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Strand-specific RNA-Seq analysis provides first insight into transcriptome response of *Vibrio alginolyticus* to phage infection

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ABSTRACT

RNA-Seq transcriptome analysis of bacteria during phage infection has been occasionally reported in last several years but little is known about how *Vibrio* bacteria respond to phage invasion on global transcriptome level. In the present study, transcriptome profiling of *V. alginolyticus* during a lytic phage infection was first investigated through strand-specific RNA-Seq analysis. The results revealed that 346 unigenes were up-regulated and 860 unigenes were down-regulated during the infection of phage Vp670. Notably, the unigenes involving in phosphorelay signal transduction system were largely down-regulated in infected *V. alginolyticus* cells. However, some unigenes in the GO terms (DNA binding and sequence-specific DNA binding transcription factor activity) were up-regulated including a *csp* gene coding cold shock protein. These transcriptome data contribute to a more comprehensive insight into cellular response of *V. alginolyticus* to phage infection and help us deeply understand the phage-host interplay on molecular levels.

1. Introduction

Bacteriophages (or phages) represent a group of viruses that is dominant in the microbial world (Guzina and Djordjevic, 2015). Phages selectively infect specific bacterial cells, which confers some unique advantage over antibiotics (Kutateladze and Adamia, 2010). In recent years, phage therapy has gained increasing attentions due to the occurrence of multi-drug resistant pathogens and a deficit in the development of new classes of antibiotics to counteract them (Kutateladze and Adamia, 2010; Cisek et al., 2017).

Lytic phages can infect and hijack host bacterium's biochemical machinery to make copy after copy of itself until the bacterium bursts. In this process, bacterial cells undergo drastic changes in cellular gene expression and host physiology (Poranen et al., 2006). In previous works, the effect of phage infection on host mRNA synthesis has been studied using microarray technology (e.g. Poranen et al., 2006; Zhao et al., 2016). However the earlier-applied technologies covered only a set of host genes selected, the development of RNA sequencing (RNA-Seq) now provides more comprehensive insight into global changes in cellular gene expression during phage infection. RNA-Seq transcriptome analysis of bacteria during phage infection have been occasionally reported in last several years (e.g. Lavigne et al., 2013; Lin et al., 2015; Couto et al., 2015), which largely help us understand molecular mechanisms of interplay between phages and their hosts.

Vibrios are widely distributed in marine and estuarine environments and there are at least 13 *Vibrio* species pathogenic to humans including *Vibrio alginolyticus* (Daniels and Shafaie, 2000). *V. alginolyticus* and some other *Vibrio* members are also notorious pathogens to aquatic animals and have caused huge economic losses (Pridgeon and Klesius, 2012). Controlling *Vibrio* infections by phages is considered as a promising method no matter for humans (Fazil and Singh, 2011) or for aquatic animals (Richards, 2014). A lot of *Vibrio* phages have been isolated and characterized (e.g. Yu et al., 2013; Kalatzis et al., 2016), however until now little is known about how *Vibrio* cells respond to phage invasion on global transcriptome level. In the present study, transcriptome profiling of *V. alginolyticus* during a lytic phage infection was first investigated through strand-specific RNA-Seq analysis. This work will contribute to our comprehensive understanding on the molecule mechanism of phage infection and the interaction between phages and hosts.

2. Data description

2.1. Bacterial culture and phage infection

V. alginolyticus E06333 and its lytic phage Vp670 were stocked in our laboratory and the strain E06333 was cultured in Luria-Bertani (LB)

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1874-7787/

Table 1
MIxS descriptors.

Item	Description
Investigation_type	Bacteria
Project_name	*Vibrio alginolyticus* transcriptomes with and without phage infection
lat_lon	22.56 N 114.51 E
Geo_loc_name	China: Daya Bay
Collection_date	2006-08-27
Collected_by	Peng Luo
Organism	*V. alginolyticus* E06333
isolation_source	*Epinephelus coioides*
Biome	Marine environment (ENVO:01000320)
Feature	Microbial feature (ENVO:01000007)
Material	Fishpond (ENVO:00000056)
Env_package	Aquaculture process (ENVO:01000906)
seq_meth	Illumina
Transcriptome platform	HiSeq4000
Assembly method	Trinity (trinityrnaseq_r20140717) and TGICL (v2.6)
Assembly name	*V. alginolyticus* E06333 transcriptome
Sample material	*V. alginolyticus* samples with and without phage infection
Experimental factors	Cultivation with or without phage Vp670
Experimental features	Transcriptome response of *V. alginolyticus* to the infection of phage Vp670

broth at 30 °C for all experiments. The strain was cultured overnight and then inoculated into fresh LB medium (1%, *V*/V) in two groups. When OD_{600nm} of experimental group (EG, triplicate samples) and control group (CG, triplicate samples) reached at 0.9–1.0, each of EG samples was added with phage extract (2%, V/V) while CG samples were left without any treatment. The samples in both groups were incubated for 2 h and then mixed respectively followed by centrifugation at 10,000*g* for 2 min. Mixed EG sample was additionally washed twice with LB medium to remove extracellular phage particles.

2.2. RNA isolation, library construction and sequencing

Total RNA was extracted using RNA Isolation Kit (Tiangen, Beijing, China) according to the manufacturer's protocol. Total RNA was treated with RNase-free DNase (TaKaRa, Dalian, China) to remove traces of genomic DNA. mRNA was enriched using MagJET mRNA Enrichment Kit (ThermoFisher, Waltham, USA). The enriched mRNA was processed to a RNA-Seq library using the mRNA-Seq sample preparation kit (Illumina, San Diego, USA) following the manufacturer's instructions. The constructed sequencing library was sequenced using the Illumina HiSeq 4000 platform (Illumina, San Diego, USA) at HengChuang Gene Limited Company (TGS, Shenzhen, China).

2.3. Assembly and annotation

Raw reads were quality-filtered using a reads trimming tool Trimmomatic with default settings (Bolger et al., 2014). After quality filtering, 11.16 and 11.05 million retained clean reads were de novo assembled using a tool Trinity (Grabherr et al., 2011). The assembled transcripts were further clustered and removed the redundant sequences through using TGICL (Pertea et al., 2003) to get final 2995 unigenes that ranged from 200 bp to 30,812 bp with an N50 length of 2964 bp (Supplementary Fig. S1). The annotations for the unigenes were performed by using Blast (v 2.2.26) to align them to NT, NR, COG, KEGG, and SwissProt. Gene Ontology (GO) annotation was obtained using Blast2GO (Conesa et al., 2005) with NR annotation. MIxS descriptors are presented in Table 1.

2.4. Differential expression analysis of EG and CG samples

Differential expression genes (DEGs) of EG and CG samples were detected using the DEGSeq (Wang et al., 2010). The fold Change ≥ 2 and the Q-value ≤0.01 were set as the threshold for significantly differential expression. There were 1206 DEGs detected, in which 346 DEGs were up-regulated and 860 DEGs were down-regulated. The distribution of 1206 DEGs was shown in Fig. 1. Extensive down-regulation of transcripts during phage infection was also observed in the response of *Pseudomonas aeruginosa* to the infection of phage PaP3 (Zhao et al., 2016).

With the GO annotation result, GO functional enrichment was performed using hypergeometric tests. The most enriched GO terms (top

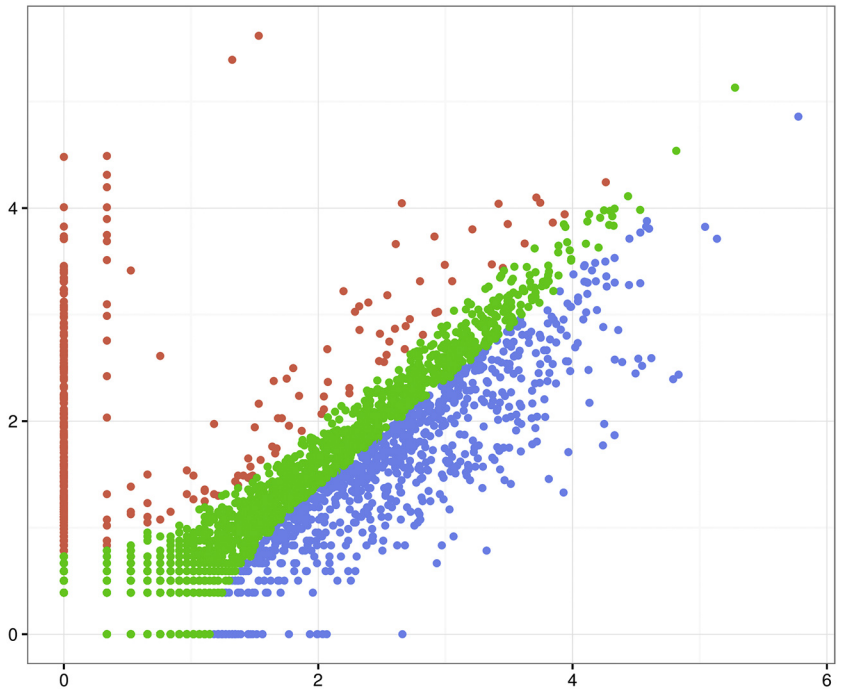

Fig. 1. Distribution of DEGs in E06333-EG compared with E06333-CG. X axis represents the expression quantity (log_{10}) of E06333-CG and Y axis represents the expression quantity (log_{10}) of E06333-EG. Every point represents a unigene. Red, blue, and green points represent up-regulated, down-regulated, and non-differential unigenes, respectively.

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