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PP-068 [Bee Health]

Genomic variations among different isolates of *Vairimorpha ceranae* reveal the risk of microsporidian adaptation to thermal stress in Taiwan

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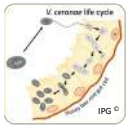
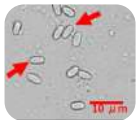
Abstract

Microsporidia are obligate intracellular parasites and *Vairimorpha ceranae* is one of the microsporidian pathogens causes microsporidiosis in *Apis mellifera*. Previous reports in 2012 suggested that mature spore counts increased as temperatures decreased in *A. mellifera* colonies. However, according to the monitoring data from 2020–2021 in Taichung, Taiwan, the mature spore load showed the different prevalence trend, spore counts increasing at higher temperatures. To better understand the potential genomic differences of *V. ceranae*, which may increase the thermotolerance, the genome of *V. ceranae* in Taichung, Taiwan (T*Vc*) was compared to the *V. ceranae* isolate from Chiang Mai, Thailand (Thai*Vc*) in this study. Besides, two publicly available genomes from NCBI were also included. By using a combination of NGS and ONT sequencing, we assembled the genomes of T*Vc* and Thai*Vc*. Comparative genomic analysis identified 162 distinctive genomic regions (DGRs) in T*Vc*, including 87 DGRs longer than 450 bp, which were absent in Thai*Vc* and previously published *V. ceranae* genomes. Furthermore, the transcriptomic data showed that 112 genes overlapped with these DGRs. Among these genes, nine genes were selected for further investigation. Of these selected genes, three genes were highly expressed during the early stage of T*Vc* infection, while the remaining six exhibited lower sequence identity to Thai*Vc* than to other isolates, assumed these genes might contribute to the different fitness between T*Vc* and Thai*Vc*. Our findings first proposed the potential role of genomic variations in *V. ceranae*. However, the correlation between these DGRs and environmental fitness still need to be further investigated.

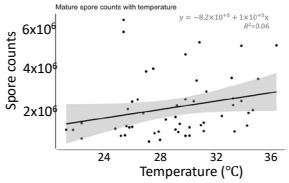
Background

- *Vairimorpha ceranae* infections of honey bees.





- Monitoring the Season–Prevalence of *V. ceranae*



In previous (Chen et al., 2012) showed the negative correlation between temperature and mature spore load, but our survey (2020-2021) showed positive correlation (Li et al., 2024).

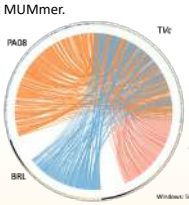
Genome sequencing and assembly

V. ceranae mature spores were collected from honey bees midgut tissues from apiaries in Taichung, Taiwan (T*Vc*) and Chiang Mai, Thailand (Thai*Vc*), respectively . Oxford Nanopore Technology (ONT) and NGS was performed to re-sequence. The assembled genome was filted by orf identity.

	T*Vc* filted genome	Thai*Vc* filted genome
Contigs	37	499
Total length	7,889,013 bp	5,864,918 bp
GC (%)	25.87	25.10
N50	310,165 bp	48,305 bp
N90	117,423 bp	4,366 bp
L50	6	36
L90	22	196
Complete BUSCO	94%	93.4%

Genome comparison

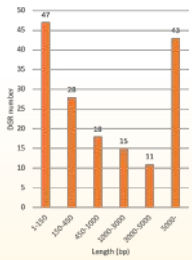
The assembled genomes and 2 published genomes (BRL, PA08; Accession number: PRJNA514060, PRJNA209464) was compared by using MUMmer.



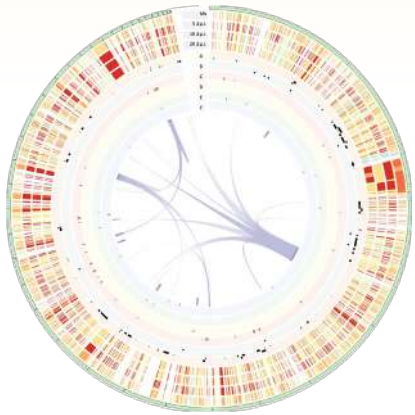
	T*Vc*	BRL	PA08
Avg Identity with T*Vc* (ANI)	98.83%	98.01%	99.02%
Aligned Seqs with T*Vc*	5,801,366 (98.92%)	8,146,464 (92.40%)	5611925 (98.61%)

Distinctive genomic regions (DGRs)

The comparisons identified 162 distinctive genomic regions (DGRs), 87 of which are longer than 450 bp.

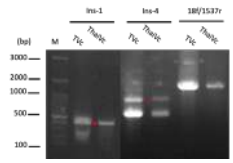


Gene expression and structure variant



The T*Vc* structure variant (SV) were compared to Thai*Vc* and identify by using **Mum&co**. To validated the correction of SV especially insertion, we design primers to check the length of insertion region.

SV	Number
Total SV	71
Insertion	10
Deletion	32
Inversion	1
Duplication	10
Contractions	8
Translocations	10



	Ins 1	Ins-4	18f/1537r
Insertion length (bp)	415	885	+
No insertion length (bp)	206	560	+

Within these DGRs, transcripts were generated using **StringTie v2.2.3** from our previous transcriptome data (Accession number: PRJNA814859)

	No.	GeneID	Diamond annotation		Transcriptome gene expression			Identity with isolates (%)		
			Protein ID	protein name	Mature spore	5 d.p.i.	10 d.p.i.	20 d.p.i.	ThaiVc	BRL	PA08
High gene expression of TVc early stage	1	MSTRG.862	EEQ83004.1	hypothetical protein	1278	1332	281	244	99.239	99.459	99.459
	2	MSTRG.594	XP_024331945.1	-	770	947	237	221	97.5	95.652	95.652
	3	MSTRG.838	KAF5140173.1	hypothetical protein	116	457	108	74	98.523	98.297	97.689
Lower identity than other isolates	4	MSTRG.1021	XP_024329322.1	endonuclease-reverse transcriptase	355	22	20	18	97.677	99.209	99.342
	5	MSTRG.1011	XP_024330535.1	hypothetical protein	641	619	269	197	98.771	99.005	99.005
	6	MSTRG.981	EEQ81296.1	hypothetical protein	169	49	21	20	96.296	99.098	99.351
	7	MSTRG.916	XP_024329462.1	hypothetical protein	183	25	11	10	95.698	98.78	98.438
	8	MSTRG.156	XP_024329611.1	hypothetical protein	146	77	29	23	95.008	98.479	98.463
	9	MSTRG.514	XP_024329364.1	endonuclease-reverse transcriptase, partial	398	0	4	0	83.859	99.83	99.838

Conclusions

1. We created high-quality genome assemblies for two *V. ceranae* isolates, achieved by combining NGS and ONT sequencing technologies.
2. Comparative genomic analysis between four genomes and identified 162 DGRs unique to the T*Vc*, 87 DGRs over 450 bp.
3. Transcriptomic profiling revealed 112 genes that overlap with these DGRs.

Future works

1. Check the relation between DGR, SV and gene expression between T*Vc* and Thai*Vc*.
2. Validate the DGR uniq of T*Vc* than Thai*Vc*.

Acknowledgments

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Reference

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