building, each fraction were subjected to 90 min gradient LC-MS/MS in data dependent acquisition mode. For quantitative analysis, 4 ug

of C18 desalted peptides from each sample were acquired in triplicate in a DIA mode. Peptides were separated using NanoViper C18 separation column (75 µm x 250 mm, 3 µm, 100 Å: Acclaim Pep Map, Thermo Scientific) at flow rate of 300 nl/min in a 90 min linear gradient of 35% acetonitrile. Using a 54 overlapping (1 Da) variable windows, TOF/MS servery scan (400-1250 m/z) was performed with accumulation time of 250 ms, and fragmentation spectrum was collected with 50 ms (100-1500 m/z).

Protein Characterization: MaxQuant version 1.6 where was used for peptide and protein identification (1% FDR) against UniProtKB N16961 protein database (3783 entries, Sep 2019). The resulting peptide and protein lists were used to generate the spectral library using Spectronaut (Biognosys) with default settings which were further used for SWATH processing and quantification. Proteins with a ratio >1.5 was considered differentially expressed. In all cases,

Experiment Type: SWATH MS

Species: Vibrio cholerae serotype o1 (strain atcc 39315 / el tor inaba n16961)

Tissue: Cell suspension culture (bto:0000221)

Cell Type: Cell suspension culture

Disease: Unknown

Instrument Details: TripleTOF 5600 (MS:1000932)

Protein Modifications: monohydroxylated residue, acetylated residue, iodoacetic acid derivatized residue

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