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RESEARCH ARTICLE

Transcriptome profiling suggests roles of innate immunity and digestion metabolism in purplish Washington clam

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Abstract

The purplish Washington clam (*Saxidomus purpuratus*) in the family Veneridae is distributed widely along the intertidal zones of northeast Asia and is increasingly being utilized as a commercially important food resource. Bivalves maintain homeostasis by regulating their food intake and digestion, innate immunity, and biotransformation in a mollusk-specific organ, the digestive gland. To understand digestive gland-specific pathways, we generated a high-quality de novo assembly of the digestive gland transcriptome of this clam using the Illumina Miseq platform. A total of 9.9 million raw reads were obtained and assembled using the Oases assembly platform, resulting in 27,358 contigs with an N50 of 433 bp. Functional gene annotations were performed using Gene Ontology, Eukaryotic Orthologous Groups, and Kyoto Encyclopedia of Genes and Genomes pathway analyses. In the transcriptome, many crucial genes involved in innate immunity and digestion metabolism were detected. A number of enzymes associated with drug metabolism were annotated, as much as that identified from the whole transcriptome of the Pacific oyster *Crassostrea gigas*. We provide valuable sequence information of *S. purpuratus* to predict functional understandings of the bivalve-specific digestive gland. This resource will be valuable for researchers comparing gene compositions and their expression levels in the digestive glands of bivalves.

Keywords Washington clam · *Saxidomus purpuratus* · Transcriptome · Digestive gland · Bivalve

Introduction

Most bivalves (e.g., clams, mussels, oysters, scallops) are filter feeders and generally ingest suspended nutrients and food particles from water bodies and/or sediments. The bivalve digestive gland is an invertebrate analogue of the vertebrate liver and consists of digestive and basophilic cells, which line numerous blind-end epithelium tubules (Cajaraville et al. 1990; Dimitriadis et al. 2004). The organ undergoes a number of daily and annual changes that are affected by cyclic environmental conditions (e.g., seasonal change and tidal rhythm) (Morton 1971; Owen 1974). The digestive tract is a key metabolic organ due to its crucial role in the secretion of digestive enzymes, intracellular digestion, storage of waterborne molecules, excretion of endogenous metabolites, and innate immunity (Livingstone et al. 1992). There are two specialized cell types (digestive cells and immunocytes) that maintain homeostasis and key physiological functions independently or synergistically in the organ. The digestive cells develop a lysosomal system for intra-cellular digestion and nutrient accumulation for gametogenesis, while immunocytes are responsible for endocytosis and phagocytosis

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of numerous endogenous and exogenous molecules (Canesi et al. 2012). Hence, bivalve digestive glands are utilized widely in the biomonitoring of environmental perturbations. Beyond the general nutritive function of the organ, the vast majority of relevant literature has focused on the bioaccumulation and metabolism of aquatic hazardous elements in bivalve digestive glands, as numerous waterborne pollutants can be absorbed directly into the organ and metabolized by diverse enzyme systems (Marigómez and Baybay-Villacorta 2003).

Veneridae is a large family of marine bivalve mollusks consisting of 12 subfamilies and more than 500 living species (Keen 1969). Most species are edible, and many of them are commercially important, contributing significantly to global mariculture. *Saxidomus purpuratus* (Veneroida: Veneridae), commonly referred to as the purplish Washington clam, is an edible marine bivalve species. Where the geographical distribution of the genus *Saxidomus* spans the globe, *S. purpuratus* is only found in the intertidal zones of northeast Asia (e.g., coastal regions of South Korea, Japan, and China), and it has a high economic value. Thus, this species is a promising model organism for characterizing the potential impact of environmental changes and anthropogenic activities. Although genomic studies have been performed in several model bivalves, studies on the genomics of *S. purpuratus* remain scarce, except for the documentation of its entire mitochondrial genome (Bao et al. 2016).

This study analyzed the digestive gland transcriptome of purplish Washington clam using basic bioinformatics tools. Genomic information on *S. purpuratus* will allow future investigation of digestive gland-specific pathways in bivalves, with a particular focus on the metabolism of nutrients, endogenous and exogenous metabolites, and innate immunity.

Materials and methods

Sample collection and RNA sequencing

One specimen (34.7 mm in length) of *S. purpuratus* was collected from the coastal region of Jumunjin (37°54′27.23″N, 128°49′38.95″E; South Korea) (Suppl. Table 1). The region was not polluted by organic contaminants; e.g., polychlorinated biphenyls (PCBs; total concentrations of 21 congeners) and polycyclic aromatic hydrocarbons (PAHs; total concentrations of 14 compounds) were 0.08 ± 0.04 ng g^{-1} and 18.54 ± 9.17 ng g^{-1}, respectively, in May 2017. Previously, the concentrations of environmental pollutants such as PCBs (0.10 ± 0.14 ng g^{-1}), dichlorodiphenyltrichloroethanes (DDTs; 0.04 ± 0.07 ng g^{-1}), PAHs (34.98 ± 23.68 ng g^{-1}), tributyltin (TBT: 3.76 ± 7.20 ng Sn g^{-1}), and polychlorinated dibenzodioxins (PCDD)/Fs (0.09 ± 0.07 pg-TEQ g^{-1}) measured at the Jumunjin coast were found to be lower than the mean value in Korean coastal waters (2.26 ± 2.01 for PCBs; 1.06 ± 0.46 for DDTs; 302.95 ± 97.31 for PAHs; 69.03 ± 61.58 for TBT; 2.43 ± 0.53 for PCDD)/Fs) (Choi et al. 2011). In addition, a recent study showed that most metals measured in Jumunjin (i.e., As, Cd, Cr, Cu, Hg, Pb, Zn) were relatively lower than the mean value in Korean coastal waters (Hwang et al. 2016). Thus, we assumed that the Jumunjin area was not polluted by environmental pollutants when we sampled *S. purpuratus*.

Species identification was confirmed by assessing morphological characteristics and the mitochondrial cytochrome oxidase subunit 1 (*CO1*) sequence. The digestive gland tissue was sampled immediately, transferred to RNAlater (Qiagen Inc., Valencia, CA, USA), and stored at − 20 °C until RNA extraction. Total RNA was extracted using the RNeasy® Micro Kit (Qiagen) according to the manufacturer's protocol. Extracted RNA was stored in RNA stable® (Biometrica, San Diego, CA, USA) to prevent RNA degradation. Total RNA was qualified and quantified using the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). High-quality mRNA was isolated from 2 µg total RNA for double-stranded cDNA library construction by poly A selection. NuGEN Encore® Complete RNA-Seq Library Systems (NuGEN, San Carlos, CA, USA) was employed for the construction of paired-end libraries (PE500) with sheared cDNA consisting of 500 bp fragments. The Illumina MiSeq System platform was used for library sequencing with 300 × 2 paired-end reads using a MiSeq™ Reagent Kit ver. 3 (Illumina, San Diego, CA, USA). Index sequencing primer and adaptor sequences were trimmed using Trimmomatic (Bolger et al. 2014), and low-quality reads were removed using the FASTX tool kit (Gordon and Hannon 2010) with the parameters -t 20, -l 70, and -Q 33.

De novo assembly

Sequencing with the Illumina MiSeq System platform produced 9.9 million (M) reads for the digestive gland tissue of *S. purpuratus*. Low-quality reads (an average quality score < 10), adapters, linkers, and PCR primers were omitted from the dataset during the quality-filtering step. Prokaryotic DNAs (e.g., bacteria, archea, viruses) were detected by Glimmer (Delcher et al. 2007) and removed from the raw data. After quality-filtering, the high-quality reads were assembled de novo using Oases software (ver. 0.2.08; Schulz et al. 2012) with the default parameters. TransDecoder (http://transdecoder.github.io) was used to identify coding regions from the assembled dataset. The unclustered transcripts and the longest sequences were considered unigenes. All unigenes were aligned against the NCBI non-redundant (NR) database using the BLASTx program with an E-value

threshold 1.00E−04 for the identification of functional transcripts.

Transcriptome annotation

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of the contigs were performed using the Blast2GO sequence annotation tool (ver. 4.0; Conesa et al. 2005). The specific GO term composition of each category was calculated and presented as a percentage at level 2. Three main categories (biological process, cellular component, and molecular function) were analyzed using the default parameters after aligning the contigs. KEGG results were compared directly with the results of the whole transcriptome of the Pacific oyster (*Crassostrea gigas*) registered with the NCBI NR database. The assembled data were arranged including read length, gene annotation, GenBank number, E-value, species, and the species accession number. The mRNA expression level of the *S. purpuratus* digestive gland transcriptome was calculated using the reads per kilobase transcriptome per million mapped reads (RPKM) method (Mortazavi et al. 2008).

Results

RNA sequencing and de novo assembly

We performed RNA-seq with the digestive gland of purplish Washington clam using the Illumina RNA-seq platform. Approximately 9.9 million raw reads with 52% GC content were obtained from the specimen. The results indicated a total of 27,358 contigs with an average length of 411 bp and N50 length of 433 bp (Suppl. Table 2). Based on BLAST searches and functional domain annotation with InterProScan in the Blast2GO software, a total of 14,874 contigs were assigned to at least one GO term (Suppl. Tables 3–5). The obtained raw RNA-seq data were deposited in the NCBI Sequence Read Archive (SRA; accession number SRP133745) under bioproject number PRJNA436489.

In silico functional annotation

The overall distribution of top BLAST hit species indicated that approximately 4380 contigs of *S. purpuratus* showed sequence similarity to the transcripts of *C. gigas*, followed by 1891 transcripts of the owl limpet (*Lottia gigantean*) (Fig. 1). Among the top-hit species, 79% and 8% of transcript contigs showed homology to the phyla Mollusca and Annelida, respectively. All of the results of GO analyses were analyzed over the second level. In the biological process category, many genes were categorized as cellular processes (20%), metabolic processes (16%), and single-organism processes (13%) (Fig. 2a). In the molecular function category, most genes were related to binding (46%), followed by catalytic activity (36%) (Fig. 2b). The vast majority of genes in the cellular component category were involved in cells (37%) and organelles (30%) (Fig. 2c).

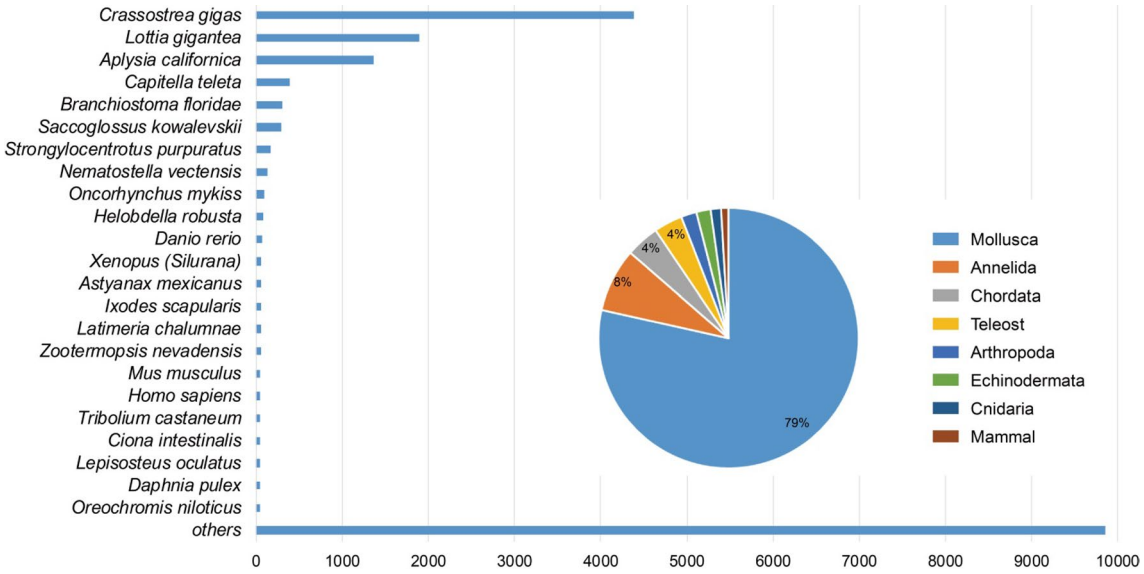

Fig. 1 Number of top BLAST-hit species matched to the *Saxidomus purpuratus* transcripts corresponding to phylum and species levels. Each number represents the number of orthologous gene families shared by the indicated genomic database

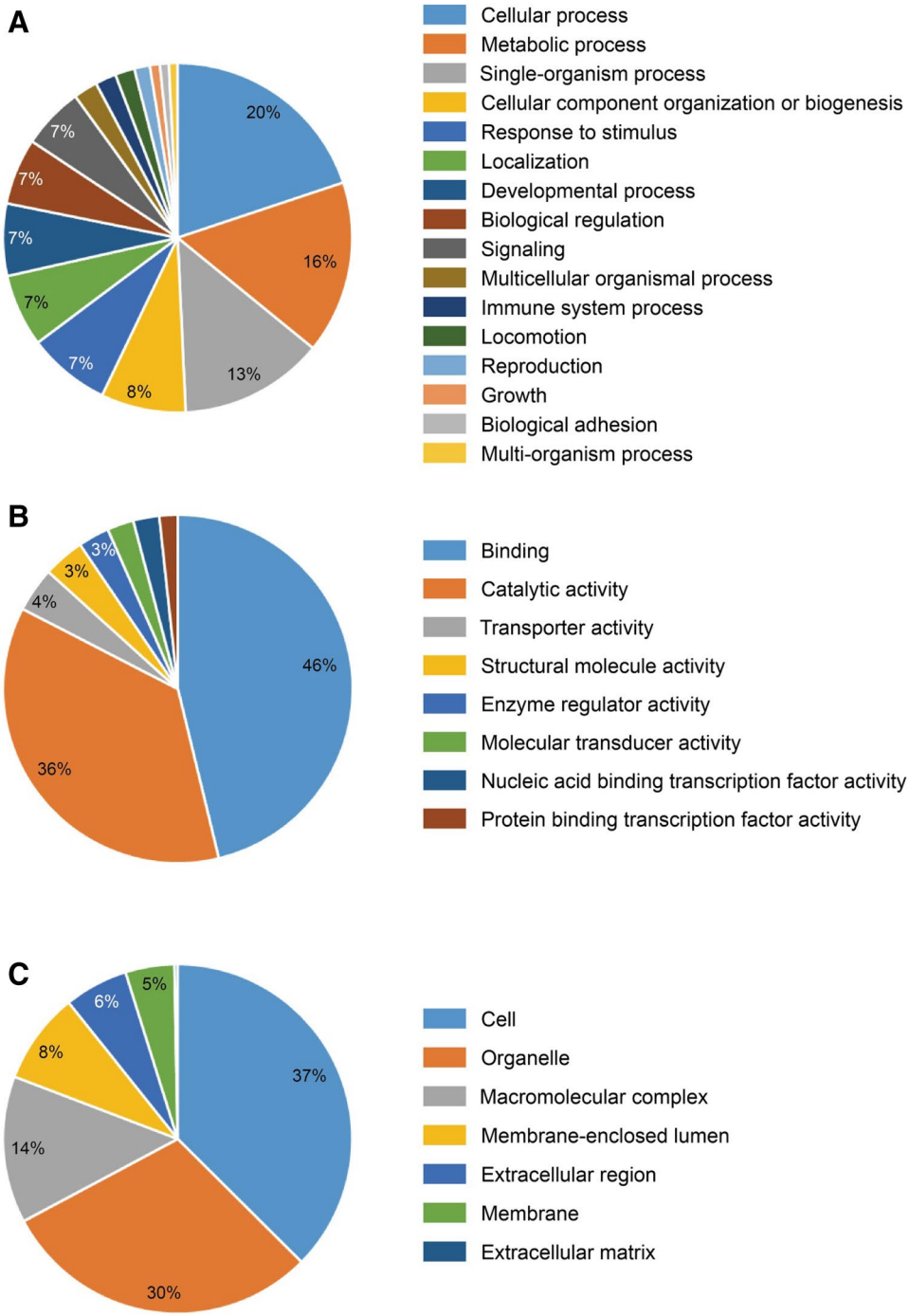

Fig. 2 Analysis of Gene Ontology (GO) in terms of **a** biological process, **b** cellular component, and **c** molecular function that are enriched in the digestive gland transcriptome of *Saxidomus purpuratus*

Prediction of digestive gland-specific metabolisms based on KEGG pathway analysis

In the *S. purpuratus* digestive gland transcriptome, we retrieved annotated sequences of many innate immune system and digestive enzymes, although our dataset does not represent a full complement of the transcripts. Of the innate immune system sequences, four major signal pathways corresponded to vertebrate canonical signaling: NF-κB (KEGG pathway number: 04064), Toll-like receptor (TLR; #04620), JAK-STAT (#04630), and TNF (#04668). Transcriptional involvement of immune-relevant genes on each pathway are provided in Suppl. Table 6. When the highly expressed subpathways from each major pathway were merged (Fig. 3), the most crucial members of innate immune pathways were mapped in the *S. purpuratus* digestive gland.

KEGG analyses revealed that the most vital members involved in the production of digestive enzymes (e.g.,

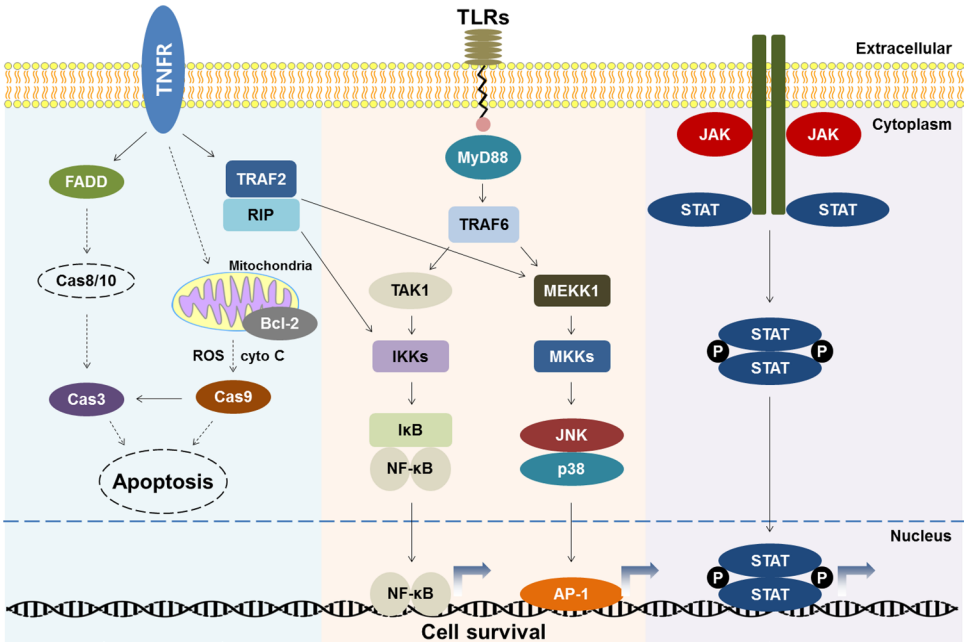

Fig. 3 Schematic diagram of innate immune pathways observed in the digestive gland transcriptome of *Saxidomus purpuratus*. Information on full components and their involvement in each pathway is provided in Suppl. Table 6. Arrow indicates the direction of signaling routes. Absence of transcript or pathway is marked with dashed line or circle

protease, lipase, amylase; Suppl. Table 2) as well as the absorption of food molecules were annotated: salivary secretion (#04970), gastric acid secretion (#04971), pancreatic secretion (#04972), and bile secretion (#04976) (Fig. 4, Suppl. Table 7).

In the case of drug metabolism, we directly compared three major pathways, metabolism of xenobiotics by CYP (#00980), drug metabolism-CYP (#00982), and drug metabolism-other enzyme (#00983), to those annotated from the whole transcriptome of *C. gigas* (http://www.genome.jp/kegg-bin/show_organism?menu_type=pathway_maps&org=crg). Members involved in each pathway and their composition patterns were very similar between the two bivalves (Fig. 5, Suppl. Table 8).

Discussion

The overall sequencing quality and de novo assembly parameters (i.e., average read size, largest length, gene density, and GC ratio) are comparable with the de novo assembly information of previously published bivalves (Gerdol et al. 2014; Zhao et al. 2014; Moreira et al. 2015; Lim et al. 2016; Kim et al. 2017). The overall distribution of top BLAST-hit species suggests that sample preparation and sequencing were conducted successfully in *S. purpuratus*. Numerous GO assignments evaluated via Blast2GO analyses suggest a complex biological function of the digestive gland. The bivalve digestive gland tissue is exposed consistently to numerous endogenous and/or exogenous molecules. Thus, we assume that these challenges may trigger a diverse gene composition for maintaining physiological homeostasis.

The four major innate immunity signaling pathways are important for responding to exogenous pathogens and maintaining homeostasis in bivalves (Venier et al. 2011). KEGG analyses suggested that the four immune system pathways were actively expressed in the organ and might serve as a host defense modulator against exogenous infections (e.g., bacteria and other pathogens). In particular, signal pathways from the TLRs to the NF-κB/IκB complex or JNK/p38-mediated AP1 regulation via the MyD88-dependent pathway and JAK-STAT were highly conserved in the gland, while we were not able to determine the signal pathway from TNFR to apoptosis due to transcriptional absence of caspase proteins (e.g., caspase 8 and caspase 10). Because the organ used in this study was not influenced by environmental factors or infection, further study with immune challenges would be helpful to measure the response and sensitivity of the digestive gland.

This gland plays a central role in metabolism by aiding the intracellular digestion of waterborne food sources through the synthesis of numerous enzymes and secretions. In invertebrates, numerous enzymes and molecules are secreted by different organs (e.g., bile from the liver, intestine, pancreas, salivary gland, stomach), while their secretion for digestion is far less localized. The bivalve organ, normally referred to as the hepatopancreas, is multifunctional: it corresponds with the functions performed using distinct organs in the mammalian body (e.g., liver and pancreas). In fact, the metabolic processes involved in digestive function and secretion are complex in bivalves, and are controlled by two or more organs, such as the digestive gland and salivary glands. However, studies on the estimation of enzymatic activity or gene expression have suggested that

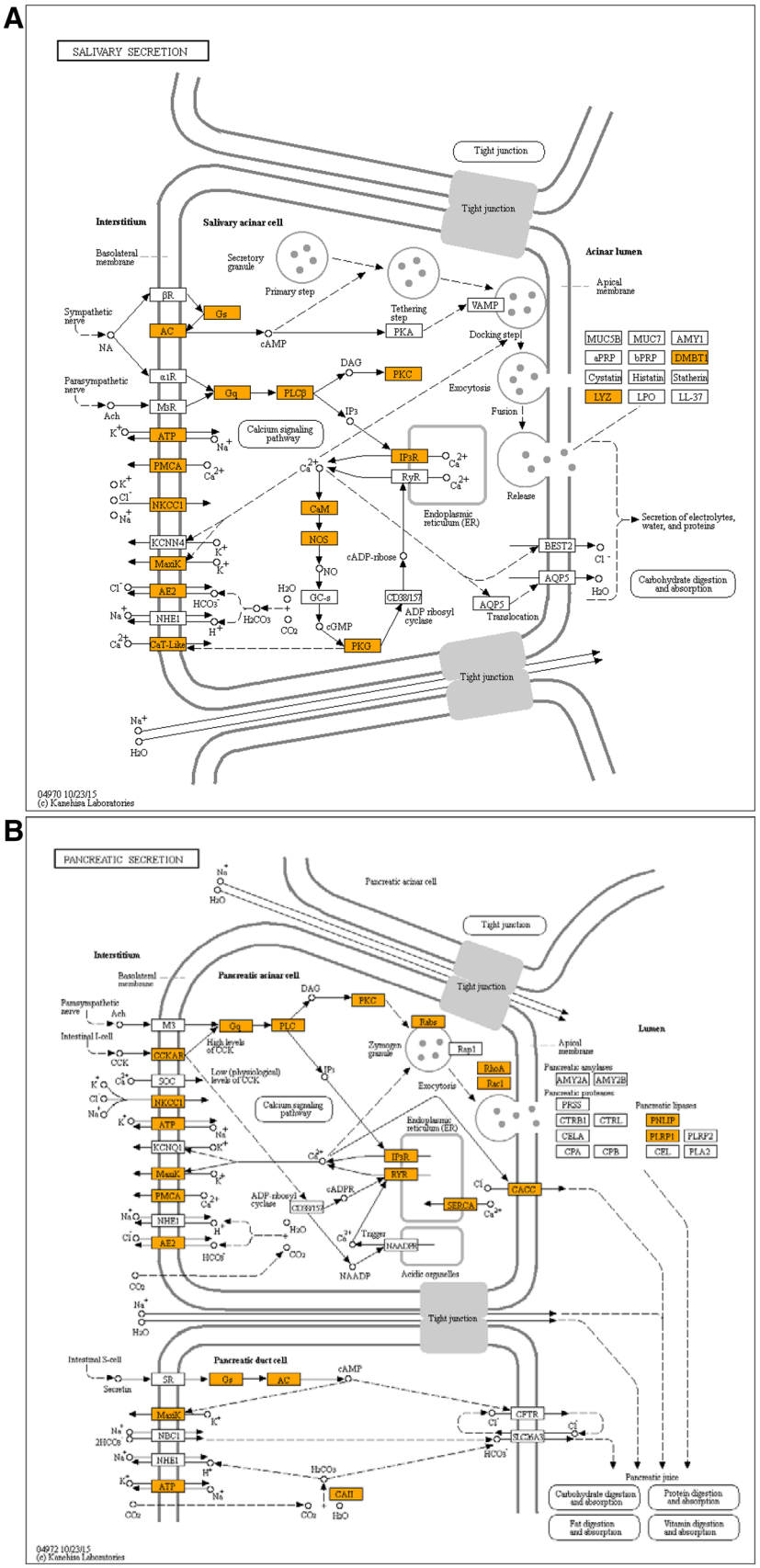

Fig. 4 Transcriptional coverage of *Saxidomus purpuratus* transcripts on the vertebrate salivary secretion (#04970) and pancreatic secretion (#04972) signaling pathways. Matched homologues are highlighted with lighter shade of orange color. (Color figure online)

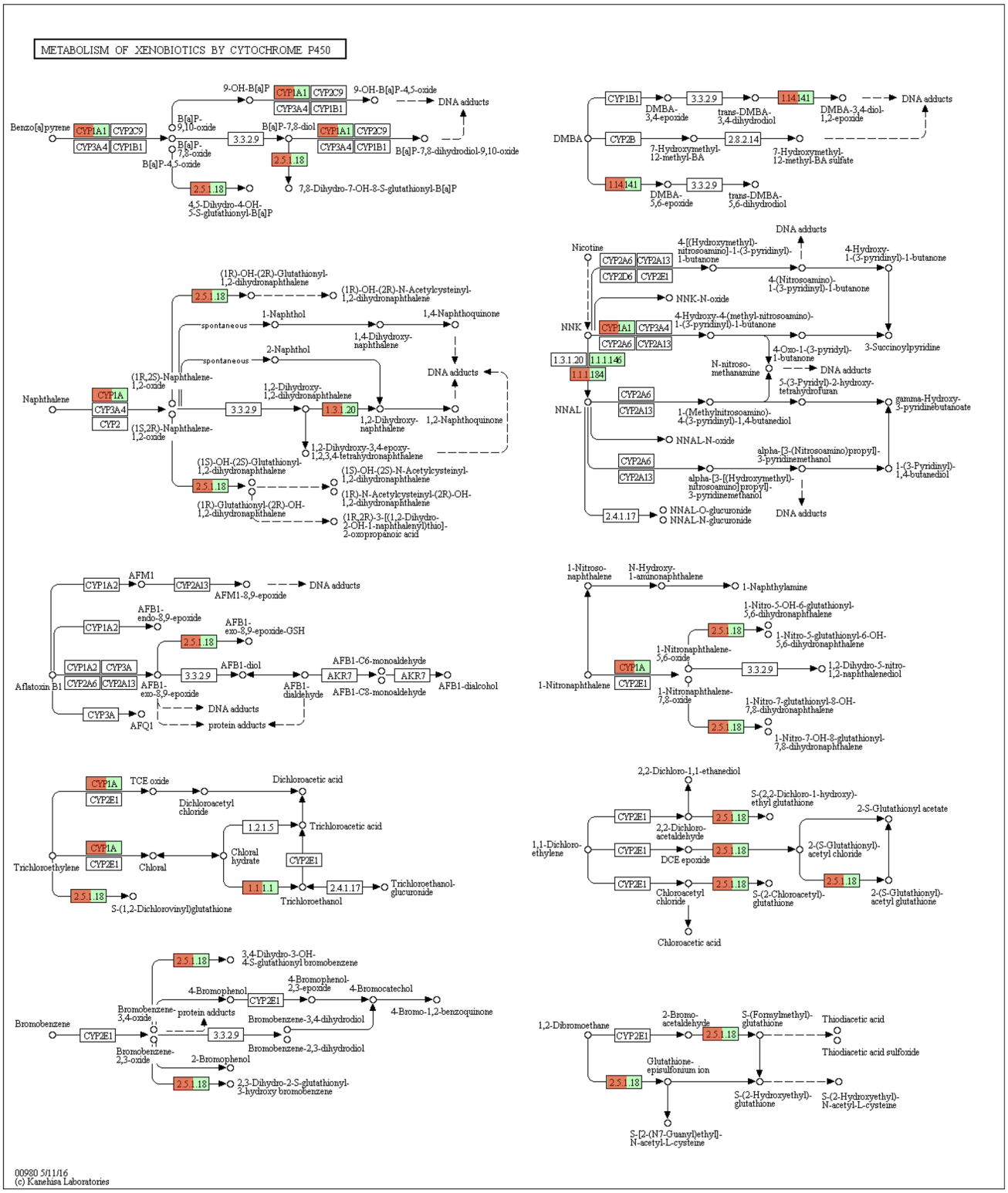

Fig. 5 Transcriptional coverage of *Saxidomus purpuratus* transcripts on the KEGG pathway (#00980), entitled with metabolism of xenobiotics by CYP, and comparative analysis with the mapped transcripts from entire transcriptome of the Pacific oyster, *Crassostrea gigas*. Pathway components with homologues (green color) in the *C. gigas* transcriptome are highlighted in a lighter shade of red. (Color figure online)

the organs share many functions via connecting ducts (Owen 1974; Fretter and Graham 1994; Barkalova et al. 2016; Ponte and Modica 2017). Several functions such as receptor activation in gland cells, ion channels, and membrane potential controls, and the secretion of enzymes, ions, and fluids would be employed commonly throughout the digestive tract. Many digestive enzymes, peptidases, proteinases, and acids are secreted from salivary glands. Bile secretion is involved in the absorption and digestion of fatty acids and fats. Given that most important genes were matched to vertebrates' major digestive system as analyzed by KEGG, we suppose that a conserved digestive mechanism with key regulators is likely conserved in the bivalve digestive gland at the molecular level. Taken together, these results suggest the intactness and high quality of the *S. purpuratus* digestive gland transcriptome corresponding to its basic function.

CYPs are one of the largest gene families and play an important role in the molecular detoxification mechanism of the phase I oxidative metabolic system (Guengerich 2008). In the pathway of metabolism of xenobiotics by CYP (#00980), entire members for the matched *C. gigas* genes were observed in the *S. purpuratus* digestive gland transcriptome, except for one member of nicotine metabolism. Thus, our digestive gland transcriptome would be sufficient to cover the essential genes involved in drug metabolism, as a similar composition was observed in the entire set of *C. gigas* transcriptomes. Interestingly, one contig was matched to the vertebrate homolog *CYPA1* in both bivalves at default parameters. In invertebrates, transcription factor-mediated regulation of the *CYP1* family has not been investigated thoroughly yet due to absence of the CYP1 family, while several *CYP1-like* genes or other relevant *CYPs* of aquatic invertebrates including bivalves have been reported to be clustered with vertebrate *CYP1* (Yadetie et al. 2012; Kim et al. 2013; Zanette et al. 2013). Thus, we assume that a potential compensatory role of the *CYP1-like* genes would be regulated via a xenobiotic metabolism in bivalves.

Conclusion

Library construction and assembly were successfully conducted with the digestive gland of *S. purpuratus*, although the digestive gland-specific sequence information at the transcription level provides a limited view of the whole complement of transcripts, which are expressed in other tissues. However, our transcriptome data are sufficient to provide sequence information to predict functional understandings of the bivalve-specific digestive gland. RNA-seq is a good source to obtain novel gene information from non-model animals. The digestive gland transcriptome is likely the best resource for future comparative studies across bivalve species, as well as a reference for bivalve-specific high-throughput mRNA expression profiling.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflicts of interest.

Ethical approval All animal handling and experimental procedures were approved by the Animal Welfare Ethical Committee and Animal Experimental Ethics Committee of Korea Polar Research Institute (KOPRI, Incheon, South Korea).

References

Bao X, He C, Gao X, Li Y, Gao L, Jiang B, Liu W (2016) The complete mitochondrial genome of *Saxidomus purpuratus* (Veneroida: Veneridae). Mitochondrial DNA A 27:3648–3649

Barkalova VO, Fedosov AE, Kantor YI (2016) Morphology of the anterior digestive system of tonnoideans (Gastropoda: Caenogastropoda) with an emphasis on the foregut glands. Molluscan Res 36:54–73

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120

Cajaraville MP, Diez G, Marigoḿez I, Angulo E (1990) Responses of the basophilic cells of the digestive gland of mussels to petroleum hydrocarbon exposure. Dis Aquat Org 9:221–228

Canesi L, Ciacci C, Fabbri R, Marcomini A, Pojana G, Gallo G (2012) Bivalve molluscs as a unique target group for nanoparticle toxicity. Mar Environ Res 76:16–21

Choi HG, Moon HB, Choi M, Yu J (2011) Monitoring of organic contaminants in sediments from the Korean coast: spatial distribution and temporal trends (2001–2007). Mar Pollut Bull 62:1352–1361

Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21:3674–3676

Delcher AL, Bratke KA, Powers EC, Salzberg SL (2007) Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23:673–679

Dimitriadis VK, Domouhtsidou GP, Cajaraville MP (2004) Cytochemical and histochemical aspects of the digestive gland cells of the mussel *Mytilus galloprovincialis* (L.) in relation to function. J Mol Histol 35:501–509

Fretter V, Graham A (1994) British prosobranch molluscs. Ray Society, London

Gerdol M, De Moro G, Manfrin C, Milandri A, Riccardi E, Beran A, Venier P, Pallavicini A (2014) RNA sequencing and de novo assembly of the digestive gland transcriptome in *Mytilus galloprovincialis* fed with toxinogenic and non-toxic strains of *Alexandrium minutum*. BMC Res Notes 7:722

Gordon A, Hannon G (2010) Fastx-toolkit. FASTQ/A short-reads pre-processing tools. http://hannonlab.cshl.edu/fastx_toolkit/ (unpublished)

Guengerich FP (2008) Cytochrome p450 and chemical toxicology. Chem Res Toxicol 21:70–83

Hwang DW, Kim SG, Choi M, Lee IS, Kim SS, Choi HG (2016) Monitoring of trace metals in coastal sediments around Korean Peninsula. Mar Pollut Bull 102:230–239

Keen AM (1969) Superfamily Veneracea. In: Moore RC (ed) Treatise on invertebrate paleontology. Geological Society of America and University of Kansas Press, Lawrence, pp 670–690

Kim R-O, Kim B-M, Jeong C-B, Nelson DR, Lee J-S, Rhee J-S (2013) Expression pattern of entire cytochrome P450 genes and response of defensomes in the benzo[*a*]pyrene-exposed monogonont rotifer *Brachionus koreanus*. Environ Sci Technol 47:13804–13812

Kim B-M, Kim K, Choi I-Y, Rhee J-S (2017) Transcriptome response of the Pacific oyster, *Crassostrea gigas* susceptible to thermal stress: a comparison with the response of tolerant oyster. Mol Cell Toxicol 13:105–113

Lim H-J, Kim B-M, Hwang IJ, Lee J-S, Choi I-Y, Kim Y-J, Rhee J-S (2016) Thermal stress induces a distinct transcriptome profile in the Pacific oyster *Crassostrea gigas*. Comp Biochem Physiol D 19:62–70

Livingstone D, Lips F, Martnez P, Pipe R (1992) Antioxidant enzymes in the digestive gland of the common mussel (*Mytilus edulis* L.). Mar Biol 112:265–276

Marigómez I, Baybay-Villacorta L (2003) Pollutant-specific and general lysosomal responses in digestive cells of mussels exposed to model organic chemicals. Aquat Toxicol 64:235–257

Moreira R, Pereiro P, Canchaya C, Posada D, Figueras A, Novoa B (2015) RNA-Seq in *Mytilus galloprovincialis*: comparative transcriptomics and expression profiles among different tissues. BMC Genom 16:728

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

Morton B (1971) The daily rhythm and the tidal rhythm of feeding and digesting in *Ostrea edulis*. Biol J Linn Soc 3:329–342

Owen G (1974) Feeding and digestion in the bivalvia. Adv Comp Physiol Biochem 5:1–35

Ponte G, Modica MV (2017) Salivary glands in predatory mollusks: evolutionary considerations. Front Physiol 8:580

Schulz MH, Zerbino DR, Vingron M, Birney E (2012) Oases: robust de novo RNA-seq assembly across the dynamic range of expression levels. Bioinformatics 28:1086–1092

Venier P, Varotto L, Rosani U, Millino C, Celegato B, Bernante F, Lanfranchi G, Novoa B, Roch P, Figueras A et al (2011) Insights into the innate immunity of the Mediterranean mussel *Mytilus galloprovincialis*. BMC Genom 12:69

Yadetie F, Butcher S, Førde HE, Campsteijn C, Bouquet JM, Karlsen OA, Denoeud F, Metpally R, Thompson EM, Manak JR, Goksøyr A, Chourrout D (2012) Conservation and divergence of chemical defense system in the tunicate *Oikopleura dioica* revealed by genome wide response to two xenobiotics. BMC Genom 13:55

Zanette J, Jenny MJ, Goldstone JV, Parente T, Woodin BR, Bainy AC, Stegeman JJ (2013) Identification and expression of multiple *CYP1-like* and *CYP3-like* genes in the bivalve mollusk *Mytilus edulis*. Aquat Toxicol 128/129:101–112

Zhao X, Yu H, Kong L, Liu S, Li Q (2014) Comparative transcriptome analysis of two oysters, *Crassostrea gigas* and *Crassostrea hongkongensis* provides insights into adaptation to hypo-osmotic conditions. PLoS ONE 9:e111915