RESEARCH ARTICLE

Phytophthora palmivora RPA1, a Homolog of *Phytophthora infestans RPA190*, is Irrelevant to Metalaxyl Resistance in *Phytophthora palmivora* Causing Root and Stem Rot of Durian in Thailand

Kamonwan Sichai, Patcharin Nianwichai, Nutsuda Taraput, Veeranee Tongsri, and Pattavipha Songkumarn*

Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

*Corresponding author fagrpps@ku.ac.th

ABSTRACT

Root and stem rot caused by *Phytophthora palmivora* is one of the most serious diseases affecting durian production in Thailand where metalaxyl, an effective fungicide against oomycetes, has been used extensively for a long period to control this disease. Although field isolates of *P. palmivora* resistant to metalaxyl exist in Thailand, a molecular basis for *P. palmivora* has not yet been elucidated regarding metalaxyl resistance. The current study tested whether *P. palmivora RPA1* (the DNA-directed RNA polymerase I subunit gene), a homolog gene of *RPA190* associated with metalaxyl resistance in some isolates of *Phytophthora infestans*, had a role in the resistance mechanism toward metalaxyl. In total, 40 durian-derived isolates of *P. palmivora* were assessed for metalaxyl sensitivity using a mycelial growth inhibition assay. The effective concentrations for 50% mycelial growth inhibition values for all isolates tested were in the range 0.01-872.88 mg/L. The isolates were clustered into three groups: sensitive (n=23), moderately resistant (n=11), and resistant (n=6) groups. No polymorphism was revealed based on multiple alignment analysis of the amino acid sequences translated from the corresponding DNA sequences in the region of *RPA1* of the metalaxyl-sensitive (n=5), moderately resistant (n=2), and resistant isolates (n=6). Furthermore, investigation of the *RPA1* expression among these representative isolates (n=3, each group) indicated that *RPA1* expression may not be involved in the regulation of *P. palmivora* resistance to metalaxyl. Based on this line of evidence, there was no detected relationship regarding metalaxyl resistance and *P. palmivora RPA1*.

Keywords: DNA directed RNA polymerase I subunit gene, Durian, Metalaxyl resistance, *Phytophthora palmivora*

OPEN ACCESS

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2023 December, 52(2): 73-96
https://doi.org/10.4489/kjm.520201

Received: November 13, 2023
Revised: February 05, 2024
Accepted: April 22, 2024

INTRODUCTION

Durain (*Durio zibethinus* L.), known as the king of fruits [1], ranks as the most valuable crop in the fruit industry of Thailand, which is ranked the world's number one durian exporter with an export value of USD

1.36 billion in the first quarter of 2023 [2]. Unfortunately, one of major challenges in durian production in Thailand is controlling plant disease, including root and stem rot caused by *Phytophthora palmivora*, a destructive oomycete plant pathogen [3]. This pathogen can affect different parts of the plant leading to several symptoms, including root and stem rot, leaf and stem blight, trunk canker, and pre- and post-harvest fruit rot [1,4]. Indeed, fruit damage losses of 10-20% have been noted, caused by *P. palmivora* postharvest fruit rot [1]. Primarily, durian root and stem rot disease management in Thailand relies on recommended fungicide treatment using phenylamides (PAs), quinone outside inhibitors, and carboxylic acid amides [3]. In fact, PAs, such as metalaxyl, are commonly used in the major durian-producing areas, including the Eastern and Southern parts of Thailand. Long-term and extensive usage of fungicide accelerate the risk of fungicide resistance, as evidenced by the discovery of metalaxyl-resistant isolates of *P. palmivora* in commercial durian orchards in Southern [3] and Eastern [5] Thailand, where metalaxyl has been regularly applied at increasingly higher doses.

Metalaxyl, a systemic fungicide belonging to the phenylamides, was reported to be effective against oomycete pathogens [6]. This fungicide acts as a single site-specific inhibitor toward ribosomal RNA synthesis through interfering with RNA polymerase activity [7], and consequently it affects mycelial growth and zoospore germination [8]. Being a site-specific fungicide, metalaxyl is categorized as high risk regarding resistance development [9]. Soon after the release of the fungicide in 1977 [10], fungicide resistance of oomycete pathogens was reported in the genus *Phytophthora* of distinct hosts, such as *P. infestans* on potato [11], *P. citricola* and *P. parasitica* on ornamental hosts [12], *P. capsici* on peppers [13], *P. cactorum* and *P. nicotianae* on strawberry [14], and *P. palmivora* on durian [3]. The molecular basis underlying the metalaxyl resistance mechanism has been dissected in some species of *Phytophthora*. Sequence variation in gene coding of the large subunit of RNA polymerase I, *RPA190*, of *P. infestans* was shown to be associated with metalaxyl resistance [15,16]. Furthermore, the expression of *RPA190-pc*, a homolog gene of *P. infestants RPA190*, was shown to play role in the regulation of the metalaxyl resistance of *P. capsici*, by which gene upregulation may be a consequence of the sequestration of metalaxyl, resulting in the unavailability of metalaxyl to target the activity of RNA polymerase activity inside the pathogen [17]. In addition, other proteins besides the large subunit of RNA polymerase I may contribute to metalaxyl resistance. As shown in the study of Vogel et al. [18], a number of single nucleotide polymorphism (SNP) markers associated with mefenoxam (metalaxyl-m) sensitivity of *P. capsici* anchored to a region of scaffold 62, from where gene encoding a homolog of yeast ribosome synthesis factor Rrp5 was identified among the candidate genes for mefenoxam sensitivity. Indeed, Rrp5 was shown to associate with the formation of 18S and 5.8S rRNA during the yeast ribosome biogenesis process [19]. Conversely, no gene encoding subunit of RNA polymerase I was found from such region. A similar result was also observed in the study by of Marin et al. [8], in which a number of *P. cactorum* genes with unknown function that had been identified through SNPs were possibly responsible for mefenoxam-resistance. However, no RNA polymerase subunit genes were presented in the list of candidate genes. In recent years, although there have been comprehensive reports of *P. palmivora*-resistant isolates associated with durian disease under field conditions, the metalaxyl resistance mechanism in *P. palmivora* has not been dissected yet. As such, knowledge on the molecular mechanism of metalaxyl resistance in *P. palmivora* may facilitate monitoring the resistant strains in durian cultivation, leading to an overall design for a proper disease management program.

Based on the above background, the current study was conducted to assess whether *RPA1*, a homolog gene of *P. infestans RPA190*, influenced metalaxyl resistance in *P. palmivora*, the isolate associated the durian disease. The findings obtained should help to establish the basis of the molecular mechanism of *P. palmivora* resistance to metalaxyl.

MATERIALS AND METHODS

Phytophthora palmivora isolates

In total, 40 isolates of *P. palmivora*, obtained in 2021 and previously used in the studies of Nianwichai et al. [20] and Thongsri et al. [21], were used as the study samples in the current study. We reconfirmed the species using DNA-barcode based polymerase chain reaction (PCR) with the *P. palmivora*-specific primers, FM35 and FMPhy-10b [22,23], designed from the *CoxII*/Internal spacer/*CoxI* (mitochondrial cytochrome c oxidase subunit II, Internal spacer, and cytochrome c oxidase subunit I) region. Details of the primer sequences are provided in Table 1. All sequences from the *CoxII*/Internal spacer/*CoxI* were deposited in the GenBank database under accession numbers OP204950–OP204989 (Supplementary Material Table S1).

Table 1. List of primers used in this study

Primer name	Sequence (5'-3')	Annealing temperature (°C)	Reference
FM35	CAGAACCTTGGCAATTAGG	47	[22]
FMPhy-10b	GCAAAAGCACTAAAAATTAAATATAA	47	[23]
Pal -RPA1ups4-F	CCAGGCTTACAAGTGAATGTAGCC	60	This current study
P al-RPA1midR	GTCGCTGTTGGATCCGTGTACG	60	This current study
P al-RPA1midF	CGCCGCTGCGTGGTCTTATTC	60	This current study
Pal- RPA1down3-R	ACCGAGCACTCAAACTCTGCC	60	This current study
qR Tpal-RPA1F1	ATGGCGCCACCTTAAGACAG	64	This current study
qR Tpal-RPA1R1	GAAATCGGCGTTGAACGTGT	64	This current study
Lili -ActinHE-F1	GTACTACGGGCTGTGTGCTT	64	[25]
Lili -ActinHE-R1	ACGCACAATAGCGTGAGGAA	64	[25]

In vitro determination of sensitivity to metalaxyl

The 40 isolates of *P. palmivora* were cultured on half-strength potato dextrose agar (PDA) (HiMedia; Bangalore, India) for 5 days at room temperature. The mycelial plugs (5 mm in diameter) of each isolate were transferred into the center of the Petri dishes (90 mm in diameter) containing PDA amendment with different concentrations of metalaxyl (Table 2) and were incubated at room temperature for 6 days. The experiment was arranged in a completely randomized design with at least 3 replications. Subsequently, the growth diameters of each colony on the culture plate were measured and calculated as the percentage of mycelial growth inhibition using the formula: % growth inhibition=[diameter of colony in control - diameter of treated plate]/diameter of control×100]. Then, the fifty percent effective concentration (EC_{50}) values were calculated using a probit model by linear regressing the probit value corresponding to the percentage of growth inhibition against the logarithm of the fungicide concentration. Next, the EC_{50} mean values of metalaxyl across repeated experiments of each isolates were calculated, with each *P. palmivora* isolate clustered into different groups of metalaxyl sensitivity response, as described by Kongtragoon et al. [3], where the isolates with EC_{50} values<1 mg/L were considered as sensitive (S), isolates with EC_{50} values of 1 to 100 mg/L were classified as moderately resistant (MR), and isolates with EC_{50} values greater than 100 mg/L were considered as resistant (R) toward metalaxyl. The dataset of mean EC_{50} values of metalaxyl for all isolates was used to construct histograms of multimodal datasets from which the frequency distribution of metalaxyl sensitivity was established based on equal proportions of $\log_{10} EC_{50}$ ranges.

Table 2. Different ranges of metalaxyl concentration used for determination of metalaxyl sensitivity in *Phytophthora palmivora* population

Series	Concentration range (mg/L)
1	0, 100, 200, 300, 400, 500, 600, 700, 800, 900
2	0, 5, 10, 15, 20, 25, 30, 35, 40, 45
3	0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5
4	0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45

Identification of a homolog gene of *Phytophthora infestans RPA190* in *Phytophthora palmivora* genome

As *P. infestans RPA190* is known to be associated with metalaxyl resistance in some studies [15,16], the amino acid sequence of RPA190 from the *P. infestans* strain T30-4 resistant isolate (accession number: PITG_03855) was used as a reference sequence to search for relative amino acid sequence similarity against the genome of *P. palmivora* var. *palmivora* strain sbr112.9, available in the FungiDB database (https://fungidb.org/fungidb/app) using a BLASTP search. The amino acid sequence that showed the highest similarity to *P. palmivora* var. *palmivora* strain sbr112.9, with a maximum score and an E-value close to 0, was further used in this study.

Analysis of DNA and amino acid variance using DNA sequence of *Phytophthora palmivora RPA1*, the large subunit of RNA polymerase I

Due to the fact that *RPA1* (PHPALM_20058) of *P. palmivora* was identified as the best hit from the above process, we used two primer sets, named as Pal-RPA1ups4-F/Pal-RPA1midR and Pal-RPA1midF/Pal-RPA1down3-R (Table 1), to obtain the complete DNA sequence covering the *RPA1* region of *P. palmivora*. Indeed, the Pal-RPA1midR and RPA1down3-R primers were designed at the same positions reported by Chen et al. [16] for designed primer sets for DNA amplification of the *P. infestans RPA190* region, while the Pal-RPA1ups4-F and Pal-RPA1midF primers were designed from the current study using DNA sequence corresponding to the *RPA1* region of *P. palmivora* var. *palmivora* strain sbr112.9.

The DNA sequences covering the region of the *RPA1* gene from the representative metalaxyl sensitive isolates (CTT2, RKT1, RWT3, TKT3, and TML1), moderately resistant isolates (TML2 and CKKB2), and resistant isolates (CKLB1, CKLL1, TBL1, TBL2, TBL3, and TKL3) of of *P. palmivora* (n=13 in total) were amplified using the two primer sets described above. Genomic DNA of each isolate was subjected to PCR amplification with a total reaction volume of 60 μL, consisting of 500 ng DNA template, 1X PCR Master mix Solution i-StarTaq™ (iNtRON Bio, Seongnam, Korea), 0.5 μM forward primer, and 0.5 μM reverse primer. The PCR reaction was performed under conditions consisting of an initial denaturation at 95℃ for 4 min, followed by 32 cycles of denaturation at 95℃ for 30 s, annealing at 60℃ for 30 s, and extension at 72℃ for 3.30 min, with a final extension at 72℃ for 10 min. The PCR products were electrophoresed through 1% (w/v) agarose gels containing RedSafe™ (iNtRON Bio; Gyeonggi-do; Korea) and visualized under UV light. Then, the PCR products were sequenced with an Illumina BTSeq™ (barcode taq sequencing) by Celemics, Inc. (Seoul; Korea). The sequences of *P. palmivora* RPA1 were submitted to GenBank under the GenBank accession numbers OQ282371–OQ282380 and PP261190–PP261192.

The heterozygous sites of *RPA1* in 10 representative *P. palmivora* isolates were examined using the Integrative Genomics Viewer (version 2.12.3) [24] with the default parameters. The sequences were translated to amino acids using the 'translate' tool (https://web.expasy.org/translate/) and SNPs and various amino acids were analyzed using alignment MUSCLE algorithm implemented in ClustalW2 (https://www.ebi.ac.uk/Tools/msa/clustalw2/).

Phytophthora palmivora RPA1 gene expression analysis

The representative isolates of *P. palmivora*—either resistant (CKLB1, TBL2, and TKL3) or sensitive (RKT1, RWT3, and TML1) to metalaxyl—were selected for total RNA preparation. All above isolates were selected due to their stable growth in the culture media, both in PDA and in potato dextrose broth (PDB; HiMedia, Bangalore, India). All isolates were cultured on half strength PDA for 7 days at room temperature. Subsequently, each isolate was transferred into half strength PDB and cultured for 2 days with 250 rpm shaking. Subsequently, the *P. palmivora* culture mycelia of each isolate were subjected to a metalaxyl treatment. In detail, metalaxyl was added to the PDB medium at a final concentration based on the EC_{50} value for each *P. palmivora* isolate (872.88 mg/L for TBL2, 326.22 mg/L for CKLB1, 103.36 mg/L for TKL3, 0.08 mg/

L for RWT3, 0.03 mg/L for TML1, and 0.01 mg/L for RKT1). The metalaxyl treated and non-treated (as control) samples were cultured under the same conditions at room temperature and 250 rpm shaking for 2 h. Subsequently, mycelia from each sample were collected and washed thrice with sterilized water. Total RNA of each sample was extracted using an RNeasy® Plant Mini Kit (Qiagen Ltd., Hilden, Germany), according to the manufacturer's protocol. The concentration and integrity of the RNA were determined using NanoDrop™ (Thermo Fisher Scientific Inc., Waltham, MA, USA). The first-strand cDNA was prepared using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA), following the manufacturer's protocol and subsequently used as the template for gene expression analysis.

The *P. palmivora RPA1* samples of each isolates obtained from either the metalaxyl treatment or the non-treatment were quantified using real-time quantitative reverse transcription PCR (qRT-PCR) using the primer set (qRTpal-RPA1F1/qRTpal-RPA1R1) that was designed based on the study by Wang et al. [17]. In addition, the primer set (Lili-ActinHE-F1/Lili-ActinHE-R1) designed from the *P. palmivora Actin* gene [25] was used as the internal reference. The details of all primer sequences used in this experiment are provided in Table 1. The qRT-PCR was performed in a total reaction volume of 10 µl, consisting of 10 ng cDNA template, 1x HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis BioDyne, Tartu, Estonia), 0.05 µM forward primer and 0.05 µM reverse primer using a Bio-Rad CFX96 q-PCR system (Bio-Rad Corporation; Hercules, CA, USA) with SYBR Green I fluorescent dye detection. Each analysis consisted of two biological replicates and three technical replicates per biological replicate. The relative *RPA1* mRNA levels were normalized with the relative expression levels of the internal reference *Actin* gene using the $2^{-\Delta\Delta Ct}$ method [26]. The *RPA1* expression levels were analyzed using one-way ANOVA statistical analysis and Tukey's test at $p<0.05$, with the *RPA1* expression levels between control non-treated metalaxyl samples and treated metalaxyl samples analyzed using Tukey's *t*-test ($p<0.05$). The correlation between the $\log_{10}$ transformed EC_{50} values *in vitro* and the relative expression levels of *RPA1* was analyzed using Pearson's correlation coefficient (*r*).

RESULTS

In vitro sensitivity to metalaxyl of *Phytophthora palmivora* isolates

In vitro sensitivity to metalaxyl assessment among a total of 40 *P. palmivora* isolates had EC_{50} values in the range 0.01-872.88 mg/L (Fig.1, Supplementary Material Table S2), representing a ratio between EC_{50} of the least and most sensitive isolate tested of 87,288. The large value of this ratio indicated a risk of resistance to metalaxyl in this natural *P. palmivora* population. According to their metalaxyl sensitivity responses, they were categorized in to 3 groups: sensitive (n=23), moderately resistant (n=11), resistant (n=15). The frequency distribution of the EC_{50} values formed a multimodal curve (Fig. 2), which confirmed that there were distinct subpopulations based on the metalaxyl sensitivity response levels among all the individuals tested.

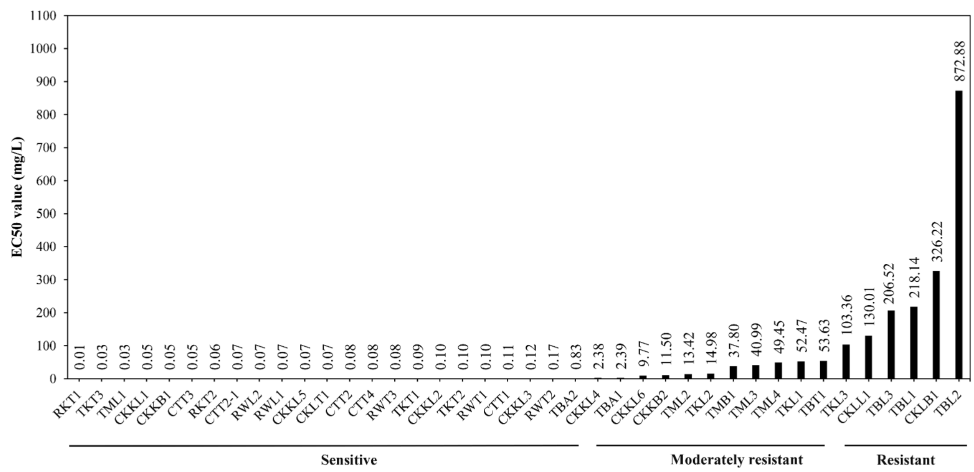

Fig. 1. Sensitivity of *Phytophthora palmivora* (n=40 isolates) to metalaxyl. Metalaxyl sensitivity of each isolate represented as EC_{50} value. Grouped responses to metalaxyl sensitivity based on Kongtragoul et al. [3], based on isolates with EC_{50} values<1 mg/L classified as sensitive (S), isolates with EC_{50} values of 1-100 mg/L classified as moderately resistant (MR), and isolates with EC_{50} values greater than 100 mg/L classified as resistant (R) toward metalaxyl.

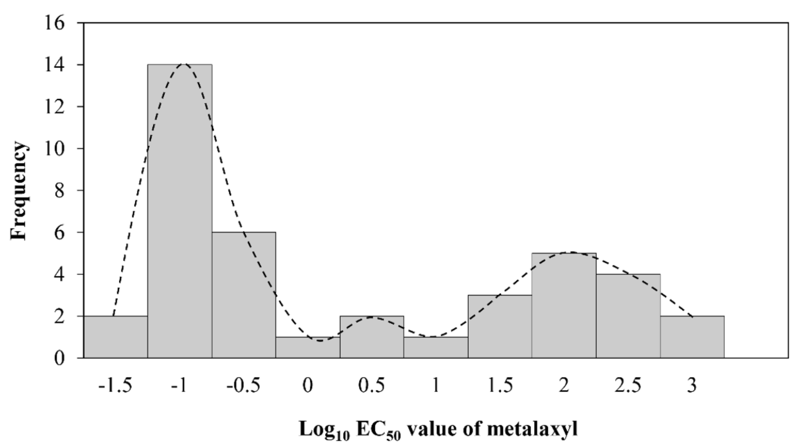

Fig. 2. Frequency distribution of metalaxyl sensitivity in *Phytophthora palmivora* isolates (n=40) versus $\log_{10} EC_{50}$ value.

Identification and sequence analysis of *Phytophthora palmivora* RPA1

BLASTP analysis of the protein sequences of *P. infestans* RPA190 (PITG_03855) against the *P. palmivora* sbr112.9 annotated protein database (https://fungidb.org/fungidb/app) resulted in the greatest similarity with *P. palmivora* DNA-directed RNA polymerase I subunit RPA1 (PHPALM_20058), with a maximum score of 3,482, an E value of 0.0, and 94% identity. The gene structures of *P. infestans RPA190* and *P. palmivora RPA1* are shown in Fig. 3. *RPA1* was 5,745 bps in length (including intron), encoding 1,810 amino acids. DNA sequence analysis of the *RPA1* regions among the 13 representative isolates of *P. palmivora* including the sensitive, moderately resistant, and resistant isolates to metalaxyl revealed no deletion/insertion in this region.

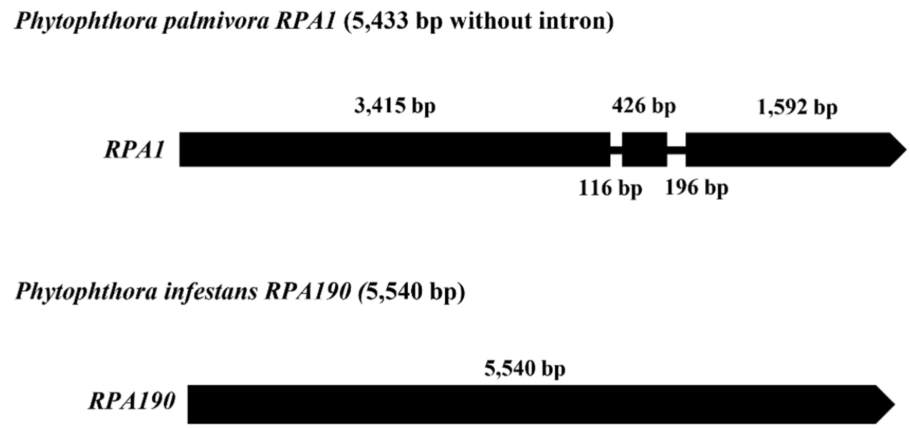

Fig. 3. Gene structures of *Phytophthora palmivora RPA1* and of *Phytophthora infestans RPA190*. Black lines represent intron segments.

Only one isolate of *P. palmivora* (TML1) had a single heterozygous synonymous SNP at the A2484T site, which did not produce any change in the amino acid sequence (data not shown). In addition, 100% identical amino acid alignments of RPA1 among the 13 representative isolates were observed (Supplementary Material Fig. S1), inferring that *P. palmivora RPA1*, a gene homolog of *P. infestans RPA190*, may not play a role in resistance to metalxyl.

Phytophthora palmivora RPA1 expression analysis

Analysis of the *P. palmivora RPA1* gene expression level based on qRT-PCR among the representative metalaxyl-resistant and -sensitive isolates (n=3, each group) displayed the upregulation of the *RPA1* gene in some isolates (CKLB1, RKT1, TKL3, and TML1) (Fig. 4). In addition, there was negligible correlation between the relative expression level of *RPA1* and the phenotype responsible for the resistance to metalaxyl, with a Pearson's correlation coefficient of 0.15 (r^2=0.02; p=0.78), as shown in Fig. 5. Overall, the results suggested that the *P. palmivora RPA1* gene may not play a role in resistance to metalaxyl at a transcriptional control level.

DISCUSSION

P. palmivora was recognized as the most devastating pathogen of durian plants in Thailand as it causes a variety of symptoms on the plant, including seedling dieback, leaf blight, root rot, trunk cankers, and preharvest and postharvest fruit rot [1] and has been shown to be the predominant causal agent associated with fruit, root, and stem rot of durian cultivated in Southern and Eastern Thailand [3,5,20,21].

Metalaxyl has been recommended to combat durian canker disease caused by *P. palmivora* for more than the past three decades in Southeast Asia [1]. In oomycetes, resistance to phenylamide fungicides evolves rapidly [27]. The intensive use over a long period fungicidal applications has resulted in the development of *P. palmivora* isolates that are resistant to metalaxyl as evidenced in other studies, where an increase in

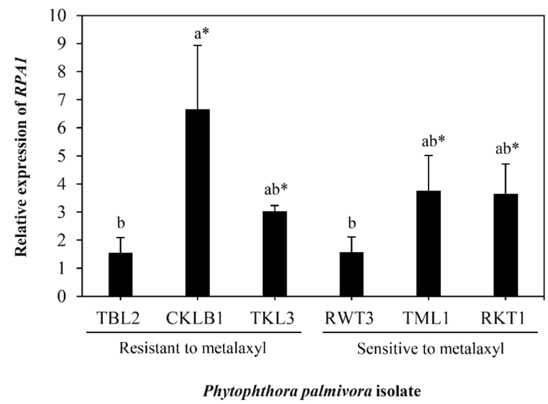

Fig. 4. Relative expression analysis of *RPA1* among *Phytophthora palmivora* representative isolates, sensitive and resistant to metalaxyl (n=3, each group), using real-time quantitative reverse transcription polymerase chain reaction. The relative expression levels of *RPA1* in each isolate were normalized to *P. palmivora* Actin gene. Error bars represent mean±standard deviation (SD) from two independent biological experiments with three technical replicates. Asterisks indicate significant difference at $p<0.05$ between control samples (non-treated with metalaxyl) and tested samples (treated with metalaxyl) as determined using a t-test. Means followed by different letters are significantly different among representative isolates tested based on Tukey's test at $p<0.05$.

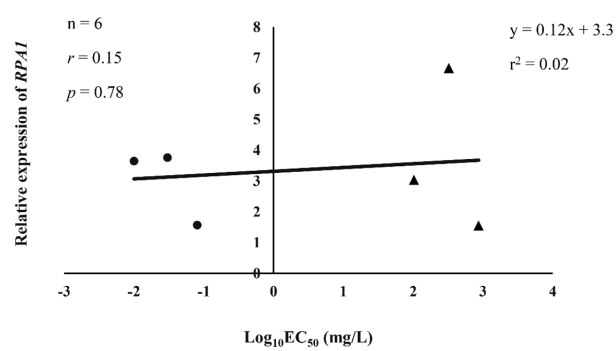

Fig. 5. Pairwise correlation between $\log_{10}$ EC_{50} value of metalaxyl and relative *RPA1* expression level among *Phytophthora palmivora* representative isolates, resistant (represented as filled triangles) and sensitive (represented as filled circles) to metalaxyl (n=3, each group).

the proportion occurred in metalaxyl-resistant isolates of *P. palmivora* from 30% in 2014 [28] to 70% in 2017 [3] in the durian orchards in Southern Thailand where metalaxyl has been used regularly for disease management. In addition, Somnuek et al. [5] reported a greater frequency of metalaxyl-resistant isolates of *P. palmivora* in proportion to sensitive isolates in the *P. palmivora* population recently obtained from the durian orchards in Thailand, implying that this pathogen is under selection pressure to evade a negative outcome following exposure to the metalaxyl fungicide. Despite the emergence of metalaxyl resistance among the field isolates of *P. palmivora*, the molecular mechanism of metalaxyl resistance of *P. palmivora* has not yet been clarified. *P. palmivora RPA1* is a homolog gene of *P. infestans RPA190* and of *P. capsici RPA190-pc*, previously known to be associated with metalaxyl resistance [15-17]. Therefore, to establish a first perspective on such a mechanism, the current study investigated whether *P. palmivora RPA1*, a putative gene encoding the DNA-directed RNA polymerase I subunit, had a role in metalaxyl resistance through the analysis of the amino acid sequences decoded from *RPA1* and of the *RPA1* expression levels between the metalaxyl-sensitive and -resistant isolates of *P. palmivora*.

Herein the current study successfully obtained representative *P. palmivora* isolates from both metalaxyl-sensitive and -resistant subpopulation groups for further examination of the *RPA1* gene function associated with metalaxyl resistance. Indeed, in this study, we defined the types of metalaxyl response (sensitive, moderately resistant, or resistant) of each individual based on a range of EC_{50} values described by Kongtragoul et al. [3]. Although, there was a concern that the data obtained through the same standard protocol may have varied between the different laboratories due to experimental variation [29], there was a considerable range in the variation factor (87,288) between the least-sensitive and the most-sensitive isolates among the current *P. palmivora* population; furthermore, the metalaxyl multimodal curve could be used to confirm that there was great diversity in metalaxyl sensitivity among the current population. Therefore, the representative *P. palmivora* isolates selected from either the low or high ranges of EC_{50} values should be reliable for further assessment of the association between the *RPA1* gene and the metalaxyl resistant phenotype.

As mentioned above, the *P. palmivora RPA1* gene was a prime focus for the elucidation of a molecular mechanism underlying the metalaxyl resistance in the current study. However, herein we reported that the *RPA1* gene may not be involved in metalaxyl resistance in the *P. palmivora* population derived from diseased durian plants in Thailand. Until now, it was debatable whether the RNA polymerase I subunit (*RPA*) gene was involved in metalaxyl resistance, since some studies provided evidence supporting such an association [15-17], while other studies failed to demonstrate the *RPA* gene had such a role [8,18,30]. Although, in the current study there was only a single synonymous heterozygous SNP in a single isolate (TML1) among the whole *P. palmivora* population, overall, there was no amino acid-based variant of protein encoded by *RPA1* among the representative *P. palmivora* isolates from the distinct groups of the metalaxyl sensitivity phenotype. This finding contradicted the results reported by Randall et al. [15] and Chen et al. [16], where SNP-based variants of *RPA190* were found in the *P. infestans* populations obtained in such studies. Furthermore, SNP T1145A located in the *RPA190* gene region could associate with the metalaxyl-resistant phenotype in a majority of the isolates with some particular genotype [15], and multiple SNPs of the AA genotype in *RPA190* in relation to the metalaxyl resistant level [16]. No polymorphism in the *RPA1* region in the representative *P. palmivora* isolates in the current study may more likely suggest a non-involvement of the *RPA1* gene in the resistance toward metalaxyl. However, it could not be ruled out that *RPA1* may be associated with this function in other *P. palmivora* populations obtained elsewhere, as shown in the study by Randall et al. [15], where the existence of a base T at position 1145 (genotype corresponding to metalaxyl sensitivity) in *P. infestans* isolates (accounting for 14% of the overall isolates used in that study) was not correlated with the metalaxyl-sensitive phenotype, but demonstrated intermediate sensitivity or resistance toward metalaxyl. Indeed, all isolates showing such a phenomenon were from the same original background genotype. Therefore, additional isolates of *P. palmivora* obtained from different countries should be included for an association analysis of the *RPA1* genotype and metalaxyl resistance function in the future.

In the current study, other evidence to support our postulation on the non-involvement function in metalaxyl resistance of *P. palmivora RPA*1 was the lack of a correlation between the *RPA1* gene expression and the EC_{50} levels of the representative isolates. This result was inconsistent with the results presented in the study by Wang et al. [17], where *RPA190-pc* gene expression participated in the regulation of metalaxyl resistance in *P. capsici*. Since the representative *P. palmivora* field isolates showing resistance and sensitive to metalaxyl used in the gene expression analysis were from different origins, they were probably not from the same background genotype. Therefore, it cannot be ruled out that other genes, perhaps existing in some individuals, may affect the metalaxyl resistance function, which may have caused the inconclusive result. In addition, due to the fact that *RPA1* expression observed in this study was shown to be constitutively expressed in all the samples tested either with or without the metalaxyl treatment. This may because of its crucial role in cellular protein biogenesis. Since *RPA190*, a homolog gene of *RPA1*, encoding of the large subunit of RNA polymerase I, plays role in the synthesis of ribosomal RNA. It is important constituent of the cellular protein synthetic machinery. As shown in the study of Wittekind et al. [31]., conditional expression of *RPA190* in *Saccharomyces cerevisiae* brought about deprivation of RNA polymerase I leading to a decrease of rRNA synthesis and such that caused the growth defect of the yeast.

Notably, metalaxyl affects pathogens through interference of RNA polymerase I template complex DNA [7,32,33], whereas, in the current study, the focus was only on *RPA1* (accession number: PHPALM_20058) for the characterization of metalaxyl resistance. Therefore, other predicted proteins of DNA-directed RNA polymerase subunits of *P. palmivora* should be further characterized for such function.

Although the association of DNA-directed RNA polymerase subunit encoding genes and metalaxyl resistance has been clearly substantiated in the above studies, there was evidence showing that these genes have a negligible function in metalaxyl resistance. The association between the SNP T1145A genotype in *RPA190* and metalaxyl resistance was more ambiguous in the study by Matson et al. [30], where such association could not be addressed in some clonal lineages of *P. infestans*, and co-segregation analysis of the variants derived from the cross of some resistant isolates failed to provide the conclusive result. This result led to their suggestion that *RPA190* may not be appropriate for verifying the metalaxyl sensitivity level among the *P. infestans* population. In the study by Vogel et al. [18], some SNP markers were suggested as candidate genes for mefanoxam resistance associated with mefenoxam sensitivity of *P. capsici* anchored to a region of scaffold 62 from where a homolog gene of yeast ribosome synthesis factor Rrp5 was identified. Notably, genes encoding the subunit of RNA polymerase I were not found in such a region. Likewise, genome sequencing analysis and SNP variant calling of metalaxyl-resistant and -sensitive *P. cactorum* isolates in the study by Marin et al. [8] succeeded in identifying potential candidate genes related to metalaxyl resistance, but the RNA polymerase subunit genes were not included.

In conclusion, our study has provided an initial perspective on the molecular mechanism underlying the metalaxyl resistance of *P. palmivora*, in which the *RPA1* gene may be not responsible for the metalaxyl-resistant function in *P. palmivora* populations associated with root and stem rot of durian in Thailand. A comprehensive perspective on such a mechanism requires further research via a genome-wide association study to identify the key proteins affecting metalaxyl sensitivity, which may lead to the development of potential markers for monitoring and controlling *P. palmivora* metalaxyl-resistant strains and the design of novel inhibitors for future use.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENTS

This research was partially supported by the Agricultural Research Development Agency (ARDA), grant number PRP6405031160.

REFERENCES

1. Drenth A, Guest DI. Diversity and management of *Phytophthora* in Southeast Asia. Canberra: Australian Centre for International Agricultural Research; 2004. p. 238.
2. OAE. Monthly export value of durians in Thailand form January to April 2023 (in million Thai baht) [Internet]. New York: Statista Research Department; 2023 [cited 2023 Sept 5]. Available from https://www.statista.com/statistics/1319645/thailand-monthly-export-value-of-durians/.
3. Kongtragoul P, Ishikawa K, Ishii H. Metalaxyl resistance of *Phytophthora palmivora* causing durian diseases in Thailand. Horticulturae 2021;7:375.
4. Tongon R, Soytong K, Kanokmedhakul S, Kanokmedhakul K. Nano-particles from *Chaetomium brasiliense* to control *Phytophthora palmivora* caused root rot disease in durian var Montong. Int J Agric Technol 2018;14:2163-70.
5. Somnuek S, Jaenaksorn T, Kongtragoul P. Fungicide resistance of *Phytophthora palmivora* causing durian diseases in Eastern and Southern Thailand and the *in vitro* alternative control by cajeput leaf extracts. Int J Agric Technol 2023;19:703-20.
6. Sukul P, Spiteller M. Metalaxyl: Persistence, degradation, metabolism, and analytical methods. Rev Environ Contam Toxicol 2000;164:1-26.
7. Davidse LC, Hofman AE, Velthuis GCM. Specific interference of metalaxyl with endogenous RNA polymerase activity in isolated nuclei from *Phytophthora megasperma* f. sp. *medicaginis*. Exp Mycol 1983;7:344-61.
8. Marin MV, Baggio JS, Oh Y, Han H, Chandra S, Wang NY, Lee S, Peres NA. Identification of sequence mutations in *Phytophthora cactorum* genome associated with mefenoxam resistance and development of a molecular assay for the mutant detection in strawberry (*F.×ananassa*). Sci Rep 2023;13:7385.
9. FRAC. FRAC code list 2022 [Internet]. Fungicide Resistance Action Committee; 2022 [cited 2023 Sept 5]. Available from https://www.frac.info/.

10. Leadbeater AJ. Plant health management: Fungicides and antibiotics. In: Van Alfen NK, editor. Encyclopedia of agriculture and food systems. London: Academic Press; 2014. p. 408-24.
11. Davidse LC, Danial DL, Van Westen CJ. Resistance to metalaxyl in *Phytophthora infestans* in the Netherlands. Neth J Plant Pathol 1983;89:1-20.
12. Ferrin DM, Kabashima JN. *In vitro* insensitivity to metalaxyl of isolates of *Phytophthora citricola* and *P. parasitica* from ornamental hosts in Southern California. Plant Dis 1991;75:1041-4.
13. Oh JS, Kim CH. Varying sensitivity to metalaxyl of Korean isolates of *Phytophthora capsici* from red pepper fields. Plant Pathol J 1992;8:29-33.
14. Marin MV, Seijo TE, Zuchelli E, Peres NA. Resistance to mefenoxam of *Phytophthora cactorum* and *Phytophthora nicotianae* causing crown and leather rot in Florida strawberry. Plant Dis 2021;105:3490-5.
15. Randall E, Young V, Sierotzki H, Scalliet G, Birch PR, Cooke DE, Csukai M, Whisson SC. Sequence diversity in the large subunit of RNA polymerase I contributes to mefenoxam insensitivity in *Phytophthora infestans*. Mol Plant Pathol 2014;15:664-76.
16. Chen F, Zhou Q, Xi J, Li DL, Schnabel G, Zhan J. Analysis of *RPA190* revealed multiple positively selected mutations associated with metalaxyl resistance in *Phytophthora infestans*. Pest Manag Sci 2018;74:1916-24.
17. Wang W, Liu D, Zhuo X, Wang Y, Song Z, Chen F, Pan Y, Gao Z. The *RPA190-pc* gene participates in the regulation of metalaxyl sensitivity, pathogenicity and growth in *Phytophthora capsici*. Gene 2021;764:145081.
18. Vogel G, Gore MA, Smart CD. Genome-wide association study in New York *Phytophthora capsici* isolates reveals loci involved in mating type and mefenoxam sensitivity. Phytopathology 2021;111:204-16.
19. Venema J, Tollervey D. RRP5 is required for formation of both 18S and 5.8S rRNA in yeast. EMBO J 1996;15:5701-14.
20. Nianwichai P, Tongsri V, Taraput N, Srisopha W, Sichai K, Bussabong N, Songkumarn1 P, Koohapitagtam M. Mancozeb resistance of *Phytophthora palmivora*, a causal agent of stem rot and leaf blight of durian in eastern Thailand. King Mongkut's Agric J 2022;40:225-35.
21. Tongsri V, Nianwichai P, Sichai K, Songkumarn P, Suttiviriya P, Kongtragoul P. Sensitivity tests of dimethomorph, ethaboxam and etridiazole on *Phytophthora palmivora* causing stem rot and leaf blight of durian in eastern Thailand. Agr Nat Resour 2023;57:559-68.
22. Martin FN. Phylogenetic relationships among some *Pythium* species inferred from sequence analysis of the mitochondrially encoded cytochrome oxidase II gene. Mycologia 2000;92:711-27.
23. Martin FN, Tooley PW, Blomquist C. Molecular detection of *Phytophthora ramorum*, the causal agent of sudden oak death in California, and two additional species commonly recovered from diseased plant material. Phytopathology 2004;94:621-31.
24. Robinson JT, Thorvaldsdottir H, Turner D, Mesirov JP. igv.js: An embeddable JavaScript implementation of the Integrative Genomics Viewer (IGV). Bioinformatics 2023;39:btac830.
25. Ochoa JC, Herrera M, Navia M, Romero HM. Visualization of *Phytophthora palmivora* infection in oil palm leaflets with fluorescent proteins and cell viability markers. Plant Pathol J 2019;35:19.
26. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. Methods 2001;25:402-8.

27. Gisi U, Sierotzki H. Fungicide modes of action and resistance in downy mildews. Eur J Plant Pathol 2008;122:157-67.
28. Kongtragoul P. *In vitro* fungicidal effect of chitosan with different molecular weights on fungicide-resistant *Phytophthora* fruit rot on durian from the export market. Acta Hortic 2018;1210:65-72.
29. Russell PE. Sensitivity baselines in fungicide resistance research and management. Brussels: Crop Life International; 2004. p. 54.
30. Matson ME, Small IM, Fry WE, Judelson HS. Metalaxyl resistance in *Phytophthora infestans*: Assessing role of *RPA190* gene and diversity within clonal lineages. Phytopathology 2015;105:1594-600.
31. Wittekind M, Kolb JM, Dodd J, Yamagishi M, Mémet S, Buhler, JM, Nomura M. Conditional expression of *RPA190*, the gene encoding the largest subunit of yeast RNA polymerase I: effects of decreased rRNA synthesis on ribosomal protein synthesis. Mol Cell Biol 1990;10:2049-59.
32. Davidse LC, Gerritsma OCM, Velthuis GCM. A differential basis of antifungal activity of acylalanine fungicides and structurally related chloroacetanilide herbicides in *Phytophthora megasperma* f. sp. *medicaginis*. Pestic Biochem Physiol 1984;21(3):301-8.
33. Wollgiehn R, Bräutigam E, Schumann B, Erge D. Effect of metalaxyl on the synthesis of RNA, DNA and protein in *Phytophthora nicotianae*. Z Allg Mikrobiol 1984;24:269-79.

Supplementary data Table S1. Nucleotide sequence blast hit result on the mitochondrial cytochrome c oxidase subunit II, Internal spacer, and cytochrome c oxidase subunit I region of forty isolates of *Phytophthora palmivora* causing durian disease collected form eastern Thailand in GenBank and details of isolates used in this study

No .	Isolate code	Location	Source	Blast hit identity (Accession number)	Sequence similarity (%)	Accession number[a]
1.	CKKB1	Chanthaburi 12° 47'35.8"N, 102 °04'47.8"E	Branch	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204950
2.	CKKB2	Chanthaburi 12° 47'35.8"N, 102 °04'47.8"E	Branch	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204951
3.	CKKL1	Chanthaburi 12° 47'35.8"N, 102 °04'47.8"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204952
4.	CKKL2	Chanthaburi 12° 47'35.8"N, 102 °04'47.8"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .80	OP204953
5.	CKKL3	Chanthaburi 12° 47'35.8"N, 102 °04'47.8"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204954
6.	CKKL4	Chanthaburi 12° 47'35.8"N, 102 °04'47.8"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204955
7.	CKKL5	Chanthaburi 12° 47'35.8"N, 102 °04'47.8"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204956
8.	CKKL6	Chanthaburi 12° 47'35.8"N, 102 °04'47.8"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204957
9.	CKLB1	Chanthaburi 12° 27'17.6"N, 102 °16'37.0"E	Branch	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .80	OP204958
10.	CKLL1	Chanthaburi 12° 39'50.6"N, 102 °19'30.9"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204959
11.	CKLT1	Chanthaburi 12° 39'50.6"N, 102 °19'30.9"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204960
12.	CTT1	Chanthaburi 12° 38'49.7"N, 102 °00'10.1"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204961
13.	CTT2	Chanthaburi 12° 38'49.7"N, 102 °00'10.1"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204962
14.	CTT2-1	Chanthaburi 12° 38'49.7"N, 102 °00'10.1"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204963
15.	CTT3	Chanthaburi 12° 39'41.8"N, 101 °59'59.1"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204964
16.	CTT4	Chanthaburi 12° 39'41.8"N, 101 °59'59.1"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204965
17.	RKT1	Rayong 12° 45'10.4"N, 101 °33'06.2"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .69	OP204966
18.	RKT2	Rayong 12° 45'10.4"N, 101 °33'06.2"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204967
19.	RWL1	Rayong 12° 54'18.9"N, 101 °31'38.5"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204968
20.	RWL2	Rayong 12° 54'18.9"N, 101 °31'38.5"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204969
21.	RWT1	Rayong 12° 53'32.7"N, 101 °33'38.2"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204970
22.	RWT2	Rayong 12° 53'32.7"N, 101 °33'38.2"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204971
23.	RWT3	Rayong 12° 53'32.7"N, 101 °33'38.2"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204972
24.	TBA1	Trat 12°30 '10.5"N102 °34'27.2"E	Apical	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204973
25.	TBA2	Trat 12° 42'15.8"N, 102 °25'34.5"E	Apical	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204974
26.	TBL1	Trat 12° 30'10.5"N, 102 °34'27.2"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204975

Supplementary data Table S1. (continued)

No .	Isolate code	Location	Source	Blast hit identity (Accession number)	Sequence similarity (%)	Accession number[a]
27.	TBL2	Trat 12° 42'15.8"N, 102 °25'34.5"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204976
28.	TBL3	Trat 12° 42'15.8"N, 102 °25'34.5"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204977
29.	TBT1	Trat 12° 30'43.1"N, 102 °36'12.1"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204978
30.	TKL1	Trat 12° 30'10.5"N, 102 °34'27.2"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204979
31.	TKL2	Trat 12° 30'10.5"N, 102 °34'27.2"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204980
32.	TKL3	Trat 12° 30'10.5"N, 102 °34'27.2"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204981
33.	TKT1	Trat 12° 21'53.4"N, 102 °26'21.2"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204982
34.	TKT2	Trat 12° 21'53.4"N, 102 °26'21.2"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204983
35.	TKT3	Trat 12° 21'53.4"N, 102 °26'21.2"E	Trunk	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204984
36.	TMB1	Trat 12° 03'56.3"N, 102 °34'15.5"E	Branch	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204985
37.	TML1	Trat 12° 03'56.3"N, 102 °34'15.5"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204986
38.	TML2	Trat 12° 03'56.3"N, 102 °34'15.5"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204987
39.	TML3	Trat 12° 03'56.3"N, 102 °34'15.5"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204988
40.	TML4	Trat 12° 04'01.2"N, 102 °33'38.8"E	Leaf	*Phytophthora palmivora* culture ICMP:14517 (MT032128)	99 .70	OP204989

[a] Accession numbers of each individual generated in this study and have been deposited in National Center for Biotechnology Information (NCBI).

Supplementary data Table S2. EC_{50} values of metalaxyl in *Phytophthora palmivora* (n=40 isolates) with their linear regression equation and regression coefficient (r^2)

No.	Isolate code (sensitivity type[a])	EC_{50}	Regression equation	r^2
1.	CKKB1 (S)	0.05	y=3.24x+9.32	0.96
2.	CKKB2 (MR)	11.50	y=0.41x+4.57	0.95
3.	CKKL1 (S)	0.05	y=3.23x+9.34	0.92
4.	CKKL2 (S)	0.10	y=3.58x+8.56	0.75
5.	CKKL3 (S)	0.12	y=2.59x+7.43	0.94
6.	CKKL4 (MR)	2.38	y=1.50x+4.44	0.74
7.	CKKL5 (S)	0.07	y=3.94x+9.53	0.87
8.	CKKL6 (MR)	9.77	y=1.03x+3.98	0.89
9.	CKLB1 (R)	326.22	y=0.99x+2.51	0.72
10.	CKLL1 (R)	130.01	y=0.86x+3.19	0.85
11.	CKLT1 (S)	0.07	y=3.41x+8.84	0.81
12.	CTT1 (S)	0.11	y=2.37x+7.29	0.96
13.	CTT2 (S)	0.08	y=2.89x+8.25	0.96
14.	CTT2-1 (S)	0.07	y=3.66x+9.30	0.92
15.	CTT3 (S)	0.05	y=3.43x+9.48	0.84
16.	CTT4 (S)	0.08	y=1.85x+7.05	0.92
17.	RKT1 (S)	0.01	y=2.04x+8.90	0.99
18.	RKT2 (S)	0.06	y=4.37x+10.30	0.89
19.	RWL1 (S)	0.07	y=3.38x+8.91	0.78
20.	RWL2 (S)	0.07	y=3.83x+9.42	0.85
21.	RWT1 (S)	0.10	y=2.32x+7.27	0.96
22.	RWT2 (S)	0.17	y=8.42x+11.45	0.97
23.	RWT3 (S)	0.08	y=7.73x+13.53	0.91
24.	TBA1 (MR)	2.39	y=1.28x+4.52	0.77
25.	TBA2 (S)	0.83	y=1.66x+5.13	0.97
26.	TBL1(R)	218.14	y=0.80x+3.14	1.00
27.	TBL2 (R)	872.88	y=0.32x+4.06	0.82
28.	TBL3 (R)	206.52	y=0.56x+3.69	0.82
29.	TBT1 (MR)	53.63	y=1.26x+2.81	0.86
30.	TKL1 (MR)	52.47	y=1.03x+3.23	0.88
31.	TKL2 (MR)	14.98	y=2.45x+2.12	0.91
32.	TKL3 (R)	103.36	y=0.63x+3.74	1.00
33.	TKT1 (S)	0.09	y=4.11x+9.30	0.80
34.	TKT2 (S)	0.10	y=2.07x+7.03	0.82
35.	TKT3 (S)	0.03	y=2.75x+9.36	0.84
36.	TMB1 (MR)	37.80	y=0.45x+4.29	0.93
37.	TML1 (S)	0.03	y=3.73x+10.55	0.79
38.	TML2 (MR)	13.42	y=2.03x+2.72	0.90
39.	TML3 (MR)	40.99	y=1.07x+3.27	0.97
40.	TML4 (MR)	49.45	y=0.69x+3.83	0.99

[a] Sensitive (S): EC_{50} values<1 mg/L, moderately resistant (MR): EC_{50} values of 1 to 100 mg/L and resistant (R): EC_{50} values>100 mg/ L (Kongtragoul et al., [3]).

```
TBL2(OQ282376)(R)       MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
CKLB1(OQ282372)(R)      MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
TBL1(PP261191)(R)       MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
TBL3(PP261192)(R)       MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
CKLL1(PP261190)(R)      MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
TKL3(OQ282377)(R)       MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
TML2(OQ282380)(MR)      MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
CKKB2(OQ282371)(MR)     MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
RWT3(OQ282375)(S)       MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
CTT2(OQ282373)(S)       MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
TML1(OQ282379)(S)       MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
TKT3(OQ282378)(S)       MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
RKT1(OQ282374)(S)       MDSATDQTILRHEVAEVAFGFYSDAEIRDLSVKQLTSRLSFDHLNNPVVGGLYDPALGPV
                        ************************************************************

TBL2(OQ282376)(R)       DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
CKLB1(OQ282372)(R)      DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
TBL1(PP261191)(R)       DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
TBL3(PP261192)(R)       DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
CKLL1(PP261190)(R)      DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
TKL3(OQ282377)(R)       DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
TML2(OQ282380)(MR)      DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
CKKB2(OQ282371)(MR)     DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
RWT3(OQ282375)(S)       DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
CTT2(OQ282373)(S)       DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
TML1(OQ282379)(S)       DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
TKT3(OQ282378)(S)       DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
RKT1(OQ282374)(S)       DFNMICPTCHQTQKECPGHLGHLELPVPVYNPVLFGQMLNLLKRKCFTCHKFRVASARSR
                        ************************************************************

TBL2(OQ282376)(R)       VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
CKLB1(OQ282372)(R)      VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
TBL1(PP261191)(R)       VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
TBL3(PP261192)(R)       VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
CKLL1(PP261190)(R)      VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
TKL3(OQ282377)(R)       VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
TML2(OQ282380)(MR)      VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
CKKB2(OQ282371)(MR)     VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
RWT3(OQ282375)(S)       VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
CTT2(OQ282373)(S)       VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
TML1(OQ282379)(S)       VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
TKT3(OQ282378)(S)       VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
RKT1(OQ282374)(S)       VIRVKILLLDNGFENEAAQLSELLEQRNGVEDEPTQRTFQRQQAILDEYERLALSKSSSS
                        ************************************************************

TBL2(OQ282376)(R)       TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
CKLB1(OQ282372)(R)      TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
TBL1(PP261191)(R)       TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
TBL3(PP261192)(R)       TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
CKLL1(PP261190)(R)      TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
TKL3(OQ282377)(R)       TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
TML2(OQ282380)(MR)      TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
CKKB2(OQ282371)(MR)     TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
RWT3(OQ282375)(S)       TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
CTT2(OQ282373)(S)       TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
TML1(OQ282379)(S)       TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
TKT3(OQ282378)(S)       TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
RKT1(OQ282374)(S)       TNGKTQLLRPLPRLAEVIREKLAAEFLKGMKNKCENCGAISPALRQDANAKIFLKGLSAR
                        ************************************************************

TBL2(OQ282376)(R)       SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
CKLB1(OQ282372)(R)      SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
TBL1(PP261191)(R)       SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
TBL3(PP261192)(R)       SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
CKLL1(PP261190)(R)      SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
TKL3(OQ282377)(R)       SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
TML2(OQ282380)(MR)      SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
CKKB2(OQ282371)(MR)     SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
RWT3(OQ282375)(S)       SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
CTT2(OQ282373)(S)       SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
TML1(OQ282379)(S)       SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
TKT3(OQ282378)(S)       SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
RKT1(OQ282374)(S)       SRKVNRSKNLTVTSALDTIRGNVSDKDDESMNGDDSESEMDDDENTYATTEDSSSRSKYL
                        ************************************************************
```

```
TBL2(OQ282376)(R)      PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
CKLB1(OQ282372)(R)     PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
TBL1(PP261191)(R)      PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
TBL3(PP261192)(R)      PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
CKLL1(PP261190)(R)     PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
TKL3(OQ282377)(R)      PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
TML2(OQ282380)(MR)     PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
CKKB2(OQ282371)(MR)    PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
RWT3(OQ282375)(S)      PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
CTT2(OQ282373)(S)      PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
TML1(OQ282379)(S)      PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
TKT3(OQ282378)(S)      PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
RKT1(OQ282374)(S)      PPLEVQSQLQLMWQNEDGLMELLYGDRNIASGRVSGRKPEGWRKFFLNVIPVAPSRFRPP
                       ************************************************************

TBL2(OQ282376)(R)      VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
CKLB1(OQ282372)(R)     VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
TBL1(PP261191)(R)      VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
TBL3(PP261192)(R)      VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
CKLL1(PP261190)(R)     VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
TKL3(OQ282377)(R)      VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
TML2(OQ282380)(MR)     VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
CKKB2(OQ282371)(MR)    VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
RWT3(OQ282375)(S)      VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
CTT2(OQ282373)(S)      VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
TML1(OQ282379)(S)      VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
TKT3(OQ282378)(S)      VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
RKT1(OQ282374)(S)      VFMGDKQFEHAQNAHLSKIMTLSESIVQSDYYKRQAATTSDEDDAEKEDQINLSRKLALW
                       ************************************************************

TBL2(OQ282376)(R)      TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
CKLB1(OQ282372)(R)     TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
TBL1(PP261191)(R)      TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
TBL3(PP261192)(R)      TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
CKLL1(PP261190)(R)     TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
TKL3(OQ282377)(R)      TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
TML2(OQ282380)(MR)     TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
CKKB2(OQ282371)(MR)    TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
RWT3(OQ282375)(S)      TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
CTT2(OQ282373)(S)      TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
TML1(OQ282379)(S)      TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
TKT3(OQ282378)(S)      TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
RKT1(OQ282374)(S)      TELQTAVNLLVDSSKAKPGTDVAQGIKQVIEKKEGLFRKHMMGKRVNYAARSVISPDPYI
                       ************************************************************

TBL2(OQ282376)(R)      STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
CKLB1(OQ282372)(R)     STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
TBL1(PP261191)(R)      STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
TBL3(PP261192)(R)      STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
CKLL1(PP261190)(R)     STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
TKL3(OQ282377)(R)      STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
TML2(OQ282380)(MR)     STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
CKKB2(OQ282371)(MR)    STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
RWT3(OQ282375)(S)      STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
CTT2(OQ282373)(S)      STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
TML1(OQ282379)(S)      STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
TKT3(OQ282378)(S)      STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
RKT1(OQ282374)(S)      STSQIGVPLRFAKTLTYPQPVTPWNVEEMRQLVINGPDVHPGANFVESENGRLIDLSKRT
                       ************************************************************

TBL2(OQ282376)(R)      PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
CKLB1(OQ282372)(R)     PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
TBL1(PP261191)(R)      PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
TBL3(PP261192)(R)      PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
CKLL1(PP261190)(R)     PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
TKL3(OQ282377)(R)      PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
TML2(OQ282380)(MR)     PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
CKKB2(OQ282371)(MR)    PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
RWT3(OQ282375)(S)      PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
CTT2(OQ282373)(S)      PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
TML1(OQ282379)(S)      PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
TKT3(OQ282378)(S)      PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
RKT1(OQ282374)(S)      PHQREAISKTLLTRSASAQGTSKNRVKRVWRHLKTGDVVLMNRQPTLHKPSIMAHTTRVL
                       ************************************************************
```

```
TBL2(OQ282376)(R)        TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
CKLB1(OQ282372)(R)       TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
TBL1(PP261191)(R)        TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
TBL3(PP261192)(R)        TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
CKLL1(PP261190)(R)       TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
TKL3(OQ282377)(R)        TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
TML2(OQ282380)(MR)       TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
CKKB2(OQ282371)(MR)      TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
RWT3(OQ282375)(S)        TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
CTT2(OQ282373)(S)        TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
TML1(OQ282379)(S)        TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
TKT3(OQ282378)(S)        TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
RKT1(OQ282374)(S)        TNPKMQTIRMHYANCNTFNADFDGDEMNMHFPQNELARSEAYNIASNDNQYIVPTDGSPL
                         ************************************************************

TBL2(OQ282376)(R)        RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
CKLB1(OQ282372)(R)       RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
TBL1(PP261191)(R)        RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
TBL3(PP261192)(R)        RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
CKLL1(PP261190)(R)       RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
TKL3(OQ282377)(R)        RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
TML2(OQ282380)(MR)       RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
CKKB2(OQ282371)(MR)      RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
RWT3(OQ282375)(S)        RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
CTT2(OQ282373)(S)        RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
TML1(OQ282379)(S)        RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
TKT3(OQ282378)(S)        RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
RKT1(OQ282374)(S)        RGLIQDHVDSGVKLTQRDTFLNKDMYMQLLYNAWASMEDAGVEKAHIETVPPAILKPEPL
                         ************************************************************

TBL2(OQ282376)(R)        WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
CKLB1(OQ282372)(R)       WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
TBL1(PP261191)(R)        WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
TBL3(PP261192)(R)        WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
CKLL1(PP261190)(R)       WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
TKL3(OQ282377)(R)        WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
TML2(OQ282380)(MR)       WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
CKKB2(OQ282371)(MR)      WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
RWT3(OQ282375)(S)        WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
CTT2(OQ282373)(S)        WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
TML1(OQ282379)(S)        WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
TKT3(OQ282378)(S)        WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
RKT1(OQ282374)(S)        WTGKQVITSVLKLLTKGLPPLNLDSKAKIKGDLYGSANNEHVVIFRDGELLQGVLDKSQF
                         ************************************************************

TBL2(OQ282376)(R)        GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
CKLB1(OQ282372)(R)       GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
TBL1(PP261191)(R)        GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
TBL3(PP261192)(R)        GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
CKLL1(PP261190)(R)       GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
TKL3(OQ282377)(R)        GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
TML2(OQ282380)(MR)       GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
CKKB2(OQ282371)(MR)      GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
RWT3(OQ282375)(S)        GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
CTT2(OQ282373)(S)        GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
TML1(OQ282379)(S)        GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
TKT3(OQ282378)(S)        GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
RKT1(OQ282374)(S)        GASMYGMVHACYEVYGARIAADFLSALGRLFTCYLQFAGHTCAMEDLTLNTAAEKRRHKL
                         ************************************************************

TBL2(OQ282376)(R)        VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
CKLB1(OQ282372)(R)       VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
TBL1(PP261191)(R)        VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
TBL3(PP261192)(R)        VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
CKLL1(PP261190)(R)       VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
TKL3(OQ282377)(R)        VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
TML2(OQ282380)(MR)       VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
CKKB2(OQ282371)(MR)      VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
RWT3(OQ282375)(S)        VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
CTT2(OQ282373)(S)        VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
TML1(OQ282379)(S)        VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
TKT3(OQ282378)(S)        VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
RKT1(OQ282374)(S)        VKDSEVMGEEAYAEFAGLSELLEKKRASEKNGKKRRMNEEERVQIRDRMRTLLSGPDRDD
                         ************************************************************
```

```
TBL2(OQ282376)(R)      NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
CKLB1(OQ282372)(R)     NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
TBL1(PP261191)(R)      NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
TBL3(PP261192)(R)      NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
CKLL1(PP261190)(R)     NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
TKL3(OQ282377)(R)      NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
TML2(OQ282380)(MR)     NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
CKKB2(OQ282371)(MR)    NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
RWT3(OQ282375)(S)      NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
CTT2(OQ282373)(S)      NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
TML1(OQ282379)(S)      NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
TKT3(OQ282378)(S)      NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
RKT1(OQ282374)(S)      NAKALDAHMMGCVHGSNSDIIKTCLPSGQSKAFPKNNFSLMVLTGAKGSMVNHSQISCGL
                       ************************************************************

TBL2(OQ282376)(R)      GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
CKLB1(OQ282372)(R)     GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
TBL1(PP261191)(R)      GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
TBL3(PP261192)(R)      GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
CKLL1(PP261190)(R)     GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
TKL3(OQ282377)(R)      GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
TML2(OQ282380)(MR)     GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
CKKB2(OQ282371)(MR)    GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
RWT3(OQ282375)(S)      GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
CTT2(OQ282373)(S)      GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
TML1(OQ282379)(S)      GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
TKT3(OQ282378)(S)      GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
RKT1(OQ282374)(S)      GQQALEGRRVPILCSGRSLPSFEPFDPAPRAGGYVTDRFLTGLRPQEYYHHCMAGREGLV
                       ************************************************************

TBL2(OQ282376)(R)      DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
CKLB1(OQ282372)(R)     DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
TBL1(PP261191)(R)      DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
TBL3(PP261192)(R)      DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
CKLL1(PP261190)(R)     DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
TKL3(OQ282377)(R)      DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
TML2(OQ282380)(MR)     DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
CKKB2(OQ282371)(MR)    DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
RWT3(OQ282375)(S)      DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
CTT2(OQ282373)(S)      DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
TML1(OQ282379)(S)      DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
TKT3(OQ282378)(S)      DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
RKT1(OQ282374)(S)      DTAVKTSRSGYLQRCLIKHLEDIQVGYDHTVRSSDGNVIQFLYGEDGIDPVQSAMLSGKD
                       ************************************************************

TBL2(OQ282376)(R)      AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
CKLB1(OQ282372)(R)     AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
TBL1(PP261191)(R)      AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
TBL3(PP261192)(R)      AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
CKLL1(PP261190)(R)     AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
TKL3(OQ282377)(R)      AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
TML2(OQ282380)(MR)     AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
CKKB2(OQ282371)(MR)    AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
RWT3(OQ282375)(S)      AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
CTT2(OQ282373)(S)      AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
TML1(OQ282379)(S)      AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
TKT3(OQ282378)(S)      AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
RKT1(OQ282374)(S)      AQFDFQAMNHRSISHKYGINAKFFEKTKLDIMKPLKLHQEVREAKENNFASTSSVLKAGV
                       ************************************************************

TBL2(OQ282376)(R)      KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
CKLB1(OQ282372)(R)     KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
TBL1(PP261191)(R)      KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
TBL3(PP261192)(R)      KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
CKLL1(PP261190)(R)     KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
TKL3(OQ282377)(R)      KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
TML2(OQ282380)(MR)     KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
CKKB2(OQ282371)(MR)    KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
RWT3(OQ282375)(S)      KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
CTT2(OQ282373)(S)      KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
TML1(OQ282379)(S)      KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
TKT3(OQ282378)(S)      KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
RKT1(OQ282374)(S)      KVMARRLKQGETKWKRGSIELGFHAAEIVAVAEENEDGRECKYDIKYLDTGLKSFGVPRT
                       ************************************************************
```

```
TBL2(OQ282376)(R)        AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
CKLB1(OQ282372)(R)       AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
TBL1(PP261191)(R)        AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
TBL3(PP261192)(R)        AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
CKLL1(PP261190)(R)       AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
TKL3(OQ282377)(R)        AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
TML2(OQ282380)(MR)       AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
CKKB2(OQ282371)(MR)      AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
RWT3(OQ282375)(S)        AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
CTT2(OQ282373)(S)        AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
TML1(OQ282379)(S)        AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
TKT3(OQ282378)(S)        AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
RKT1(OQ282374)(S)        AKFKANKPTNATRAMSGRVDIIKVSLEDPVMQNLPLNEHVGLISERIQDKLRNYNKRNPS
                         ************************************************************

TBL2(OQ282376)(R)        NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
CKLB1(OQ282372)(R)       NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
TBL1(PP261191)(R)        NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
TBL3(PP261192)(R)        NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
CKLL1(PP261190)(R)       NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
TKL3(OQ282377)(R)        NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
TML2(OQ282380)(MR)       NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
CKKB2(OQ282371)(MR)      NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
RWT3(OQ282375)(S)        NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
CTT2(OQ282373)(S)        NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
TML1(OQ282379)(S)        NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
TKT3(OQ282378)(S)        NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
RKT1(OQ282374)(S)        NCLELMKKSKKSKKSKRAEDDGEVDREVMQSKHVVSPDAFQLLVWVNYLRSMCAPGENVG
                         ************************************************************

TBL2(OQ282376)(R)        ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
CKLB1(OQ282372)(R)       ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
TBL1(PP261191)(R)        ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
TBL3(PP261192)(R)        ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
CKLL1(PP261190)(R)       ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
TKL3(OQ282377)(R)        ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
TML2(OQ282380)(MR)       ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
CKKB2(OQ282371)(MR)      ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
RWT3(OQ282375)(S)        ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
CTT2(OQ282373)(S)        ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
TML1(OQ282379)(S)        ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
TKT3(OQ282378)(S)        ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
RKT1(OQ282374)(S)        ILAAQGIGEPSTQMTLNTFHLAGHGAANVTLGIPRLREIIMTASQKMSTPMMTIPLRDDV
                         ************************************************************

TBL2(OQ282376)(R)        ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
CKLB1(OQ282372)(R)       ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
TBL1(PP261191)(R)        ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
TBL3(PP261192)(R)        ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
CKLL1(PP261190)(R)       ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
TKL3(OQ282377)(R)        ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
TML2(OQ282380)(MR)       ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
CKKB2(OQ282371)(MR)      ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
RWT3(OQ282375)(S)        ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
CTT2(OQ282373)(S)        ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
TML1(OQ282379)(S)        ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
TKT3(OQ282378)(S)        ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
RKT1(OQ282374)(S)        ESSRAQEVEQHLNQVALSELINSTNGVRVKDQFHSSENGILWVRDYHIRLTFFQLKEIKR
                         ************************************************************

TBL2(OQ282376)(R)        VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
CKLB1(OQ282372)(R)       VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
TBL1(PP261191)(R)        VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
TBL3(PP261192)(R)        VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
CKLL1(PP261190)(R)       VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
TKL3(OQ282377)(R)        VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
TML2(OQ282380)(MR)       VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
CKKB2(OQ282371)(MR)      VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
RWT3(OQ282375)(S)        VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
CTT2(OQ282373)(S)        VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
TML1(OQ282379)(S)        VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
TKT3(OQ282378)(S)        VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
RKT1(OQ282374)(S)        VFGLSADQVFSSVGRGFVGKLLTLISREMKKSGVTVSAVEEKNNFSTPSGSSRKKKGDDD
                         ************************************************************
```

```
TBL2(OQ282376)(R)          DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
CKLB1(OQ282372)(R)         DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
TBL1(PP261191)(R)          DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
TBL3(PP261192)(R)          DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
CKLL1(PP261190)(R)         DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
TKL3(OQ282377)(R)          DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
TML2(OQ282380)(MR)         DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
CKKB2(OQ282371)(MR)        DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
RWT3(OQ282375)(S)          DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
CTT2(OQ282373)(S)          DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
TML1(OQ282379)(S)          DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
TKT3(OQ282378)(S)          DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
RKT1(OQ282374)(S)          DDDDDEQGTLRFGSRGEVQGYGEMDEEDEKIRKSQMVDDSDSDSDDETSGNKKSTDANIS
                           ************************************************************

TBL2(OQ282376)(R)          DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
CKLB1(OQ282372)(R)         DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
TBL1(PP261191)(R)          DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
TBL3(PP261192)(R)          DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
CKLL1(PP261190)(R)         DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
TKL3(OQ282377)(R)          DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
TML2(OQ282380)(MR)         DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
CKKB2(OQ282371)(MR)        DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
RWT3(OQ282375)(S)          DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
CTT2(OQ282373)(S)          DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
TML1(OQ282379)(S)          DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
TKT3(OQ282378)(S)          DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
RKT1(OQ282374)(S)          DDESMENGSGEMPNKDEKKSFATSGMESLEVPTSVRKNPYFVFCGRNDKKNYVELHLRFP
                           ************************************************************

TBL2(OQ282376)(R)          THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
CKLB1(OQ282372)(R)         THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
TBL1(PP261191)(R)          THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
TBL3(PP261192)(R)          THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
CKLL1(PP261190)(R)         THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
TKL3(OQ282377)(R)          THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
TML2(OQ282380)(MR)         THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
CKKB2(OQ282371)(MR)        THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
RWT3(OQ282375)(S)          THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
CTT2(OQ282373)(S)          THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
TML1(OQ282379)(S)          THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
TKT3(OQ282378)(S)          THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
RKT1(OQ282374)(S)          THSKTLLMVPLVEKVAKQVLVQHCPGISRCYLINQRIGESQDEKPCVQTEGLNFQEIWGF
                           ************************************************************

TBL2(OQ282376)(R)          DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
CKLB1(OQ282372)(R)         DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
TBL1(PP261191)(R)          DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
TBL3(PP261192)(R)          DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
CKLL1(PP261190)(R)         DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
TKL3(OQ282377)(R)          DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
TML2(OQ282380)(MR)         DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
CKKB2(OQ282371)(MR)        DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
RWT3(OQ282375)(S)          DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
CTT2(OQ282373)(S)          DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
TML1(OQ282379)(S)          DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
TKT3(OQ282378)(S)          DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
RKT1(OQ282374)(S)          DDILDVNNLATNDIYQVLQTYGVEAARASISKQITDVFGVYGISVDPRHLSLLADYMTAQ
                           ************************************************************

TBL2(OQ282376)(R)          GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
CKLB1(OQ282372)(R)         GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
TBL1(PP261191)(R)          GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
TBL3(PP261192)(R)          GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
CKLL1(PP261190)(R)         GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
TKL3(OQ282377)(R)          GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
TML2(OQ282380)(MR)         GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
CKKB2(OQ282371)(MR)        GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
RWT3(OQ282375)(S)          GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
CTT2(OQ282373)(S)          GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
TML1(OQ282379)(S)          GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
TKT3(OQ282378)(S)          GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
RKT1(OQ282374)(S)          GGYMPLNRMGMNNKGSSLQQITFETSMKFLSQAALGGLVDKLESPSARLVLGQPPRVGTG
                           ************************************************************
```

```
TBL2(OQ282376)(R)        SFSLLQPIAL
CKLB1(OQ282372)(R)       SFSLLQPIAL
TBL1(PP261191)(R)        SFSLLQPIAL
TBL3(PP261192)(R)        SFSLLQPIAL
CKLL1(PP261190)(R)       SFSLLQPIAL
TKL3(OQ282377)(R)        SFSLLQPIAL
TML2(OQ282380)(MR)       SFSLLQPIAL
CKKB2(OQ282371)(MR)      SFSLLQPIAL
RWT3(OQ282375)(S)        SFSLLQPIAL
CTT2(OQ282373)(S)        SFSLLQPIAL
TML1(OQ282379)(S)        SFSLLQPIAL
TKT3(OQ282378)(S)        SFSLLQPIAL
RKT1(OQ282374)(S)        SFSLLQPIAL
                         **********
```

Supplementary data Fig. S1. Multiple amino acid sequence alignments of RPA1 in 13 representative isolates of *Phytophthora palmivora* analyzed in this study. The isolate name, accession number and sensitivity type are shown in the left of the column and asterisk indicates the positions of identical amino acids in the sequence.