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An updated molecular basis for mussel immunity

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ABSTRACT

Non-self recognition with the consequent tolerance or immune reaction is a crucial process to succeed as living organisms. At the same time the interactions between host species and their microbiome, including potential pathogens and parasites, significantly contribute to animal life diversity. Marine filter-feeding bivalves, mussels in particular, can survive also in heavily anthropized coastal waters despite being constantly surrounded by microorganisms. Based on the first outline of the *Mytilus galloprovincialis* immunome dated 2011, the continuously growing transcript data and the recent release of a draft mussel genome, we explored the available sequence data and scientific literature to reinforce our knowledge on the main gene-encoded elements of the mussel immune responses, from the pathogen recognition to its clearance. We carefully investigated molecules specialized in the sensing and targeting of potential aggressors, expected to show greater molecular diversification, and outlined, whenever relevant, the interconnected cascades of the intracellular signal transduction.

Aiming to explore the diversity of extracellular, membrane-bound and intracellular pattern recognition receptors in mussel, we updated a highly complex immune system, comprising molecules which are described here in detail for the first time (e.g. NOD-like receptors) or which had only been partially characterized in bivalves (e.g. RIG-like receptors). Overall, our comparative sequence analysis supported the identification of over 70 novel full-length immunity-related transcripts in *M. galloprovincialis*. Nevertheless, the multiplicity of gene functions relevant to immunity, the involvement of part of them in other vital processes, and also the lack of a refined mussel genome make this work still not-exhaustive and support the development of more specific studies.

1. Introduction

The study of animal species often reveals taxon-specific patterns of evolutionary diversification, according to the organism life style and related environmental niches. In particular, the evolution of innate defense systems exposes the never-ending race between the animal host and more quickly evolving microorganisms, with the development of specialized host-pathogen (or host-parasite) interactions, independent events of gene loss or gene expansion and fast diversification of molecules essential for pathogen sensing and targeting [1]. As a matter of fact, the study of species unable to mount long-term adaptive responses has highlighted fascinating aspects of animal diversity and physiology in a changing environment [2,3].

Molecules and pathways of the innate immune response have been more extensively studied in invertebrates such as the fruit fly [4], the sea urchin [2] and cnidarians [5,6]. In comparison, the repertoire of gene-encoded elements composing the lophotrochozoan immunity has still to be revealed, particularly in molluscs, which represent the second most species-rich metazoan group with about 100,000 estimated extant species [7,8]. The first molluscan genome to be sequenced, pertaining to the gastropod *Lottia gigantea*, was released only in 2007, 7 and 9 years later than the genomes of *Drosophila melanogaster* and *Caenorhabditis elegans*, respectively. As regards bivalve molluscs, a class comprising species of great ecological and commercial importance, only in recent years the increasing accessibility of next generation sequencing (NGS) technologies permitted significant advances [9]. So far, just two bivalve draft genomes (*Crassostrea gigas* and *Pinctada fucata*) have been released but RNA-seq datasets for more than 40 different species have been already produced (NCBI SRA, accessed in November 2014).

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Abbreviations

AMP	antimicrobial peptide
BD	big defensin
BIR	baculovirus inhibitor of apoptosis protein repeat
BPI	bactericidal/permeability increasing protein
C1qDC	C1q domain-containing
CARD	caspase recruitment domain
CpG-DNA	CpG oligodeoxynucleotides
CLECT	C-type lectin domain
CRD	carbohydrate recognition domain
CS-αβ	cystine-stabilized alpha-beta motif
CTL	C-type lectin
Gram+	Gram positive [staining]
Gram−	Gram negative [staining]
GNBP	Gram-negative binding protein
iE-DAP	γ-D-Glu-meso-diaminopimelic acid
IFN	interferon
IPS-1	IFN-beta promoter stimulator
IRF	interferon regulatory factors
JNK	c-JUN N-terminal kinase
LGBP	lipopolysaccharide and β-1, 3-glucan binding proteins
LRR	leucine-rich repeats
MAP3K	mitogen-activated protein kinase kinase kinase
MAMP	microbe associated molecular pattern
MAPK	mitogen-activated protein kinase
MAPKK	mitogen-activated protein kinase kinase
MKK	mitogen-activated protein kinase kinase
MDP	muramyl dipeptide
NGS	next generation sequencing
NLR	NOD-like receptor
PAMP	pathogen associated molecular pattern
PGN	peptidoglycan
PGRP	peptidoglycan recognition protein
PO	prophenoloxidase
PRR	pattern recognition receptors
RLR	RIG-like receptor
SRCR	scavenger receptor cysteine-rich
STING	stimulator of interferon genes
TIMP	tissue inhibitor of metalloproteinases
TIR	Toll-interleukin-1-receptor
TNF	tumor necrosis factor
TLR	Toll-like receptor

The sequence data currently available for the common mussel (*Mytilus* spp.) are summarized in Table 1. The first glimpse on the complex mussel immune system was provided by Sanger EST sequencing [10], an approach which was followed by 454 Life Sciences sequencing [11–15] and by high throughput Illumina sequencing, a technology allowing a better full-length reconstruction of transcripts [16]. In 2014, a non-annotated set of genomic sequences of *Mytilus galloprovincialis* was released, a real landmark for the progression of genomic studies in this bivalve [17].

Mussels are rather tolerant to environmental changes and they are therefore used as pollution sentinels in coastal waters but, more intriguingly, they appear less affected or not harmed by syndromes and infectious agents distressing other bivalves [18,19]. How mussels govern microorganisms associations with their seasonally varying amounts of microbe-associated molecular patterns (MAMPs) and virulence factors remains to be established. For these reasons, we have undertaken a revision of sequence and literature data to update our knowledge on the gene-encoded molecules shaping the strength and peculiarities of the innate responses of mussels in the context of their fluctuating holobiome. Starting from the first "immunome" description [20] and expanding the analysis to NGS datasets related to the blue mussel [13,21] and other bivalve species [22–24] we propose a step forward in the understanding of pathogen recognition and clearance in *M. galloprovincialis*. Since the *de novo* assembly of RNA-seq data can provide only a partial view of the genes involved in mussel immune responses, we have often used the Pacific oyster *C. gigas* genome for comparison. Considering possible drawbacks inherent to the *de novo* assembly (transcript fragmentation, misassembly, etc.) we have deposited in GenBank only selected sequences of novel full length transcripts, highly supported either in terms of read coverage or confirmed by genomic sequences.

The functional validation of the novel mussel transcripts goes beyond the purpose of this work. As well, the comprehensive characterization of single genes or gene families (especially the analysis of regulatory gene elements and splicing patterns) is not affordable in a single paper nor it is feasible in the absence of a finished genome. While updating the available knowledge on the various molecules participating in the mussel immunity, we have paid more attention to receptors and effectors which likely undergo

Table 1
Overview of the sequence resources available for *Mytilus* spp. in Nov 2014. Species, samples and sequencing details, including the total sequencing effort, are reported for each study.

Species	Sample	Sequencing technology	Sequencing strategy	Sequencing effort (Gbp)	Year of release	Reference[b]
M. galloprovincialis	mixed tissues	Sanger	EST-seq	<0.1	2009	[10]
M. galloprovincialis	mixed tissues	454	RNA-seq	<0.1	2010	[14]
M. edulis	mixed tissues	454	RNA-seq	1.1	2012	[13]
M. galloprovincialis	digestive gland	454	RNA-seq	1.5	2013	[11]
M. edulis	mantle	454	RNA-seq	0.3	2014	[12]
M. galloprovincialis	foot	454	Targeted genome sequencing	0.6	2014	[15]
M. galloprovincialis	digestive gland	Illumina	RNA-seq	8.1	2014	[16]
M. galloprovincialis	whole body	Illumina	RNA-seq	12.4	2014	PRJNA249058
M. edulis	whole body	Illumina	RNA-seq	10.9	2014	PRJNA249058
M. trossulus	whole body	Illumina	RNA-seq	5.8	2014	PRJNA249058
M. californianus	whole body	Illumina	RNA-seq	3.9	2014	PRJNA249058
M. edulis	larvae	Illumina	RNA-seq	32.8	2014	[21]
M. galloprovincialis	mantle	Illumina	Whole genome sequencing	1.6[a]	2014	[17]

[a] This number is referred to the assembled genome size.
[b] For unpublished data, the Bioproject accession ID is reported.

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