Identification and characterization of carboxyl esterases of gill chamber-associated microbiota in the deep-sea shrimp *Rimicaris exoculata* using functional metagenomics

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Supplemental Material:

Supplemental Materials and Methods

Supplemental Figures

Supplemental Materials and Methods

Substrate fingerprints. The following ester substrates where used to examine the reactivity profiles: *p*NP-acetate, *p*NP-propionate, *p*NP-butyrate, *p*NP-valerate, *p*NP-octanoate, *p*NP-decanoate, *p*NP-myristate, triacetin, tripropionin, tributyrin, tricaprin, (*R*)-menthyl acetate, caproyl acetate, ethyl 4-bromobutyrate, γ-butyrolacton, methyl (±)-α-bromophenylacetate, methyl 2-bromopropionate, methyl bromoacetate, methyl chloroacetate, methyl-(*D*)-lactate, methyl-(*L*)-lactate, methyl-3-Br-propionate, tri-*O*-acetyl-D-glucal, vinyl benzoate, α-D-glucose pentaacetate, methyl-(*R*)-mandelate, (*R*)-glycidyl 4-nitrobenzoate, methyl-2-Br-2-butenoate, methyl-(*R*)-3-Br-2-methyl propionate, ethyl chloroacetate, (*S*)-glycidyl 4-nitrobenzoate, methyl-(*S*)-mandelate, ethyl bromoacetate, methyl-2-Br-propionate, ethyl fluoroacetate, methyl-(*S*)-3-Br-2-methyl propionate, phenyl acetate, ethyl-2-bromopropionate, methyl α-bromoisobutyrate, ethyl iodoacetate, α-naphthyl acetate, α-naphthyl propionate, α-naphthyl butyrate, methyl glycolate, methyl-2-chloropropionate, methyl-2-chloro-3-hydroxypropionate, ethyl-4-bromobutyrate, methyl butyrate, ethyl-3-bromopropionate, methyl-2-Br-caproate, ethyl-trans-cinnamate, ethyl-α-isobromobutyrate, butyl acetate, methyl benzoate, ethyl trifluoroacetate, ethyl tribromoacetate, ethyl (*S*)-(−)-4-chloro-3-hydroxybutyrate, methyl-3-bromopropionate, methyl 4-bromobenzoate, 2-bromoethyl acetate, ethyl propionylacetate, ethyl octanoate, ethyl hexanoate, ethyl butyrate, propyl acetate, isopropenyl acetate, vinyl decanoate, ethyl benzoate, geranyl acetate, methyl cinnamate, methyl trans-cinnamate, methyl *p*-coumarate, methyl sinapinate, tert-butyl cinnamate, isobutyl cinnamate, phenethyl cinnamate, vinyl cinnamate, (*R*)-(−)-glycidyl butyrate, (*S*)-(+)-glycidyl butyrate, butyl acetate, methyl octanoate, methyl 2,3-dibromo propionate, ethyl decanoate, methyl-3-hydroxybutyrate, N-benzyl-(*L*)-proline ethyl ester, methyl ferulate, E-3-b-2(bromoethyl)propionate, ethyl 2-chloropropionate, vinyl laurate, ethyl 4-chloroacetoacetate, methyl pyruvate, vinyl acetate, vinyl crotonate, vinyl methacrylate, vinyl pivalate, (-)-ethyl-(*L*)-lactase, ethyl caproate, ethyl caprylate, vinyl butyrate, vinyl propionate, ethyl acetoacetate, ethyl-2-ethylacetoacetate, ethyl-2-methylacetoacetate, ethyl-3-oxohexanoate, ethyl-propionylacetate, HOPHD (2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate), HOHD (2-hydroxy-6-oxohepta-2,4-dienoate), (+)-methyl (*R*)-2-chloropropionate, (-)-methyl (*S*)-2-chloropropionate, γ-valerolacton, dimethyl phthalate, methyl phenanthrene-3-carboxylate, anthracene-2-carboxylic acid methyl ester, anthracene-9-carboxylic acid methyl ester, 2,5-dihydroxycinnamic acid methyl ester, naphthalene carboxylic acid methyl ester, 3-hydroxy-naphthalene-2-carboxylic acid methyl ester, naphthalene-1-carboxylic acid phenyl ester, protocatechuic acid ethyl ester, naphthalene-2,3-dicarboxylic acid diethyl ester, naphthalene-1,4,5,8-tetracarboxylic acid tetramethyl ester, methyl

phenanthrene-9-carboxylate, benzoic acid 4-formyl-phenylmethyl ester, benzoic acid 2-benzoyl-methyl ester, benzyl butyl phthalate, Propyl propionate, (*S*)-menthyl acetate, (*R*)-panto-lactone, (*S*)-(+)-pantolactone, (*S*)-neomenthylacetate, and (*R*)-neomenthylacetate.

FIG S1 A Coomassie-stained SDS-PAGE gel showing the purity level of proteins as overexpressed in the active form in *E. coli*. As shown, purity higher than 98% after a single His_6-tag purification step was obtained. Abbreviation: MW, theoretical molecular weight marker.

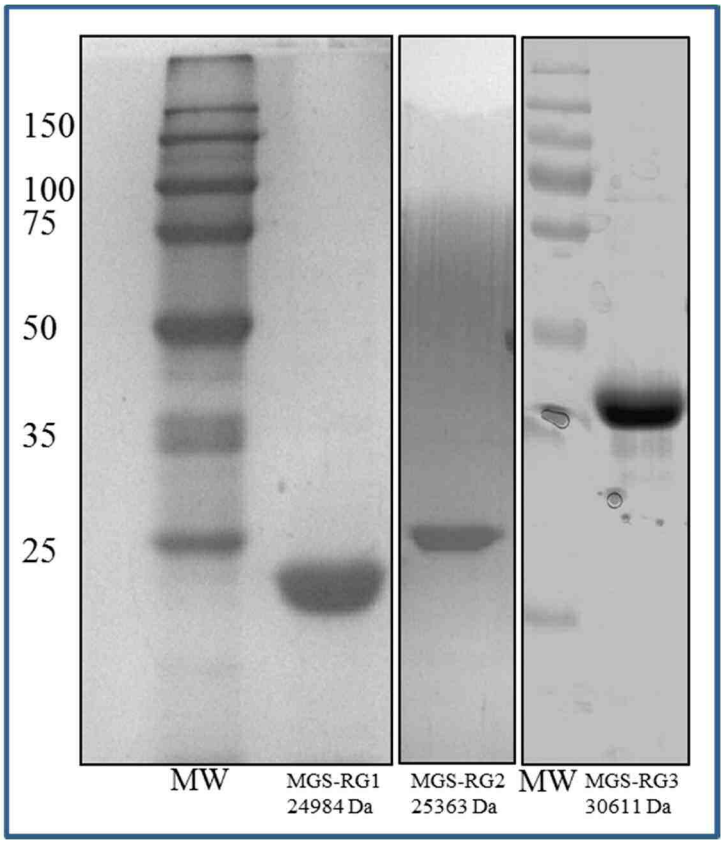

FIG S2 The phylogenetic relationship of the carboxyl esterases from the gill chamber of *R. exoculata*. The unrooted tree was constructed based on multiple sequence alignment using MUSCLE program by Poisson method with 1,000 bootstrap replicates. Sub-families of esterase/lipase are marked at the right part of the tree. Sequences of the enzymes used for comparative purposes in this study are also depicted in the tree and are marked in bold. MGS-RG enzymes are also marked in bold shadowed font.

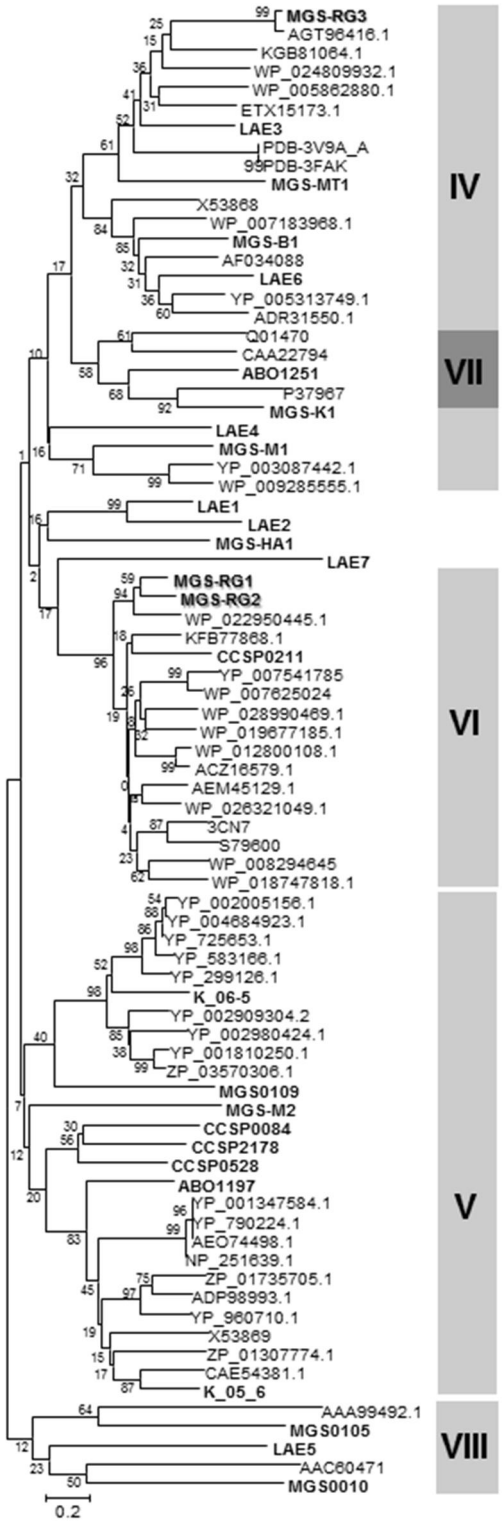

FIG S3 pH profiles of the enzymes. The data represent the relative percentages of specific activity (unit mg^{-1}) compared with the maximum (100%; MGS-RG1: 0.756; MGS-RG2: 0.097; MGS-RG3: 0.159 unit mg^{-1}). The specific activities were calculated, using 2 μg proteins and *p*NP-propionate (1 mM) as assay substrate, at the optimal temperature (MGS-RG1: 45°C; MGS-RG2: 50°C; MGS-RG3: 30°C) in 50 mM buffers as described in the Materials and Methods. SD of triplicate assays is provided.