

# POLY- $\beta$ -HYDROXYBUTYRATE (PHB) REGULATES THE IMMUNE RESPONSES IN MUSSEL LARVAE CHALLENGED WITH *Vibrio coralliilyticus*

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## INTRODUCTION

Poly- $\beta$ -hydroxybutyrate (PHB) is a natural bacterial polymer that is thought to be intestinally depolymerized into water soluble 3-hydroxybutyrate. This monomer can deliver energy and/or act as a microbial control agent. The current poster describes the effect of PHB on innate immune system and phenoloxidase activity of blue mussel larvae (*Mytilus edulis*) challenged with pathogenic *Vibrio coralliilyticus*.

Aims of study: whether PHB regulates innate immune response and phenoloxidase (PO) activity of blue mussel larvae challenged with *V.coralliilyticus*

## MATERIALS & METHODS

**Challenge test:** Sterile D-larvae (rifampicin) Challenge in Tissue Culture Dish for survival Challenge in 1 L bottle for both RNA extraction gene expression and PO activity

4 treatments (n=3)

-Larvae (L)

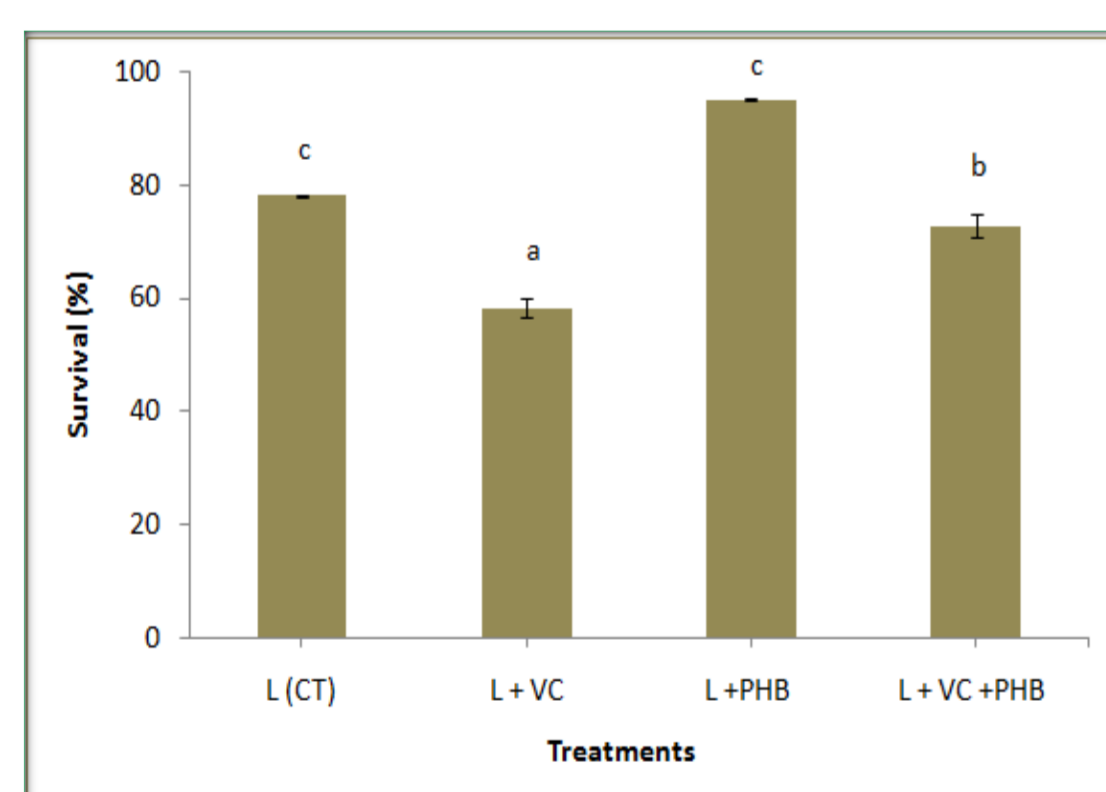
-Larvae + PHB (L +PHB)

-Larvae + *V.coralliilyticus*(L + VC)

-Larvae + *V.coralliilyticus* + PHB (L+ VC + PHB)

PHB (1mg L<sup>-1</sup>) fed the larvae for 6 h then exposure to *V. coralliilyticus* at 10<sup>5</sup> CFU mL<sup>-1</sup>

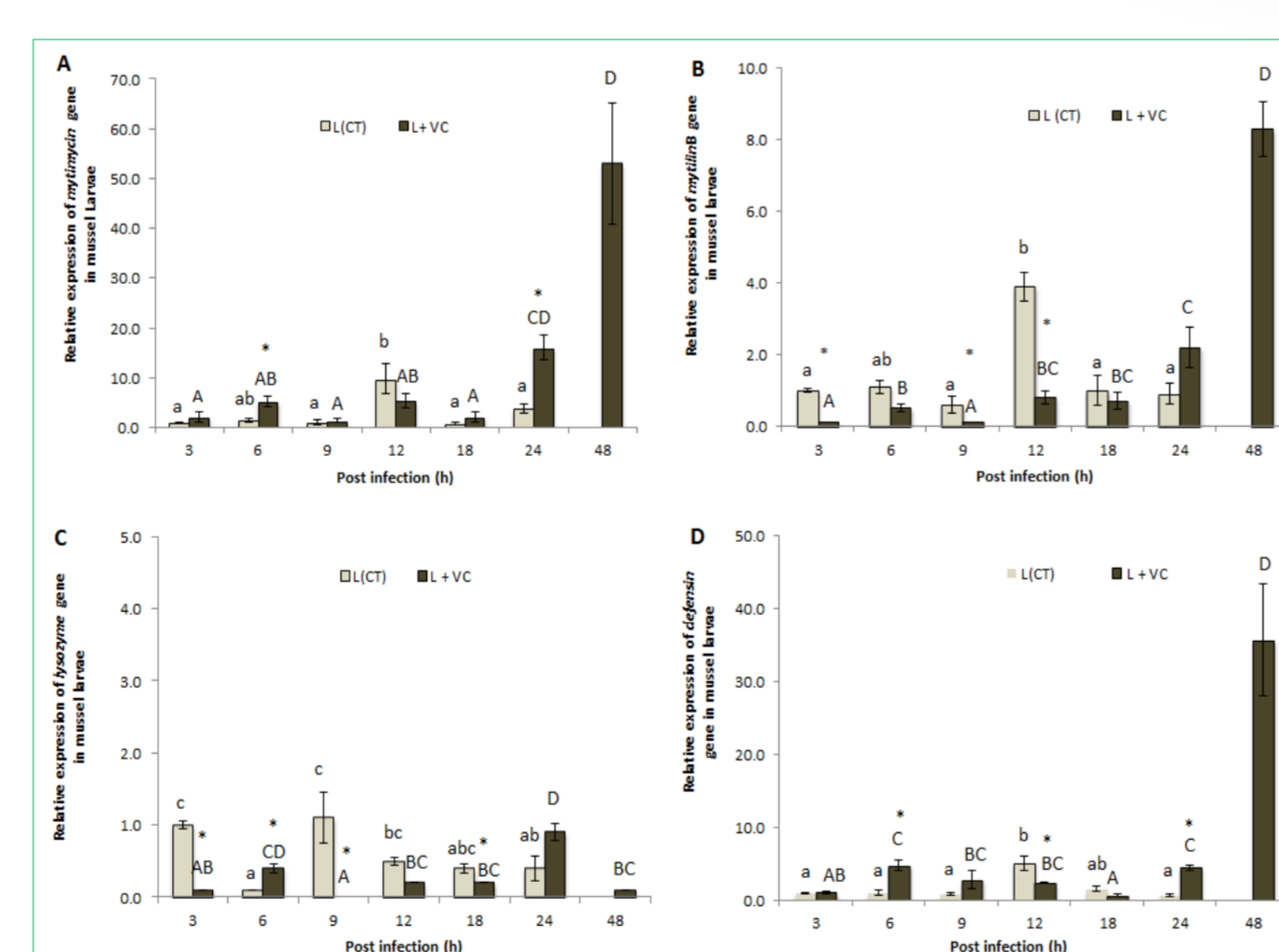
Survival was scored at 48 h after challenged.



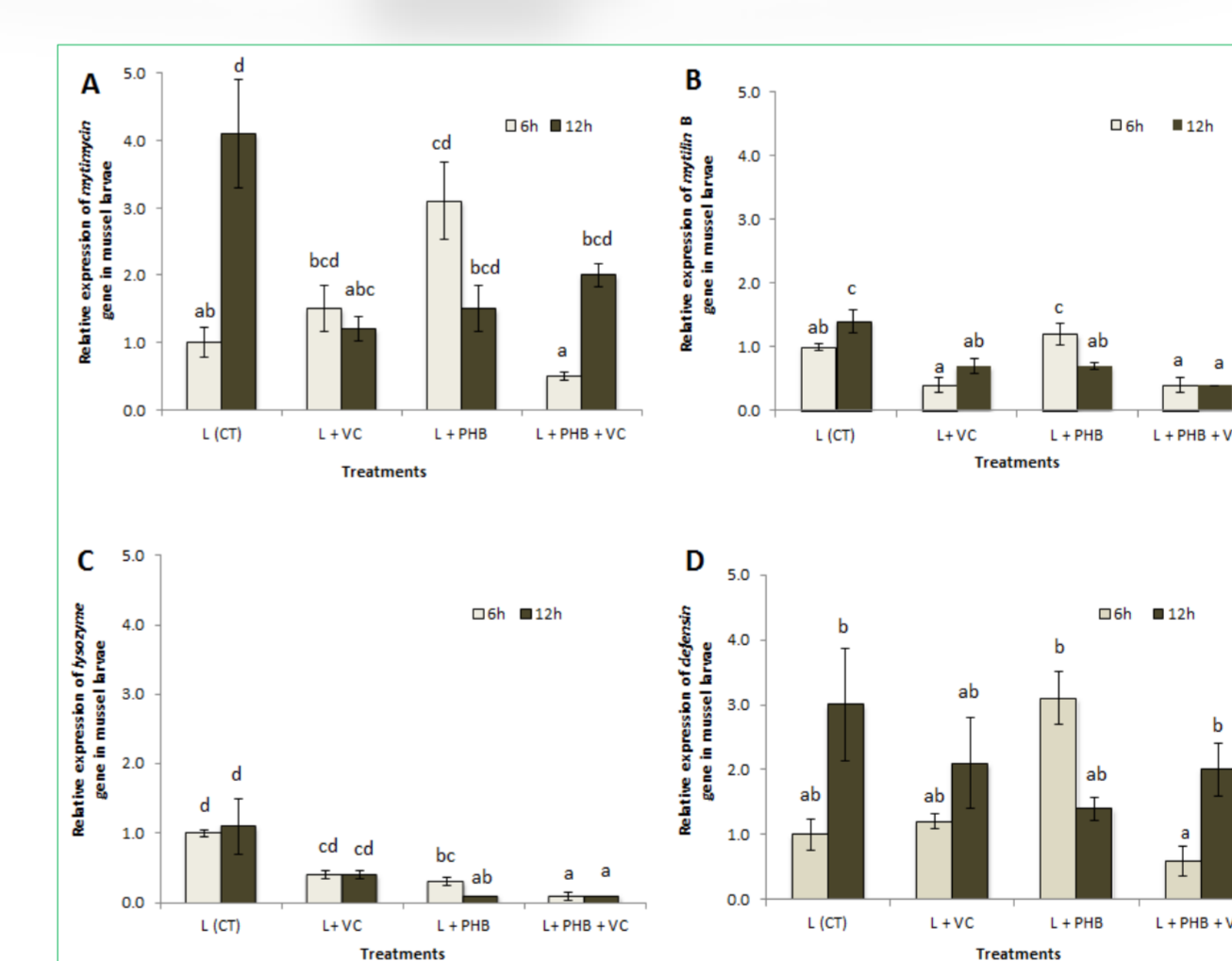
**Fig. 1.** Survival (%) of mussel larvae after 48h exposure to *V.coralliilyticus* (10<sup>5</sup> CFU mL<sup>-1</sup>)

**Table 1:** Characteristics of the primers used for expression analysis with RT-qPCR

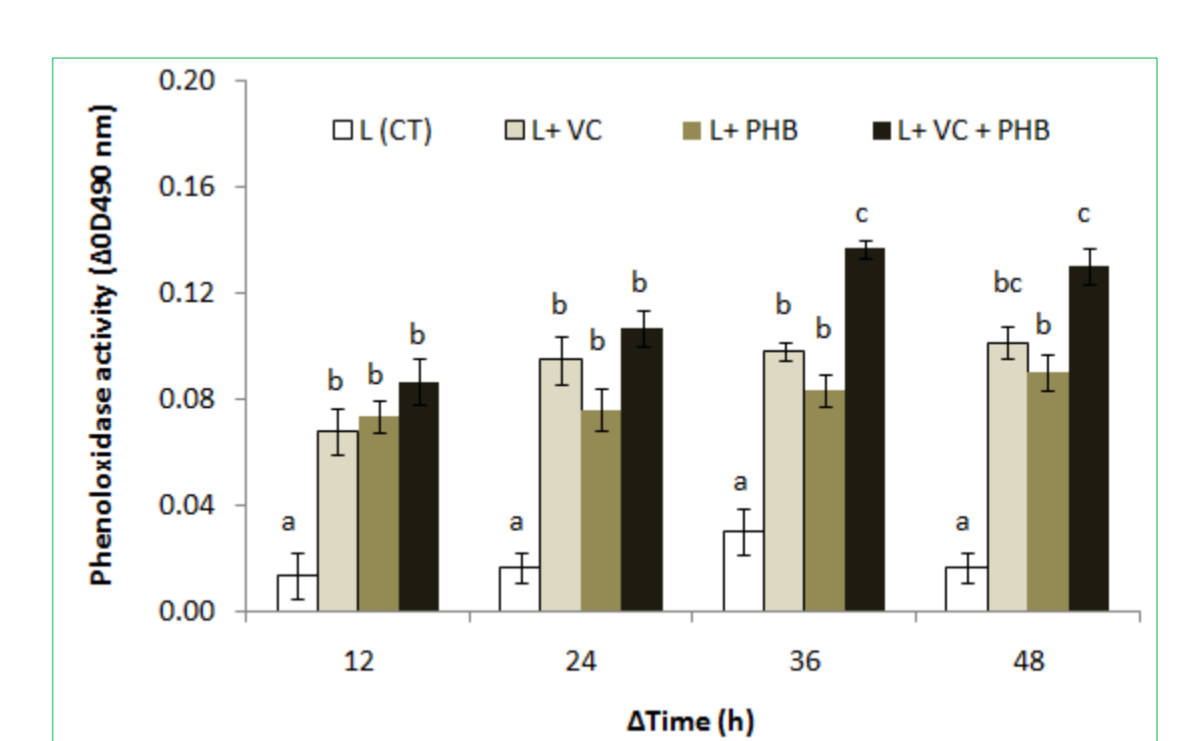
No	Gene	GenBank accession no.	Primer	Amplicon length (bp)	Annealing temperature
1	18sRNA	L33448.1	F: TTAAGAGGACTGACGGGG R: TTGGCAATGCTTCGCACT	93	60 °C
2	MytilinB	JN825739.1	F: CCATTGTGGGACTGCACT R: CGGTCCCACTTCATAACA	123	63 °C
3	MytilinB	AF177540.1	F: CAGAGGCAAGTTGTGCTCC R: GGAATGCTCACTGGAACAACG	125	63 °C
4	Lysozyme	AF334662.1	F: CCAAGCACTATCATGTGCT R: TCCCTTGACCTCATTTGT	122	58 °C
5	Defensin	JN935271.1	F: CCCAGCAGCAATCTAGGAC R: AAGCCCATATGCTGCTACT	140	58 °C



**Fig. 2.** Expression of immune genes (A) mytilinB, (B) mytilinB (C) lysozyme (D) defensin of mussel larvae no challenges L (CT) and Challenge (L+VC) with *V.coralliilyticus* (10<sup>5</sup> CFU L<sup>-1</sup>). Asterisks denote a significant difference (p<0.05). A sample of unchallenged treatment at 48h was not determined.



**Fig. 3.** PHB at 1 mg L<sup>-1</sup> regulates the expression of immune genes (A) mytilinB, (B) mytilinB (C) lysozyme (D) defensin in mussel larvae challenged with *V.coralliilyticus*. Different letters denote significantly difference treatment in pool for each gene.



**Fig. 4.** Phenoloxidase activity (PO) in the mussel larvae (mean  $\Delta$ OD $\pm$ SE, n=3). Data of PO quantify the optical density increase ( $\lambda$ abs=490 nm) compared to the control treatment L (CT). Different letters indicate significant difference (p < 0.05).

1. PHB had a significantly different increase survival in blue mussel larvae challenged with *V.coralliilyticus*
2. The level of expression is stable in first life cycle stage but rapidly increases from 24h to 48h post-infection
3. PO activity was not only triggered by the presence of the pathogen, but also by the presence of amorphous PHB.

## ACKNOWLEDGEMENTS

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