

Research Article



CrossMark

Open Access

등검은말벌과 꿀벌의 장내 세균 군집 비교

김익연,[†] 서정원,[†] 양소희, 김인선, 구연종*

Intestine Bacterial Microbiota of Asian Hornet (*Vespa Velutina Nigrithorax*) and Honey Bee

Euyeon Kim,[†] Jeongwon Seo,[†] So Hee Yang, In-Seon Kim and Yeonjong Koo* (Department of Agricultural Chemistry, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 61186, Korea)

Received: 15 June 2018/ Revised: 21 June 2018/ Accepted: 25 June 2018
Copyright © 2018 The Korean Society of Environmental Agriculture

This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID

Yeonjong Koo

<http://orcid.org/0000-0002-0147-1758>

Abstract

BACKGROUND: The Asian hornet (*Vespa velutina nigrithorax*), a wasp species, has attacked honey bee populations and affected the beekeeping industry in Korea over the past 15 years. However, little research has been done with this invasive species. In this study, we investigated the intestine bacterial microbiota of Asian hornets and honey bees to design an attractive trap for Asian hornets.

METHODS AND RESULTS: Genomic DNAs isolated from the intestine microorganisms of Asian hornets and honey bees were utilized to amplify bacterial 16S rDNA for the comparative sequence analysis. The next generation sequencing analysis identified that the orders *Flavobacteriales* as the most abundant intestinal microorganisms in Asian hornets, showing a clear difference compared to honey bees in which *Aeromonadales* are dominant. We also report five newly identified 16S rDNA sequences of Asian hornet intestinal bacteria. According to the sequence blast search, these five bacteria belong to the genera *Thalassomonas*, *Caedobacter*, *Vampirovibrio*, *Alkaliphilus* and *Calothrix*.

CONCLUSION: While Asian hornets and honey bees show similar intestine bacterial diversity, the relative ratio of bacterial populations is different. providing useful information to design pest control agents specifically targeting Asian hornets.

Key words: Asian hornet, Honey bee, Intestine bacterial microbiota

서론

가 가 (Seebens et al., 2017). (Asian hornet, *Vespa velutina*), 가 (Monceau et al., 2014; Budge et al., 2017). (Darrouzet et al. 2015; Poidatz et al. 2017) (Sauvard et al. 2018), (Barbet-Massin et al. 2018).

[†]These authors contributed equally this work

*Corresponding author: Yeonjong Koo

Phone: +82-62-530-2133; Fax: +82-62-530-2139;

E-mail: yeonjong@jnu.ac.kr

Table 1. Community richness and diversity indexes

SampleName	OTUs	Chao1	Shannon	Simpson
Asian hornet	49	51	3.9760567	0.9069076
Honey bee	51	51.8571	3.4534622	0.8189556

가
 (Choi *et al.*, 2015; Kang *et al.*, 2016).

(D'Argenio and Salvatore, 2015).

Qiagen Plasmid Purification kit (Qiagen, Germany) multiplex index PCR 8 cycle normalization MiSeq™ platform (Illumina, San Diego, USA) (Macrogen, Korea).

Sequencing 결과 분석

Adaptor 99,721,194 가
 Sequencing read 439,378 ,
 417,848 8715 OTU 6718
 OTU bacterial 16S rDNA가 (Table 1).

결과 및 고찰

count Table 2

Table 2

(Fig. 1).

(order) *Flavobacteriales*

Sphingobacterales

Aeromonadales *Alteromonadales* *Flavobacteriales*

Sphingobacterales Bacteroidetes (Phylum)

Aeromonadales

Proteobacteria

가 (genus)

(Table 3).

Sphingobacterales *Pedobacter* 가
Pedobacter nyackensis

Pedobacter oryzae (species) 가

Proteobacteria

Novosphingobium aromaticivorans

가 가 *Aeromonadales*

가 *Aeromonas hydrophila*

재료 및 방법

Genomic DNA 추출 및 PCR 반응

70% EtOH
 30% glycerol 500 µL -8
 0°C Genomic DNA
 glycerol

DNA (Kouduka *et al.*, 2012). 100 mM NaOH 가 95°C 15
 Tris-HCl (pH 7.0)

가 10000 rpm 5

PCR

Illumina 16S Metagenomic sequencing library protocols

PCR ex-Taq polymerase (Takara, Japan)

annealing 60°C 25 cycle

16S rDNA primer 16S Amplicon PCR

Forward Primer 5'-TCGTCGGCAGCGTCAGATGTG

TATAAGAGACAGCCTACGGGNGGCWGAAG-3'

16S Amplicon PCR Reverse Primer 5'-GTCTCGTG

GGCTCGGAGATGTCTATAAGAGACAGGACTACHV

GGGTATCTAATCC-3' 16S rDNA

V3-V4 (Petrosino *et al.*, 2009). PCR

Table 2. Bacterial microorganism in Asian hornet and Honey bee

Order	Species	Asian hornet	Honey bee
	Unidentified	809	199
Flavobacteriales	<i>Flavobacterium anhuiense</i>	1124	876
	<i>Flavobacterium aquaticum</i>	14	9
	<i>Flavobacterium brevivitae</i>	4	3
	<i>Flavobacterium cauense</i>	49	20
	<i>Flavobacterium fluvii</i>	23	9
	<i>Flavobacterium glycines</i>	12	10
	<i>Flavobacterium hauense</i>	3	0
	<i>Flavobacterium hydatis</i>	2	1
	<i>Flavobacterium macrobrachii</i>	389	290
	<i>Flavobacterium notoginsengisoli</i>	2	1
	<i>Flavobacterium rakeshii</i>	32	35
	<i>Flavobacterium urocaniciphilum</i>	2	1
	Sphingobacteriales	<i>Pedobacter kyungheensis</i>	7
<i>Pedobacter nyackensis</i>		516	340
<i>Pedobacter oryzae</i>		298	136
Cytophagales	<i>Arcicella aurantiaca</i>	1	2
Bacillales	<i>Paenibacillus aestuarii</i>	104	53
Clostridiales	<i>Clostridium carboxidivorans</i>	1	2
Sphingomonadales	<i>Novosphingobium aromaticivorans</i>	33	15
	<i>Sphingomonas astaxanthinifaciens</i>	1	2
Burkholderiales	<i>Acidovorax delafieldii</i>	18	26
	<i>Comamonas aquatica</i>	189	305
	<i>Comamonas testosteroni</i>	77	107
	<i>Massilia suwonensis</i>	3	7
	<i>Massilia varians</i>	218	339
Neisseriales	<i>Vogesella amnigena</i>	4	6
	<i>Vogesella fluminis</i>	135	221
Nitrosomonadales	<i>Methylophilus flavus</i>	2	1
	<i>Methylothermobacter mobilis</i>	78	94
Enterobacteriales	<i>Enterobacter kobei</i>	0	3
Aeromonadales	<i>Aeromonas hydrophila</i>	3	9
	<i>Aeromonas taiwanensis</i>	1181	3341
Alteromonadales	<i>Shewanella chilikensis</i>	0	10
	<i>Shewanella xiamenensis</i>	306	575
Chromatiales	<i>Rheinheimera arenilitoris</i>	15	37
	<i>Rheinheimera baltica</i>	54	96
	<i>Rheinheimera mesophila</i>	7	6
Pseudomonadales	<i>Acinetobacter johnsonii</i>	92	94
	<i>Acinetobacter tandoii</i>	124	125
	<i>Pseudomonas guineae</i>	677	956
	<i>Pseudomonas linyingensis</i>	130	169
	<i>Pseudomonas migulae</i>	113	151
	<i>Pseudomonas panipatensis</i>	5	5
Xanthomonadales	<i>Lysobacter ruishenii</i>	1	2
	<i>Stenotrophomonas pavanii</i>	20	23
	Total	6878	8715

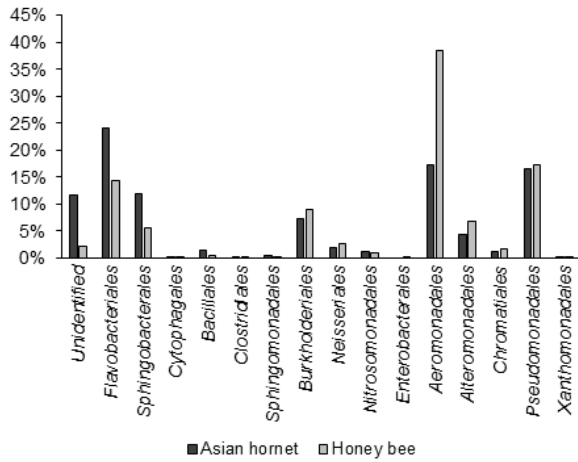


Fig 1. Graphic comparison of bacterial population ratio of Asian hornet and honey bee in the order level.

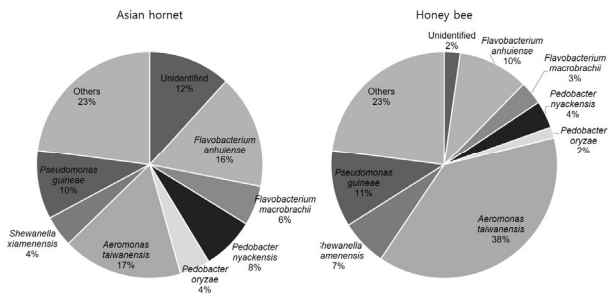


Fig 2. The population ratio of major bacteria species in the Asian hornet and honey bee intestine.

Table 3. Bacterial microbiome comparison between Asian hornet and honey bee in the genus level

Genus	Asian hornet (%)	Honey bee (%)
Unidentified	12	2.3
<i>Flavobacterium</i>	24	14
<i>Pedobacter</i>	12	5.5
<i>Arcicella</i>	0.01	0.02
<i>Paenibacillus</i>	1.5	0.61
<i>Clostridium</i>	0.01	0.02
<i>Novosphingobium</i>	0.48	0.17
<i>Sphingomonas</i>	0.01	0.02
<i>Acidovorax</i>	0.26	0.3
<i>Comamonas</i>	3.9	4.7
<i>Massilia</i>	3.2	4.0
<i>Vogesella</i>	2.0	2.6
<i>Methylophilus</i>	0.03	0.01
<i>Methylotenera</i>	1.1	1.1
<i>Enterobacter</i>	0.00	0.03
<i>Aeromonas</i>	17	38
<i>Shewanella</i>	4.5	6.7
<i>Rheinheimera</i>	1.1	1.6
<i>Acinetobacter</i>	3.1	2.5
<i>Pseudomonas</i>	13	15
<i>Lysobacter</i>	0.01	0.02
<i>Stenotrophomonas</i>	0.29	0.26

Aeromonas taiwanensis 2 가 .
4%
7 ,

Aeromonas taiwanensis,
Favobacterium anhuiense, *Pseudomonas guineae*, *Pedobacter nyyackensis*, *Flavobacterium macrobrachii*가 5%

가 70% . *Aeromonas taiwanensis*, *Pseudomonas guineae*, *Flavobacterium anhuiense*, *Shewanella xiamenensis* 4 5%

(Fig. 2). 가

40% *Aeromonas waiwanensis*가

가

가 ,

가

. *Thalassomonas*, *Caedobacter*, *Vampirovibrio*,

Alkaliphilus *Calothrix*
16S rDNA 가 가
Table 4 .
가

(*Vespa velutina nigrithorax*)

요 약

16S rDNA 가 DNA
V3, V4
(order)

Flavobacteriales 가
, *Aeromonadales* *Pseudomonadales*가

Table 4. Unidentified bacterial 16S rDNA sequences from Asian hornet intestine

Family	16S rDNA	The closest species (Identities %)
Caedobacter	CCTACGGGAGGCAGCAGTGAAGAATCTTGGGCAATGGGCTAACCGCTGACCCAGTGAGAACACA TGCATGATGAATGCGCACAGTTTTCTGTAAAGTGTATTTCGGGGATCAAGAAAACGACACAAGC CCGAGATGAAGCGCCGCCAACTCCGTGCCAGCAGCCCGGTAAGACGGAGGGCGCGAGCGTTA TTCGTTTTGATTGGGTGTAAGGGTATGTAGGCGGCCCTAGTTTTTTGGCTAAAAAAGCGGAGTG TTCCTATGCTATATGGCCTTTGAAAAAGGGGGCTTGTGTTGATGATAGGTCGGGGCAAGTCTTAT GTAGGGGTAGAATCCCACCATATGAGAACGAACGACAGCTGGCGAAGGCGCCCTCCCGCTTCA ACAGACGCTAAGGTACGGAAGCTTAGGTAGCAAACGGGATTAGATACCCTA	<i>Caedibacter caryophilus</i> (77%)
Alkaliphilus	CCTACGGGGGGCTGCAGTAGGAAATCTCCACAATGGACGAAAGTCTGATGGAGCAACTCCCGCT GCAGGATGAAGGCCTTAGGGTCGTAAACTGCTTTTATGAGTGAAGAATATGACGGTAACTCATGA ATAAGGGTCGGCTAACTACGTGCCAGCAGCCCGGTCATACGTAGGACCCAAGCGTTATCCGGA GTGACTGGGCGTAAAGAGTTGCGTAGGTGGTTTGTAAAGTGAATAGTGAATCTGGCGGCTCAAC CGTACAGGCTATTATTCAAACTGGCAAACTCGAGAATGGTAGAGTAACTGGAATTTCTTGTGTA GGAGTGAATCCGTAGATATAAGAAGGAACACCAATGGCGTAGGCAGGTTACTGGACCATTCT GACACTGAGGCACGAAAGCGTGGGGAGCGAAACCGGATTAGATACCCAGTAGTC	<i>Alkaliphilus metalliredigens</i> (81%)
Calothrix	CCTACGGGAGGCAGCAGTGAAGAATTTTCTGCAATGGGCGAAAGCCTGACAGAGCAATACCGCG TGAGGGATGAAGGCCTGTGGGTGTAAAACCTCTTTTCTCAGGAAAGAAGATCTGACGTTACTTGA GGAATAAGCATCGGCTAACCCGTGCCAGCAGCCCGGTAATACAGAGGATGCAAGTGTATTTCG GAATTAATGGGCTAAAGCGTCTGTAGTGGCAATTAAGTCTTTTGTCAAATCTTTGGGCTTAAC CCAAAGCGTGCAAAAAGAACTGTTTGTCTAGAGTCTGTGAGAGGTAAGGGAATTTCCAGTGGAG CCGTAAAATGCGTAGATATTGGAAGGAACACCAAAGCGAAAGCACTTTACTGGCCAGTACTG AACTGAGAGACGAAAGCTAGGGGAGCAAACAGGATTAGATACCCAGTAGTC	<i>Calothrix desertica</i> (85%)
Thalassomonas	CCTACGGGGGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCGTGATGCAGCCATGCCGCG TGTGTGAAGAAGGCCTTCGGGTGTAAAGCACTTTCAGTAGGGAGGAAAGGGTGAGTCTTAATAC GGCTCATCTGTGACGTTACCTACAGAAGAAGGACCCGGCTAACTCCGTGCCAGCAGCCCGGTAAT ACAGAGGATGCAAGTGTATTTCGGAATTAATGGGCGTAAAGCGTCTGTAGTGGGCAATTAAGTC TTTTGTCAAATCTTTGGGCTTAACCAAAGCGTGCAAAAAGAACTGTTTGTCTAGAGTCTGTTAGA GTAAAGGGGAATTTCCAGTGGAGCGGTAATAATGCGTAGATATTGGAAGGAACACCAAAGCGAA AGCACTTTACTGGCCAGTACTGACACTGAGAGACGAAAGCTAGGGGAGCA	<i>Thalassomonas agariperforans</i> (84%)
Vampirovibrio	CCTACGGGTGGCAGCAGTGGGGAATTTACGCAATGGGGGAAACCCGTGACGTAGCGACACCGCG TGAGCGAAGAAGCCCTTTGGGTGTAAAGCTCTGTGACGTGGAACGAAAACAATGACGGTACCA GCAGAGGAAGCATCGGCTAACTACGTGCCAGCAGCCCGGTAAGACGTAGGATGCGAGCGTTGT CCGGATTTATTGGGCGTAAAGAGTTCGTAGGTGGTTTGTAAAGTTGGTGTAAAGATCGGGGCTC AACCCCTGGGACTGCACTGAATACTGGCAGACTCGAGTGTGGTAGAGGCTAGTGAATTCAGTG TAGCGGTGAAATGCGTAGATATTGGGAAGAACCCGTGGCGTAGCGACTAGCTGGCCATAA CTGACGCTGAGGAACGAAAGCCAGGGGAGCGAATGGGATTAGATACCCAGTA	<i>Vampirovibrio chlorellavorus</i> (84%)

*Flavobacteriales*가, *Aeromonadales* *Pseudomonadales*

16S rDNA

Thalassomonas,*Caedobacter*, *Vampirovibrio*, *Alkaliphilus* *Calothrix***Note**

The authors declare no conflict of interest.

Acknowledgement

This work was supported by Korea national research foundation NRF-2017R1D1A1B03036241.

References

- Barbet-Massin, M., Rome, Q., Villemant, C., & Courchamp, F. (2018). Can species distribution models really predict the expansion of invasive species?. *PLoS One*, 13(3), e0193085.
- Budge, G. E., Hodgetts, J., Jones, E. P., Ostojá-Starzewski, J. C., Hall, J., Tomkies, V., Semmence, N., Brown, M., Wakefield, M., & Stainton, K. (2017). The invasion, provenance and diversity of *Vespa velutina* Lepeletier (Hymenoptera: Vespidae) in Great Britain. *PLoS One*, 12(9), e0185172.
- Choi, Y. S., Lee, M. L., Lee, M. Y., Kim, H. K., Yoon, M. Y., & Kang, A. R. (2015). Trapping of vespa *velutina nigrithorax* Buysson (Hymenoptera: Vespidae) queen near apiaries honeybee comb extract in south region of Korea. *Journal of Apicultural Research*, 30(4), 281-285.
- Darrouzet, E., Gévar, J., Guignard, Q., & Aron, S. (2015). Production of early diploid males by European colonies of the invasive hornet *Vespa velutina nigrithorax*. *PLoS One*, 10(9), e0136680.
- D'Argenio, V., & Salvatore, F. (2015). The role of the gut microbiome in the healthy adult status. *Clinica Chimica Acta*, 451(part A), 97-102.
- Kang, E. J., Lee, M. L., Lee, M. Y., Kim, H. K., & Choi, Y. S. (2016). Field evaluation of Honeybee Pheromone traps for the Hornet *Vespa velutina nigrithorax* Buysson (Hymenoptera: Vespidae). *Proceeding of the Apicultural Society of Korea*, 116.
- Kouduka, M., Suko, T., Morono, Y., Inagaki, F., Ito, K., &

- Suzuki, Y. (2012). A new DNA extraction method by controlled alkaline treatments from consolidated subsurface sediments. *FEMS Microbiology Letters*, 326(1), 47-54.
- Monceau, K., Bonnard, O., & Thiéry, D. (2014). *Vespa velutina*: a new invasive predator of honeybees in Europe. *Journal of Pest Science*, 87(1), 1-16.
- Petrosino, J. F., Highlander, S., Luna, R. A., Gibbs, R. A., & Versalovic, J. (2009). Metagenomic pyrosequencing and microbial identification. *Clinical chemistry*, 55(5), 856-866.
- Poidatz, J., Bressac, C., Bonnard, O., & Thiéry, D. (2017). Delayed sexual maturity in males of *Vespa velutina*. *Insect Science*, 1-11.
- Sauvard, D., Imbault, V., & Darrouzet, É. (2018). Flight capacities of yellow-legged hornet (*Vespa velutina nigrithorax*, Hymenoptera: Vespidae) workers from an invasive population in Europe. *PloS one*, 13(6), e0198597.
- Sauvard, D., Imbault, V., & Darrouzet, É. (2018). Flight capacities of yellow-legged hornet (*Vespa velutina nigrithorax*, Hymenoptera: Vespidae) workers from an invasive population in Europe. *PloS one*, 13(6), e0198597.