Honey bee ectoparasitic mite, *Tropilaelaps mercedesae* TRPA1, a novel target for developing mite control method

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Abstract

Tropilaelaps mercedesae is one of the most important honey bee ectoparasitic mites causing the large colony losses of honey bee in China. Commonly used miticides have the negative effects on honey bees, and have been resisted by the mites. Therefore, the effective and safe methods are required to control honey bee mites. Transient receptor potential channel, subfamily A, member 1 (TRPA1) functions as a nocisensor to induce avoidance behavior against noxious stimuli. A set of natural compounds capable of specially activating the *T. mercedesae* TRPA1 (TmTRPA1) but not AmHsTRPA* have been screened with calcium imaging, and they can be used as potential repellents to develop novel control method for *T. mercedesae*.

*AmHsTRPA is a functional counterpart of TRPA1 in honey bee.

Introduction

- Mite parasitization is one of the most important causes inducing colony losses of honey bee.
- *T. mercedesae* is a new ectoparasitic mite of *A. mellifera* (Figure 1) (Original host: *Apis dorsata*) and restricted in Asia (except Japan) to date but may diffuse all over the world due to global trade of honey bee and bee products (ex. *Varroa* mites).
- We still lack knowledge of chemical control targets of *T. mercedesae.*
- TRPA1 expressed in sensory neurons is one of the evolutionarily conserved non-selective cation channels, and functions as a nocisensor to induce avoidance behaviors.
- Natural compounds (extracted from plant essential oil) capable of specifically activating the TmTRPA1 but not AmHsTRPA can be used as potential safe and effective repellents to develop novel control methods for *T. mercedesae.*

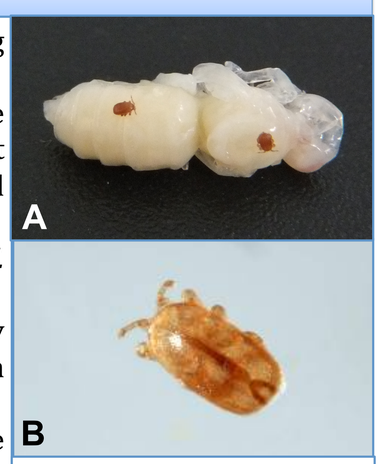

Figure 1 (A) *T. mercedesae* on honey bee larva. (B) A female *T. mercedesae.*

Methods

1.*TmTRPA1* cDNA isolation

- *TmTRPA1* cDNA was not identified, so the first step of this project was to isolate the full length *TmTRPA1* cDNA from total RNA of *T. mercedesae* (Figure 2).

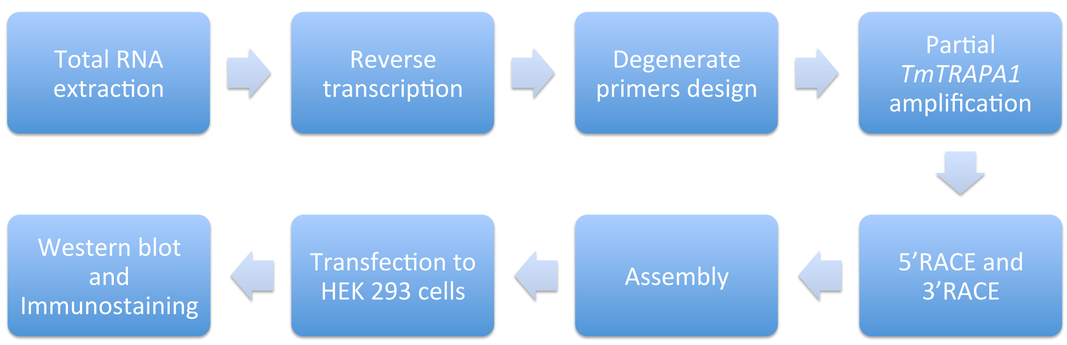

Figure 2. RT-PCR was used to amplify a partial *TmTRPA1* cDNA at 5' end with degenerate primers designed based on TRPA1 sequences of three mite/tick species. Then, 5'RACE and 3'RACE were used to isolate the full length *TmTRAPA1* cDNA. TmTRAPA1 protein expression was confirmed by Western blot and Immunostaining, after transfection into HEK 293 cells.

2. Calcium imaging

- Ca^{2+} imaging method was used to screen natural compounds that can specifically activate the TmTRPA1 but not AmHsTRPA. (Figure 3)

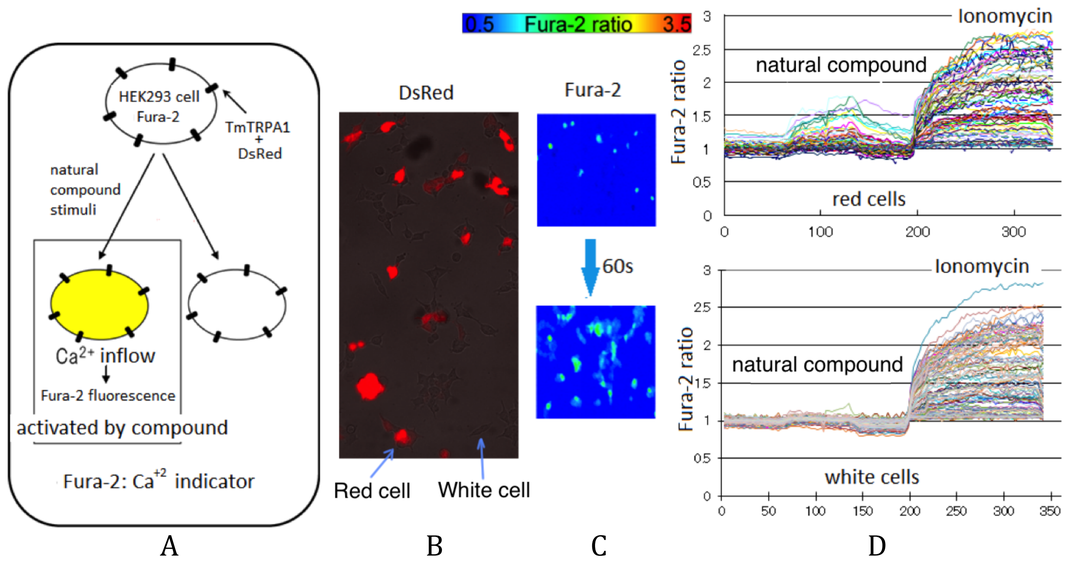

Figure 3.

A. HEK293 cells were transfected by *TmTRPA1* cDNA and DsRed which is a transfection marker. Fura-2 loaded in the cells is a free intracellular calcium indicator.
B. The transfected cells were identifed by DsRed, showing red colour fluorescence at 488nm.
C. The fluorescence of Fura-2 increased at 340 nm and 380 nm of light, indicating the increase of intracellular Ca^{2+} level.
D. When the activating compound was added to the cells, the Fura-2 ratio increased in the *TmTRPA1* transfected cells (red cells) but not in the untransfected cells (white cells).

* Ionomycin was added later to test the plasma membrane integrity as well as viability of cells.

Conclusions

- TmTRPA1 channel expressed in HEK293 cells functions as chemical sensor. **Compound 1, Compund 7** and **compound 8** could specifically activate one or more differrent TmTRPA1s but not AmHsTRPA channels, suggesting they could be used as potential repellents for *T. mercedesae* mites.

My current works

- Human are still lacking knowledge about biological and ecological characters of *T. mercedesae*, such as habitats, life cycles, and reproductions. A comparative genomic analysis within species and with other sequenced genomes can help us to deeply understand this species and finally control it. The genome and transcriptome have been sequenced and daftly assembled.

Results

1.*TmTRPA1* cDNA isolation

- Three isoforms of *TmTRPA1* mRNA were identified, and named as TmTRPA1-L1, TmTRPA1-L2, and TmTRPA1-S.
- The expression of V5-epitope tagged TmTRPA1 proteins in the transfected HEK293 cells was confirmed by both Western blot and immunostaining.
- TmTRPA1-L1 and -L2 proteins had larger molecular weight than the TmTRPA1-S (Figure 4).
- The TmTRPA1 proteins were predominantly found at the plasma membrane and endoplasmic reticulum in HEK293 cells by confocal microscopy (Figure 5).

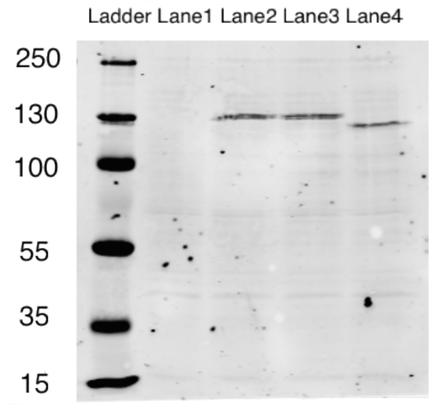

Figure 4 The TmTRPA1 proteins with molecular weight of ~130kDa were detected by Western blot. Numbers on the left side of the blot are the molecular weights (kDa) of the protein ladder. Lane 1, Negative control; Lane 2, TmTRPA1-L1; Lane 3, TmTRPA1-L2; Lane 4, TmTRPA1-S.

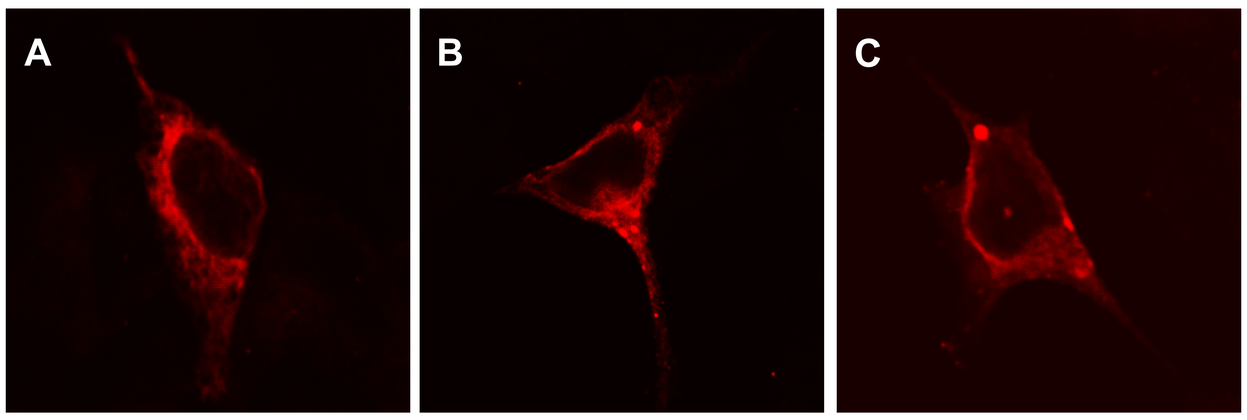

Figure 5. Localization of V5 epitope-tagged TmTRPA1 proteins in HEK293 cells visualized by Immunofluorescence A, TmTRPA1-L1; B, TmTRPA1-L2; C, TmTRPA1-S.

2. Calcium imaging

Different natural compounds were screened using Ca^{2+} imaging for the activation of each isoform of TmTRPA1 (Table 1).

Natural compounds	TmTRPA1-L1	TmTRPA1-L2	TmTRPA1-S	AmHsTRPA
Compound 1	Yes	Not tested	Yes	No
Compound 2	Yes	Not tested	Not tested	Yes
Compound 3	Yes	Yes	Yes	Yes
Compound 4	Yes	Yes	Yes	Yes
Compound 5	Yes	Not tested	Not tested	yes
Compound 6	Yes	Not tested	Not tested	Yes(week)
Compound 7	Yes	Yes	Yes	No
Compound 8	Yes	Not tested	Not tested	No
Compound 9	Yes (week)	Yes	Yes	Yes
Compound 10	Yes	Not tested	Yes	Yes
Compound 11	Yes	Not tested	Not tested	Yes
Compound 12	No	Yes	Yes	Yes
Compound 13	No	Yes	Not tested	Yes
Compound 14	No	Not tested	Yes	Yes
Compound 15	No	Not tested	Not tested	No
Compound 16	No	Not tested	Not tested	No
Compound 17	No	Yes	Yes	Not tested
Compound 18	No	Yes	Not tested	Not tested
Compound 19	No	Not tested	Not tested	Not tested
Compound 20	No	Not tested	Not tested	Not tested
Compound 21	No	Not tested	Yes	Not tested
Compound 22	No	Not tested	Yes	Not tested
Compound 23	No	Yes (week)	Not tested	Not tested
Compound 24	No	Not tested	Yes	Not tested

Table 1. Summary of activation of three isoforms of TmTRPA1 by different natural compounds. Yes, the TmTRPA1 could be activated by corresponding compound; No, the TmTRPA1 could not be activated by corresponding compound; Not tested, the corresponding compound has not been tested yet. The concentration of these compounds used for testing were almost 1mM.