Insect Biochemistry and Molecular Biology 52 (2014) 33–50

Contents lists available at ScienceDirect

Insect Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/ibmb

Horizontal gene transfer and functional diversification of plant cell wall degrading polygalacturonases: Key events in the evolution of herbivory in beetles

Roy Kirsch [a, *], Lydia Gramzow [b], Günter Theißen [b], Blair D. Siegfried [c], Richard H. ffrench-Constant [d], David G. Heckel [a], Yannick Pauchet [a, **]

[a] Department of Entomology, Max Planck Institute for Chemical Ecology, Hans-Knoell-Str. 8, 07745 Jena, Germany
[b] Department of Genetics, Friedrich Schiller University Jena, Philosophenweg 12, 07743 Jena, Germany
[c] Department of Entomology, University of Nebraska, 312A Entomology Hall, Lincoln, 68583-0816 NE, United States
[d] Biosciences, University of Exeter, Penryn TR10 9EZ, United Kingdom

ARTICLE INFO

Article history:
Received 28 April 2014
Received in revised form
12 June 2014
Accepted 19 June 2014
Available online 28 June 2014

Keywords:
Horizontal gene transfer
Plant cell wall degradation
Glycoside hydrolase
Leaf beetles
Weevils

ABSTRACT

Plant cell walls are the largest reservoir of organic carbon on earth. To breach and utilize this carbohydrate-rich protective barrier, microbes secrete plant cell wall degrading enzymes (PCWDEs) targeting pectin, cellulose and hemicelluloses. There is a growing body of evidence that genomes of some herbivorous insects also encode PCWDEs, raising questions about their evolutionary origins and functions. Among herbivorous beetles, pectin-degrading polygalacturonases (PGs) are found in the diverse superfamilies Chrysomeloidea (leaf beetles, long-horn beetles) and Curculionoidea (weevils). Here our aim was to test whether these arose from a common ancestor of beetles or via horizontal gene transfer (HGT), and whether PGs kept their ancestral function in degrading pectin or evolved novel functions. Transcriptome data derived from 10 beetle species were screened for PG-encoding sequences and used for phylogenetic comparisons with their bacterial, fungal and plant counterparts. These analyses revealed a large family of PG-encoding genes of Chrysomeloidea and Curculionoidea sharing a common ancestor, most similar to PG genes of ascomycete fungi. In addition, 50 PGs from beetle digestive systems were heterologously expressed and functionally characterized, showing a set of lineage-specific consecutively pectin-degrading enzymes, as well as conserved but enzymatically inactive PG proteins. The evidence indicates that a PG gene was horizontally transferred ~200 million years ago from an ascomycete fungus to a common ancestor of Chrysomeloidea and Curculionoidea. This has been followed by independent duplications in these two lineages, as well as independent replacement in two sublineages of Chrysomeloidea by two other subsequent HGTs. This origin, leading to subsequent functional diversification of the PG gene family within its new hosts, was a key event promoting the evolution of herbivory in these beetles.

1. Introduction

Living plant cells enclose themselves in primary walls containing cellulose and hemicellulose fibers embedded in a pectin matrix (Cosgrove, 1997, 2005; Keegstra, 2010; McNeil et al., 1984; Willats et al., 2001). This complex polysaccharide network was likely a prerequisite for land plant evolution (Sorensen et al., 2010) as it provides structural integrity, intercellular signaling, cell–cell adhesion and mechanical protection (Carpita and Gibeaut, 1993; Hadfield and Bennett, 1998; Lagaert et al., 2009; Somerville et al., 2004). Plant cell walls represent the most abundant repository of organic carbon on earth (Gilbert, 2010), thus playing an important role in plant–heterotroph interactions (Bellincampi et al., 2004; Cervone et al., 1989; Cosgrove, 2005; Keegstra, 2010; Ridley et al., 2001). To breach this carbohydrate rich physical barrier and to utilize the energy stored in it, phytopathogenic or saprotrophic bacteria, fungi and protists secrete a multiplicity of plant cell wall degrading enzymes (PCWDEs). Most PCWDEs belong to various glycoside hydrolase (GH)

* Corresponding author. Tel.: +49 (0) 3641 571562; fax: +49 (0) 3641 57 1502.
** Corresponding author. Tel.: +49 (0) 3641 571507; fax: +49 (0) 3641 57 1502.
E-mail addresses: rkirsch@ice.mpg.de (R. Kirsch), ypauchet@ice.mpg.de (Y. Pauchet).

http://dx.doi.org/10.1016/j.ibmb.2014.06.008
0965-1748/

families targeting the polysaccharide network with cellulase, hemicellulase and pectinase activities (Gilbert, 2010).

Besides microbes, insects comprise the highest number of plant consuming species as they make up 25% of all known multicellular organisms, and nearly half feed on living plants (Schoonhoven et al., 2005; Strong et al., 1984). Insects have thus developed some adaptive strategies not available to microbes, i.e. grinding mandibles, alkaline gut pH and specific gut structures (Barbehenn, 1992; Hochuli, 1996; Watanabe and Tokuda, 2010). However the enzymatic cleavage of plant cell wall polysaccharides has long been regarded as the province of microbes, and it has been assumed that endogenous PCWDE activities are absent from herbivorous insects and are instead provided by their microbial symbionts (Martin, 1991). This view was challenged in 1998 when Watanabe et al. (1998) identified the first insect gene encoding a functional cellulase from a lower termite. Since then, advances in sequencing technology and bioinformatic analysis of genomes and transcriptomes have revealed many more endogenous PCWDE genes in different herbivorous insect lineages (Calderon-Cortes et al., 2012; Pauchet et al., 2010, 2009; Watanabe and Tokuda, 2010).

As endogenous PCWDE gene families in some groups of leaf- or wood-feeding insects are rare enough to have eluded discovery until recently, questions naturally arise concerning their origin and evolutionary history. Were they present in a distant common ancestor, and subsequently lost in most present-day insects except for certain specialized groups? Or is the current restriction to specialized groups due to horizontal gene transfer (HGT) from an unrelated source to a more recent common ancestor, thus accounting for their absence in most other insects?

Some PCWDEs are widespread enough to be fully consistent with the first scenario (Calderon-Cortes et al., 2012; Watanabe and Tokuda, 2010). Cellulases in the GH9 family are found in most insect orders and are thus considered to have been present in the most recent common ancestor of all insects (Davison and Blaxter, 2005). On the other hand, polygalacturonases (PGs) of the GH28 family (Henrissat, 1991; Jenkins and Pickersgill, 2001; Markovic and Janecek, 2001) have a much more restricted distribution in insects. Enzyme assays and transcriptome data indicate the presence of PGs in the orders Hemiptera and Coleoptera, the latter in superfamilies Chrysomeloidea (leaf beetles, long-horn beetles) and Curculionoidea (weevils) (Allen, 2007; Allen and Mertens, 2008; Celorio-Mancera et al., 2008, 2009; Girard and Jouanin, 1999; Hull et al., 2013; Kirsch et al., 2012; Pauchet et al., 2010, 2009; Shen et al., 1996). In the following we use the term “Phytophaga” for Chrysomeloidea plus Curculionoidea referring to a recent beetle phylogeny that revealed their sister group relationship (Marvaldi et al., 2009). To avoid confusion, the terms herbivorous and herbivory instead of phytophagous and phytophagy will be used in the text to describe plant feeding habits in general. Genome sequences from a weevil and a leaf beetle recently confirmed that PGs are encoded in their genomes (Keeling et al., 2013; Pauchet et al., 2014b). However, genome sequences of Hymenoptera, Lepidoptera and Diptera appear to lack PG genes ((Calderon-Cortes et al., 2012); *Drosophila* PG ESTs therein are due to contamination from beetle cDNA). Moreover, the genome sequence of the model beetle species *Tribolium castaneum* also lacks PG genes.

PGs cleave the 1,4-linkages of α-D-galacturonic acid in the homogalacturonan polymer (Markovic and Janecek, 2001), which is the main constituent of pectin in the primary cell wall (Mohnen, 2008; Ridley et al., 2001; Willats et al., 2006). Galacturonic acid in pectin can be esterified with methyl and acetyl groups. As a component of other pectic polysaccharides, it can be decorated with side branches of sugars or alternate with rhamnose in the polymer backbone (Mohnen, 2008). Thus esterases, lyases and rhamnogalacturonases also contribute to degrading pectins (Gilbert, 2010; Henrissat et al., 1995; Jayani et al., 2005; Schols et al., 1990). Degradation of pectin is necessary for further breakdown of other cell wall polysaccharides (Calderon-Cortes et al., 2012; De Lorenzo and Ferrari, 2002; Idnurm and Howlett, 2001). PGs and other pectin-degrading enzymes would thus be expected to play an important role in the exploitation of the plant cell wall by herbivorous insects, but little functional information is available and a comprehensive phylogenetic analysis is lacking.

Here we combine phylogenetic and functional approaches to investigate the origin and evolution of the PG gene family in insects. Previous work on a restricted set of beetle PGs implicated HGT from microbes, but only a limited diversity of potential donors was considered (Pauchet et al., 2010). We have extended this approach by comparing a much larger set of PG genes from 10 species from three beetle families in the context of PGs from heteropteran mirid bugs, nematodes, plants, bacteria and fungi. First, we examine the question of whether PGs found in the superfamilies Chrysomeloidea and Curculionoidea descended from an ancient common ancestor of beetles, or more recently by HGT from an evolutionarily unrelated source. Second, we explore functional diversification of the family by expressing 50 beetle PGs and screening them for different pectolytic activities. Our aim was thus to provide the first comprehensive phylogenetic as well as functional analysis of insect PGs to understand the importance of endogenous pectolytic enzymes for the evolution of herbivory in the hyper-diverse beetle superfamilies Chrysomeloidea and Curculionoidea.

2. Material and methods

2.1. Beetle transcriptomes, manual curation and full length sequencing of cDNAs

The generation of cDNA libraries and transcriptome sequencing for *Chrysomela tremula*, *Gastrophysa viridula*, *Callosobruchus maculatus*, *L. decemlineata*, *Sitophilus oryzae*, *Phaedon cochleariae* and *Apriona japonica* were previously described (Kirsch et al., 2012; Pauchet et al., 2014a, 2010, 2009). A normalized cDNA library was generated for larval *Diabrotica virgifera* guts and further sequenced by 454 pyrosequencing following the procedure described by Pauchet et al. (2009). Publicly available EST datasets for *Dendroctonus ponderosae* (185,437 ESTs) and *Pissodes strobi* (11,089 ESTs) were retrieved from the dbEST public database (NCBI) as FASTA files. These datasets were then assembled using the SeqMan Pro assembler of the Lasergene software package v10.2.1 (DNASTAR, Madison, USA) with the following program parameters: match size, 50 bp; minimum match percentage, 80%; minimum sequence length, 40 bp; gap length penalty, 0.70 and maximum mismatch end bases, 15.

Contigs corresponding to sequences of interest, which were not already published, were retrieved from the above described transcriptomes. From these contigs, the sequences of cDNAs encoding full-length transcripts were confirmed by designing specific primers used to re-amplify the complete open reading frame (ORF). cDNA sequences encoding only a partial ORF were used to design specific primers to perform 5′- and 3′-Rapid Amplification of cDNA ends (RACE) PCRs. For these we used the SMARTer RACE cDNA Amplification Kit (BD Clontech), according to the manufacturer's instructions. The resulting PCR products were then cloned into pCR2.1 TOPO/TA vector (Invitrogen) for sequencing. Finally, all full-length cDNA sequences were annotated and submitted to Genbank under accession numbers KF724628–KF724640 (Table S1).

2.2. Phylogeny reconstruction

PG sequences were obtained from transcriptome data, the literature (Danchin et al., 2010; Markovic and Janecek, 2001;