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Research Article

Host-insect and host-plant associated diversity in microbiota isolated from most important Oriental-Australian region egg parasitoid

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ABSTRACT: Host-insect and host-plant associated differentiation of genetically divergent microbiota were recorded from economically important egg parasitoid collected from 26 locations in India, *Trichogramma chilonis* constituted 86.8% of the populations collected. It was recorded from 14 host-insects, 14 different crops and weed plants from 12 states. Nine species of yeast were recorded from parasitoid from 5 host-insects with *Wickerhamomyces anamalus* was isolated from 36.4% samples and highest numbers were recorded from parasitoid collected on sugarcane. *Bacillus cereus*, *Pseudomonas* sp. and *Stenotrophomonas maltophilia* from *T. chilonis* constitute 64.3% of bacterial diversity based on their 16S rDNA sequences. For taxonomic identification using 16s rDNA and ITS sequences, we performed taxonomic classification of total 33 ITS isolates against UNITE Fungal ITS database and assigned taxonomy hierarchy to the sequences. Also, a total of 13 isolates 16s rDNA sequences were taxonomically assigned against RDP 16s rDNA database using RDP Naive Bayesian rRNA Classifier Version 2.1. Most of the species are correctly identified in the respective species members with high confidence threshold value support.

KEY WORDS: Egg parasitoid, evolutionary relationship, host-insects, host-plants, Microbiota

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INTRODUCTION

The insect gut is known to harbor diverse microbiota that often perform beneficial role, though some are known to have opportunistically harmful role (Bignell, 1984; Kaufman and Klug, 1991; Dillon and Dillon, 2004; Bernays and Klein, 2002; Tagami *et al.,* 2006; Bourtzis and Miller, 2003; Broderick *et al.,* 2006). Several yeasts have been recorded in insects, mainly those feeding on fruiting bodies, flowers and nectar (Lachance and Bowles, 2002; Suh *et al.,* 2004; Nguyen *et al*., 2006). Ferrari *et al*., (2006) reported host-associated differentiation in divergent microbiota isolated from pea aphid. Host-Insect and host-plant associated divergence was proposed as a mechanism promoting greater than before species range (Stireman *et al.,* 2005). It has been proposed that the microbiota diversity of insects play a crucial role in host-insect or host-plant associated diversity by allowing insects to use different plant species (Ferrari and Vavre, 2011; Gauthier *et al*., 2015). The microbiota of the insects has been quantified using culture-dependent and culture-independent methods (Mrázek *et al*., 2008; Chandler *et al.,* 2011; Toju and Fukatsu, 2011; Ngo *et al.,* 2015; Montagna *et al*., 2015). Culture-dependent methods often produce results based on microbes that can be cultured on artificial media. In metagenomics approach, culture-independent molecular analyses of the ITS gene for yeast species and 16S rRNA gene for bacteria has produced a enhanced and more complete representation of communities of insects. The latest advances in the molecular biological use through next generation sequencing technologies have resulted in quantification of the significantly larger number of microbiota than previously estimated by traditional culture-based method (Yun *et al*., 2014). The analysis of microbial diversity with its link to host insect and host-

plant is vital for a better understanding of the ecological aspects. However, most studies carried out so far focused on insect pests or predators, but none of them have been on parasitoids like *Trichogramma*, except for work done by Srinatha *et al*., (2015).

The relationship of insect populations with various host-plants may depend on suitable microbiota harbored by them, in such cases there is the possibility of a divergence of various species and or strains (Medina *et al*., 2011). Bilodeau *et al*., (2013) reported that insect parasitoids and their host-insects represent a wide range of parasitic trophic relationship and observed a strong variation of the predictive power of intrinsic (body color) and extrinsic traits (symbionts, host plant). Host variables considered as key predictors of outcomes strongly interact and cannot be considered in isolation.

Among various natural enemies used for suppression of insect pests, egg parasitoids trichogrammatids are the most widely used, particularly for lepidopteran pests on several crops such as rice, corn, sugarcane, cotton, several vegetable and fruit crops, and forest trees, covering more than 33 crops and 52 insect species (Hassan, 1997) because of their ability to manage several insect pests and are easily mass-produced. *Trichogramma chilonis* Ishii is the most widely used species in an integrated pest management programme in South, Southeast Asia, Far-east, Indo-China region and Pacific region. It has also been reported as exotic in Kenya, Spain, South Africa and Australia (Jalali *et al*., 2006). It is often cited that compared to the laboratory-bred population, field-collected populations of *Trichogramma* are robust and have a higher biological fitness (Nagarkatti and Nagaraja, 1978; Jalali and Singh, 1993).

In the present work, we investigated the association of microbiota isolated from egg parasitoids collected from fourteen crops including weed plants. There is no work done to date on the diversity and composition of microbes associated with trichogrammatids and corresponding host-plants. The present study, therefore, aimed to investigate the diversity of the microbiota through extensive sampling approach and sequencing technique.

MATERIALS AND METHODS

Insect collection

At least fifty eggs and about 20 eggs-masses of insects were collected from 14 insect species, including unknown insect eggs on 14 crops, including weed plants, 12 different states covering 26 locations in India, ranging from GPS coordinates of 11.018° to 34.090°N and 73.200° to 94.220°E (Table 1). These eggs were brought to Molecular Entomology laboratory, ICAR-NBAIR, Bangalore, for observing the emergence of the egg parasitoids. After emergence, the parasitoids were identified by a renowned *Trichogramma* taxonomist, Dr. H. Nagaraja, for their identity and were immediately used for isolation of microbiota.

Isolation of endosymbionts from different Trichogramma species collected from various locations, host-insects and crops

Adult wasps of *Trichogramma* were released in sterile Eppendorf vials (1.5 ml) and were frozen at –70°C deep freezer (M/s New Brunswick Ultra Low Freezer CFC free Model U41085). Such frozen wasps were allowed to thaw for 10 min and were first washed with 70% ethanol for 1 min followed by surface sterilization with 10% sodium hypochlorite for 5 minutes to remove the adhering contaminants especially the external microflora and washed with distilled water five times (Meyer and Hoy, 2008). This method of surface sterilization prior to extraction of microbiota is standard as endorsed by Hammer *et al*., (2015), who used five different methods such as freezing, ethanol, Dimethyl Sulfoxide (DMSO), Cetrimonium Bromide (CTAB), and room-temperature storage without preservative and found that storage method had little to no effect on assessments of microbiota composition. Five to 10 *Trichogramma* adults were transferred into sterile Eppendorf vials (1.5 ml), using a camel hair brush (0 number) and were homogenized with a micro-pestle in 50µl sterile distilled water in a Laminar Flow Chamber (M/s Alpha Linear, Bangalore, India). 50µl of the supernatant was pipetted out on Yeast Extract Peptone, Dextrose (YEPD) agar amended with and without chloramphenicol/cycloheximide @ 100ppm in each plate and spread using a L shaped spreader and the plates were incubated for 48 hours in a BOD incubator (M/s Remi Instruments, Bangalore, India) maintained at 26±0.5°C and 60±2% RH.

DNA isolation, amplification, sequencing, and identification

Yeast DNA isolation and amplification

Yeast colonies obtained on chloramphenicol amended YEPD medium were sub cultured on yeast extract peptone, dextrose broth and incubated for 48 hours in a BOD incubator maintained at 26±0.5°C and 60±2% RH for 48 h. One ml of the cell suspension was transferred to a fresh 1.5ml eppendorf tube and centrifuged at 7000 rpm for 3 minutes. The cells were re-suspended in sorbitol buffer. Genomic DNA was extracted using bacterial and yeast genomic DNA miniprep purification kit (M/s Himedia Laboratories, India) as per manufacturer's instructions. The extracted DNA was stored at –20°C for further use. Each population of yeast was maintained individually.

The fungus-specific universal primers ITS1 and ITS4 were used to amplify the ITS region as described by White *et al.,* (1990). The primers used for YITS-PCR: YITS Forward: 5' TCCGTAGGTGAACCTGCGG3' YITS Reverse: 5'TCCTCCGCTTATTGATATGC3'. PCR was performed in a total volume of 50 µl consisting of 10X Taq buffer, 10mM dNTP's, 1U of Taq DNA polymerase, 20 pmol each of the primer and 50µg of template DNA. PCR was performed by employing the following conditions: samples were subjected to 94°C for initial denaturation for 3minutes; followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute and extended elongation step was performed at 72°C for 3 min. With each run a negative control was performed with nuclease free water in place of template DNA in the PCR mixture.

Bacterial DNA isolation and amplification

Similar to yeast cultures, bacterial colonies obtained on cycloheximide amended medium were sub cultured on Nutrient Agar consisting of (mass/volume): 0.5% Peptone, 0.3% yeast extract, 1.5% agar, 0.5% Nacl and distilled water and finally pH is adjusted to neutral. For DNA extraction, the cultures were grown on a nutrient broth for 48 hours. The primers used for 16S rDNA: 16S Forward: 5'AGAGTTTGATCCTGGCTCAG 3' and 16S Reverse: 5'CGGTGTGTACAAGACCC 3'. PCR was performed in a total volume of 50 µl consisting of 10X Taq buffer, 15mM $MgCl_2$, 10mM dNTP's, 1U of Taq DNA polymerase (M/s MBI Fermentas, Germany), 20 pmol each of the 16S rDNA primers and 50µg of template DNA. PCR was performed by employing the following conditions: samples were subjected to 94°C for initial denaturation for 3min; followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute and extended elongation step was performed at 72°C for 3 minutes. With each run a negative control was performed with nuclease free water in place of template DNA in the PCR mixture. PCR products were electrophoresed on a 1.8% low melting point agarose gel (M/s Genei, Bangalore, India). Gels were stained using ethidium bromide @ 0.5µg/ml. A 100 bp molecular weight marker (M/s MBI Fermentas, Germany) was run along with the samples for reference.

DNA isolation and *Wolbachia* amplification

The primers used for wsp and ftsZ were as follows: Initially the general primer 81F-5' TGGTCCAAGTGATGAAGAAAC 3' and 691R- 5' AAAAATTAAACGCTACTCCA 3' was used to know the presence of *Wolbachia* endosymbiont and then screened with the group-specific ftsz-A and ftsZ-B: 308F- 5' TTAAAGATGTAACATTTG 3' and 691R-5' AAAAATTAAACGCTACTCCA 3'. The B group gene primer used was 183F-5' AAGGAACCGAAGTTCATG 3' and 691R- 5' AAAAATTAAACGCTACTCCA 3'. PCR was performed in a total reaction volume of 50 µl consisting of 10X Taq buffer, 15mM $MgCl_2$, 10mM dNTP's, 1U of Taq DNA polymerase (M/s MBI Fermentas, Germany), 20 pmol each of the wsp and ftsZ-A and ftsZ-B primers and 50µg of template DNA. PCR was performed by employing the following conditions: samples were subjected to 94°C for initial denaturation for 3 minutes; followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 55-60°C for 1 min, and extension at 72°C for 1 minute and extended elongation step was performed at 72°C for 3 minutes. With each run a negative control was performed with nuclease free water in place of template DNA in the PCR mixture. PCR products were electrophoresed on a 1.8% low melting point agarose gel (M/s Genei, Bangalore). Gels were stained using ethidium bromide @ 0.5µg/ml. A 100 bp molecular weight marker (M/s MBI Fermentas, Germany) was run along with the samples for reference.

Yeast and bacterial DNA sequencing and identification

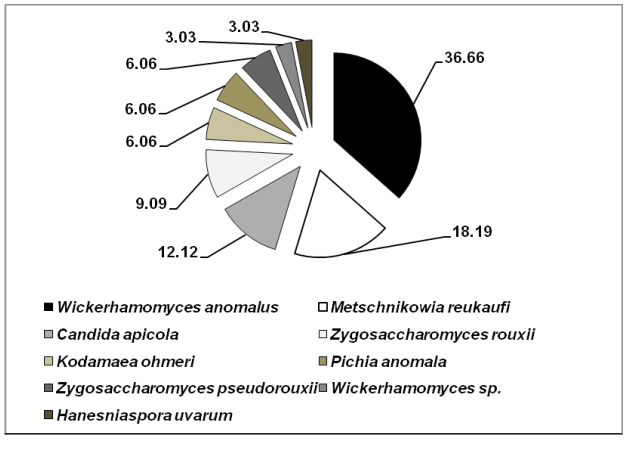
The amplification products were purified with the QIA-Quick PCR purification kit (M/s Qiagen, Hilden, Germany). DNA fragments were extracted from the gel using the Qia Quick Gel extraction kit (M/s Qiagen, Hilden, Germany). The DNA fragments were sequenced using an ABI prism 310 DNA sequencer using Big Dye Terminator reaction. Yeast and bacterial species were identified by searching databases using the BLAST sequence analysis tool. The ITS1-5.8S-ITS2 region and 16s rDNA sequences were compared with sequences acquired from GenBank ITS and 16s rDNA sequences for yeast and bacteria, respectively, using nucleotide BLAST (Blastn). Species identification was determined from the lowest E-value of the BLAST output.

Taxonomic classification of endosymbionts cultured from different populations of *Trichogramma* using RDP classifier

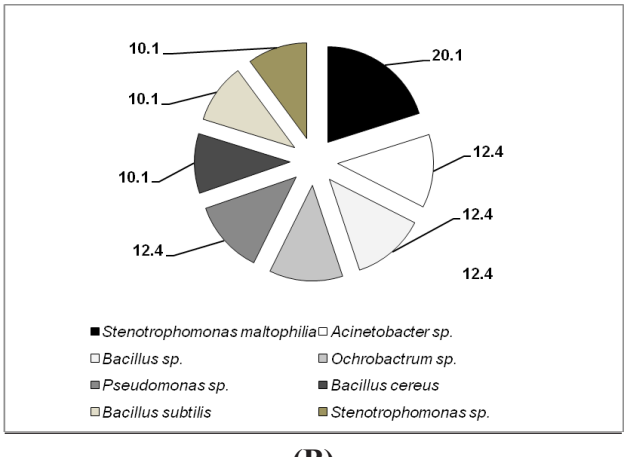
Our 13 Bacterial 16S rDNA sequence classification was performed against the RDP 16S rRNA reference database (release accessed 7th October 2016) in RDP Naive Bayesian rRNA Classifier Version 2.11 (http://rdp.cme.msu.edu/classifier/classifier.jsp) (Wang *et al.,* 2007) and all 33 isolates of fungal ITS sequences were searched against UNITE Fungal ITS database (trainset 07/04/2014) using RDP Naive Bayesian rRNA Classifier Version 2.1 (Deshpande *et al*., 2016). We used procedural tutorials for RDP Classifier to analyse 16S rDNA and ITS sequences. Taxonomic hierarchical classification is done with confidence threshold 80%.

RESULTS AND DISCUSSION

Out of 53 *Trichogramma* specimens obtained from 26 locations across 12 states, 46 were identified as *T. chilonis* Ishii, 3 as *T. achaeae* Nagaraja and Nagarkatti and 4 as *T. danausicida* Nagaraja (Table 1). Among three species, *T. chilonis* was recorded from eight host-insect eggs in nine crops and, *T. achaeae* from three host-insects on two crops and *T. danausucida* from 4 unknown host-insect eggs on weed plants. *Trichogramma chilonis* was found to occur more frequently from all the states from which collections were made, however, *T. achaeae* and *T. danausicida* was recorded from 2 and 1 states, respectively. These were purified by singe colony isolation by repeating the process thrice. *Wolbachia* was not amplified from any of 53 samples in the present study by two primers used, indicating its absence in samples collected from different places in India.



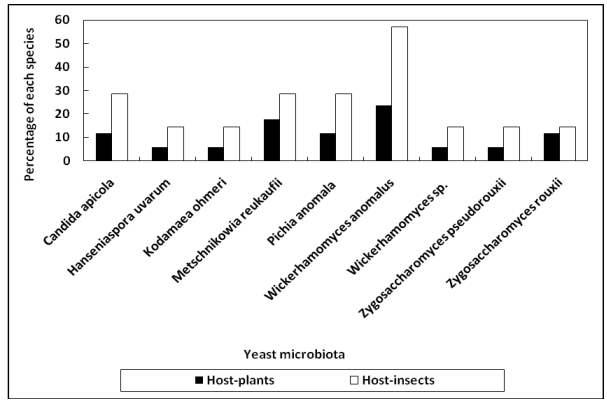
(A)



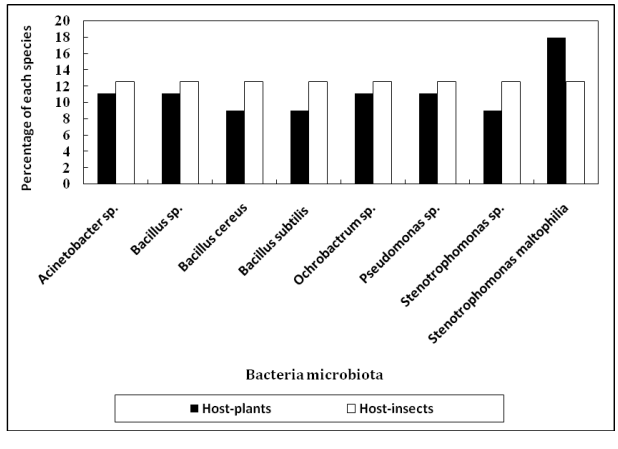
(B)

Fig. 1. Diversity of yeast (A) and bacteria (B) microbiota recorded from *Trichogramma* spp.

Nine species of yeast were recorded from 5 host insects, *Wickerhamomyces anomalus* was recorded from 36.4%, *Metschnikowia reukaufii* from 18.2%, *Candida apicola* from 12.1% samples and *Zygosaccharomyces rouxii* from 9.1% samples, constituting 75.8% of the yeast diversity based on their ITS sequences (Fig. 1). Highest yeast species were recorded from parasitoid collected from sugarcane (7 species) and five species from tomato and other 4 host-plant contributed 1 each (Table 2). Eight bacterial species were recorded from three crops and from 4 states. *Bacillus cereus* was recorded from 28.6%, *Pseudomonas* from 21.4% and *Stenotrophomonas maltophilia* from 14.3% samples, while other species like *Acinetobacter* sp., *Bacillus* sp., *Bacillus subtilis*, *Ochrobactrum* sp., *Stenotrophomonas* sp. were recorded from 7.14% samples based on their 16S rDNA sequences (Table 1). *Wickerhamomyces anomalus* was recorded from 23.5% of the host-plants and from 57.1% of host-insects, thus making it the most abundant yeast species and other important species was *Metschnikowia reukaufii*, which was recorded from 17.6 and 28.6% of host-plants and host-insects, respectively. In contrast, bacterial species were observed consistently across host-plants and host-insects (Fig. 1 and 2).



(A)



(B)

Fig. 2. Percentage composition of all yeast (A) and bacteria (B) on different host-plants and different host-insects.

Table 1. List of locations, host-plant and host-insects and populations of *Trichogramma* collected in India, microorganisms isolated from them and GenBank accession numbers of ITS and 16s rDNA sequences of yeasts and bacteria, respectively

L. No.	Species	Host-plants	Host-Insects	Location	State	GPS Coordinates	Organism	GenBank accn. no.
Cultivable yeast isolated from *Trichogramma*								
1	*T. chilonis*	Tomato	*Helicoverpa armigera*	Coimbatore	Tamil Nadu	11.018° N, 76.973° E	*Zygosaccharomycespseudorouxii*	JF803489
							Zygosaccharomycespseudorouxii	JF803490
2	*T. chilonis*	Sugarcane	*Chilo infuscatellus*	Cuddalore	Tamil Nadu	11.750° N, 79.750° E	*Kodamaea ohmeri*	HQ696999
							Zygosaccharomyces rouxii	HQ696996
3	*T. chilonis*	Sugarcane	*Chilo infuscatellus*	Mandya	Karnataka	12.520° N, 76.900° E	*Kodamaea ohmeri*	HQ696998
4	*T. chilonis*	Tomato	*Helicoverpa armigera*	Bangalore	Karnataka	12.967° N, 77.567° E	*Wickerhamomyces anomalus*	JF416789
							Zygosaccharomyces rouxii	JF416790
	T. achaeae	Sun hemp	Unknown eggs				*Wickerhamomyces anomalus*	JF728870
	T. chilonis	Rose	*Helicoverpa armigera*				*Pichia anomala*	FJ599744
	T. danausicida	Weed	Unknown eggs				*Wickerhamomyces anomalus*	JN210893
							Wickerhamomyces anomalus	JN210892
							Wickerhamomyces anomalus	JF803491
5	*T. chilonis*	Tomato	*Helicoverpa armigera*	Malur	Karnataka	13.021° N, 77.938° E	*Metschnikowia reukaufii*	HM222406
6	*T. chilonis*	Tomato	*Helicoverpa armigera*	Kolar	Karnataka	13.133° N, 78.133° E	*Metschnikowia reukaufii*	HM222405
7	*T. chilonis*	Tomato	*Helicoverpa armigera*	Rajankunte	Karnataka	13.181° N, 77.554° E	*Metschnikowia reukaufii*	HM222408
8	*T. chilonis*	Tomato	*Helicoverpa armigera*	Doddaballapur	Karnataka	13.292° N, 77.543° E	*Metschnikowia reukaufii*	HM222407
9	*T. chilonis*	Tomato	*Helicoverpa armigera*	Vikarabad	Telangana	17.330° N, 77.900° E	*Wickerhamomyces anomalus*	HM601458
10	*T. chilonis*	Tomato	*Helicoverpa armigera*	Hyderabad	Telangana	17.370° N, 78.480° E	*Candida apicola*	FJ713025
11	*T. chilonis*	Sugarcane	*Chilo infuscatellus*	Anakapalli	Andhra Pradesh	17.680° N, 83.020° E	*Wickerhamomyces sp.*	HQ696994
12	*T. chilonis*	Sugarcane	*Chilo infuscatellus*	Pune	Maharashtra	18.520° N, 73.857° E	*Wickerhamomyces anomalus*	HQ696991
	T. achaeae	Sun hemp	Unknown eggs				*Pichia anomala*	FJ224365
13	*T. chilonis*	Sugarcane	*Chilo infuscatellus*	Bhubaneshwar	Odisha	20.270° N, 85.840° E	*Wickerhamomyces anomalus*	HQ696992
14	*T. chilonis*	Sugarcane	*Chilo infuscatellus*	Navasari	Gujarat	20.949° N, 72.914° E	*Wickerhamomyces anomalus*	HQ615929
							Zygosaccharomyces rouxii	HQ696997
15	*T. chilonis*	Sesame	Unknown eggs	Amreli	Gujarat	21.620° N, 71.230° E	*Pichia anomala*	FJ224365
16	*T. chilonis*	Sugarcane	*Chilo infuscatellus*	Vadodara	Gujarat	22.300° N, 73.200° E	*Hanseniaspora uvarum*	HM601459
17	*T. chilonis*	Sugarcane	*Chilo infuscatellus*	Lucknow	Uttar Pradesh	26.800° N, 80.900° E	*Candida cf. apicola*	HQ697001
	T. chilonis	Sugarcane	*Chilo auricilius*				*Wickerhamomyces anomalus*	HQ615930

18	*T. chilonis*	Sugarcane	*Chilo auricilius*	Shahjahan-pur	Uttar Pradesh	28.000° N, 79.833° E	*Candida cf. api-cola*	HQ697000
							Metschnikowia reukaufii	HM601457
19	*T. chilonis*	Sugarcane	*Chilo infuscatel-lus*	Uchani	Haryana	29.690° N, 76.980° E	*Candida cf. api-cola*	HQ615928
20	*T. chilonis*	Sugarcane	*Chilo auricilius*	Nawansha-har	Punjab	31.125° N, 76.116° E	*Wickerhamomyces anomalus*	HQ696995
21	*T. chilonis*	Sugarcane	*Chilo auricilius*	Jalandhar	Punjab	31.326° N, 75.576° E	*Wickerhamomyces anomalus*	HQ696993
22	*T. chilonis*	Maize	*Chilo partellus*	Srinagar	Jammu & Kashmir	34.090° N, 74.790° E	*Metschnikowia reukaufii*	HQ221884
Cultivable bacteria isolated from *Trichogramma*								
23	*T. chilonis*	Cabbage	*Plutella xylo-stella*	Bangalore	Karnataka	12.967° N, 77.567° E	*Bacillus cereus*	JF728871
	T. chilonis	Tomato	*Helicoverpa armigera*				*Bacillus subtilis*	GU391355
							Pseudomonas sp.	JF266598
							Bacillus sp	HQ651056
							Acinetobacter sp.	HM629807
							Ochrobactrum sp.	HM629806
							Stenotrophomonas maltophilia	HM629805
							Pseudomonas sp.	HM629804
24	*T. chilonis*	Cotton	*Helicoverpa armigera*	Davanagere	Karnataka	14.467° N, 75.924° E	*Stenotrophomonas sp.*	JF266601
							Stenotrophomonas maltophilia	JF266599
25	*T. chilonis*	Cabbage	*Plutella xylo-stella*	Pune	Maharashtra	18.520° N, 73.857° E	*Bacillus cereus*	JF736842
26	*T. chilonis*	Cabbage	*Plutella xylo-stella*	Navsari	Gujarat	20.949° N, 72.914° E	*Bacillus cereus*	JF736840
27	*T. chilonis*	Cabbage	*Plutella xylos-tella*	Ludhiana	Punjab	30.910° N, 75.850° E	*Bacillus cereus*	JF736841
Locations from where no cultural microbes were obtained								
28	*T. danau-sicida*	Weed	Unknown eggs	Bangalore	Karnataka	12.967° N, 77.567° E		
	T. chilonis	Cotton	*Helicoverpa armigera*	Bangalore	Karnataka	12.967° N, 77.567° E		
29	*T. achaeae*	Castor	*Acanthodelta janata*	Nelaman-gala	Karnataka	13.500° N, 77.230° E		
30	*T. chilonis*	Castor	*Acanthodelta janata*	Jorhat	Assam	26.750° N, 94.220° E		
31	*T. chilonis*	Sugarcane	*Chilo auri-cilius*	Jalandhar	Punjab	31.326° N, 75.576° E		
32	*T. chilonis*	Pomegranate	Unknown eggs	Srinagar	Jammu & Kashmir	34.090° N, 74.790° E		

Table 2. Composition of yeast and bacteria microbiota obtained of parasitoid, *Trichogramma chilonis* from different host-plants and host-insects

Microbiota diversity	Host-plant	Host-insect
Yeast microbiota		
Candida apicola	Tomato, sugarcane	*Helicoverpa armigera*, *Chilo auricilius, C. infuscatellus*
Hanseniaspora uvarum	Sugarcane	C. infuscatellus
Kodamaea ohmeri	Sugarcane	C. infuscatellus
Metschnikowia reukaufii	Tomato, Sugarcane, Maize	*Helicoverpa armigera*, *Chilo auricilius*, *C. partellus*
Pichia anomala	Rose	*Helicoverpa armigera,* unknown eggs

Wickerhamomyces anomalus	Tomato, Sugarcane	*Helicoverpa armigera*, *Chilo auricilius, C. infuscatellus*, unknown eggs
Wickerhamomyces sp.	Sugarcane	*C. infuscatellus*
Zygosaccharomyces pseudorouxii	Tomato	*Helicoverpa armigera*
Zygosaccharomyces rouxii	Tomato, sugarcane	*Helicoverpa armigera, C. infuscatellus*
Bacterial microbiota		
Acinetobacter sp.	Tomato	*Helicoverpa armigera*
Bacillus cereus	Cabbage	*Plutella xylostella*
Bacillus sp	Tomato	*Helicoverpa armigera*
Bacillus subtilis	Tomato	*Helicoverpa armigera*
Ochrobactrum sp.	Tomato	*Helicoverpa armigera*
Pseudomonas sp.	Tomato	*Helicoverpa armigera*
Stenotrophomonas maltophilia	Tomato	*Helicoverpa armigera*
Stenotrophomonas sp.	Cotton	*Helicoverpa armigera*

Zygosaccharomyces rouxii was obtained only from Tamil Nadu and Karnataka (southern states) that too only from Sugarcane and tomato. Zygosaccharomyces pseudorouxii has been observed only in samples from Tamil Nadu.

All 33 cultures of yeast recorded from different host-plants belonged to class Saccharomycetes, whereas in bacteria from different host-plants belonged to the class Bacilli (42.85%), Gammaproteobacteria (50.0%) and Alphaproteobacteria (7.14%) of diversity. The class Saccharomycetes was recorded from seven host-insects, *viz*., *Helicoverpa armigera*, *Chilo auricilius*, *C. infuscatellus*, *C. partellus*, *Acanthodelta janata*, *Plutella xylostella* and unknown insect eggs. The Class Bacilli was recorded from *Plutella xylostella and Helicoverpa armigera*, Class Gammaproteobacteria from *Plutella xylostella* and *Helicoverpa armigera* and Class Alphaproteobacteria from *Helicoverpa armigera* only. *Bacillus cereus* was observed only in specimens from *Plutella xylostella* cabbage field and not from specimens from *Helicoverpa* on cotton.

A total of 33 yeast ITS sequences were used for the taxonomic identification. The taxonomic composition of the Fungi is shown in Supplementary Table 1. Prediction Confidence threshold is given in brackets. All the ITS sequences of *Candida cf. apicola* (4), Kodamaea ohmeri (2), *Pichia anomala* (2), *Wickerhamomyces anomalus* (12), Z*ygosaccharomyces rouxii* (3) species correctly identified to corresponding species. In contrast, there were a total of five sequences of *Metschnikowia reukaufii isolate and Zygosaccharomyces pseudorouxii* (2) were matched with the *Metschnikowia_cibodasensis* and *Zygosaccharomyces_rouxii* species/, whereas *Metschnikowia reukaufii* isolate TCE2 is showed close association with another genus Naumovozyma. Also, *Hanseniaspora uvarum* strain Tcy19 (HM601459) sequence could not match with same species.

A total of 13 Bacterial 16s rDNA sequences were used for the taxonomic identification. The taxonomic composition of the Bacteria is shown in Supplementary Table 2. Prediction Confidence threshold is given in brackets. Our results revealed that there were total 6 sequences represented within the *Bacillus* genus. Among the most abundant genera were *Stenotrophomonas* (3), *Pseudomonas* (2), *Acinetobacter* (1), and *Ochrobactrum* (1).

In the insects, their diversity and ecological niche they occupy makes it difficult to generalize microbiota associated with them. The microbiota generally varies with feeding habits, like sap feeders (sucking pests) to termites (wood or cellulose feeder) to insects which feed on foliage or tissue / fruit borers (Rizzi *et al*., 2013). Another group of insects, parasitoids, which essentially are surviving on its host and as a free-living adults feed on fruits, flowers and nectar, acquire generally yeast and sometimes bacterial microbiota (Suh *et al.,* 2004; Nguyen et al., 2006; Srinatha *et al*, 2015). In insects, microbiota-insect host-plant-host interactions are formed by different factors because insects lack a 'classical' adaptive protected system (McFall-Ngai, 2007). Colman *et al*., (2012) observed that it is one of the reasons that some insects contain fewer microbiota communities. In the present study, most interesting aspect was that at least three yeast species obtained from parasitoid, *T. chilonis*, *viz*., *Wickerhamomyces* sp., *Kodamaea ohmeri* and *Hanseniasopra uvarum* were recorded from *Chilo infuscatellus* on sugarcane but completely absent from any other host-plant, which suggests that these microbiota could be involved in the host-plant association. In contrast to yeast, 6 bacteria from parasitoid, *T. chilonis* were found associated with host-insect, *H. armigera* on tomato and *B. cereus* was recorded from host-insect, *P. xylostella* on cabbage. In an earlier study, Medina *et al*., (2011) also observed that association of bacteria with their host confers the trait, which allows them to differentially exploit distinct host-plants. The studies shown earlier that there are documented proof that such traits are involved in different roles such as nutritional advantages (Bernays and Klein,

2002; Engel *et al*., 2012), defenses against natural enemies (Oliver *et al*., 2005) and enhancing biological traits in insect natural enemies (Srinatha *et al*., 2015; Hagen *et al*., 1970). It may be inferred that in similar parasitoids recorded from different host-insects, an association of yeast or bacteria may be caused by different host-plant species (Hansen and Moran, 2014). It appears that most of these species may have been transferred to parasitoid through their host-insects and host-plants, which is in agreement with Priya *et al*., (2012), who reported a host-plant induced variation in gut bacteria of *H. armigera.*

In the present study, wolbachia was not recorded from any of 53 populations of *Trichogramma* species collected from all over India. No primary bacterial or yeast species were identified in *T. chilonis* collected from any host-insect or any host-plant, which is in agreement with the previous reports (Medina *et al*., 2011; Priya *et al*., 2012). All microbiota associated with *T. chilonis* have been found associated with other insects. Of all microbes recorded on parasitoid, the most abundant crop was tomato and *H. armigera* was the host-insect on which 48% of diversity was recorded. *Wickerhamomyces anomalus* and *M. reukaufii* were the most prominent of all microbial diversity in the present study.

Host associated demarcation may be defined as the presence of genetically different, host associated populations have been studied earlier for the microbes associated with the insect hosts. Microbial symbionts, especially of herbivore insects, help in allowing the insect species to choose diverse plant species (Vorwerk *et al*., 2007; Leonardo and Muiru, 2003; Simon *et al.,* 2003; Tsuchida *et al.,* 2004; Ferrari *et al.,* 2007). Some bacterial symbionts are known to develop the immune system of insects (de Souza *et al*., 2009).

Some species of symbionts may be present in higher population due to their occurrence on the floral parts of the host plants on which the insects obtain nectar as food. Though some species of symbionts may occur in low percentage of samples they may play an important role by providing specific amino acids to the insect. The differences in the species isolated from insects collected from different host-plants may be explained as allochronic isolations. Allochronic species do not occur simultaneously in same geological time, vice-versa also may be true that is in alliance of insect population with other plant species may be dependent on the suitable symbionts harbored by them.

Microbial symbionts in insect guts help in regulating host metabolism and they provide efficient digestion of food, extraction of maximum energy from ingested food besides protecting the host from other potentially harmful pathogens. In the sucking pests like aphids, thrips and whitefly the symbionts may interfere with the transmission of plant viruses due to the interference in the multiplication of viruses inside the insect gut, while in egg parasitoids, which harbor symbionts, may be helped in getting better biological fitness, enhanced fecundity, more female population, insecticide resistance, etc.

In conclusion, *T. chilonis*, most important egg parasitoid was recorded as most dominant parasitoid in India. It harbored vast diversity of microbiota, both yeast and bacteria from different host-insects and host-plants. The yeast, *Wickerhamomyces anamalus* was dominant yeast species isolated, while *Bacillus cereus*, *Pseudomonas* sp. and *Stenotrophomonas maltophilia* were most frequently recorded bacteria species. The taxonomic identification using RDP classifier correctly identified in the respective species with high higher confidence threshold percentage (>80) support. Results revealed that most of the species among yeast representing 9 species belong to class Saccharomycetes. Bacterial taxonomic analysis suggested that major seqeunces representing *Bacillus* species.

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