Mol Genet Genomics (2012) 287:803–817
DOI 10.1007/s00438-012-0713-9

ORIGINAL PAPER

Transcriptome analyses of *Bactericera cockerelli* adults in response to "*Candidatus* Liberibacter solanacearum" infection

Punya Nachappa · Julien Levy · Cecilia Tamborindeguy

Received: 10 April 2012 / Accepted: 20 July 2012 / Published online: 4 September 2012

Abstract The potato/tomato psyllid, *Bactericera cockerelli* (Šulc) is an economically important crop pest that not only causes damage through its feeding but also transmits the bacterium, "*Candidatus* Liberibacter solanacearum" (CLs), which causes zebra chip disease in potato. There is some information about the phenotypic effects of phytopathogenic bacteria on their insect vectors; however, there are no published reports of the molecular mechanisms underlying phytopathogenic bacteria–insect vector interaction. In order to investigate the effects of CLs infection on *B. cockerelli*, transcriptomic analyses of CLs-infected and uninfected adult psyllids that were reared on potato were performed. De novo assembly of cDNA sequences generated 136,518 and 109,983 contigs for infected and uninfected insect libraries with an average contig length of 514 bp. BlastX analysis against the NCBI-nr database revealed that 33.33 % had significant matches. Gene ontology data illustrated that the majority of the expressed psyllid genes are involved in metabolic process, biological regulation, binding and catalytic activity. The psyllid transcriptome had an abundance of genes such as vitellogenin, heat shock protein, ejaculatory bulb-specific protein, ferritin, and cytochrome oxidase. Notably absent in the psyllid transcriptome were innate immunity genes induced in response to Gram-negative bacteria (IMD pathway). Several functionally diverse contigs related to symbiotic bacteria including the primary endosymbiont *Carsonella ruddii*, *Wolbachia*, and CLs in the psyllid transcriptome were identified. A total of 247 contigs showed differential expression in response to CLs infection including immune and stress-related genes and vitellogenins. Expression analyses of selected psyllid genes were performed on psyllids that were exclusively reared on potato (host of the insects used for RNAseq) and psyllids exclusively reared on tomato (alternative host of psyllids). These genes showed similar expression patterns irrespective of the host plant on which the psyllids were reared, which suggests that host–plant association may not modulate expression of these genes. Our findings suggest that the impact of CLs on psyllid transcriptome was to a large extent on genes involved in metabolic processes and to a small extent on immune and stress response genes. This study is the first description of transcriptomic changes in an insect vector in response to infection with a naturally occurring bacterial plant pathogen. Data from this study provide new sequence and gene expression resources for functional genomics of potato psyllids.

Keywords Zebra chip · Potato psyllid · Liberibacter · RNASeq

Communicated by A. Hirsch.

P. Nachappa and J. Levy have contributed equally to this paper.

P. Nachappa · C. Tamborindeguy (✉)
Department of Entomology, 412 Heep Center,
Texas A&M University, College Station,
TX 77843, USA
e-mail: ctamborindeguy@ag.tamu.edu

Present Address:
P. Nachappa
Department of Biology, Science Bldg,
Indiana University-Purdue University,
2101 E. Coliseum Blvd, Fort Wayne, IN 46805, USA
e-mail: nachappa@ipfw.edu

J. Levy
Department of Horticultural Science,
202 HSF Building, Texas A&M University,
College Station, TX 77843, USA
e-mail: julienlevy@tamu.edu

Introduction

A large majority of plant pathogens are transmitted by insects, of which phloem-feeding insects in the order Hemiptera comprise a majority of the vectors (Hogenhout et al. 2008). Further, these insects harbor several species of Gram-negative symbiotic bacteria that supplement their dietary deficiencies (Baumann et al. 2006) and confer protection against abiotic and biotic stresses (Oliver et al. 2010); however, very little is known about how these insects modulate their defenses against pathogens and symbionts. Plant pathogens have been shown to affect their arthropod vectors either directly (pathogen has an effect on the vector upon infection), or indirectly (pathogen has an effect on the vector through modification of the host plant) (Stout et al. 2006; and references therein). The direct interactions between pathogen and vector includes activation of the insect's innate immune system (Wu et al. 2001; De Gregorio et al. 2001; Daborn et al. 2001) and other genes involved in vital biological processes that are affected due to the pathogen's presence and multiplication in the insect. The indirect or plant-mediated interactions between pathogen and vector involve the activation of plant defense-related signaling pathways and modification of primary and secondary metabolisms (Stout et al. 2006). Further, these interactions can be mutualistic, parasitic, and commensal, depending on the relative effects of the pathogen on the fitness of the vector (Purcell 1982a, b). Although there is abundant information on the phenotypic effects of phytopathogens on their insect vectors, the molecular basis of the interactions is less well-studied. Molecular evidence is starting to accumulate for plant virus–vector interaction, but those for bacteria, fungi and other pathogens are still lacking.

Research so far has focussed on immunological responses in invertebrate model organisms such as *Drosophila melanogaster* to non-vectored pathogen infection. In *D. melanogaster*, recognition of a specific pathogen can lead to the activation of four signaling cascades including the Toll pathway, the immunodeficiency (IMD) pathway, the c-Jun N-terminal kinase (JNK) pathway and the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway (Govind 2008). However, very little is known about immunity in hemipterans. For instance, several key members of the IMD pathway, which is activated against Gram-negative bacteria, were not identified in the pea aphid, *Acyrthosiphum pisum* genome (Gerardo et al. 2010).

The potato/tomato psyllid (*Bactericera cockerelli*) is a hemipteran insect that feeds almost exclusively on solanaceous plants (Wallis 1955; Pletsch 1947; Pack 1930). The potato psyllid is an economically important insect pest that damages crop plants through feeding resulting in yellowing of leaves, shortened thickened internodes, stunted plant growth, and reduced fruit size; additional symptoms in potato include aerial tubers and reduced tuber size (Munyaneza et al. 2007; Cranshaw 2001). These symptoms are associated with a plant disorder called "psyllid yellows" and are thought to be caused by a salivary toxin (Carter 1939). In the past, the potato psyllid was found in Mexico, the southwestern and central United States (Pletsch 1947); more recently, the geographical range of the insect has increased to include western, and northwestern US, as well some countries in Central America (Crosslin et al. 2010) and New Zealand (Liefting et al. 2009). The observed expansion in the geographical range of potato psyllids coincided with the discovery of its vectoring ability of a bacterial plant pathogen.

In 2008, it was discovered that in addition to direct feeding, the potato psyllid transmits the bacterium, "*Candidatus* (*Ca.*) Liberibacter solanacearum" (CLs) also known as "*Ca*. Liberibacter psyllaurous" the causal agent of Zebra Chip (ZC) disease in potatoes (Liefting et al. 2008, 2009; Hansen et al. 2008; Crosslin and Bester 2009; Abad et al. 2009; Secor and Rivera-Varas 2004). The bacterium, CLs, not only exacerbates psyllid feeding symptoms but also affects the potato tubers causing internal browning of raw tubers and very dark stripes when the tubers are fried. The coloration of the fried tubers shows as rays or stripes, hence the common name "zebra chip". Ultimately, ZC disease significantly reduces yield, quality and crop longevity. Currently, ZC disease has been detected in the southwestern, northwestern and central US, Mexico (Crosslin et al. 2010), several Central American countries (MAFBNZ 2008), and New Zealand (Liefting et al. 2008). The pathogen has been shown to infect tomato, pepper, and other solanaceous crop species (Liefting et al. 2009).

Recently, it was demonstrated that population growth rate of potato psyllids infected with the CLs (hereafter called infected psyllids) was significantly reduced (approximately 1.5 times) compared to psyllids with undetectable levels of CLs (uninfected psyllids), but there was no significant difference in adult longevity (Nachappa et al. 2012). Further, CLs titer in the insect was negatively correlated with several life-history traits of the psyllids including 7-day fecundity, maximum nymphs, maximum adults and nymph survival (Nachappa et al., in review). It is hypothesized that fecundity reduction due to pathogen infection may be a result of the trade-off in order to conserve energy for pathogen defense rather than egg production (Hurd 2003). However, the molecular mechanism(s) underlying fecundity reduction in infected psyllids is not known. Moreover, there is no published information regarding immune responses in potato psyllids.

Despite being an agriculturally important pest and a major vector of a bacterial plant pathogen, there are few

genomic resources available for potato psyllids. Transcriptomic analysis provides an efficient and feasible method of discovering genes involved in pathogen–vector interaction that may serve as potential targets for insect vector control. Potato psyllids–CLs interaction provides an excellent opportunity to study the biological processes of the insect vector in response to a naturally occurring bacterial plant pathogen, which has been less-studied compared to plant virus–vector interactions. To date, there have been only two published studies investigating transcriptomics of psyllids, including transcriptomics of the Asian citrus psyllid, *Diaphorina citri* (Hunter et al. 2009) and a pilot study of transcriptomics of potato psyllids (Hail et al. 2010). There are no published reports of transcriptomic analysis of CLs–potato psyllid interaction. In the current study, data from transcriptomic high-throughput sequencing analyses of infected and uninfected adult potato psyllids using a 105 paired-end Illumina sequencing are presented. In combination with quantitative real-time RT-PCR validation, expression profiles of genes that may play important roles in CLs–potato psyllid interaction are characterized.

Materials and methods

Potato psyllid colonies

The CLs-infected and uninfected psyllids were obtained from Dr. Joe Munyaneza and from Drs. Charlie Rush and Don Henne, respectively. These colonies have been maintained in the laboratory for over 3 years (approximately 36 generations). In the laboratory, the insect colonies were maintained on either potato or tomato plants that were initially healthy in separate 0.35 × 0.35 × 0.35 m insect cages (BioQuip, Rancho Dominguez, CA, USA) at a temperature of 24 ± 1 °C and photoperiod of 16:8 (L:D) h. Diagnostic PCR analyses are performed routinely to confirm the presence or absence of CLs in psyllid colonies reared on potato and tomato host plants as previously described (Nachappa et al. 2011, 2012). The infection rate in the uninfected colony and infected colony was 0 and 80 %, respectively, as detected using PCR (Nachappa et al. 2011, 2012).

For cDNA library construction, adult psyllids of both sexes were obtained from uninfected and infected colonies that were solely maintained on uninfected potato plants (cv Atlantic) under laboratory conditions mentioned above. For confirmatory RT-qPCR, adult psyllids were also extracted from uninfected and infected psyllid colonies that were reared separately on tomato plants (cv Moneymaker) under similar laboratory conditions.

cDNA library construction and multiplexed RNA-seq

About 20 psyllids from infected and uninfected colonies were used for RNA extraction using RNeasy Plant kit (Qiagen, Valencia, CA, USA) following manufacturer's instructions. Prior to cDNA library construction, diagnostic PCR analysis was performed to confirm presence and absence of CLs, respectively (Nachappa et al. 2011). Total RNA was then treated with DNase (Turbo DNase, Ambion, Austin, TX, USA) to remove genomic DNA contamination, and subjected to quality control. RNA quality and integrity was confirmed using NanoDrop spectrophotometer (Eppendorf, Hauppauge, NY, USA) and Bioanalyzer (Agilent Technologies, Inc., Palo Alto, CA, USA). About 1.2 μg of resulting RNA was subjected to multiplex library construction performed using TruSeq RNA Sample Preparation kit following manufacturer's instructions (Illumina). Libraries were prepared and sequenced at the Texas AgriLife Genomics and Bioinformatics Core Facility in Texas A&M University. For the two, infected and uninfected psyllid RNAseq libraries, 105 paired end sequencing was performed using the Illumina Genome Analyzer II (GAII) Platform.

Bioinformatic data analysis

Sequence data were first processed using Illumina pipeline programs: SCS 2.8/RTA 1.8 for real time basecalling, Bcl-Converter-1.7.1 to produce qseq.txt files, CASAVA-1.7.0 to sort libraries, remove barcode tags and remove adaptor sequences. Total assembly was performed using CLC Genomics Workbench 4.8 platform to create a unigene set.

The unigene set was compared to the NCBI non-redundant (nr) protein database using the BLASTx program with an expected E value cut-off of $<10^{-5}$, to determine the homology of sequences with known genes. Similarities with E value $>10^{-5}$ were considered non-significant and not used for further analysis. Gene ontology and annotation were performed on all assembled contigs greater than 200 bp length by BLAST2GO software http://www.blast2go.org.

To identify differentially expressed transcripts between the CLs-infected and the CLs-uninfected libraries, each library was separately assembled using Trinity RNA-Seq Assembly (trinityrnaseq_r2011-08-20). Minimum assembled contig length was set to 200 nucleotides. To compare expression levels, the expression score of each gene transcript represented in RPKM (reads per kilobase of transcript per million mapped reads), which takes into account the relative size of the transcripts and only uses the mapped-read datasets (i.e. excludes the non-mapped reads), was determined (Mortazavi et al. 2008). RPKM was computed by TopHat and Cufflinks (Trapnell et al. 2010) pipeline. The TopHat program aligned quality-filtered

Illumina reads to the general assembly. The RPKM value of each gene transcript was computed by Cufflinks, based on the alignment resulting from TopHat.

Cloning and sequencing

Primer pairs for a subset of six differentially expressed genes obtained from RNASeq were designed using Primer 3 program (Table 6) and RT-PCR was performed using cDNA obtained from uninfected and infected psyllids (described in detail in the next section). Cloning and sequencing of the seven genes (six candidate genes plus 28S rDNA) were performed using the pGEM®-T Easy vector (Promega, Madison, WI, USA) systems. Briefly, 3 μL of purified PCR products were ligated into the pGEM®-T Easy vector overnight at 4 °C followed by heat-shock transformation of JM109 High efficiency competent cells (Promega). The transformed cells were allowed to grow on LB plates containing ampicillin overnight at 37 °C for colony development. Plasmid DNA purifications were performed using PureLink™ Quick Plasmid Miniprep Kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol and the samples were sent to sequencing. Sequences obtained from cloning were trimmed of vector and their similarity with the RNAseq contig was verified by Blastn.

Confirmatory quantitative real-time PCR

Quantitative real-time PCRs were performed on RNA samples from two pools of ten adult psyllids each from uninfected and infected colonies that were maintained either on potato or on tomato host plants. Reactions for all samples were performed in duplicates with a negative control in each run. RNA extractions were performed on pooled insect samples using Trizol® (Invitrogen, Carlsbad, CA, USA) method. RNA concentrations were determined by the NanoDrop spectrophotometer (Eppendorf). In addition, the quality of the RNA was assessed by visualizing the samples on a 1 % agarose gel. The RNA was subsequently treated with DNase using the rigorous DNA removal procedure of the Turbo DNAse (Ambion). Complete removal of DNA was tested by PCR for the amplification of the internal control 28S rDNA. cDNA was synthesized from 500 ng DNA-free RNA using the Verso cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA). The qPCR Mastermix for SYBR Green (Applied Biosystems, Foster City, CA, USA) was used according to the manufacturer's instructions. Each reaction contained 1 μL cDNA, 0.5 μL of 0.25 μM of each primer, 5 μL of SYBR Green Master Mix, 3 μL of water. The real-time PCR program was incubated at 50 °C for 2 min and 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and followed by 60 °C for 1 min. Real-time PCR assays were performed using an Applied Biosystems ABI 7300 real-time PCR Thermocycler (Applied Biosystems) according to the manufacturer's recommendations. The mean threshold cycles (C_t) values and standard deviation (SD) were automatically calculated by the 7300 system SDS software version 1.3.1 (Applied Biosystems). PCR efficiencies (E) of target and internal control genes were determined using the LinRegPCR software (Ruijter et al. 2009). The relative expression of each gene in the uninfected and infected psyllid samples was estimated using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001) by normalizing transcript levels of genes of interest to the internal control gene (28S rDNA), followed by normalization to the lowest value of the uninfected psyllid sample. T tests were performed to examine differences in gene expression between uninfected and infected psyllids using Microsoft Excel program.

Sequence data deposition

The Illumina RNASeq data of uninfected and infected adult potato psyllids were deposited in the http://psyllid.org/node/10 website.

Results and discussion

Transcriptome profile of adult potato psyllids

To analyze the transcriptome of infected and uninfected potato psyllids, RNA samples isolated from adult psyllids of both sexes were submitted for RNA sequencing

Table 1 Summary statistics of assembly and annotation of potato psyllid transcriptome

Parameters	Total sequences	Infected psyllids	Uninfected psyllids
Assembly			
Total number of reads	84,460,368	50,276,150	34,184,218
Total de novo assembled contigs	158,242	109,983	136,518
Longest assembly (bp)	17,011	16,994	17,011
Average sequence length (bp)	514.35	526.5	502.2
Average RPKM	5.32	8.28	6.35
N50 (bp)	322	343	326
Annotation			
Contigs used for BlastX search	47,399		
Significant matches (E value $< 10^{-5}$)	15,788		
Non-significant matches	31,611		
GO annotations	15,934		

(RNASeq). A total of 84.5 million paired reads were obtained (50.3 and 34.2 million paired-end reads from infected and uninfected psyllid cDNA libraries, respectively) (Table 1). Approximately 1.5 times more reads were obtained from the infected psyllid library compared to the uninfected library; however, quality of both libraries was similar (data not shown). To annotate this resource, both libraries were assembled together using CLC Genomic Workbench 4.8 platform. CLC Genomic Workbench produced 47,399 contigs with an average contig size of 514 bp (Table 1). Contig size ranged from 200 to 17,011 bp, which suggests that many transcripts may not be full length and may be split into more than one contig.

The contigs were compared against the NCBI non-redundant database using BlastX analysis. BlastX searches yielded a significant match ($E < 10^{-5}$) for 33.3 % of the contigs (15,788 contigs) (Table 1). Comparison of the potato psyllid and Asian citrus psyllid transcriptomic data sets shows similar gene annotation distributions in that around 35 % sequences had a putative match to proteins in GenBank (Table 1) (Hunter et al. 2009). The majority of the potato psyllid transcripts had significant match to arthropod proteins in the database (13,558); remaining transcripts were similar to bacteria (147), viruses (13), and others (2070). Overall, a greater number of psyllid sequences had significant matches to *A. pisum* (Hemiptera) proteins, followed by *Tribolium castaneum* (Coleoptera), *Pediculus humanus* (Psocodea) and *Nasonia vitripennis* (Hymenoptera) sequences (Fig. 1). This is not surprising, since *A. pisum*, the pea aphid is presently the only hemipteran with a sequenced genome; further, *P. humanus* is a part of the hemipteroid assemblage along with Thysanoptera (thrips). It is expected that once the genome of the Asian citrus psyllid is released and annotated, the percentage of sequences from this dataset with similarities to proteins will increase.

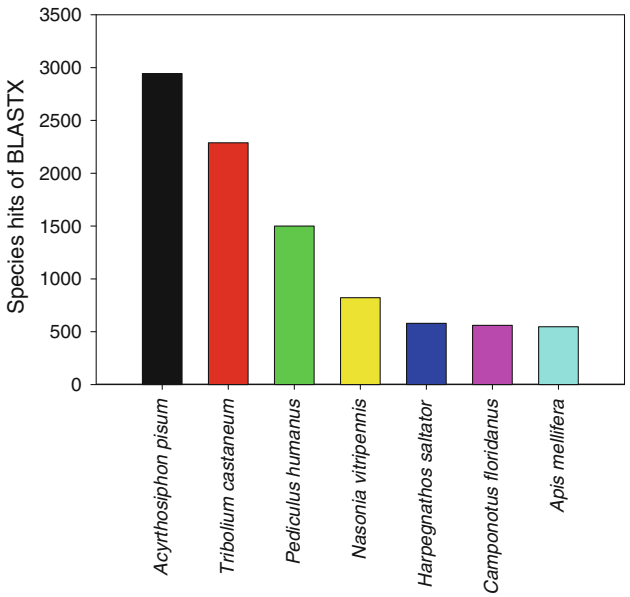

Fig. 1 The most frequent species distribution of the BlastX hits of potato psyllid transcripts against the non-redundant (nr) protein database

The contig set was assigned Gene Ontology (GO) annotations using Blast2Go analyses (Table 1). A total of 15,934 GO terms were assigned: 5,219 for biological process, 6,257 for molecular function, and 4,458 for cellular component. Metabolic process, cell localization, and catalytic activity were the most abundant categories in the psyllid transcriptome (Fig. 2). Pyrosequencing of the potato psyllid transcriptome identified sequences related to metabolism, binding and transport, ribosomal functions, and organelle construction (Hail et al. 2010), which are similar to our findings. Response to stimulus accounted for approximately 10 % of the sequences and was among the third most abundant biological process term. In this category, there was an abundance of heat shock protein (hsp) 70 and 90 transcripts relative to other genes. Further, five sequences assigned to the term "response to bacterium" including ejaculatory bulb-specific protein 3, tak1-associated binding protein 2, forkhead box subgroup o, wiskott-aldrich syndrome-like, and pr-10 type pathogenesis-related protein were identified. Tak-1-associated binding protein 2 is a gene involved in the IMD pathway, which as mentioned previously has been shown to protect against Gram-negative bacteria. Since CLs is a Gram-negative bacterium, it is probable that this gene was induced in response to CLs infection in the infected insects.

Comparison of the transcriptome pattern of potato psyllid for eight different GO terms (biological process-level 2) with those of Asian citrus psyllid (ESTs deposited in GenBank) showed significant differences in several categories, notably development process, response to stimulus and reproduction in the distribution of genes across GO categories (Fig. 3). The Asian citrus psyllid transcriptome comprised uninfected psyllids (Hunter, personal communication), hence it is not surprising that the response to stimulus term had fewer sequences compared to the current study, which included infected psyllids in the analyses. The inclusion of both nymphs and adults in the construction of Asian citrus psyllid libraries may explain the lack of reproduction terms in the Asian citrus psyllid transcriptome.

Among the 30 most-abundant transcripts in the psyllid transcriptome, 19 sequences were annotated by BlastX, 3 sequences encoded ribosomal RNA while the rest shared no similarity to proteins or nucleotides in the databases. The annotated sequences are presented in Table 2. There was some commonality between the most-abundant transcripts detected in the Asian citrus psyllid transcriptome and the current study including the presence of cytochrome

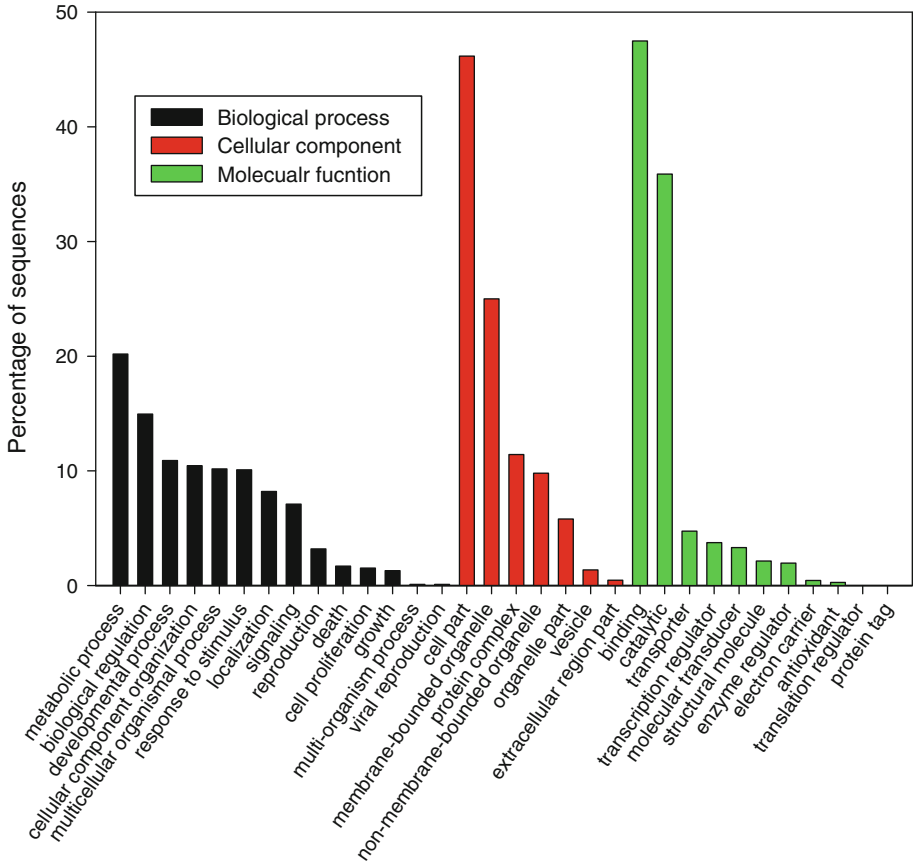

Fig. 2 Gene Ontology (GO) annotation terms, biological process (level 2), cellular component (level 3) and molecular function (level 2) of potato psyllid predicted proteins

oxidase subunit II, ejaculatory-bulb protein, and ferritin transcripts (Hunter et al. 2009). Hail et al. (2010) reported high abundance of vitellogenin (Vg) transcripts, ejaculatory-bulb protein, actin II, heat shock protein, troponin and transferrin in the potato psyllid transcriptome as well. One of the most abundant contig in the potato psyllid transcriptome was similar to Vg from the giant water bug, *Lethocerus deyrollei.* Furthermore, 5 out of the 19 contigs were also similar to Vg protein (Table 2). Vitellogenin is the major yolk protein (vitellin) precursor in insects (Wahli et al. 1981). Vitellogenins are important for growth and differentiation of oocytes and transporting metallic ions, lipids, and vitamins into the oocytes (Raikhel and Dhadialla 1981). Hence, it is not surprising that a high abundance of Vg transcripts were obtained in the adult psyllid cDNA libraries. Insect Vgs are large proteins; their transcripts are 6–7 kb long. Since none of the contigs encode full length sequences, it is likely that different contigs are from a same gene. Presently, 1–3 Vg genes have been identified in hemipterans.

Identification of reproduction-related genes

A total of 425 contigs out of 5,219 "biological process" annotated contigs were assigned a "reproduction" term by Blast2go analysis (Fig. 2). These included contigs involved in ovarian follicle cell development, ovarian follicle cell migration, ovarian follicle cell stalk formation, ovarian follicle cell adhesion, ovarian follicle development, ovarian fusome organization, ovarian nurse cell to oocyte transport, and oviposition. High Vg abundance was reported in the transcriptome of the small brown planthopper, *L. striatellus* although, adult females showed higher Vg expression compared to males (Xue et al. 2010). Since the proportion of males and female psyllids in the insect pool sample used for library construction was not determined, it is difficult to speculate about the apparent abundance of Vg transcripts in the psyllid transcriptome.

Identification of immune and stress-related genes

Several genes putatively involved in immune responses were identified in the potato psyllid transcriptome (Table 3). For the Toll pathway, contigs with similarities to the transmembrane receptor Toll, and MyD88 adaptors, the kinase pelle, the inhibitor molecule cactus, cactin, Pellino, Traf, and the transactivator dorsal with the exception of Spatzle were found (Table 3). Contigs with similarities to two proteins in the JNK pathway, basket and kayak were also detected. Three JAK/STAT proteins, including Janus

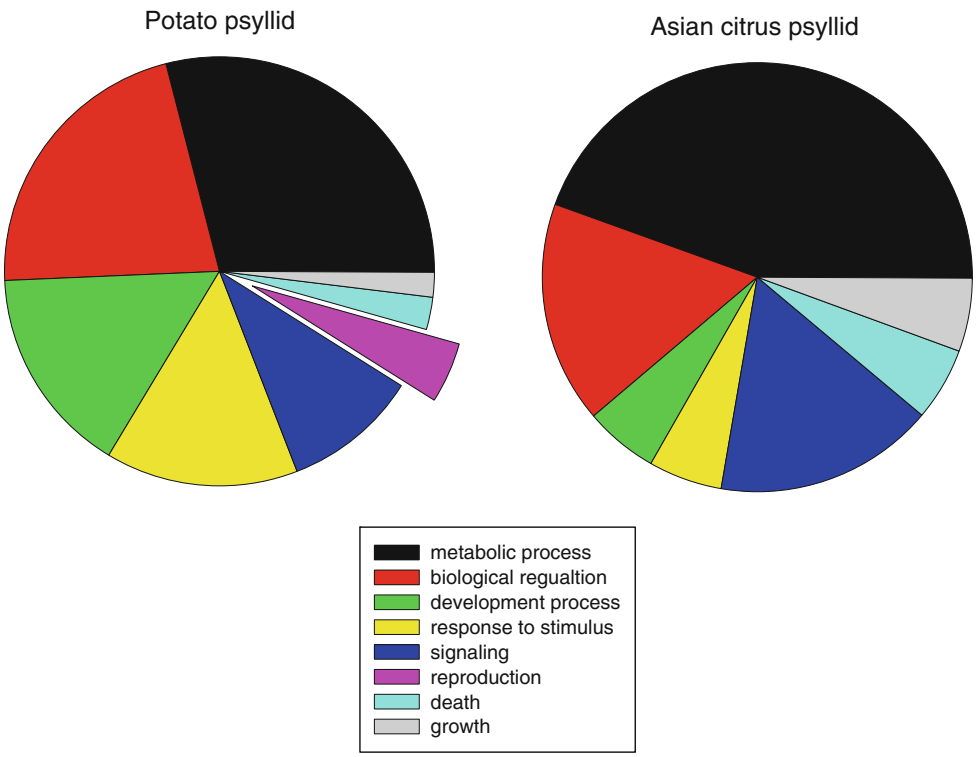

Fig. 3 Comparison of potato psyllid transcriptome pattern with that of Asian citrus psyllid, based on eight different biological process Gene Ontology (GO) terms (level 2). Asian citrus psyllid data were obtained from ESTs deposited in GenBank

Table 2 A subset of the most abundant transcripts identified in the potato psyllid transcriptome

Contig	Putative annotation	Accession number	*E* value	RPKM
Contig 42152	NADH dehydrogenase subunit 1	AAT99378	1.1E−173	25,480.59
Contig 41973	Vitellogenin	BAG12118	2.3E−73	18,767.24
Contig 26156	Hypothetical protein	AAA50836	2.38E−08	18,509.07
Contig 45015	Vitellogenin	BAJ33507	1.70E−29	14,476.40
Contig 42586	Vitellogenin	BAJ33507	2.73E−96	9,257.26
Contig 29195	Vitellogenin	BAG12118	1.51E−14	7,030.52
Contig 17406	Ferritin	ABR27877	7.64E−80	5,750.55
Contig 42587	Vitellogenin	Q9U8M0	2.27E−10	5,637.77
Contig 2033	Hypothetical protein	EFX76379	8.79E−07	5,014.68
Contig 24714	Cytochrome *c* oxidase subunit I	YP_073284	2.07E−102	4,875.50
Contig 47344	Predicted protein	XP_002431398	5.74E−15	3,491.73
Contig 29327	Ejaculatory bulb-specific protein 3 precursor	XP_002432594	1.32E−22	3,449.72
Contig 17240	Ubiquitin	AAV84266	2.76E−94	3,320.89
Contig 42766	Cytochrome *c* oxidase subunit II	YP_073285	6.57E−94	3,180.29
Contig 8050	Heat shock protein 70	ADE3417	0	2,633.93
Contig 42592	40S ribosomal protein	Q5G5C4	5.7E−142	2,502.40
Contig 11602	Cytochrome *b*	YP_073294	2.5E−102	2,080.10
Contig 9502	60S ribosomal protein	ABG81987	6.03E−23	1,972.80
Contig 15556	25S ribosomal protein	ABG18982	1.16E−32	1,842.80

kinase, JAK tyrosine kinase, and the STAT1 protein were identified. These three pathways are involved in immunity and development. Interestingly, genes from the IMD pathway were predominantly absent from the potato psyllid transcriptome, only one contig with similarity to TAK was detected (Table 3). This is particularly interesting for two reasons. First, the IMD pathway is activated in *D. melanogaster* and other holometabolous insects in response to Gram-negative bacteria. Since CLs is a Gram-negative bacterium, it is expected that genes of the IMD pathway

Table 3 Putative immune response genes identified in potato psyllid transcriptome

Contig ID	Role in immunity	Gene name	Accession number	*E* value
Contig 45232	Recognition and response	C-type lectin precursor	NP_001164139	2.47E–32
Contig 24977		Galectin 4 putative	XP_002428817	5.21E–45
Contig 45042		Galectin 4 like	ABE98245	6.08E–15
Contig 1212	Bind to lipoproteins, bacteria	Similar to scavenger receptor SR-C-like protein	XP_001812043	3.19E–14
Contig 32950	Toll pathway	Cactus	XP_002427786	1.91E–12
Contig 41619		Cactus	XP_002427786	5.85E–7
Contig 1837		Dorsa–ventral patterning protein Sog-like	XP_001952734	2.27E–49
Contig 1362		Dorsal 1C	ABU96700	7.87E–11
Contig 14247		Dorsal 1C	ABU96700	2.18E–27
Contig 36469		Neuropilin and tolloid-like protein 1	EGI63926	1.03E–22
Contig 26326		Myd88-like adapter protein	XP_314167	7.26E–25
Contig 19152		Pelle	XP_001943030	0
Contig 1179		Pellino	XP_002426083	6.71E–74
Contig 13995		Pellino-like	XP_003398776	4.75E–70
Contig 30544		Protein toll precursor, putative	XP_002431445	7.69E–63
Contig 31809		Protein toll precursor, putative	XP_002431445	7.83E–140
Contig 42921		Tolloid-like protein 2	EGI62193	0.00
Contig 5855		Protein toll-like	XP_001946943	1.08E–35
Contig 10375		Protein toll-like	XP_001946943	0
Contig 9838		Tollo	EEZ99353	7.32E–144
Contig 15388		Tollo	EEZ99353	8.70E–115
Contig 23298		TNF-receptor-associated factor 4-like isoform	XP_003394191	7.13E–81
Contig 24165		TNF-receptor-associated factor 4-like isoform	XP_003394191	2.97E–38
Contig 5795		Similar to 18 wheeler	XP_972409	2.68E–76
Contig 15277		Similar to 18 wheeler	XP_972409	7.40E–84
Contig 26300	JNK pathway	Basket	EGI62749	6.77E–153
Contig 42737		Kayak	XP_002426121	3.19E–46
Contig 12858	JAK/STAT pathway	Protein inhibitor of activated signal-transducer and activator of transcription 1	ACR77525	1.06E–19
Contig 24743		Tyrosine-protein kinase jak2, putative	XP_002425471	0
Contig 24097		Tyrosine-protein kinase jak2, putative	XP_002425471	1.18E–46
Contig 20715	IMD pathway	TAK1-associated binding protein	XP_001122664	6.52E–14

will be expressed at least in the infected psyllid library. Secondly, annotation of the pea aphid genome revealed the absence of several genes of the IMD pathway (Gerardo et al. 2010). However, IMD pathway genes including an IMD-like transcript and lap2 were identified in the brown planthopper, *Nilaparvata lugens* transcriptome (Peng et al. 2011). IMD pathway transcripts, including IMD, TAB 2, DIAPE, Dfadd, and NF-kB like protein Relish were identified in the small brown planthopper, *Laodelphax striatellus* albeit with relatively low *E* value (Zhang et al. 2010). Nevertheless, the absence of the complete IMD pathway appears to be common in the hemipteran suborder, Sterrnorhyncha, which consists of aphids, whiteflies, scale insects, and psyllids if not all hemipteran insects, and it is plausible that psyllids, as aphids, might use other mechanisms to control Gram-negative bacteria. Interestingly, the

Table 4 Putative bacterial genes identified in potato psyllid transcriptome

Contig	Description	Accession number	*E* value
P-endosymbiont			
Contig 5640	Chaperonin GroEL	YP_802448	0
Contig 9439	Chaperone protein dnak	YP_802459	1.40E−151
Contig 38583	Cold shock protein	YP_802578	1.84E−40
Contig[a]	16S rDNA gene[b]	AF211126	0
Wolbachia			
Contig 3300	Ankyrin repeat protein	ADW80235	1.10E−15
Contig 42056	Ankyrin repeat protein	ADW80235	0
Contig 22249	Heat shock protein grpe	YP_001975300	1.31E−44
Contig 22141	Hypothetical protein C1A_1038	ZP_03335073	3.99E−115
Contig 12788	Hypothetical protein wpa_1129	YP_001975868	3.86E−73
Contig 15463	Hypothetical protein wpa_0198	YP_001975009	2.39E−59
Contig 7910	Hypothetical protein	BAH22253	3.30E−49
Contig 2375	Hypothetical protein wpa_0044	YP_001974877	1.01E−33
Contig 263	Hypothetical protein wpa_0984	YP_001975734	3.78E−30
Contig 29191	Hypothetical protein wpa_0294	YP_001975095	9.83E−16
Contig 15049	Hypothetical protein wpa_0985	YP_001975735	1.18E−10
Contig 10440	Hypothetical protein	BAA89627	1.17E−08
Contig 6940	Hypothetical protein Wendoof_01000684	ZP_01314509	2.87E−54
Contig 1924	Transposase	ZP_00373323	3.00E−07
Contig 12371	Translation elongation factor Tu	YP_001975333	0
Contig[a]	16S rDNA gene[b]	GU124506	0
Liberibacter			
Contig 4021	Chaperonin GroEL	YP_004063216	6.09E−45
Contig 25098	Glycerol kinase	ACD87749	3.08E−71
Contig[a]	16S rDNA gene[b]	FJ914619	0

[a] Presence of several contigs representing the same gene

[b] Genes identified using Blastn search

IMD signaling pathway leads to activation of the JNK signaling pathway (Gerardo et al. 2010), when TAK is activated, it triggers the JNK pathway. An IMD-independent activation of JNK pathway, via the inducer Eiger, has been proposed in *D. melanogaster* (Bidla et al. 2007) and the pea aphid (Gerardo et al. 2010).

Identification of bacterial genes

Analysis of the transcriptome revealed the presence of psyllid bacterial symbiont contigs among the insect contigs. Four prokaryotic intracellular bacterial symbionts have been found associated with the potato psyllid: primary (P-) or obligate symbiont (*Carsonella ruddii*) (Thao et al. 2000b; Nachappa et al. 2011) and three facultative symbionts, a secondary (S-) symbiont (Thao et al. 2000a), *Wolbachia* (Nachappa et al. 2012; Liu et al. 2006), and the plant pathogen, CLs (Hansen et al. 2008). These symbionts are heritable and transmitted from mother to offspring (Baumann 2005). The P-symbiont influences fundamental biological processes in their insect host and is required for survival and development of the psyllid (Baumann 2005), whereas the facultative symbionts can influence ecologically relevant fitness traits of their hosts (Oliver et al. 2010).

Sequences with similarities to three symbiotic bacteria were identified in the transcriptome of potato psyllids with the exception of the S-endosymbiont which is not present in the psyllids used for this study (Nachappa et al. 2011). Contigs with similarities to P-endosymbiont proteins identified included: chaperones GroEL, and DnaK, cold shock protein (Table 4). GroEL is a chaperone protein and is one the most abundant proteins expressed by P-endosymbiont such as *Buchnera aphidicola,* the P-endosymbiont of aphids (Baumann et al. 1996). GroEL is hypothesized to protect plant viruses against their vector immune responses and thereby increase transmission (Hogenhout et al. 1998; Morin et al. 1999). The function of GroEL expressed by the psyllid P-endosymbiont is unknown. Several contigs with similarities to *Wolbachia* proteins, including translation elongation factor Tu, ankyrin repeat protein, heat shock protein GrpE, transposase and several hypothetical proteins were identified (Table 4). The two contigs with similarity to CLs proteins obtained were glycerol kinase and chaperonin GroEL (Table 4). In addition, Blastn analysis identified 34 bacterial transcripts in the psyllid transcriptome with

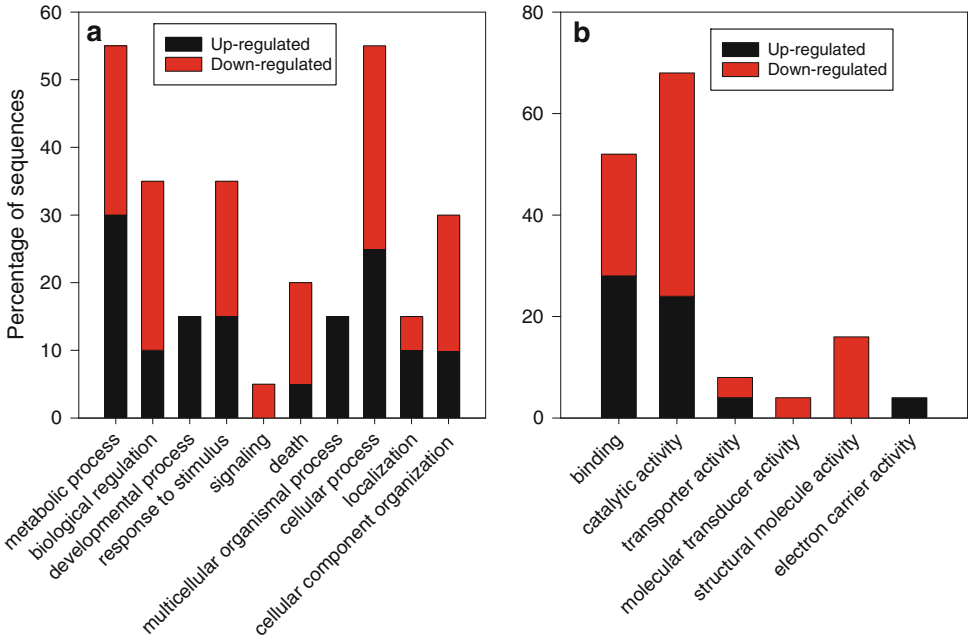

Fig. 4 The percentage of sequences for some selected GO terms (level 2). **a** Biological process and **b** molecular function for differentially expressed transcripts in the infected psyllids

E value: $>E^{-100}$. These primarily included the 16S ribosomal genes of the P-endosymbiont, *Wolbachia,* and CLs (Table 4). Even though RNASeq targets mRNA, bacterial sequences were obtained in the psyllid transcriptomic data sets probably because these genes are AT rich; hence, RNASeq may serve as a resource to identify bacterial endosymbiont genes expressed in their insect host for investigating host–endosymbiont interaction.

Comparative analyses of infected and uninfected psyllids

To investigate differentially expressed contigs between the infected and uninfected psyllid libraries, each library was analyzed separately. RPKM values were compared to identify putative differentially expressed genes between infected and uninfected psyllids. Overall, there were 247 contigs that were differentially expressed between the infected and uninfected psyllids. Among these contigs, 84 had significant matches with putative proteins in the database. The majority of the contigs (66 %) had no significant hit; these contigs might represent genes unique to psyllids, or yet unidentified genes involved in immune response to Gram-negative bacteria in hemipteran insects and/or genes involved in the CLs–psyllid interaction. Analysis of the differentially expressed contigs that also had a GO annotation term revealed that a large percentage of contigs were down-regulated in the "biological process" and "molecular function" GO term categories in infected insects (Fig. 4a, b). In the "response to stimulus" category, there was greater proportion of contigs that were down-regulated. The down-regulated contigs had similarities to beta-tubulin, hemolymph proteinase, c-myc-binding protein, proteasome activator complex subunit 3. The contigs that were up-regulated in the "response to stimulus" category had similarities to heat shock proteins, phenoloxidase, and lola protein isoform a.

A list of differentially expressed contigs that had a significant BlastX hit are described in Table 5. Two Vg contigs were differentially expressed between the uninfected and infected psyllids and both contigs belonged to the same ortholog (BAJ33507) (Table 5). Changes in Vg in response to pathogen infection have been reported in literature. Vitellogenin expression was significantly reduced 24 h after blood meal in the mosquito, *Anopheles gambiae* following *Plasmodium yoeii nigeriensis* infection (Ahmed et al. 2001), but no significant difference was observed after that period. In addition, Vg hemolymph concentration began to increase to a greater extent in the infected insects after 24 h, probably due to reduced uptake, since ovarian vitellin (yolk protein) was significantly decreased by infection in the infected insects. In the current study, the level of Vg transcripts was higher in the infected psyllids than the uninfected psyllids; however, the infected psyllids have significantly lower fecundity (1.5 times) compared to uninfected psyllids (Nachappa et al. 2012). Interestingly, Vg has been associated with anti-bacterial responses in several fish species (Shi 2006). Further, it was demonstrated that in *Plasmodium*-infected mosquitoes Lipophorin and Vg, both transporters of nutrients to developing oocytes reduced the efficiency of parasite killing by the mosquito immune system. Conversely, in the absence of the nutrient transporters, the immune system was at its maximal capacity for parasite killing but there was reduced

Table 5 Subset of differentially expressed transcripts identified in the infected and uninfected potato psyllid transcriptome

Contig	Putative annotation	Accession number	RPKM	
			Infected	Uninfected
Contig 12608	Beige/BEACH domain containing protein	XP_001301665	15.51	84.34
Contig 26340	Cathepsin B preproprotein	BAE44111	23.21	71.47
Contig 17213	C-Myc-binding protein	EGD82253	61.56	346.86
Contig 8759	Cuticular protein 97Ea	NP_651529	30.56	141.44
Contig 11997	Cysteinyl-tRNA synthetase, cytoplasmic-like	XP_003396062	61.47	14.77
Contig 5466	Cytochrome *c* oxidase subunit I	YP_086802	116.49	0.31
Contig 11235	Gamma-glutamyl hydrolase precursor, putative	XP_002425321	60.14	279.80
Contig 39964	General odorant-binding protein lush-like	XP_001603472	117.35	31.56
Contig 29274	Glycosyl hydrolase family 31 protein	ABF71570	24.38	127.59
Contig 40206	Glucose dehydrogenase-like	XP_001943395	22.64	68.78
Contig 11012	GRIP and coiled-coil domain containing 2	XP_002192480	41.27	205.50
Contig 44489	Heat shock protein 70	ADK39311	230.51	39.08
Contig 11613	Heat shock protein 70	ADO14473	130.05	24.25
Contig 11977	Hemolymph proteinase 5	AAV91003	101.93	444.22
Contig 29257	Predicted protein CREG2-like	XP_001950884	105.18	26.36
Contig 16868	Predicted protein p8 MTCP-1-like	XP_002930296	93.01	338.34
Contig 42647	Lysosomal aspartic protease	EGI64243	133.28	956.73
Contig 24874	Out at first protein, putative	XP_002433056	100.58	27.70
Contig 5610	Peroxidase-like	XP_001951217	129.64	31.05
Contig 25104	Peroxidase-like	XP_001951217	118.18	33.33
Contig 31742	Phenoloxidase subunit A3-like	XP_001949307	55.13	12.96
Contig 32830	Probable adenylate kinase isoenzyme F38B2.4	ACO14567	22.49	171.37
Contig 8519	Putative sodium-coupled neutral amino acid transporter 9-like	XP_001946160	25.63	84.36
Contig 7951	Putative gag-pol protein	ABP48077	31.33	9.43
Contig 25101	Serine protease easter	EFN75743	69.09	218.28
Contig 11524	Transferrin	AAN03488	1245.20	132.74
Contig 41679	Transient receptor potential protein-like	XP_003240303	6.59	32.51
Contig 12963	Tubulin alpha chain-like	XP_625145	86.80	462.86
Contig 143474	Tubulin beta 2C-like	NP_001171814	128.19	627.72
Contig 8775	Uncharacterized protein C8orf41-like	XP_003240602	20.83	416.07
Contig 7716	Vitellogenin	BAJ33507	55.43	0.23
Contig 45984	Vitellogenin	BAJ33507	64.05	0.18
Contig 25098	Glycerol kinase	ACD87749	162.65	1.17

reproductive efficiency in terms of mosquito egg development (Rono et al. 2010). Hence, it is plausible that the observed increase in Vg expression level in the infected insects was correlated with CLs infection and the apparent trade-off with fecundity in the infected insects. The authors are currently investigating the relationship between CLs level and Vg expression in adult psyllids in time course experiments (Nachappa et al., unpublished). Future studies may also be aimed at investigating the relationship between CLs infection level and the vitellin protein. In contrast to the above reports, viruliferous small brown planthopper infected with rice stripe virus showed an abundance of Vg transcripts compared to naïve insects (Zhang et al. 2010). The authors hypothesized that rice stripe virus may be exploiting the Vg "traffic pathway" to overcome the barrier between the follicle cells and oocytes thereby facilitating its persistent transmission via maternal germ line. These studies highlight the potential effects of pathogen infection on egg development in their insect vectors.

There was differential expression of another transcript involved in reproduction, lysosomal asparatic protease, which showed higher expression in the uninfected psyllids than the infected psyllids (Table 5). In insects, lysosome enzymes mediate fat body remodeling during metamorphosis and termination of vitellogenesis in adult females (Raikhel 1986). In mosquito fat body, a rise in the activities

Table 6 Primer pair sequences designed for validation of differentially expressed contigs in uninfected and infected psyllid libraries using real-time quantitative reverse transcriptase PCR

Gene name	Contig number	Mean PCR efficiencies[a]	Primer sequence (5′–3′, forward/reverse)
Lysosomal aspartic protease	Contig 42647	1.91	TTATATCACTGGGCACACCA
			TGGAATCCACAAGTCTGAAGAA
Cuticle protein	Contig 8759	1.91	TCATTCTCTGCGCCACCT
			TGCGTTCTCAGAGGTCAAGA
Serine protease	Contig 25101	1.95	CAACTTACTTCCTGCCTTGGA
			CGTCTCATAATGACTTATCAGCCTA
Heat shock protein 70	Contig 11613	1.99	GGCTCGTTCACAATCCTCAT
			AGGCCACGAAGGATGCAG
Odor-binding protein	Contig 39964	1.94	GTTCGAACCCAAGCTCTCAC
			CCAGCCTACGGATTGAACAT
Phenoloxidase	Contig 31742	1.92	AACCGAACACGGTTATCGAG
			GAGTCCAGGTCACGGAATGT
28S rDNA[b]	–	1.99	AGTTTCGTGTCGGGTGGAC
			AACATCACGCCCGAAGAC

[a] Mean PCR efficiencies were calculated using the LinRegPCR software (Ruijter et al. 2009)

[b] Primer sequence for 28S rDNA internal control gene, which was designed based on the gene sequence (accession no. EU812555) deposited in GenBank

of several lysosomal enzymes coincides with a decline in the synthesis of Vg (Raikhel 1986). Taken together, psyllid transcriptomic analyses confirm the increased expression of Vg and subsequent reduction in lysosomal aspartic protease in infected psyllids.

Confirmatory real-time quantitative reverse transcriptase PCR

To confirm RNASeq results, six putative differentially expressed genes were selected from the infected and uninfected psyllid libraries for RT-qPCR analyses to verify differential expression: lysosomal aspartic protease, cuticle protein, serine protease, heat shock protein, odor-binding protein, and phenoloxidase. These genes were selected because of their potential involvement in key functions such as reproduction, stress and immunity. Prior to RT-qPCR analysis, amplifications obtained by species-specific primers for all six genes were cloned and sequenced to verify their identity following procedures described in "Cloning and sequencing" in "Materials and methods". The sequences showed 100 % similarity to their respective contigs in the transcriptome (data not shown). Real-time PCR primer sequences and PCR efficiencies are displayed in Table 6. In the psyllid transcriptome, contigs with similarity to lysosomal aspartic protease, cuticle protein and serine protease showed higher expression levels in the uninfected insects, whereas contigs with similarity to heat shock protein, odor-binding protein, and phenoloxidase were highly expressed in the infected insects. To confirm these expression patterns, RT-qPCR analyses were performed on psyllids that were exclusively reared on potato (host of the insects used for RNAseq) and psyllids exclusively reared on tomato (alternative host of psyllids). This was done in order to determine if host plant association would influence the magnitude and direction of expression of psyllid genes. The RT-qPCR results were in agreement with the RNASeq results showing similar trends in up- and down-regulation of genes (Fig. 5; Table 5). There was high variability in expression levels of certain genes specifically in the infected samples (Fig. 5), which may be related to CLs infection level in the infected insects. Overall, RT-qPCR analyses confirmed expression results from RNAseq and also showed that plant host association did not affect gene expression of the selected genes.

Conclusions

The current study provides the first in depth analysis of the transcriptome of the potato psyllid, *B. cockerelli* following infection with a bacterial plant pathogen, CLs. The key features identified in the psyllid transcriptome were an abundance of genes involved in metabolism, reproduction, development, stress and immune responses. There was differential expression of a suite of genes in response to CLs infection in psyllids including genes involved in reproduction, stress response and several genes that are yet to be annotated. Interestingly, several bacterial gene sequences specifically that of CLs, which was highly expressed in the infected psyllids as expected were

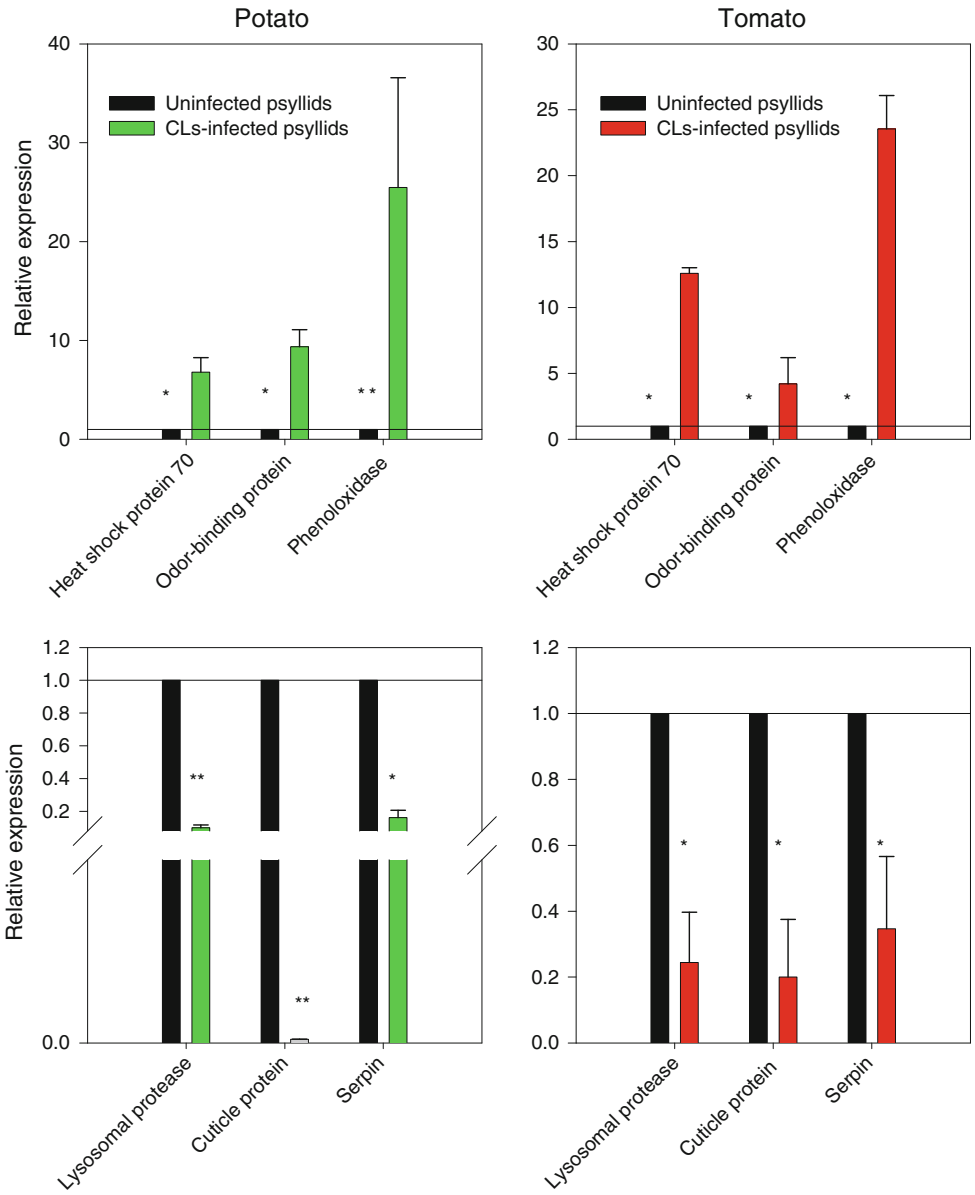

Fig. 5 Mean ± SE levels of six potato psyllid genes that showed differential expression in the potato psyllid transcriptome determined using RT-qPCR analyses. The *top panel* indicates genes that were up-regulated in response to CLs infection and the *bottom panel* indicates genes that were down-regulated. *Green colored bars* denote psyllids that were reared on potato (host of the insects used for RNAseq), whereas *red color* denotes psyllids that were reared on tomato (alternate host). *Asterisks* indicate statistically significant differences in expression levels between uninfected and infected psyllids (*$P \leq 0.05$, **$P \leq 0.01$; *t* test) (color figure online)

obtained. Confirmatory RT-qPCR on differentially expressed psyllid genes confirmed the trends observed in RNASeq results. In addition, data showed that host plant association did not affect expression of the selected differentially expressed psyllid genes, which suggests that sequencing results may be applied to study potato psyllid–CLs interaction in different host plant systems. Our results will help unraveling the molecular interaction between psyllids and the bacterial pathogen, CLs. The gene expression and sequence data obtained from this study will provide a valuable resource for further functional genomics studies of potato psyllids, and provide insights into molecular mechanisms underlying phytopathogen effects on its insect vector, which has not been explored previously. Identification of transcripts from infected insects may serve as potential targets for the development of transgenic technologies for insect pest and vector control.

Acknowledgments We thank Drs. Rush, Henne and Munyaneza for supplying psyllid stocks that were used to establish the colonies used in this study.

References

Abad JA, Bandla M, French-Monar RD, Liefting LW, Clover GRG (2009) First report of the detection of '*Candidatus* Liberibacter' species in zebra chip disease-infected potato plants in the United States. Plant Dis 93(1):108

Ahmed AM, Maingon R, Romans P, Hurd H (2001) Effects of malaria infection on vitellogenesis in *Anopheles gambiae* during two gonotrophic cycles. Insect Mol Biol 10(4):347–356

Baumann P (2005) Biology bacteriocyte-associated endosymbionts of plant sap-sucking insects. Annu Rev Microbiol 59:155–189

Baumann P, Baumann L, Clark MA (1996) Levels of *Buchnera aphidicola* Chaperonin GroEL during growth of the aphid *Schizaphis graminum*. Curr Microbiol 32(5):279–285

Baumann P, Moran N, Baumann L (2006) Bacteriocyte-associated endosymbionts of insects. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes. Springer, New York, pp 403–438

Bidla G, Dushay MS, Theopold U (2007) Crystal cell rupture after injury in *Drosophila* requires the JNK pathway, small GTPases and the TNF homolog Eiger. J Cell Sci 120:1209–1215

Carter W (1939) Injuries to plants caused by insect toxins. Botan Rev 5:273–326

Cranshaw WS (2001) Diseases caused by insect toxin: psyllid yellows. In: Stevenson WR, Loria R, Franc GD, Weingartner PD (eds) Compendium of potato diseases, 2nd edn. APS, St. Paul, pp 73–74

Crosslin JM, Bester G (2009) First report of '*Candidatus* Liberibacter psyllaurous' in Zebra Chip symptomatic potatoes from California. Plant Dis 93(5):551

Crosslin JM, Munyaneza JE, Brown JK, Liefting LW (2010) Potato zebra chip disease: a phytopathological tale. Plant Health Progr. doi:10.1094/PHP-2010-0317-1001-RV

Daborn PJ, Waterfield N, Blight MA, Ffrench-Constant RH (2001) Measuring virulence factor expression by the pathogenic bacterium *Photorhabdus luminescens* in culture and during insect infection. J Bacteriol 183:5834–5839

De Gregorio E, Spellman PT, Rubin GM, Lemaitre B (2001) Genome-wide analysis of the *Drosophila* immune response by using oligonucleotide microarrays. Proc Natl Acad Sci 98:12590–12598

Gerardo N, Altincicek B, Anselme C, Atamian H, Barribeau S, de Vos M, Duncan E, Evans J, Gabaldon T, Ghanim M, Heddi A, Kaloshian I, Latorre A, Moya A, Nakabachi A, Parker B, Perez-Brocal V, Pignatelli M, Rahbe Y, Ramsey J, Spragg C, Tamames J, Tamarit D, Tamborindeguy C, Vincent-Monegat C, Vilcinskas A (2010) Immunity and other defenses in pea aphids *Acyrthosiphon pisum*. Genome Biol 11(2):R21

Govind S (2008) Innate immunity in *Drosophila*: pathogens and pathways. Insect Sci 15(1):29–43

Hail D, Hunter WB, Dowd SE, Bextine B (2010) Expressed sequence tag (EST) survey of life stages of the potato psyllid, *Bactericera cockerelli*, using 454 pyrosequencing. Southwest Entomol 35(3):463–466

Hansen AK, Trumble JT, Stouthamer R, Paine TD (2008) A new Huanglongbing Species, "*Candidatus* Liberibacter psyllaurous," found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). Appl Environ Microbiol 74(18): 5862–5865

Hogenhout SA, van der Wilk F, Verbeek M, Goldbach RW, van den Heuvel JFJM (1998) Potato leafroll virus binds to the equatorial domain of the aphid endosymbiotic GroEL homolog. J Virol 72(1):358–365

Hogenhout S, el Ammar D, Whitfield A, Redinbaugh M (2008) Insect vector interactions with persistently transmitted viruses. Annu Rev Phytopathol 46:327–359

Hunter WB, Dowd SE, Katsar CS, Shatters RGJ, McKenzie CL, Hall DG (2009) Psyllid biology: expressed genes in adult Asian citrus psyllids *Diaphorina citri* Kuwayama. Open Entomol J 3(18):18–29

Hurd H (2003) Manipulation of medically important insect vectors by their parasites. Annu Rev Entomol 48:141–161

Liefting LW, Perez-Egusquiza ZC, Clover GR, Anderson JAD (2008) A new '*Candidatus* Liberibacter' species in *Solanum tuberosum* in New Zealand. Plant Dis 92:1474

Liefting LW, Southerland PW, Ward LI, Paice KL, Weir BS, Clover GRG (2009) A new "*Candidatus* Liberibacter" species associated with diseases of solanaceous crops. Plant Dis 92:208–214

Liu D, Trumble JT, Stouthamer R (2006) Genetic differentiation between eastern populations and recent introductions of potato psyllid (*Bactericera cockerelli*) into western North America. Entomol Exp Appl 118(3):177–183

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2 - \Delta\Delta CT$ method. Methods 25(4):402–408

MAFBNZ (2008) http://www.biosecurity.govt.nz/pestdiseases/plants/potato-tomato-psyllid.htm (accessed 18 September 2008)

Morin S, Ghanim M, Zeidan M, Czosnek H, Verbeek M, van den Heuvel JFJM (1999) A GroEL homologue from endosymbiotic bacteria of the whitefly *Bemisia tabaci* is implicated in the circulative transmission of tomato yellow leaf curl virus. Virology 256:75–84

Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B (2008) Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Meth 5(7):621–628

Munyaneza JE, Crosslin JM, Upton JE (2007) Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with "zebra chip," a new potato disease in southwestern United States and Mexico. J Econ Entomol 100(3):656–663

Nachappa P, Levy J, Pierson E, Tamborindeguy C (2011) Diversity of endosymbionts in the potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), vector of zebra chip disease of potato. Curr Microbiol 62:1510–1520

Nachappa P, Shapiro A, Tamborindeguy C (2012) Effect of "*Candidatus* Liberibacter solanacearum" on fitness of its vector, *Bactericera cockerelli* (Hemiptera: Triozidae) on tomato. Phytopathology 102(1):41–46

Oliver K, Degnan PH, Burke GR, Moran NA (2010) Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. Annu Rev Entomol 55:247–266

Pack HJ (1930) Potato psyllid. Utah Agric Expt Stn Bull 216:21

Peng X, Zha W, He R, Lu T, Zhu L, Han B, He G (2011) Pyrosequencing the midgut transcriptome of the brown planthopper, *Nilaparvata lugens*. Insect Mol Biol 20(6):745–762

Pletsch DJ (1947) The potato psyllid *Paratrioza cockerelli* (Sulc), its biology and control. Montana Agric Exp Stn Bull 446:95

Purcell AH (1982a) Evolution of the insect vector relationship. In: Mount MS, Lacey GH (eds) Phytopathogenic prokaryotes. Academic Press, New York, pp 121–156

Purcell AH (1982b) Insect vector relationships with procaryotic plant pathogens. Annu Rev Phytopathol 20:397–417

Raikhel AS (1986) Role of lysosomes in regulating of vitellogenin secretion in the mosquito fat body. J Insect Physiol 32:597–604

Raikhel A, Dhadialla T (1981) Accumulation of yolk proteins in insect oocytes. Ann Rev Entomol 37:217–251

Rono MK, Whitten MMA, Oulad-Abdelghani M, Levashina EA, Marois E (2010) The major yolk protein vitellogenin interferes with the anti-*Plasmodium* response in the malaria mosquito *Anopheles gambiae*. PLoS Biol 8(7):e1000434

Ruijter J, Ramakers C, Hoogaars W, Karlen Y, Bakker O, Van Den Hoff M, Moorman A (2009) Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res 37(6):e45

Secor GA, Rivera-Varas VV (2004) Emerging diseases of cultivated potato and their impact on Latin America. Revista Latinoamericana de la Papa (Suplemento) 1:1–8

Shi X (2006) Vitellogenin is a novel player in defense reactions. Fish Shellfish Immunol 20(5):769

Stout MJ, Thaler JS, Thomma BPHJ (2006) Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. Annu Rev Entomol 51(1):663–689

Thao ML, Clark MA, Baumann L, Brennan EB, Moran NA, Baumann P (2000a) Secondary endosymbionts of psyllids have been acquired multiple times. Curr Microbiol 41(4):300–304

Thao ML, Moran NA, Abbot P, Brennan EB, Burckhardt DH, Baumann P (2000b) Cospeciation of psyllids and their primary prokaryotic endosymbionts. Appl Environ Microbiol 66(7): 2898–2905

Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ, Pachter L (2010) Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotech 28(5):511–515

Wahli W, Dawid I, Ryffel G, Weber R (1981) Vitellogenesis and the vitellogenin gene family. Science 212:298–304

Wallis RL (1955) Ecological studies on the potato psyllid as a pest of potatoes, vol 1107. Tech Bull, US Dep Agric, pp 1–24

Wu LP, Choe KM, Lu Y, Anderson KV (2001) *Drosophila* immunity: genes on the third chromosome required for the response to bacterial infection. Genetics 159:189–199

Xue J, Bao Y–Y, Li B-l, Cheng Y-B, Peng Z-Y, Liu H, Xu H-J, Zhu Z-R, Lou Y-G, Cheng J-A, Zhang C-X (2010) Transcriptome analysis of the brown planthopper *Nilaparvata lugens*. PLoS One 5(12):e14233

Zhang F, Guo H, Zheng H, Zhou T, Zhou Y, Wang S, Fang R, Qian W, Chen X (2010) Massively parallel pyrosequencing-based transcriptome analyses of small brown planthopper (*Laodelphax striatellus*), a vector insect transmitting rice stripe virus (RSV). BMC Genomics 11(1):1–13