Survival, Virulent Characteristics, and Transcriptomic Analyses of *Vibrio mimicus* Exposed to Starvation

Ziyan Jiang*, Xiaojun Zhang**

College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, China
* Jiangziyan1223@163.com; ** zxj9307@163.com

INTRODUCTION

Vibrio mimicus is a gram-negative bacterial pathogen distributing widely in both freshwater and estuarine environments. It is one of the most prevalent pathogens in aquaculture, which could induce high mortality among aquatic animals, impeding healthy and sustainable development of aquaculture. Bacteria can be subject to multiple environmental stresses during their life, including nutrient limitation, extreme temperature, osmotic concentrations, oxygen, copper stress and organic pollutants. Among them, nutrient deficiency is the most common stress which microorganisms routinely encounter in natural ecosystems. It has been reported that *Vibrio* spp. can differentiate into the viable but nonculturable (VBNC) state to maintain viability and survive several years in food deprived condition, which could contribute to the spreading of *V. mimicus* in the environment and hazarding public health. A common feature of bacteria subjected to starvation is the 'rounding up' phenomenon, which cells become rounder and adopt a coccus shape. In addition, the growth, survival rate, and pathogenicity were also reported to be reduced compared to the non-starved cells. These phenotypic signs induced by starvation were found in *Vibrio* spp., but the response of *V. mimicus* in survival and pathogenicity under starvation stress are not clear.

This study assessed the potential of *V. mimicus* to survive under starvation stress and further investigated the changes in morphology, motility, and pathogenicity induced by starvation. Besides, to reveal the molecular mechanism behind phenotypic changes, transcriptomic analyses between starved and wild cells were examined at the whole transcriptional level. These results will shed new light on understanding of the stress response mechanisms of *V. mimicus* under starvation.

METHODS

- *V. mimicus* Y4 was isolated from *Macrobrachium nipponense* with red body disease in Jiangsu province (Jiang et al., 2021)
- The wild and 4-week starved strain was incubated at 28℃ to the initial exponential (OD_{570} = 0.2). The values of OD_{570} were recorded at 0-24 h. Morphological changes between 4-week starved and wild cells were monitored under scanning electronic microscope (SEM) as previously described with minor modification (Arias et al., 2012). After 4-week starvation, the motility of *V. mimicus* strain Y4 was measured as described previously (Xu et al., 2014).
- Biofilm formation in microplates was quantified by crystal violet staining according to a previously described method (Dueholm et al., 2013).
- Healthy prawns were divided into 11 groups and exposed with five different doses, and prawns in the rest tank were maintained in freshwater as control group. The mortality of prawns were checked during 7 d post infection.
- Transcriptome analysis was constructed between wild and starved strain.

RESULTS AND DISCUSSION

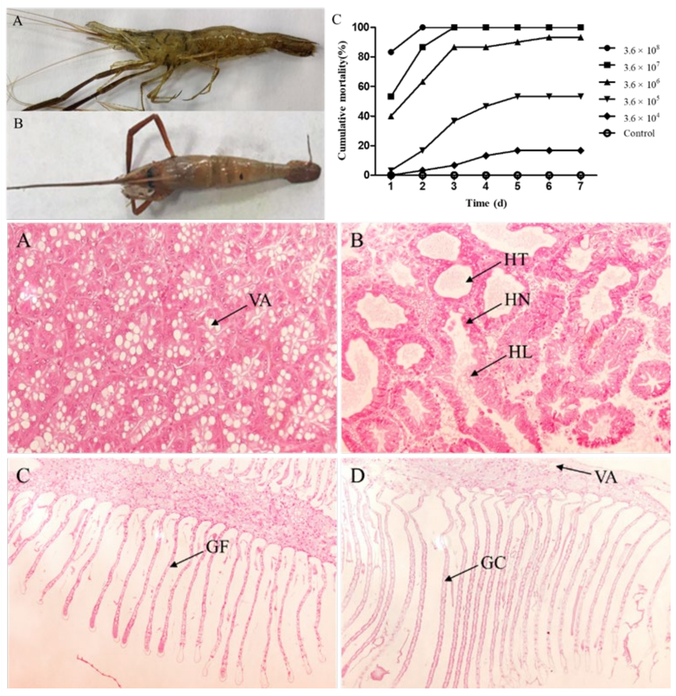

Figure 1 The symptom and histological changes of red body disease in *M. nipponense.*

- *M. nipponense* culture in Jiangsu has been suffering from red body disease with significant economic loss, and *V. mimicus* is recognized to be the etiological agent of this disease. The LD_{50} of *V. mimicus* Y4 to prawns was 3.28×10^5 CFU/mL after 7 d post-infection.
- The hepatopancreas of the infected *M. nipponense* were found that the basal laminae of the hepatopancreatic tubules rupture, the star shape of the lumen wear off, vacuolation decrease and the hepatopancreatic tubules gap dilatate.
- The gill filament cells of the infected prawn were obviously enlarged, the tissue boundary was not complete, the majority of the gill filament cells were broken, and the gill filament thickness was not uniform.

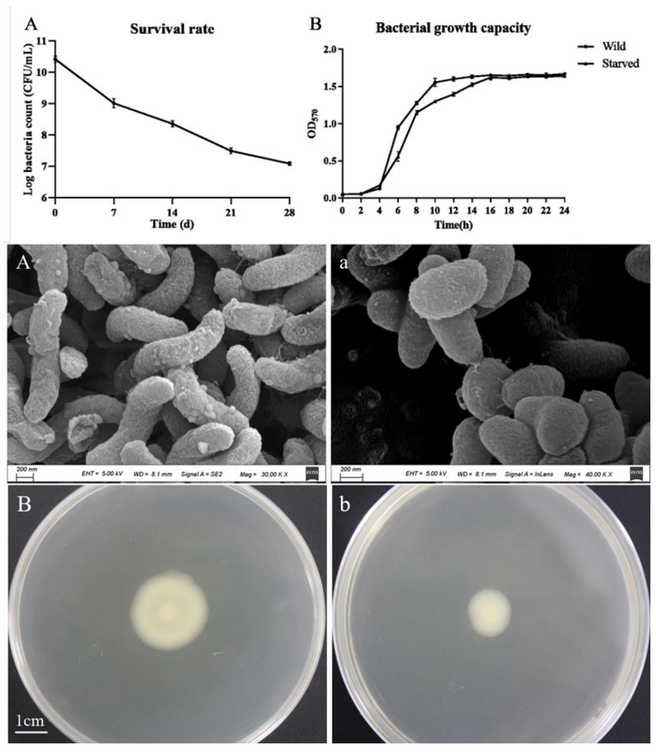

Figure 2 Survival, growth curves, morphological and motility changes of *V. mimicus* cells after starvation.

- *V. mimicus* Y4 was still culturable after 4-week starvation. The percentage of the strain Y4 culturable bacterial cells was reduced to 68.13% of their initial level.
- The mild *V. mimicus* cells were changed from rods shape to short rods, and the average cell length of the initial *V. mimicus* had significantly decreased from 1.6 ± 0.2 μm to 1.0 ± 0.2 μm by using scanning electron micrograph.
- *V. mimicus* had significantly decreased in motility (from 2.2 ± 0.2 μm to 1.2 ± 0.2 μm) after starvation.

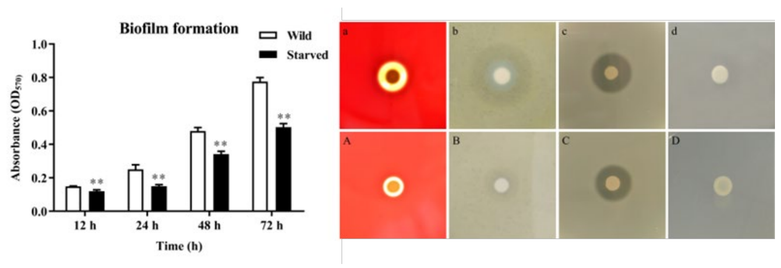

Figure 3 Biofilm production, virulence factors and pathogenicity of the starved strain

Table 1 Pathogenicity of wild and starved *V. mimicus* in *M. nipponense*

Group	Infected amount	Bacterial concentration (CFU/mL)	Time (d) and amount of mortality								Mortality(%)
			1	2	3	4	5	6	7	total	
Non-starved cells	30	3.6×10^8	25	5	0	0	0	0	0	30	100%
	30	3.6×10^7	16	10	4	0	0	0	0	30	100%
	30	3.6×10^6	12	7	7	0	1	1	0	28	84%
	30	3.6×10^5	1	4	6	3	2	0	0	16	53.3%
	30	3.6×10^4	0	1	1	2	1	0	0	5	16.7%
Starved cells	30	3.6×10^8	22	6	2	0	0	0	0	30	100%
	30	3.6×10^7	8	8	5	3	0	0	0	24	80%
	30	3.6×10^6	6	4	2	1	1	0	0	14	46.7%
	30	3.6×10^5	0	4	1	1	1	1	0	8	26.7%
	30	3.6×10^4	1	2	0	1	0	0	0	4	13.3%
Control	30	0	0	0	0	0	0	0	0	0	0

- Biofilm production of the starved cells was decreased with 0.24, 0.67, 0.40 and 0.54 fold than wild cells over a period of 12-72 h, respectively.
- Consistent with the wild strain, 4-week-starved *V. mimicus* still could produce β-hemolysis, lecithinase and caseinase, and did not produce lipase.
- The virulence of starved cells decreased with an LD_{50} value (2.45×10^6 CFU/mL), compared with wild cells (3.09×10^5 CFU/mL).

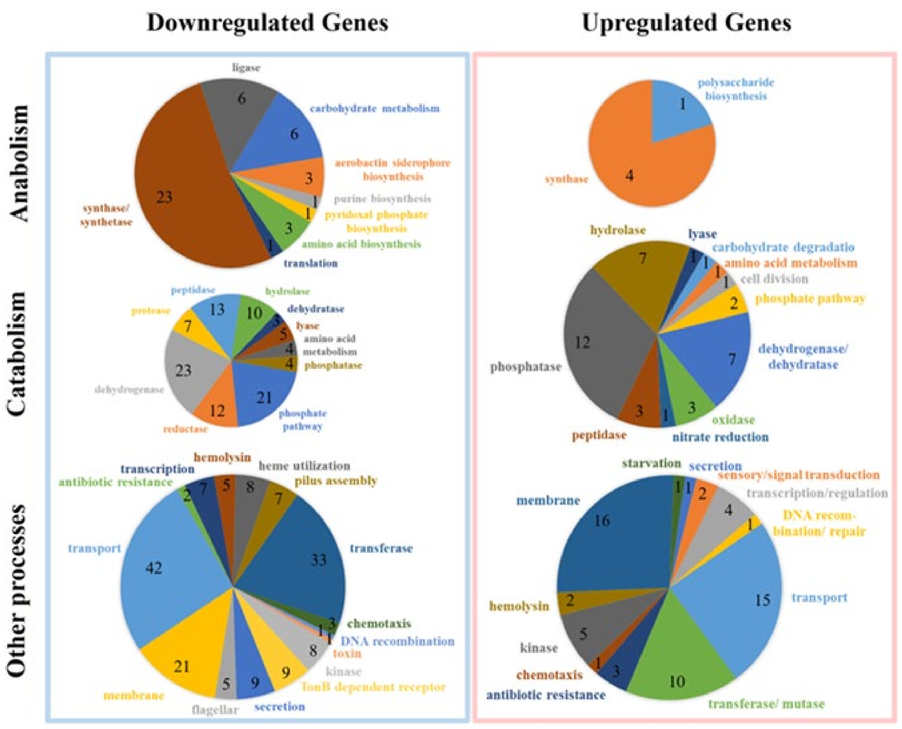

Figure 4 DEGs analysis in the wild and starved *V. mimicus.*

DEGs analysis in the wild and starved *V. mimicus*. Most of the anabolism related genes (44 out of 49, 89.8%) showed decreased expression level under starvation stress, and similar expression changes were found in catabolism related genes with 102 down-regulated and 39 up-regulated. Further, most of virulent-related genes involved in hemolysin, hume utilization, pilus assembly, toxin, secretion, etc. were also downregulated, which is consistent with the weakened virulence described above.

A large number of flagellar assembly protein-related genes showed significantly down-regulated expression after starvation, including *flgD* gene, *flgF* gene and *fliF* gene, etc., which might reduce the flagella synthesis, affect the motility of *V. mimicus*, and further decrease the bacterial adhesion and invasion ability to the host.

CONCLUSIONS

- *V. mimicus* cells were still culturable after 4-week starvation, but showed reduction in size, changed morphology from rods to short rods, and weakened pathogenicity.
- Genes involved in metabolism, virulence and adaptive evolution were differently expressed which might contribute to the survival and virulent changes under starvation.

References:
[1] Jiang, Z., Gao, X., et al. (2021). Genomic characterization and pathogenicity analysis of the *Vibrio mimicus* Y4 causing red body disease in *Macrobrachium nipponense*. Aquaculture. 548 (2), 737701.
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