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Expression profiles of genes involved in mannose metabolism are modulated during experimental infection of *Drosophila melanogaster* males with *Pseudomonas aeruginosa*

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MIHAELA RALUCA MIHALACHE (RADU)[1]*, ATTILA CRISTIAN RATIU[2]*, ALINA NEAGU[1], VERONICA LAZAR[1], MARIANA CARMEN CHIFIRIUC[3], ALEXANDRU AL. ECOVOIU[2#]

[1] University of Bucharest, Faculty of Biology, Department of Botany and Microbiology, Intrarea Portocalelor Street, No. 1-3, 060101, Bucharest, Romania

[2] University of Bucharest, Faculty of Biology, Department of Genetics, Intrarea Portocalelor Street, No. 1-3, 060101, Bucharest, Romania

[3]Research Institute of the University of Bucharest – ICUB, Life, Environmental and Earth Sciences, Spl. Independentei 91-95, Bucharest, Romania

*[#]**Address correspondence to:** Dr. Alexandru Al. Ecovoiu, University of Bucharest, Faculty of Biology, Department of Genetics, Intrarea Portocalelor Street, No. 1-3, 060101 Bucharest, Romania.*

Email: alexandru.al.ecovoiu@gmail.com

**These authors equally contributed to this work.*

Abstract

Herein we report the transcriptional profiles of Lysosomal α-mannosidase V (*LManV*) *and LManVI genes in wild-type and gammaCop* (*γCop*) *mutant Drosophila melanogaster males upon experimental infection with Pseudomonas aeruginosa, by either feeding or pricking. LManV and LManVI genes are involved in mannose metabolism and were selected consecutive to a preliminary microarray experiment performed on D. melanogaster males infected by feeding. The transcriptional signatures analyzed by qRT-PCR revealed that the expression change of LManVI was statistically significant in both categories of males, regardless of the infection approach. On the other hand, the variations of LManV transcription levels are significant in wild-type males infected by either feeding or pricking, and in mutant males infected by pricking. Our results indicate that LManV and LManVI are modulated during the response of D. melanogaster to P. aeruginosa infection and also suggest a role of γCop in the development of the anti-infectious process.*

Keywords: *D. melanogaster*, mannose metabolism, *P. aeruginosa*, pricking, ingestion, infection model

Introduction

Drosophila melanogaster is a well-established experimental model used for deciphering the complex host-pathogen interactions, including the genetic mechanisms involved in triggering and modulating the immune response against human pathogens, such as *Pseudomonas aeruginosa* (HOFFMANN & al. [1]; BRANDT & al. [2]; VODOVAR & al. [3]; NEHME & al. [4]; BIER & al. [5]; HALLER & al. [6]). The literature data reveal a significant homology between the mammalian's and *D. melanogaster*'s innate immune responses (IGBOIN & al. [7]).

Similar to other invertebrates, *D. melanogaster* apparently lacks an adaptive immunity. The anti-infective defense is relying on innate immune reactions, involving relatively complex cellular (e.g., phagocytosis of microorganisms by hemolymph cells) and humoral (e.g., blood coagulation

and production of antimicrobial peptides - AMPs) mechanisms (WANG & al. [8]). In *D. melanogaster*, the AMPs gene expression is regulated through two signaling mechanisms, i.e. the Toll pathway, which is activated in infections with Gram-positive bacteria, and the Imd (Immune deficiency) pathway, activated by Gram-negative pathogens (HOFFMANN [9]). Two of the most studied AMPs in *D. melanogaster* are encoded by *Drosocin* (*Dro*) and *Diptericin B* (*DptB*) genes (LEMAITRE & al. [10]). These effector molecules are produced in the fat body, which is the equivalent of the mammalian liver (LEMAITRE & al. [10]; VODOVAR & al. [11]).

P. aeruginosa is a Gram-negative opportunistic and nosocomial pathogen, responsible of severe infections in immunocompromised patients (MULCAHY & al. [12]). The chronic infection with *P. aeruginosa* is the leading cause of morbidity and mortality in patients with cystic fibrosis (ERICKSON & al. [13]; SIBLEY & al. [14]).

In this study, we exploited the genetically tractable *D. melanogaster* organism to model a bacterial infection with the human opportunistic pathogen *P. aeruginosa*, aiming to elucidate new aspects of the host-pathogen interaction. Consecutive to a preliminary microarray experiment performed on males infected by feeding (GEO/NCBI accession: GSE80084 [15]), we observed a significant downregulation of the expression level of *LManV* and *LManVI* genes following *P. aeruginosa* infection in *Oregon* males. This result was noteworthy since we didn't find any preceding records regarding an association between these two genes and the immune response in *D. melanogaster*. On the other side, mutations of *γCop* gene could alter an appropriate immunological response since it is a key component of the intracellular protein transport molecular process (GRIEDER & al. [16]).

Therefore, our specific objective was to investigate the expression variation of *LManV* and *LManVI* genes in *D. melanogaster* males with different genetic backgrounds regarding to *γCop* gene, in response to *P. aeruginosa* inoculated through alternative routes.

Materials and Methods

Fly stocks. Wild-type *Oregon* and homozygous $\gamma Cop^{14a}/\gamma Cop^{14a}$ young adult males (ECOVOIU & al. [17]) (no older than two weeks) were used for the experiments. The fly stocks were maintained on the *Nutri-Fly Bloomington Formulation* medium (Genesee Scientific, USA).

Bacterial strain and culture conditions. The *P. aeruginosa ATCC 27853* strain was first cultivated on *Plate Count Agar* at 37°C, then transferred on *Cetrimide* agar at 37°C. After 24 hours of cultivation, bacterial suspensions of 2.4 x 10^9 CFU/mL density (further used for the pricking assay) and of 9 x 10^9 CFU/mL (further used for the feeding experiments) were obtained from fresh cultures.

Infection by pricking. A total of 216 *Oregon* males and 216 $\gamma Cop^{14a}/\gamma Cop^{14a}$ males were anesthetized using carbon dioxide and consequently distributed in eight biological replicates for each distinct *D. melanogaster* genotype (27 individuals/replicate).

Four replicates of $\gamma Cop^{14a}/\gamma Cop^{14a}$ and, respectively, *Oregon* males were infected by pricking their torso with a tungsten needle dipped in a *P. aeruginosa* suspension of 2.4 x 10^9 CFU/mL density, while the corresponding four control groups were pricked using sterile physiological water. After approximately 22 hours subsequently to the bacterial inoculation, 10 surviving individuals from each of three randomly selected biological replicates of infected and, respectively, control groups of mutant and wild-type males were considered for RNA extraction from the whole body after being washed with absolute ethanol. For the total RNA extraction protocol, the *RNeasy Mini Kit* (Qiagen, 74104) was employed, and conversion of 600 ng of RNA into cDNA was performed using the *GoScript Reverse Transcription System* (Promega, A5000).

Infection by feeding assay. For oral infections, over 300 individuals were selected from each of the *Oregon* and $\gamma Cop^{14a}/\gamma Cop^{14a}$ male categories, then placed in vials with *Nutri-Fly* medium, every single vial standing for a biological replicate (27 males/vial). During the experimental feeding, half of the total biological replicates were transferred in vials with 5 mL of solidified agar containing 5% sucrose and covered with a sterile filter paper spotted with 230 µL of 5% sucrose solution containing a total of 9 x 10^9 CFU of *P. aeruginosa*. The control groups replicates were placed on sterile filter paper previously spotted with 230 µL of 5% sucrose. Before placing the flies in the experimental vials (infected and controls), every handled filter paper was allowed to dry for about one hour, whereas the males were starved for about six hours. The loaded vials were placed at 25°C for 60 to 64 hours, allowing the males to ingest the inoculated or control sucrose solution. Following the ingestion interval, the surviving flies were transferred in fresh vials containing only solidified agar incorporating 5% sucrose. For the RNA extraction we used six biological replicates of infected and, respectively, control males pertaining to the two *D. melanogaster* lines. From within each vial, 12 flies were selected, then anesthetized using carbon dioxide and washed in absolute ethanol. Randomly, the males from three of the biological replicates per experimental variant were subjected to dissection and intestine removal, while the males pertaining to the remaining three biological replicates were wholly processed.

Total RNA was extracted from either the intestine or the whole body using the *ReliaPrep RNA Tissue Miniprep System* (Promega, Z6111). For cDNA synthesis starting from 660 ng of RNA/biological replicate extracted from the gut we used the *GoScript Reverse Transcription System* (Promega, A5000), and for the cDNA conversion of 2000 ng of RNA/biological replicate extracted from whole bodies we used the *Reverse Transcription System* (Promega, A3500) kit.

qRT-PCR analysis. The relative expression of *LManV* and *LManVI* genes was investigated by qRT-PCR, using *RPL32* gene as an endogenous referential. Three biological replicates with three technical replicates for each of the target genes were used in the molecular investigation.

The qRT-PCR amplifications were performed using Maxima SYBR Green/ROX qPCR Master Mix (Fermentas/Thermo Fisher Scientific, K0221). For each single qRT-PCR reaction, we used 20 ng of cDNA as template, 1x master mix, 0.16 µM final concentration for each forward and reverse primer and nuclease-free water added up to a final reaction volume of 25 µl. The PCR amplification program was as it follows: 1 x (95°C - 10 minutes), 40 x (95°C - 30 sec; 57°C - 30 sec; 72°C - 32 sec). Data collection was performed during the extension step, and the actual Ct (cycle threshold) values were chosen at a threshold value of 1 and a 3-15 baseline interval. The nucleotide sequences of the primers are presented in Table 1. Excluding the sequences of *RPL32* primers, which were taken from literature (FIUMERA & al. [18]), the other ones were carefully designed using NCBI/Primer-BLAST (UNTERGASSER & al. [19]; YE & al. [20]) and manual annotation. The primers were designed so that the forward primer from each pair binds two neighboring exons.

The expression changes of the target genes were evaluated in *Oregon* and $\gamma Cop^{14a}/\gamma Cop^{14a}$ infected males relative to the expression levels of the matching genes from the corresponding control groups using the Livak ($2^{-\Delta\Delta Ct}$) method (LIVAK & al. [21]). The statistical analysis of qRT-PCR data was performed on $2^{-\Delta Ct}$ values, using two-tailed Student's t-test with Welch's correction run by GraphPad Prism 5.03 software (GraphPad Software, La Jolla California USA, www.graphpad.com). In the graphical representations, data are expressed as mean ± 95% CI.

Table 1. Oligonucleotide primers used in qRT-PCR.

Gene	**Forward** primer sequence	**Reverse** primer sequence
LManV	5' CTTGATCAGCGATGTCTGCA 3'	5' TGGAGTTCCGTGATAGCGA 3'
LManVI	5' CGTCCAGGAATTTCAATCCCA 3'	5' GCTTCATTCAGGTGGAGACC 3'
RPL32	5'CCGCTTCAAGGGACAGTATC 3'	5' GACAATCTCCTTGCGCTTCT 3'

Results

The main objective of this study was to determine the relative changes of the expression of selected *D. melanogaster* genes potentially involved in the host response to *P. aeruginosa* infection, but not mentioned as such in the scientific literature. *LManV* and *LManVI* genes were selected consecutive to a preliminary microarray experiment performed on males infected by feeding (GEO/NCBI accession: GSE80084 [15]), revealing a significant downregulation of the expression level of these genes following *P. aeruginosa* infection in *Oregon* males.

Two different *D. melanogaster* strains were considered for this experiment, namely *Oregon*, standing for the wild-type control, and γCop^{14a} mutant line, respectively. The individuals from the latter strain harbor the hypomorphic γCop^{14a} allele, which causes conditional sterility of the homozygous females and males (ECOVOIU & al. [22]). In distinct experimental setups, young adult males from both lines were subjected to *P. aeruginosa* infection by two different routes, i.e. pricking and ingestion. For the pricking strategy, the relative expression of *LManV* and *LManVI* genes was investigated by qRT-PCR starting from the whole-body of infected males. For infections carried out *via* ingestion, the relative expression of the target genes was independently assessed in whole-body and intestine samples. Further in the text, pricking stands for males infected by pricking; ingestion-w.b. stands for whole-body samples of males infected by ingestion, and ingestion-int. stands for intestine samples taken from males infected by ingestion.

As presented in Figure 1, the expression profiles of *LManV a*nd *LManVI* vary depending on the type of infection and the genetic background.

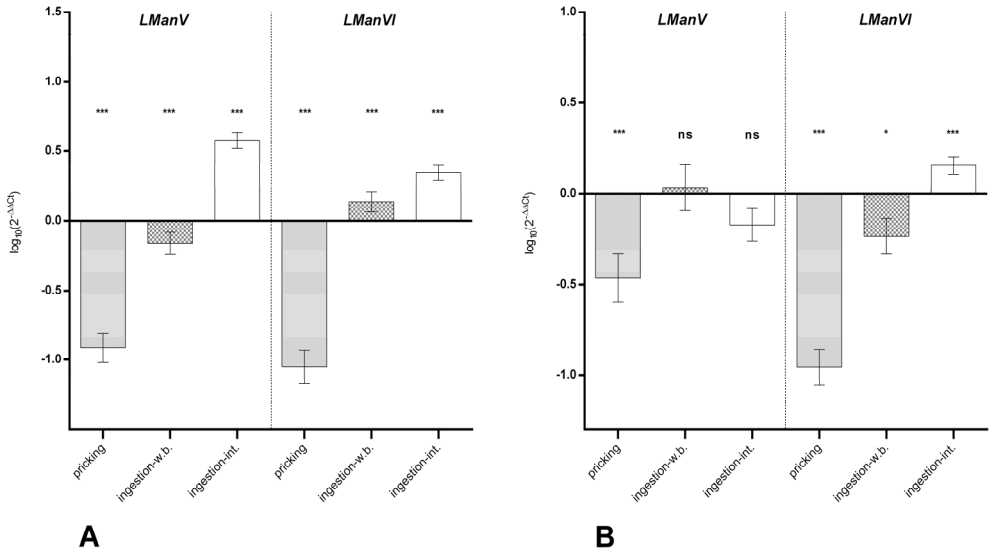

Figure 1. The relative expression of *LManV* and *LManV* genes in infected *Oregon* (A) and $\gamma Cop^{14a}/\gamma Cop^{14a}$ (B) males. The statistical significance of the changes in the expression levels of the target genes in infected males relative to their expression in the uninfected control groups is indicated by symbols (*** means a calculated p value smaller than 0.0001; ** means $p < 0.01$; * means $p < 0.05$; ns indicates not significant changes).

The males infected by pricking exhibited similar patterns of gene expression, represented by statistically significant downregulation of both genes (Fig. 2A). In *Oregon* males, *LManV* and *LManVI* are 8.12 and, respectively, 11.22-fold downregulated, while in $\gamma Cop^{14a}/\gamma Cop^{14a}$ males, the downregulation is 2.95-fold for *LManV* and 9.12-fold for *LManVI*.

The analysis of ingestion-w.b. and ingestion-int. biological samples displayed gene expression profiles that vary between *Oregon* and $\gamma Cop^{14a}/\gamma Cop^{14a}$ males (Fig. 2B and 2C). For the whole-body of *Oregon* males infected by ingestion, we found relatively minor *LManV* downregulation (1.44-fold) and *LManVI* upregulation (1.34-fold). The expression pattern of the two analyzed genes was reversed in ingestion-w.b. samples harvested from $\gamma Cop^{14a}/\gamma Cop^{14a}$ males, characterized by a non-significant upregulation of *LManV*, and a 1.7-fold downregulation of *LManVI*.

Regarding the gene expression changes recorded in ingestion-int. samples, the infected *Oregon* males shown significant upregulation of both *LManV* and *LManVI* (3.71 and 2.18-fold, respectively). In the intestines of infected $\gamma Cop^{14a}/\gamma Cop^{14a}$ males, *LManVI* is 1.41-fold upregulated, whereas apparent *LManV* downregulation has no statistical significance.

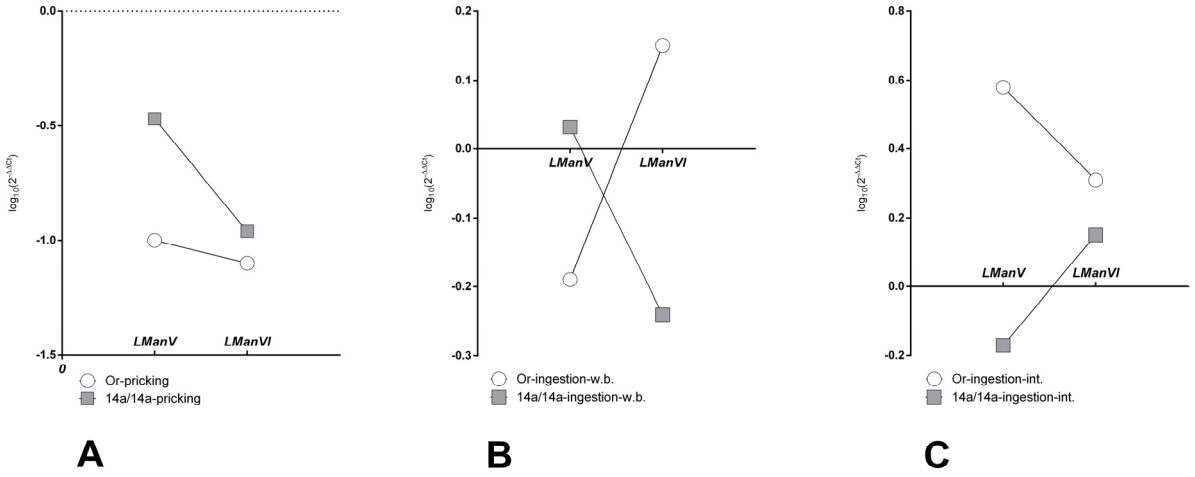

Figure 2. The comparison of the relative expressions of *LManV* and *LManVI* genes in *Oregon* (Or; white circles) and $\gamma Cop^{14a}/\gamma Cop^{14a}$ (14a/14a; gray squares) samples. The gene expression changes consecutive to pricking (A) apparently follow a similar pattern in *Oregon* and $\gamma Cop^{14a}/\gamma Cop^{14a}$ males, while distinct gene expression profiles characterize the two male categories when ingestion-w.b. (B) and ingestion-int. (C) experimental variants are considered.

LManIII, *LManIV*, *LManV* and *LManVI* are neighboring genes, being tightly located within a genomic region of approximately 14.6 Kbp on the chromosomal arm 2L of *D. melanogaster*.

The aminoacid sequences of the corresponding proteins were retrieved from NCBI database (*D. melanogaster* genome annotation version r6.07) and subjected to *in silico* analyses performed with CLC Main Workbench software v.6.9 (CLC Bio-Qiagen, Aarhus, Denmark). During an inquiry aimed to discover the Pfam domains contained by LManVI, LManV, LManIV and LManIII proteins, we found that they share a Ferritin-like domain roughly having approximatively the same amino acids coordinates, i.e. 262-455, 261-454, 262-455, and, respectively, 261-450 (Fig. 3). This search was performed by selecting specific options available within the Pfam Domain Search module of CLC Main Workbench, therefore enabling us to look for full domains and fragments of presumptive functional domains having a maximum E-value of 1. The E-values of the putative Ferritin-like domains found in LManVI-PA, LManV-PA, LManIV-PA and LManIII-PA proteins are 0.53, 0.08, 0.2, and 0.65, respectively.

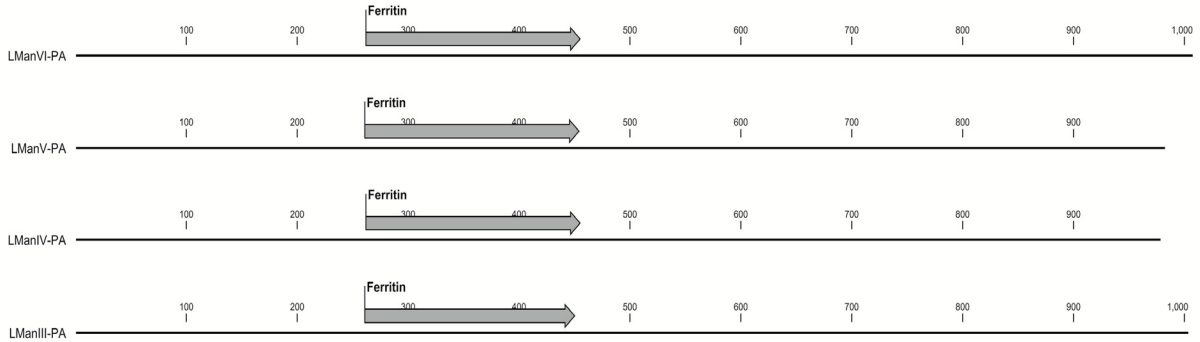

Figure 3. The representations of LManVI-PA, LManV-PA, LManIV-PA and LManIII-PA proteins harboring putative Ferritin-like functional domains. The amino acids coordinates defining these domains are as it follows: 262-455 (within LManVI), 261-454 (within LManV), 262-455 (within LManIV), and 261-450 (within LManIII).

Discussion

Currently, *D. melanogaster* model is widely used to study different features of *P. aeruginosa* pathogenesis, such as proliferation or persistence, translocation through the epithelial barrier, subversion of the phagocytic machinery, *in vivo* biofilm formation, virulence level and indirect pathogenicity provided by commensal/polymicrobial community, and assessment of novel therapeutic strategies (MULCAHY & al. [12]; D'ARGENIO & al. [23]; CHRISTOFI & al. [24]; FAUVARQUE [25]).

The purpose of the present study was to use qRT-PCR technique to investigate the expression modifications of *LManV* and *LManVI* in response to *P. aeruginosa* infection. The infected *D. melanogaster* males harbor either wild-type *γCop* alleles (*Oregon*) or γCop^{14a} mutant alleles.

Previous microarray experiments conducted by our laboratory on *D. melanogaster* wild-type *Oregon* and mutant $\gamma Cop^{14a}/\gamma Cop^{14a}$ males inoculated by ingestion of *P. aeruginosa* ATCC27853 revealed 69 genes which underwent significant ($p < 0.05$) modifications of expression in *Oregon* infected males (ECOVOIU & al. [26]). Remarkably, both *LManV* and *LManVI* gene expressions are significantly downregulated during the infection (Table 2) by 1.61 and, respectively, 2.1-folds. In *D. melanogaster*, the two genes are involved in the mannose metabolism and in the glycolytic activity, playing an important role in the host response to hypoxia conditions. Mannose is involved in the innate immunity by facilitating the recognition of bacteria by the host organism (TAKAHASHI & al. [27]). In total, *D. melanogaster* genome contains six lysosomal mannosidases (i.e. *LManI*, *LManII*, *LManIII*, *LManIV*, *LManV*, and *LManVI*), all displaying around 39-44% amino acids identity with the major human *lysosomal α-mannosidase* (*MAN2B1*) gene (ROSENBAUM & al. [28]).

Mutations affecting *MAN2B1* can lead to an autosomal recessive genetic metabolic disorder named alpha-mannosidosis, characterized by immune deficiency, hearing loss, facial and skeletal abnormalities, and intellectual disability (MALM & al. [29]). Considering the role played by *MAN2B1* in the immunity, we considered important to understand how *LManV* and *LManVI* orthologous genes are modulated in *D. melanogaster* during the infectious process.

In eukaryotes, *MAN2B1* and *γCop* directly interact during stress induced endoplasmic reticulum (ER) protein degradation (PAN & al. [30]). The ER stress sensors are responding to a large variety of stressors, including bacterial ones (PILLICH & al. [31]). Interestingly, pore-forming toxins released by *P. aeruginosa* trigger the ER stress response in humans, this process being important for controlling injurious pathogen-associated actions (LOOSE & al. [32]).

Table 2. The expression variation of *LManV* and *LManVI* genes across multiple experimental setups. The pricking and ingestion experiments employed qRT-PCR technique for gene expression evaluation. The abbreviation w.b. stands for whole body, int. stands for intestine, down stands for downregulated, up stands for upregulated, while ns means not significant.

	microarray (w.b.)		**pricking (w.b)**		**ingestion-w.b.**		**ingestion-int.**	
	Or.	**14a/14a**	**Or.**	**14a/14a**	**Or.**	**14a/14a**	**Or.**	**14a/14a**
LManV	down $p = 0.015$	ns $p = 0.29$	down $p < 0.0001$	down $p < 0.0001$	down $p < 0.0001$	ns $p = 0.421$	up $p < 0.0001$	ns $p = 0.7842$
LManVI	down $p = 0.007$	ns $p = 0.15$	down $p < 0.0001$	down $p < 0.0001$	up $p = 0.0002$	down $p = 0.014$	up $p < 0.0001$	up $p < 0.0001$

To our knowledge, here we demonstrate for the first time that *LManV* and *LManVI* genes are downregulated in both *Oregon* and in *γCop* mutant males inoculated by pricking with a *P. aeruginosa* strain with mild virulence. We consider that the pricking infection route is more suggestive for a severe infection, in which the natural barriers to infection are surpassed.

Concerning previous research using *D. melanogaster* in modelling experimental infections, Vodovar *et al*. used microarray technique and revealed that *LManV* and *LManVI* are downregulated in *Oregon* individuals infected by feeding with *P. entomophila* (VODOVAR & al. [11]). Although we used other *Pseudomonas* species, i.e. *P. aeruginosa*, our microarray data revealed similar downregulation of the same genes in the case of *Oregon* males (Table 2). A study involving whole genome mRNA sequencing and qRT-PCR demonstrated that in *Oregon* adult flies fed with symbiotic *Heterorhabditis bacteriophora* (nematodes carrying *Photorhabdus luminescens*) *LManVI* was as well downregulated (CASTILLO & al. [33]).

Ferritin is an anti-oxidant protein and the major iron storage site at molecular level (TANG and ZHOU [34]). During infection, the level of host plasma ferritin increases drastically, as a response to iron sequestration by the pathogenic agent that uses it for its own survival and pathogenicity (LAUFFER [35]). Interestingly, a *P. aeruginosa* infection model in a horseshoe crab (*Carcinoscorpius rotundicauda*) revealed that after challenged by the bacterial infection, the plasma ferritin disappears in the interval starting from 6 to 48 hours, and re-emerges thereafter (ONG & al. [36]). This characteristic of *P. aeruginosa* pathogenicity mechanisms could cause the down-regulation of *LManV* and *LManVI* during our pricking induced infections, when the gene expression levels were assessed after approximately 22 hours after infection. In *D. melanogaster*, ferritin assembly is a strictly regulated process and one of the main location for unfolding the iron-ferritin interactions is the intestine (ROSAS-ARELLANO & al. [37]). During the ingestion experiments, the time interval separating the first contact with *P. aeruginosa* inoculum and processing of *D. melanogaster* infected males in order to perform RNA extraction was over 60 hours. Therefore, especially at the gut level, the bacterial infection could have been able to induce the documented up-regulation of ferritin coding genes, including *LManV* and *LManVI*.

When comparing *LManV* and *LManVI* gene expression profiles, we found that only *LManVI* is upregulated in the intestines of both *Oregon* and mutant males infected by bacteria ingestion. At the same level, *LManV* is significantly overexpressed in *Oregon* males, while in $\gamma Cop^{14a}/\gamma Cop^{14a}$ males its variation is not significant. Surprisingly, although the two genes share functional homologies, the overall results of the feeding experiments indicate a more

significant expression variation for *LManVI* as compared to *LManV* in the $\gamma Cop^{14a}/\gamma Cop^{14a}$ mutant background.

The lack of significant variations in the expression level of *LManV* gene in $\gamma Cop^{14a}/\gamma Cop^{14a}$ males infected by ingestion compared to uninfected ones, could be caused by the genetic background particularities of these mutant males. The *γCop* gene is involved in many important biological processes; these include phagocytosis of foreign particles (STROSCHEIN-STEVENSON & al. [38]) and intracellular protein transport (GRIEDER & al. [16]). Supplementary, *γCop* gene is involved in the development of the tracheal system (GRIEDER & al. [16]). Extrapolating the latter role, *γCop* could be involved in the development of other hollow organs, such as the intestine, and concomitantly might also influence their functions. Because the mutant males bear two copies of γCop^{14a} hypomorphic allele, they could harbor inefficient *γCop* mediated intracellular protein transport processes that usually are hardly influenced by the establishing of an immune response consecutive to mild bacteria ingestion. The variation of *γCop* gene expression was insignificant when bacteria ingestion effects were evaluated within the whole body of both wild-type and mutant males (data not shown). On the other hand, regardless of the genetic backgrounds, *LManV* and *LManVI* gene expressions are significantly stimulated by acute infections within the tissues found in direct interactions with the pathogens, such as the intestines (Table 2).

Conclusions

LManV and *LManVI* may be recruited by the immune system *of D. melanoga*ster upon pricking infection with *P. aeruginosa*, possibly in strong correlation with *γCop* allelic condition. The expression of *LManV* and *LManVI* following inoculation by pricking was analyzed comparatively with the ingestion route. This study can lead us to a better understanding of the innate immune response to *P. aeruginosa* opportunistic pathogen, which is often involved in nosocomial infections. Based on experimental evidences, available scientific literature and *in silico* investigations, we conclude that *LManV* and *LManVI* genes are involved in modulating the anti-infectious defense mechanisms, possibly due to their implication in mannose metabolism and ferritin dynamics, which are important processes for triggering the immune response in *D. melanogaster*.

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References

1. J.A. HOFFMANN, J.M. REICHHART. *Drosophila* innate immunity: an evolutionary perspective. *Nature immunology,* 3(2): 121-126 (2002).
2. S.M. BRANDT, M.S. DIONNE, R.S. KHUSH, L.N. PHAM, T.J. VIGDAL, D.S. SCHNEIDER. Secreted bacterial effectors and host-produced Eiger/TNF drive death in a *Salmonella*-infected fruit fly. *PLoS Biology*, 2(12): 2067-2075 (2004).
3. N. VODOVAR, C. ACOSTA, B. LEMAITRE, F. BOCCARD. *Drosophila*: a polyvalent model to decipher host-pathogen interactions. *Trends in Microbiology*, 12(5): 235-242 (2004).
4. N.T. NEHME, S. LIEGEOIS, B. KELE, P. GIAMMARINARO, E. PRADEL, J.A. HOFFMANN, J.J. EWBANK, D. FERRANDON. A model of bacterial intestinal infections in *Drosophila melanogaster. PLoS Pathogens*, 3(11): 1694-1709 (2007).
5. E. BIER, A. GUICHARD. Deconstructing host-pathogen interactions in *Drosophila*. *Disease models & mechanisms*, 5(1): 48-61 (2011).
6. S. HALLER, S. LIMMER, D. FERRANDON. Assessing *Pseudomonas* virulence with a non-mammalian host: *Drosophila melanogaster*. *Methods in molecular biology*, 1149: 723-740 (2014).

7. C.O. IGBOIN, A.L. GRIFFEN, J.E. LEYS. The *Drosophila melanogaster* host model. J*ournal of Oral Microbiology*, 4: 10368 (2012).
8. Z. WANG, C. WILHELMSSON, P. HYRS, T.G. LOOF, P. DOBES, M. KLUPP, O. LOSEVA, M. MORGELIN, J. IKLE´, R.M. CRIPPS, H. HERWALD, U. THEOPOLD. Pathogen entrapment by transglutaminase - a conserved early innate immune mechanism*. PLoS Pathogens*, 6(2): e1000763 (2010).
9. J.A. HOFFMANN. The immune response of *Drosophila*. *Nature*, 426(6962): 33-38 (2003).
10. B. LEMAITRE, J.A. HOFFMANN. The host defense of *Drosophila melanogaster*. *Annual review of Immunology*, 25:697-743 (2007).
11. N. VODOVAR, M. VINALS, A. BASSET, J. DEGROUARD, P.T. SPELMANN, F. BOCCARD, B. LEMAITRE. *Drosophila* host defense after oral infection by an entomopathogenic *Pseudomonas* species. *PNAS*, 102(32): 11414-11419 (2005).
12. H. MULCAHY, C.D. SIBLEY, M.G. SURETTE, S. LEWENZA. *Drosophila melanogaster* as an animal model for the study of *Pseudomonas aeruginosa* biofilm infections in vivo. *PLoS Pathogens*, 7(10): e1002299 (2011).
13. D.L. ERICKSON, R. ENDERSBY, A. KIRKHAM, K. STUBER, D.D. VOLLMAN, H.R. RABIN, I. MITCHELL, D.G. STOREY. *Pseudomonas aeruginosa* quorum-sensing systems may control virulence factor expression in the lungs of patients with cystic fibrosis. *Infection and Immunity*, 70(4): 1783-1790 (2002).
14. C.D. SIBLEY, K. DUAN, C. FISCHER, M.D. PARKINS, D.G. STOREY, H.R. RABIN, M.G. SURETTE. Discerning the complexity of community interactions using a *Drosophila* model of polymicrobial infections*. PLoS Pathogens*, 4(10): e1000184 (2008).
15. http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE80084.
16. N.C. GRIEDER, E. CAUSSINUS, D.S. PARKER, K. CADIGAN, M. AFFOLTER, S. LUSCHNIG. *γCop* is required for apical protein secretion and epithelial morphogenesis in *Drosophila melanogaster. PLoS One*, 3(9): e3241 (2008).
17. A.A. ECOVOIU, M. MINK, M. GRAUR, L.M. GEORGESCU, L. GAVRILA. Generation of putative lethal and hypomorphic *γCop* gene alleles by mobilization of *P{lacW}γCop[S057302]* insertion during male germline development of *Drosophila melanogaster*. *Romanian Biotechnological Letters*, 10(1): 1985-1994 (2005).
18. A.C. FIUMERA, B.L. DUMONT, A.G. CLARK. Sperm competitive ability in *Drosophila melanogaster* associated with variation in male reproductive proteins. *Genetics*, 169(1): 243-257 (2005).
19. A. UNTERGASSER, I. CUTCUTACHE, T. KORESSAAR, J. YE, B.C. FAIRCLOTH, M. REMM, S.G. ROZEN. Primer3 - new capabilities and interfaces. *Nucleic Acids Research*, 40(15): e115 (2012).
20. J. YE, G. COULOURIS, I. ZARETSKAYA, I. CUTCUTACHE, S. ROZEN, T. MADDEN. Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics,* 13: 134 (2012).
21. K.J. LIVAK, T.D. SCHMITTGEN. Analysis of relative gene expression data using Real-Time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods*, 25(4): 402-408 (2001).
22. A.A. ECOVOIU, L. SAVU, D. OTELEA, L. GAVRILA. Analysis of a polyallelic series reveals *γCop* pleiotropy during development of *Drosophila melanogaster* [abstract]. In: 20th European Drosophila Research Conference; September 12-14; Vienna, Austria. Abstract A026 (2007).
23. D. D'ARGENIO, L. GALLAGHER, C. BERG, C. MANOIL. *Drosophila* as a model host for *Pseudomonas aeruginosa* infection. *Journal of bacteriology*, 183(4): 1466 -1471 (2001).
24. T. CHRISTOFI, Y. APIDIANAKIS. *Drosophila* immune priming against *Pseudomonas aeruginosa i*s short-lasting and depends on cellular and humoral immunity. *F1000Research*, 2: 76 (2013).
25. M.O. FAUVARQUE. Small flies to tackle big questions: assaying complex bacterial virulence mechanisms using *Drosophila melanogaster. Cellular microbiology*, 16(6): 824-833 (2014).
26. A. ECOVOIU, A. RATIU, I. CZOBOR, M.C. CHIFIRIUC. Whole genome expression profiles of *Drosophila melanogaster* consecutive to *Pseudomonas aeruginosa* infection allow selection of new model genes implicated in host-parasite relationship [abstract]. In: 24th ECCMID; May 10-13; Barcelona, Spain: ESCMID. Abstract P0130 (2014).
27. K. TAKAHASHI, A. EZEKOWITZ. The role of the mannose - binding lectin in innate immunity. *Clinical infectious diseases,* 41(Supplement 7): S440-S444 (2005).
28. E. ROSENBAUM, E. VASILJEVIC, K.S. BREHM, N.K. COLLEY. Mutations in four glycosyl hydrolases reveal a highly coordinated pathway for rhodopsin biosynthesis and *N-glycan* trimming in *Drosophila melanogaster. PLoS Genetics*, 10(5): e1004349 (2014).
29. D. MALM, O. NILSSEN. Alpha-mannosidosis. *Orphanet Journal of Rare Diseases,* 3: 21 (2008).

30. S. PAN, X. CHENG, R.N. SIFERS. Golgi-situated endoplasmic reticulum α-1, 2-mannosidase contributes to the retrieval of *ERAD* substrates through a direct interaction with *γ-COP*. *Molecular biology of the cell*, 24(8): 1111-11121 (2013).
31. H. PILLICH, M. LOOSE, K.P ZIMMER, T. CHAKRABORTY. Diverse roles of endoplasmic reticulum stress sensors in bacterial infection. *Molecular and Cellular Pediatrics,* 3: 9 (2016).
32. M. LOOSE, M. HUDEL, K.P. ZIMMER, E. GARCIA, S. HAMMERSCHMIDT, R. LUCAS, T. CHAKRABORTY, H. PILLICH. Pneumococcal hydrogen peroxide-induced stress signaling regulates inflammatory genes. *The journal of infectious diseases*, 211(2): 306-316 (2015).
33. J.C. CASTILLO, T. CREASY, P. KUMARI, A. SHETTY, U. SHOKAL, L. TALLON, I. ELEFTHERIANOS. *Drosophila* anti-nematode and antibacterial immune regulators revealed by RNA-Seq. *BMC Genomics*, 16: 519 (2015).
34. X. TANG, B. ZHOU. Iron homeostasis in insects: Insights from *Drosophila* studies. IUBMB Life, 65: 863-872 (2013).
35. R.B. LAUFFER. Iron and Human Disease. Boca Raton, FL: *CRC Press*, 162-172; 180-198 (1992).
36. D.S.T. ONG, L. WANG, Y. ZHU, B. HO, J.L. DING. The response of ferritin to LPS and acute phase of *Pseudomonas infection*. *Journal of endotoxin research*, 11: 267-280 (2005).
37. A. ROSAS-ARELLANO, J. VÁSQUEZ-PROCOPIO, A. GAMBIS, L.M. BLOWES, H. STELLER, B. MOLLEREAU, F. MISSIRLIS (2016). Ferritin assembly in enterocytes of *Drosophila melanogaster*. *International Journal of Molecular Sciences*, 17(2): 27 (2016).
38. S.L. STROSCHEIN-STEVENSON, E. FOLEY, P.H. O'FARRELL, A.D., JOHNSON. Identification of *Drosophila* gene products required for phagocytosis of *Candida albicans*. *PLoS Biology* 4(1): 0087-0099 (2006).