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등검은말벌과 꿀벌의 장내 세균 군집 비교

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

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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).

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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 Illumina sequencing adapter 가
 index PCR 8 cycle
 normalization MiSeq™ platform (Illumina, San
 Diego, USA)
 (Macrogen,
 Korea).

Sequencing 결과 분석

Adaptor 99,721,194
 93,614,940 가
 Sequencing read 439,378
 417,848 8715 OTU 6718
 OTU bacterial 16S rDNA가 (Table 1).

결과 및 고찰

count Table 2

Table 2

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

(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*

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

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

Family	16S rDNA	The closest species (Identities %)
Caedobacter	CCTACGGGAGGCAGCAGTGAAGAATCTTGGGCAATGGGCTAACCGCTGACCCAGTGAGAACACA TGCATGATGAATGCGCACAGTTTTCTGTAAAGTGTATTTCGGGGATCAAGAAAACGACACAAGC CCGAGATGAAGCGCCGCCAACTCCGTGCCAGCAGCCCGGTAAGACGGAGGGCGCGAGCGTTA TTCGTTTTGATTGGGTGTAAAGGGTATGTAGGCGGCCCTAGTTTTTTGGCTAAAAAAGCGGAGTG TTCCTATGCTATATGGCCTTTGAAAAAGGGGGCTTGTGTGATGATAGGTCGGGGCAAGTCTTAT GTAGGGGTAGAATCCCACCATATGAGAACGAACGACAGCTGGCGAAGGCGCCCTCCCGCTTCA ACAGACGCTAAGGTACGGAAGCTTAGGTAGCAAAACGGGATTAGATACCCTA	<i>Caedibacter caryophilus</i> (77%)
Alkaliphilus	CCTACGGGGGGCTGCAGTAGGAAATCTCCACAATGGACGAAAGTCTGATGGAGCAACTCCCGCT GCAGGATGAAGGCCTTAGGGTCGTAAACTGCTTTTATGAGTGAAGAATATGACGGTAACTCATGA ATAAGGGTCGGCTAACTACGTGCCAGCAGCCCGGTCATACGTAGGACCCAAGCGTTATCCGGA GTGACTGGGCGTAAAGAGTTGCGTAGGTGGTTTGTAAAGTGAATAGTGAATCTGGCGGCTCAAC CGTACAGGCTATTATTCAAACTGGCAAACTCGAGAATGGTAGAGGTAAGTGAATTTCTTGTGTA GGAGTGAATCCGTAGATATAAGAAGGAACACCAATGGCGTAGGCGAGGTTACTGGACCATTCT GACACTGAGGCACGAAAGCGTGGGGAGCGAAACCGGATTAGATACCCAGTAGTC	<i>Alkaliphilus metalliredigens</i> (81%)
Calothrix	CCTACGGGAGGCAGCAGTGAAGAATTTTCTGCAATGGGCGAAAGCCTGACAGAGCAATACCGCG TGAGGGATGAAGGCCTGTGGGTGTAAAACCTCTTTTCTCAGGAAAGAAGATCTGACGTTACTTGA GGAATAAGCATCGGCTAACCCGTGCCAGCAGCCCGGTAATACAGAGGATGCAAGTGTATTTCG GAATTAATGGGCTAAAGCGTCTGTAGTGGCAATTAAGTCTTTTGTCAAATCTTTGGGCTTAAC CCAAAGCGTGCAAAAAGAACTGTTTGTCTAGAGTCTGTGAGAGTAAAGGGAATTTCCAGTGGAG CCGTAAAATGCGTAGATATTGGAAGGAACACCAAAGCGAAAGCACTTTACTGGCCAGTACTG ACCTGAGAGACGAAAGCTAGGGAGCAAAACAGGATTAGATACCCAGTAGTC	<i>Calothrix desertica</i> (85%)
Thalassomonas	CCTACGGGGGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCGTGATGCAGCCATGCCGCG TGTGTGAAGAAGGCCTTCGGGTGTAAAGCACTTTCAGTAGGGAGGAAAGGGTGAGTCTTAATAC GGCTCATCTGTGACGTTACCTACAGAAGAAGGACCCGGCTAACTCCGTGCCAGCAGCCCGGTAAT ACAGAGGATGCAAGTGTATTTCGGAATTAATGGGCGTAAAGCGTCTGTAGTGGGCAATTAAGTC TTTTGTCAAATCTTTGGGCTTAACCAAAGCGTGCAAAAAGAACTGTTTGTCTAGAGTCTGTTAGA GTAAAGGGGAATTTCCAGTGGAGCGGTAATAATGCGTAGATATTGGAAGGAACACCAAAGCGAA AGCACTTTACTGGCCAGTACTGACACTGAGAGACGAAAGCTAGGGGAGCA	<i>Thalassomonas agariperforans</i> (84%)
Vampirovibrio	CCTACGGGTGGCAGCAGTGGGGAATTTACGCAATGGGGGAAACCCGTGACGTAGCGACACCGCG TGAGCGAAGAAGCCCTTTGGGTGTAAAGCTCTGTGACGTGGAACGAAAACAATGACGGTACCA GCAGAGGAAGCATCGGCTAACTACGTGCCAGCAGCCCGGTAAGACGTAGGATGCGAGCGTTGT CCGGATTTATTGGGCGTAAAGAGTTCGTAGGTGGTTTGTAAAGTTGGTGTAAAGATCGGGGCTC AACCCCTGGGACTGCACTGAATACTGGCAGACTCGAGTGTGGTAGAGGCTAGTGAATCCAGTG TAGCGGTGAAATGCGTAGATATTGGGAAGAACCCGTGGCGTAGGCGACTAGCTGGCCATAA 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.

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