Transcriptomic analysis of *Vibrio vulnificus* response to Wastewater Treatment Plant Effluent Exposure

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Background

Vibrio vulnificus is an opportunistic pathogen indigenous to estuarine and marine environments and associated with aquatic organisms. This bacterium is an invasive pathogen for humans and aquatic animals and in both can be transmitted by contact or ingestion (Baker-Austin *et al.*, 2017; Oliver *et al.*, 2013). However, not all strains of *V. vulnificus* are equally pathogenic with strains being grouped into clinical and environmental categories based on biotype and genotype. Previous studies have demonstrated that clinical genotypes show superior survival in human serum and possess putative virulence factors that enhance disease progression. Also, clinical *V. vulnificus* strains can exhibit different gene expression profiles depending on surrounding conditions (William, *et al.*, 2014). *V. vulnificus* is of utmost importance because of its high mortality rate (95% of the seafood-related deaths in the US) due to rapid progression of septicemia. In the United States, the most recent report from the Foodborne Diseases Active Surveillance Network (Tack *et al.*, 2019) indicated that the 2018 incidence of *Vibrio* infections increased 109% and the number of diagnosed infections by 311% as compared with 2015-2017 incidence. In urban settings, wastewater treatment plants (WWTP) act as reservoirs of antibiotic resistance given that antibiotics, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs) have been observed in the final treated effluent (Rizzo *et al.*, 2013; Xu *et al.*, 2015; Guo *et al.*, 2017). As well, the transference of ARGs from WWTP into surrounding ecosystems through the release of treated effluent has been well documented (Chu *et al.*, 2018). The combination of increasing ocean temperature and sea level, and the human activity in coastal waters are altering geographical constraints, resulting in increased *Vibrio* spread, exposure, and rates of human infection (Baker-Austin *et al.*, 2012; Vezzulli *et al.*, 2013; Froelich and Dainess, 2020). The fact that coastal population expansion is resulting in increased input of treated municipal sewage containing ARB, ARGs, and human cellular debris into areas that are also experiencing increased *Vibrio* proliferation and spread due to climate change may represent a significant public health risk due to exposure to environmental reservoirs of antibiotic-resistant *Vibrio spp* with enhanced virulence profiles.

Objectives

- ✓ The overall goal of this study was to better understand the influence of treated sewage effluent on effluent-receiving microbial communities using *Vibrio* as a model of an opportunistic pathogen.

General Methods

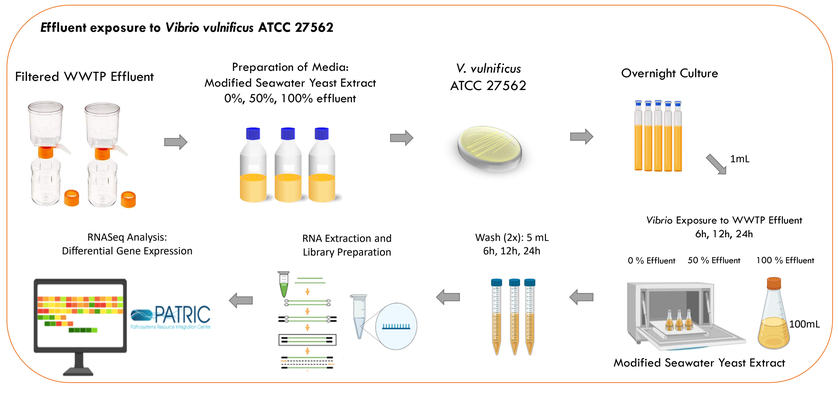

Figure 1. Graphical methodology of the Transcriptomic analysis of *V. vulnificus* ATTC 27562 response to WWTP Effluent exposure. Integrated transcriptomic approaches were used to analyze the changes in overall gene expression of a clinical *V. vulnificus* ATCC 27562 strain exposed to wastewater effluent for a period of 24 hrs using a modified seawater yeast extract media (MSWYE; Oliver and Colwell, 1973) that contains 0%, 50% and 100% filtered effluent. RNA-seq reads were mapped to the complete genome, annotated, and analyzed to find differentially expressed genes using the Tuxedo protocol in the Pathosystems Resource Integration Center (PATRIC) analysis tool (Wattam *et al.*, 2017; Trapnell *et al.*, 2012).

Results

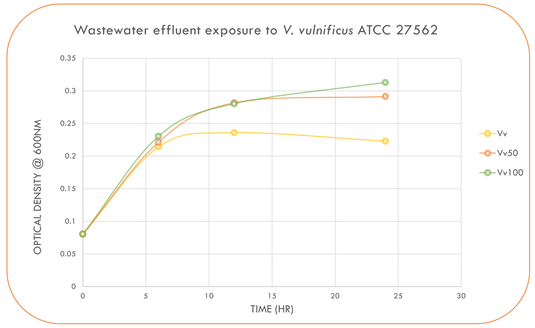

Figure 2. Growth of *V. vulnificus* ATTC 27562 in Modified Seawater Yeast Extract broth with different concentration of WWTP Effluent. *V. vulnificus* ATCC 27562 was grown on modified seawater yeast extract broth that contains 0%, 50% and 100% filtered effluent during 24 hrs. All conditions are in Log (Exponential) phase at 6 hrs. but exhibited different growth phases in the subsequence hours.

Annotation of the *Vibrio vulnificus* ATCC 27562 genome generated using the PATRIC analysis tool resulted in a total of 4,564 coding sequence regions, including 191 specialty genes that contain antibiotic resistance genes, drug target, and virulence factors. Using the generated annotation as a reference for the RNAseq analysis, this study found a significant difference in 1,789 coding sequence regions between exposure to 0% WWTP effluent and 50% effluent at 6h, where 844 are upregulated and 945 down regulated (Figure 3A). Similar trends were observed when *Vibrio* were exposed to 100% effluent, with 931 upregulated and 1087 downregulated (Figure 3B).

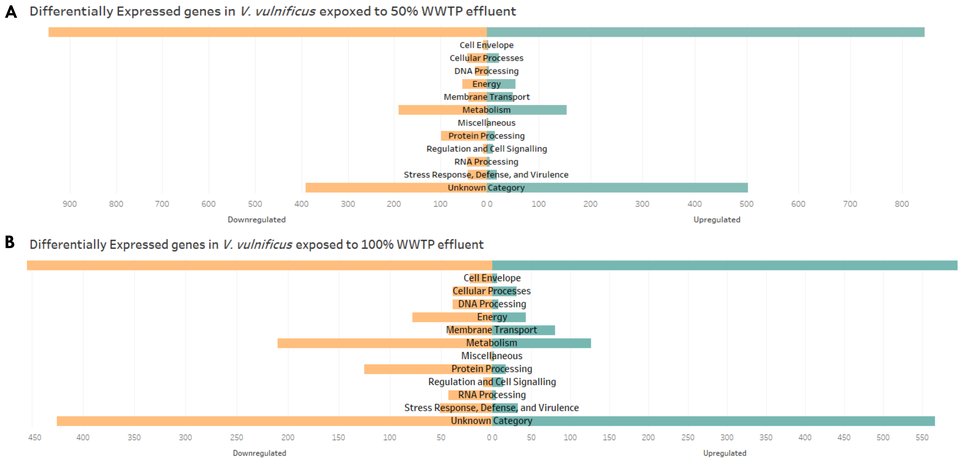

Figure 3. Differentially Expressed Genes in *V. vulnificus* ATTC 27562 exposed to 50% and 100% WWTP Effluent.

Superclasses	Condition 0%	50%	100%
Cell Envelope	9,324	10,878	5,770
Cellular Processes	20,149	31,764	23,989
DNA Processing	4,448	5,362	4,021
Energy	54,952	60,036	36,106
Membrane Transport	7,669	15,832	12,482
Metabolism	43,226	53,140	35,540
Miscellaneous	708	1,500	849
Prottein Processing	59,346	64,247	38,359
Regulation and Cell Signal..	1,240	1,437	1,281
RNA Processing	5,707	6,300	4,488
Stress Response, Defense..	17,545	21,069	15,442
Unknown Category	184,676	254,473	216,826

FPKM Mean: 708 – 254,473

Figure 4. Total Mean Fragments per kilobase of transcript per million fragments mapped (FPKM) for each super classes.

Conclusion/ Summary

- ✓ Among the differentially expressed genes, several were identified as genes involved in metabolism, antibiotic resistance, and virulence, thus indicating that exposure to wastewater effluent may impact *Vibrio* growth and virulence profiles.
- ✓ This study provides insight into the adaptive response mechanism of *V. vulnificus* to exposure to treated sewage effluent and may help further inform the developing public health predictive models of *Vibrio* exposure and virulence.

Future Work

- ✓ Determine specific genomic pathways of *Vibrio vulnificus* in the adaptive response to WWTP effluent exposure.
- ✓ Determine specific virulence pathways that can be enhanced by the WWTP effluent exposure.

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