Effects of Host Plant and Insect Generation on Shaping of the Gut Microbiota in the Rice Leaffolder, *Cnaphalocrocis medinalis*

Yajun Yang¹, Xiaogai Liu¹ ², Hongxing Xu¹, Yinhong Liu²* and Zhongxian Lu¹*

¹ State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Institute of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Sciences, Hangzhou, China, ² College of Plant Protection, Southwest University, Chongqing, China

Gut microbes in insects may play an important role in the digestion, immunity and protection, detoxification of toxins, development, and reproduction. The rice leaffolder *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Crambidae) is a notorious insect pest that can damage rice, maize, and other gramineous plants. To determine the effects of host plants and generations on the gut microbiota of *C. medinalis*, we deciphered the bacterial configuration of this insect pest fed rice or maize for three generations by Illumina MiSeq technology. A total of 16 bacterial phyla, 34 classes, 50 orders, 101 families, 158 genera, and 44 species were identified in *C. medinalis* fed rice or maize for three generations. Host plants, insect generation, and their interaction did not influence the alpha diversity indices of the gut microbiota of *C. medinalis*. The dominant bacterial taxa were *Proteobacteria* and *Firmicutes* at the phylum level and *Enterococcus* and unclassified *Enterobacteriaceae* at the genus level. A number of twenty genera coexisted in the guts of *C. medinalis* fed rice or maize for three generations, and their relative abundances occupied more than 90% of the gut microbiota of *C. medinalis*. A number of two genera were stably found in the gut of rice-feeding *C. medinalis* but unstably found in the gut microbiota of maize-feeding *C. medinalis*, and seven genera were stably found in the gut of maize-feeding *C. medinalis* but unstably found in the gut of rice-feeding *C. medinalis*. In addition, many kinds of microbes were found in some but not all samples of the gut of *C. medinalis* fed on a particular host plant. PERMANOVA indicated that the gut bacteria of *C. medinalis* could be significantly affected by the host plant and host plant \( \times \) generation. We identified 47 taxa as the biomarkers for the gut microbiota of *C. medinalis* fed different host plants by LEfSe. Functional prediction suggested that the most dominant role of the gut microbiota in *C. medinalis* is metabolism, followed by environmental information processing, cellular processes, and genetic information processing. Our findings will enrich the understanding of gut bacteria in *C. medinalis* and reveal the differences in gut microbiota in *C. medinalis* fed on different host plants for three generations.

**Keywords:** rice leaffolder, gut bacteria, host plant, Lepidoptera, rice, maize
INTRODUCTION

Insects harbor numerous microorganisms in the gut (Douglas, 2015). Gut microorganisms in insects have been shown to contribute to digestion (Anand et al., 2010; Jing et al., 2020), detoxification (Ceja-Navarro et al., 2015; Beran and Gershenzon, 2016; Blant et al. and Peterson, 2020), development (Wang et al., 2018b; Qiao et al., 2019; Pyszko et al., 2020), physiology (Engel and Moran, 2013; Xu et al., 2019; Liberati and Engel, 2020), pathogen resistance (Dillon and Dillon, 2004; Voirl et al., 2018; Moore and Aparicio, 2022), immune response (Engel and Moran, 2013; Li et al., 2020; Li et al., 2021), and the production of essential vitamins and amino acids (Hansen and Moran, 2014; Jang and Kikuchi, 2020; Jing et al., 2020). For instance, some microorganisms with metabolic characteristics could promote insect adaptation to host plants (Voirl et al., 2018). The gut microbiota was found to function in the protection of a European Bombyx species against the intestinal pathogen Crithidia bombi (Koch and Schmid-Hempel, 2011). Another example is Helicoverpa zeae (Boddi), Enterbacter ludwigi, a gut-associated bacterium, could indirectly trigger the defense of tomato (Solanum lycopersicum L.) and maize (Zea mays L.) (Wang et al., 2017, 2018a). Chung et al. (2013) documented that the Colorado potato beetle Leptinotarsa decemlineata (Say) suppressed the defenses of tomatoes by exploiting orally secreted bacteria. The gut microbiota of the pine weevil (Hylobius abietis) degrades conifer diterpenes and increases insect fitness (Bersategui et al., 2017). Gut microbes may facilitate insect herbivory to chemically defend plants (Hammer and Bowers, 2015). Gut symbionts could enhance insecticide resistance in a significant pest, the oriental fruit fly Bactrocera dorsalis (Hendel) (Cheng et al., 2017). Insect symbionts could influence insect-plant interactions at different levels through direct interactions and also through indirect plant-mediated interactions (Frago et al., 2012). Given the importance of the associated microorganisms to host fitness and feeding ecology, an effort to manipulate these partnerships and render insect pests more vulnerable to broad-scale measures of population control by targeting the bacterial symbionts was one of the important applications in gut symbiont-driven pest control (Bersategui et al., 2016). The functions of gut microbes could provide a novel concept for the application of bacteria in pest control through the restraint of the insect immune response and the induction of plant defense (Kyritis et al., 2017) and promote the understanding of gut symbiont-driven pest control (Frago et al., 2012; Bersategui et al., 2016).

Lepidoptera is one of the largest insect orders and has approximately 160,000 described species (Mitter et al., 2017). Some of them can damage agricultural crops and cause large economic losses (Wagner, 2013). However, the evidence of the fundamental function of bacteria in lepidopteran biology is scarce. Furthermore, a recent study from Hammer et al. (2017) reported that caterpillars lack a resident microbiome in the gut compared with other insect orders. The authors of this study argued that caterpillars with rough environments may prevent bacterial colonization. Lepidopteran reshaping the body structure during metamorphosis also enhances the difficulty of bacterial colonization (Hammer et al., 2014). Nevertheless, microbiota are abundant and diverse in many species of Lepidoptera. Proteobacteria and Firmicutes were found to be dominant in the gut of the diamondback moth Plutella xylostella (L.) based on the high-throughput DNA sequencing data (Xia et al., 2013). Enterococcus and Lactococcus were abundant in bacteria in a field population of Helicoverpa armigera Hubner, followed by Flavobacterium, Acinetobacter, and Stenotrophomonas (Xiang et al., 2006). The composition of microbes in the insect gut could be affected by many factors. The environmental habitat, diet, developmental stage, and phylogeny of the host could determine the bacterial diversity in the insect gut (Yun et al., 2014). In the larvae of Spodoptera littoralis (Boisduval), bacterial communities were shown to be instar-specific (Chen et al., 2016). In addition, host plants were observed to have a considerable effect on the composition of gut bacteria in Henosepilachna vigintioctopunctata (F.) (Lu et al., 2019).

The rice leaffolder Cnaphalocrocis medinalis (Guenée) (Lepidoptera: Crambidae) is an important insect pest in Asia that can damage rice (Oryza sativa L.), maize, and other gramineous plants (Barrion et al., 1991; Cheng, 1996; Yang et al., 2015). The heavy occurrence of this insect could cause serious economic loss to rice production (Yang et al., 2015). In 2015, C. medinalis damaged rice plants with an area of 15.5 million ha and caused yield losses of 0.47 million tons in China (Yang et al., 2015; Lu, 2017). Based on the traditional isolation and culture methods, 25 species of 15 phyla of gut microbiota were obtained from C. medinalis larvae (Yang, 2012). By comparison, a large number of gut microbiota were obtained from C. medinalis larvae through Illumina MiSeq technology (Liu et al., 2016). Yang et al. (2020a) analyzed the gut microbiota composition of C. medinalis across the developmental stages. Information on the host-associated changes in gut bacteria will facilitate the overall understanding of insect ecology and promote the development of novel methods for pest management. This study illustrates the composition and diversity of the gut microbiota in C. medinalis feeding on rice or maize for three generations by Illumina MiSeq technology. The findings in this study will enrich the understanding of the gut microbiota in C. medinalis and provide novel insight into the relationship between C. medinalis and its host plants.

MATERIALS AND METHODS

Insect Rearing and Sampling

Adults of C. medinalis were collected from paddy fields in Hangzhou, Zhejiang Province, East China and then cultured with 10% honey solution in the laboratory under controlled conditions of 26 ± 1°C temperature, 70 ± 10% relative humidity, and a photoperiod of 16:8 (LD) h. The neonates of the population were divided into two groups. One was reared using rice plants, and the other was reared using maize plants. Every group was reared for three generations. Rice and maize were planted in pots (one plant per pot) in the greenhouse. The leaves of plants were collected and rinsed with sterile ddH$_2$O and then air-dried before feeding them to C. medinalis, and sufficient leaves were provided for the insects.
Guts of *C. medinalis* were dissected from the fifth instar larvae of both groups from every generation. A total of fifteen guts were pooled into a biological sample, and three replicates were prepared for each treatment. Before dissection, the whole larva was rinsed with sterile ddH$_2$O, disinfected with ethanol (75%) for 90 s, and rinsed again with sterile ddH$_2$O. Following dissection, the guts were collected into a 1.5-ml sterile tube and stored at –80°C until use.

**DNA Extraction and PCR Amplification**

The dissected guts were homogenized by shaking in a sterile tube containing sterile glass beads (0.5 mm diameter) and 0.5 ml of PBS buffer (pH 7.5) for 15 min using a vortex. Total DNAs were extracted from samples using the E.Z.N.A.® bacteria DNA extract kit (OMEGA, United States) according to the instructions. The primers 515F 5’-GTGCCAGCMGCCGCGG-3’ and 907R 5’-CCGTCAATTCMTTTRAGTTT-3’ were used to amplify the V4-V5 regions of the bacterial 16S ribosomal RNA gene through PCR (95°C for 2 min, followed by 25 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s and a final extension at 72°C for 5 min). Amplicons were generated in a 20 µl reaction system containing 4 µl of 5 × FastPfu Buffer, 2 µl of 2.5 mM dNTPs, 0.8 µl of each primer (5 µM), 0.4 µl of FastPfu Polymerase, and 10 ng of template DNA. Blank DNA as a negative control was extracted, and products generated from no-template PCR were sequenced to assess what sequences are contaminants.

**Illumina MiSeq Sequencing**

Amplicons were extracted and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, United States) according to the manufacturer's instructions and quantified using QuantiFluor™-ST (Promega, United States). Then, they were pooled in equimolar amounts and paired-end sequenced (2 × 250) on an Illumina MiSeq platform according to the standard protocols.

**Bioinformatic and Statistical Analyses**

Raw FASTQ files were demultiplexed and quality-filtered using QIIME (version 1.17). According to the similarity of the sequences, effective sequences were classified into multiple operational taxonomic units (OTUs) at a similarity level of 97% using UPARSE (version 7.1), and chimeric sequences were identified and removed using UCHIME. All the sequences were annotated and blasted against the Silva (SSU115)16S rRNA database using a confidence threshold of 70% for each 16S rRNA gene sequence analyzed by RDP Classifier.

Alpha diversity was estimated through five indices: OTU number, ACE, Chao1, Shannon, and Simpson's index. The alpha diversity and relative abundance data were analyzed using one-way analysis of variance (ANOVA) with SPSS 26.0 (IBM SPSS Statistics), and multiple comparisons were analyzed using Tukey's test. Venn diagrams and stack bars were graphed by R software. Principal coordinate analysis (PCoA) based on the matrices of pairwise weighted UniFrac distances and Bray–Curtis distances was applied among all the bacterial groups. Non-metric multidimensional scaling (NMDS) plots were constructed using Bray–Curtis. Analysis of similarity (ANOSIM) was used to test the difference in the composition of microbiota among different group samples. Permutational multivariate analysis of variance (PerMANOVA) was generated using 999 permutations, and the individual repeats were included in the model as a random effect. PCoA, NMDS, ANOSIM, and PerMANOVA were analyzed and graphed using R software. Linear discriminant analysis (LDA) was used to screen the biomarkers for significant differences between different groups with LDA scores greater than two. A cladogram was drawn to show the distribution of these biomarkers at different taxonomic levels by Galaxy (accessed on 1 January 2022). Microbiota functions were predicted by annotating pathways of OTUs against the Ref99NR database using R software with the Tax4Fun2 package.

**RESULTS**

**Reads Analyzed and Taxa Generated**

We sequenced the gut microbes of *C. medinalis* fed on different host plants for three generations and obtained 1,473,836 trimmed paired reads in total (Supplementary Table 1). Blank DNA and no-template PCR sequencing were used for decontamination, and sequences of cyanobacteria or chloroplasts were found to be contaminants. After decontamination, 446 OTUs were obtained. The OTU numbers of *C. medinalis* from different samples varied from 49 to 194 (Table 1). The Ace index varied from 62.93 to 252.14, the Chao1 index varied from 57.27 to 256.25, the Shannon index varied from 0.47 to 1.36, and the Simpson index varied from 0.46 to 0.87 (Table 1). ANOVA indicated that alpha diversity indices were not significantly affected by the host plant, generation, or their interaction (Supplementary Table 2). A total of 16 bacterial phyla, 34 classes, 50 orders, 101 families, 158 genera, and 44 species were identified in *C. medinalis* fed rice or maize for three generations (Table 2).

**Gut Microbiota of *Cnaphalocrocis medinalis* Fed Rice for Three Generations**

At the phylum level, *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and unclassified *Bacteria* were found in the gut microbiota of *C. medinalis* fed on rice plants through all samples of three generations. Among them, *Firmicutes* was the absolute dominant phylum with the highest relative abundance in rice-feeding *C. medinalis* for three generations (70.62–87.53%) (Figure 1A). The relative abundance of *Proteobacteria* was 10.51–26.88%, followed by *Actinobacteria* (1.00–4.66%), *Bacteroidetes* (0.41–0.43%), and unclassified *Bacteria* (0.02–0.14%) (Figure 1A). At the family level, 18 families were found in the gut microbiota of *C. medinalis* fed rice through all samples of three generations. *Enterococcaceae* and *Enterobacteriaceae* were the two major families in the rice-feeding *C. medinalis* for three...
generations, with relative abundance ranges of 70.55–87.27% and 9.12–24.75%, respectively (Figure 1B). The relative abundance of Anaplasmataceae in the gut of the third generation of C. medinalis fed rice was higher than that of the second generation of C. medinalis, and the relative abundance of Nocardiaceae in the gut of the first generation of C. medinalis fed rice was higher than that of the other two generations of C. medinalis (Figure 1B). At the genus level, 21 genera were found in the gut microbiota of C. medinalis fed rice through all samples of three generations. Enterococcus, unclassified Enterobacteriaceae, Pectobacterium, Corynebacterium, Leucobacter, and Anaplasma occupied the top 10 in the gut microbiota of C. medinalis fed rice for three generations (Supplementary Table 3). Common genera found in all three generations occupied 93.95, 98.51, and 97.78% of the first generation to the third generation, respectively. Enterococcus and unclassified Enterobacteriaceae are the majority. In addition to the microbes found in all samples of rice-feeding C. medinalis for three generations, many kinds of microbes were found in some but not all samples of rice-feeding C. medinalis gut.

**Gut Microbiota of Cnaphalocrocis medinalis Fed Maize for Three Generations**

Similar to rice-feeding C. medinalis, the same six phyla were found in the gut microbiota of maize-feeding C. medinalis through all samples of three generations. The phylum with the highest relative abundance was Firmicutes (68.49–80.25%), followed by Proteobacteria (16.58–27.65%), Actinobacteria (1.62–2.01%), Bacteroidetes (0.41–0.83%), and unclassified Bacteria (0.004–0.29%) (Figure 2A). At the family level, 20 families found gut microbiota of C. medinalis fed maize through all samples of three generations. Enterococcaceae and Enterobacteriaceae were also the dominant families, with relative abundance ranges of 67.88–80.23% and 14.69–24.42%, respectively (Figure 2B). The relative abundance of Comamonadaceae in the gut of the third generation of C. medinalis fed maize was higher than that of the first generation of C. medinalis, the relative abundance of Micrococcaceae in the second generation of C. medinalis was higher than that of the first generation of C. medinalis, and the relative abundance of Rhodocyclaceae in the gut of the second generation of C. medinalis was higher than that of the third generation of C. medinalis (Supplementary Table 3). At the genus level, 26 genera were found in the gut microbiota of C. medinalis fed maize through all samples of three generations. Enterococcus, unclassified Enterobacteriaceae, Corynebacterium, unclassified Comamonadaceae, Leucobacter, Microbacterium, Anaplasma, and Sphingobacterium occupied the top 10 in the gut microbiota of C. medinalis fed maize for three generations (Supplementary Table 3). Common genera found in all three generations occupied 97.75, 96.34, and 99.29% of the first generation to the third generation, respectively. Enterococcus and unclassified Enterobacteriaceae are the majority. In addition to the microbes found in all samples of maize-feeding C. medinalis for three generations, many kinds of microbes were found in some but not all samples of maize-feeding C. medinalis gut.

**Influence of Host Plant and Insect Generation on the Gut Bacterial Communities of Cnaphalocrocis medinalis**

Comparing the gut microbiota between C. medinalis fed rice and maize, five phyla and 16 families were found in all samples of the three generations. At the genus level, 19 genera were found in the gut microbiota of C. medinalis fed on rice or maize plants for three generations (Supplementary Table 3). The relative abundance of these genera occupied more than 90% of the gut microbiota of C. medinalis fed rice or maize plants, and the two major genera were Enterococcus and unclassified Enterobacteriaceae (Figure 3). Seven genera, Bacillus, Empedobacter, Flavobacterium, Rhizobium, Rhodococcus,
**FIGURE 1** | Relative abundance of the gut microbiota from rice-feeding *C. medinalis* for three generations at the phylum (A) and family (B) levels. R1–R3: the first to third-generation of *C. medinalis* fed on rice.

**FIGURE 2** | Relative abundance of the gut microbiota from maize-feeding *C. medinalis* for three generations at the phylum (A) and family (B) levels. M1–M3: the first to third generation of *C. medinalis* fed on maize.
Sphingobacterium, and unclassified Beutenbergiaceae, were stably found in all samples of maize-feeding C. medinalis for three generations, whereas Tsukamurella and Ochrobactrum were stably found in all samples of rice-feeding C. medinalis for three generations (Supplementary Table 4).

Principal coordinate analysis based on the Bray–Curtis distance and weighted UniFrac distance was used to compare the community similarities between samples. The PCoA scatter plot showed that the absissa and ordinate represent the two characteristic values that contribute to the largest differences between the samples, and their influence degrees were 74.09 and 14.73% based on weighted UniFrac distance (Figure 4A) and 65.61 and 18.16% based on the Bray–Curtis (Figure 4B), respectively. PerMANOVA showed that there were significant differences in the gut microbiota of rice- and maize-feeding C. medinalis (Table 3; PerMANOVA: $R^2 = 0.35, p = 0.001$). Host plant × generation significantly affected the gut microbiota of C. medinalis (Table 3; PerMANOVA: $R^2 = 0.28, p = 0.004$). No significant differences were observed between the samples from different generations of C. medinalis (Table 3; PerMANOVA: $R^2 = 0.02, p = 0.751$).

Non-metric multidimensional scaling analysis revealed significant differences between the gut microbiota of rice- and maize-feeding C. medinalis (Figure 5). ANOSIM showed that there were significant differences in the gut microbiota of rice- and maize-feeding C. medinalis ($R = 0.5538, p = 0.001$) (Supplementary Table 5). There were no significant differences in the gut microbiota of rice- and maize-feeding C. medinalis in the same generations (Supplementary Table 5).

Venn diagrams showed overlapping OTUs of C. medinalis fed on rice or maize from the first generation to the third generation (Figure 6). The results indicated that 171 OTUs, which comprised 77.73 and 71.25% of the total OTUs of the first generation of C. medinalis fed rice or maize, were shared by C. medinalis fed rice or maize (Figure 6A). The second generation of C. medinalis fed on rice or maize shared 243 OTUs, which accounted for 85.56 and 86.17% of the total OTUs of the second generation of C. medinalis fed on rice or maize, respectively (Figure 6B). The third generation of C. medinalis fed on rice or maize shared 82 OTUs, which accounted for 40.39 and 64.57% of the total OTUs of the third generation of C. medinalis fed on rice or maize, respectively (Figure 6C).

To find the biomarkers with significant differences between different groups, LDA effect size (LEfSe) was used to screen out different taxa at various levels (kingdom, phylum, class, order, family, genus, and species) between different groups based on a standard LDA value greater than two (Figure 7). Meanwhile, the cladogram from phylum to genus was graphed to fully understand the distribution of these different taxa at various taxonomic levels (Figure 8). In the third generation of C. medinalis fed maize (M3), the gut microbiota had the most of the different taxa (LDA > 2). There were 60 taxa mainly belonging to Firmicutes, Bacteroidota, Acidobacteriota, Proteobacteria, Actinobacteria, and Ignavibacteriaceae. A total of six taxa belonging to Actinobacteria were in the gut microbiota of the first generation of C. medinalis fed rice (R1). A total of two taxa belonging to Proteobacteria and one taxon belonging to Bacteroidetes were in the gut microbiota of the second generation of C. medinalis fed rice (R2). A total of two taxa belonging to Proteobacteria were in the gut microbiota of the third generation of C. medinalis fed rice (R3). A total of five taxa belonging to Proteobacteria and one taxon belonging to Bacteroidetes were in the gut microbiota of the first generation of C. medinalis fed maize (M1). A total of three taxa belonging to Proteobacteria, five taxa exclusive to Ignavibacteriaceae, five taxa belonging to Firmicutes, and four taxa belonging to Actinobacteria were in the gut microbiota of the second generation of C. medinalis fed maize (M2). A total of fourteen taxa belonging to Proteobacteria, seven taxa belonging to Actinobacteria, and six taxa belonging to Bacteroidetes were in the gut microbiota of the third generation of C. medinalis fed on maize (M3). LEfSe was also used to find the biomarkers with significant differences between samples fed different host plants (Supplementary Figure 1). A total of forty-seven taxa were identified as the biomarkers in the gut microbiota of C. medinalis fed on different host plants (Supplementary Figure 2). A total of six taxa belonging to Actinobacteria and one taxon belonging to Proteobacteria were in the gut microbiota of C. medinalis fed rice. Nineteen taxa belonging to Proteobacteria, 10 taxa belonging to Bacteroidetes, and 11 taxa belonging to Actinobacteria were in the gut microbiota of C. medinalis fed maize.

**Functional Prediction of the Gut Microbiota of Cnaphalocrocis medinalis**

To better understand the important role of the gut microbiota of C. medinalis, we used R software with Tax4Fun2 to predict the function in samples based on 16S rDNA sequencing data and compared them with the Ref99NR database (Figure 9). The results showed that the most functional prediction...
FIGURE 4 | PCoA bacterial communities of C. medinalis fed on different host plants over generations based on weighted UniFrac (A) and Bray–Curtis (B) distances. M1–M3: the first to third generation of C. medinalis fed on maize; R1–R3: the first to third generation of C. medinalis fed on rice.

categories were related to metabolism (70.12–71.18%) followed by environmental information processing (16.17–17.08%), cellular processes (5.39–5.94%), and genetic information processing (3.87–4.06%). In the metabolism category, global and overview maps had the highest abundance (34.18–34.73%) followed by carbohydrate metabolism (14.93–15.75%), amino acid metabolism (5.08–5.45%), energy metabolism (3.00–3.10%), metabolism of cofactors and vitamins (2.16–2.40%), nucleotide metabolism (2.18–2.26%), lipid metabolism (2.02–2.17%), xenobiotics biodegradation and metabolism (1.34–1.55%), biosynthesis of other secondary metabolites (1.31–1.35%), metabolism of other amino acids (1.12–1.28%), glycogen biosynthesis and metabolism (1.08–1.14%), and metabolism of terpenoids and polyketides (0.74–0.85%). In the environmental information processing category, membrane transport had the highest abundance (12.05–12.87%) followed by signal transduction (4.08–4.26%). In the cellular processes category, the cellular community had the highest abundance (3.85–4.21%) followed by cell motility (0.88–1.06%), cell growth and death (0.49–0.52%), and transport and catabolism (0.14–0.15%). In the genetic information processing category, replication and repair had the highest abundance (1.55–1.64%), followed by translation (1.45–1.53%) and folding, sorting, and degradation (0.76–0.79%).

TABLE 3 | PERMANOVA of the bacterial communities of C. medinalis fed rice or maize for three generations.

| Source                  | df | SS    | MS    | Pseudo-$F$ | $R^2$ | p-value |
|-------------------------|----|-------|-------|------------|-------|---------|
| Host plant              | 1.17 | 1.5924 | 1.5924 | 8.66       | 0.35  | 0.001   |
| Generation              | 1.17 | 0.1086 | 0.1086 | 0.39       | 0.02  | 0.751   |
| Host plant × Generation | 1.17 | 1.2699 | 1.2699 | 6.22       | 0.28  | 0.004   |

PERMANOVA was generated using 999 permutations, and the individual repeat was included in the model as a random effect.

DISCUSSION

This study profiled the gut bacterial community in C. medinalis fed on different host plants for three generations. Considering the limited gut bacterial information isolated and cultured by traditional methods (Yang, 2012), we obtained the bacterial information of C. medinalis by MiSeq sequencing. Recently, we reported that the composition of the gut bacterial community changes across the life cycle of C. medinalis, and the phyla Proteobacteria and Firmicutes were the dominant bacterial taxa (Yang et al., 2020a). In the guts of both C. medinalis fed rice and maize, the phyla Proteobacteria and Firmicutes were also the dominant bacterial taxa. In this study, host plants, generation, and their interaction did not significantly affect the alpha diversity indices of the gut microbiota in C. medinalis. Ace and choa1 values indicated that community richness did not differ among the different groups. Shannon and Simpson values indicated that community diversity did not differ among the different groups. The experimental results provide a more comprehensive understanding of the relationship between C. medinalis and its microbiota. Our results revealed the influence of host plants and insect generation on the gut bacterial community in C. medinalis and provide a foundation for investigating gut microbe C. medinalis–host plant interactions.

Diet is one of the important factors for insect development (Karley et al., 2002; Qubaiová et al., 2021), and it also plays an important role in shaping insect phenotypes and gut microbial communities (Colman et al., 2012; Xu et al., 2019; Luo et al., 2021; Mason et al., 2021). Host diet could influence the diversity, structure, or composition of the gut in many insects (Strano et al., 2018; Lü et al., 2019; Leite-Mondin et al., 2021; Yuan et al., 2021). Leite-Mondin et al. (2021) discovered that the gut microbiota composition of Trichoplusia ni (Hubner) altered by diet may influence its polyphagous behavior. An imbalanced diet-altered variation in gut microbiota is detrimental to mirid...
bugs, *Adelphocoris suturalis* Jakovlev (Luo et al., 2021). In this study, at the family and genus levels, the composition of the gut microbiota of *C. medinalis* differed between the host plants. Among the genera found in the gut of *C. medinalis* fed different host plants, only 21 genera were found in all samples of three generations of rice-feeding *C. medinalis*, and only 26 genera were found in all samples of three generations of maize-feeding *C. medinalis*. These results indicated that most kinds of microbes are not stably colonized in the gut of *C. medinalis* fed a particular host plant. Hammer et al. (2017) reported that caterpillars lack a resident gut microbiome. Jones et al. (2019) found high variability in gut bacterial composition and abundance between the individuals of the same insect species even fed on the same food source. The reports from other lepidopteran species showed that gut microbial assemblages differed between individuals (Priya et al., 2012; Staudacher et al., 2016). In this study, only 19 genera coexisted in *C. medinalis* fed rice or maize, whereas their relative abundances occupied more than 90% of the gut microbiota of *C. medinalis* fed rice or maize. In addition, we found that two genera (*Tsukamurella* and *Ochrobactrum*) were stable in the gut of rice-feeding *C. medinalis*, but unstable in the gut microbiota of maize-feeding *C. medinalis*, and seven genera (*Bacillus*, *Empedobacter*, *Flavobacterium*, *Rhizobium*, *Rhodococcus*, *Sphingobacterium*, and unclassified *Beutenbergiaceae*) were stable in the gut microbiota of maize-feeding *C. medinalis*, but unstable in the gut of rice-feeding *C. medinalis*. For example, some genera that were stable in the gut of maize-feeding *C. medinalis* were found in some but not all samples of rice-feeding *C. medinalis*. The gut bacteria that were stable in the gut of *C. medinalis* for three generations may have an important role in shaping the microbiota community in *C. medinalis*. Through LEfSe, 47 taxa were found to be the biomarkers for the gut microbiota of *C. medinalis* fed different host plants. Stable host-related bacteria may function to help
FIGURE 7 | Bacterial taxa with LDA scores greater than two in the gut microbiota of \textit{C. medinalis} fed different host plants for three generations.
*C. medinalis* to adapt to host plants. In addition to diet, there are many factors that influence the gut microbiota in insects. Life stage and environment could shape the insect gut microbial community combined with diets as drivers (Colman et al., 2012). Host plant and population sources could drive the diversity of the microbial community in two polyphagous insects (Jones et al., 2019). Different host genotypes and microbial sources could influence the gut bacterial communities in lepidopterans (Mason et al., 2021). In this study, host plant × insect generation may be a factor influencing the gut microbiota in *C. medinalis*. In the colonization of gut microbes, the interaction of the host plant and generation may play an important role. A recent study indicated that diet is not the primary driver of gut bacterial community structure in wood- and litter-feeding cockroaches (Lampert et al., 2019). The phyllosphere microbiome in host plants contributes more than leaf phytochemicals to the variation in the gut microbiome structure in *Agrilus planipennis* (Mogouong et al., 2021). In lepidopterans, metamorphosis, which entails major morphological changes with dietary transformation, could also have a strong impact on the gut microbiota composition (Voirol et al., 2018). However, certain taxa can persist throughout all the stages of the insect (Hammer et al., 2014; Yang et al., 2020a). In insects, the gut microbiota can promote gut homeostasis (Buchon et al., 2013), and core microbes in the gut microbiota may reach homeostasis by interacting with the factors in the environment. Gut microbes coexisting in all samples of rice- and maize-feeding *C. medinalis* may compose the core microbes in *C. medinalis*.

The gut microbiota could play a crucial role in the whole life of insects. The lepidopteran gut microbiota could function in digestion and nutrient acquisition, protection against entomopathogens, and counteraction to anti-herbivore plant defenses (Voirol et al., 2018). Jing et al. (2020) found that the most dominant role of gut bacteria is essential nutrient provisioning, followed by digestion and detoxification. In this study, functional prediction indicated that the most dominant...
FIGURE 9 | Comparison of predicted GO functions of the gut bacteria of *C. medinalis* fed different host plants for three generations.
role of the gut microbiota in C. medinalis is metabolism, followed by environmental information processing, cellular processes, and genetic information processing. Distinct antimicrobials could alter gut microbial communities as a result of different mortalities of P. xylostella (Lin et al., 2015). The gut microbiota involved in P. xylostella susceptibility to Bt Cry1Ac protoxin is associated with the host immune response (Li et al., 2021).

In the guts of both C. medinalis fed rice and maize, the Proteobacteria and Firmicutes phyla were the dominant bacterial taxa. Proteobacteria and Firmicutes have also been reported as dominant taxa in many insects’ gut microbiota, especially in Lepidoptera (Chen et al., 2020; Liu et al., 2020). They may function in carbohydrate metabolism, amino acid metabolism, and membrane transport pathways of the host (Liu et al., 2020; Wang et al., 2020a; Chen et al., 2021). In particular, stably colonized gut bacteria may be crucial for insects to adapt to host plants (Yang et al., 2020b). Global and overview maps, carbohydrate metabolism, membrane transport, amino acid metabolism, signal transduction, and cellular community were the top six pathways in the functions of the gut microbiota in C. medinalis. Enterococcus is an important flora that exists in both rice- and maize-feeding C. medinalis for three generations, followed by the unclassified Enterobacteriaceae, Pectobacterium, and Corynebacterium. Enterococcus has also been reported to be stably maintained in many insects, and it can protect insects against pathogens, fix toxic molecules from plants, increase host fitness, and tolerate toxic diets (Shao et al., 2011; Johnston and Rolf, 2015; Vilanova et al., 2016; Shao et al., 2017). Enterobacteriaceae is one of the important dominant taxa in the gut microbiota of many insects (Wang et al., 2014; Yun et al., 2018; Raza et al., 2020). Enterobacteriaceae are involved in insect metabolism (Pers and Hansen, 2021; Zhou et al., 2021), insect resistance or susceptibility to parasites, and pathogens and insecticides (Oliver et al., 2003; Álvarez-Lagazzi et al., 2021; Polenogova et al., 2021) and play an important role in the host adaptability and reproduction of insects (Shi et al., 2012; Wang et al., 2020a). Pectobacterium, a clade of Enterobacteriaceae, is known as a function of nitrogen fixation (Behar et al., 2005, 2008). In addition to fixing nitrogen, the gut microbiota may help recycle nitrogenous waste products into usable compounds, such as uric acid and ammonia (Behar et al., 2005, 2008). Corynebacterium-related bacteria grow on a variety of sugars, organic acids, and alcohols as the single or combined carbon and energy sources as a workhorse for the large-scale production of amino acids (Eikmanns and Blombach, 2014). The detailed actual functions of these microbes in the gut of C. medinalis need to be proven and verified in further investigations.

CONCLUSION

In conclusion, our results indicated that the alpha diversity indices of gut microbes in C. medinalis could not be affected by the host plant, generation, or host plant × generation. PerMANOVA indicated that the gut bacteria of C. medinalis could be significantly affected by the host plant and host plant × generation. Coexisting bacteria that were found in both rice- and maize-feeding C. medinalis for three generations may play an important role in the development of insects, while stably colonized bacteria in C. medinalis fed a particular plant may function in host adaptation. The most dominant role of the gut microbiota in C. medinalis is metabolism, followed by environmental information processing, cellular processes, and genetic information processing. Furthermore, further experiments should be performed to reveal the function of these microbes, which may promote the identification of new targets for the management of C. medinalis. Our results provide a theoretical basis for the study of gut microbes in C. medinalis.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI—PRJNA785679, SRR17106748–SRR17106753

AUTHOR CONTRIBUTIONS

YY, YL, and ZL contributed to conceptualization of the study. YY and ZL contributed to funding acquisition. XL investigated the study. HX contributed to methodology. YL and ZL contributed to supervision. YY and XL visualized the study. YY wrote the original draft and contributed to writing, reviewing, and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research was funded by the Zhejiang Provincial Natural Science Foundation of China (grant no. LY20C140004), the earmarked fund for the China Agriculture Research System (grant no. CARS-01-39), and the State Key Laboratory for Managing Biotic and Chemical Treats to the Quality and Safety of Agro-products (grant nos. 2010DS700124-ZZ2007 and 2010DS700124-KF1908).

ACKNOWLEDGMENTS

We are thankful to Josie Lynn Catindig for her generous help with manuscript editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.824224/full#supplementary-material

Supplementary Figure 1 | Bacterial taxa with linear discriminant analysis (LDA) score > 2 in the gut microbiota of C. medinalis fed on different host plants.

Supplementary Figure 2 | Cladogram of bacterial biomarkers, from the phylum (innermost ring) to genus (outermost ring) level, with an LDA score > 2. Differential
REFERENCES

Álvarez-Lagazzi, A. P., Cabrera, N., Francis, F., and Ramírez, C. C. (2021). Bringing back the fruit into fruit fly–

REFERENCES

Cheng, J. A. (1996). Rice Pests, 1st Edition. Beijing: China Agricultural Press.

Supplementary Table 2 | ANOVA of Alpha diversity indices of gut bacterial communities in Cnaphalocrocis medinalis fed rice or maize for three generations.

Supplementary Table 3 | Relative abundance (%) of genus in gut microbiota of rice- or maize-feeding Cnaphalocrocis medinalis for three generations.

Supplementary Table 4 | List of genera of the gut microbiota only stable in all samples of rice- or maize-feeding Cnaphalocrocis medinalis.

Supplementary Table 5 | Analysis of similarity (ANOSIM) between gut microbial communities from within sample groups of Cnaphalocrocis medinalis.

bacterial taxa are marked by lowercase letters. Each small circle at different
taxonomic levels represents a taxon at that level, and the diameter of the circle is
proportional to the relative abundance. The coloring principle is to color the
species with no significant difference as yellow and the other different species as
the group with the highest abundance of the species. Different colors represent
different groups, and nodes with different colors represent the communities that
play an important role in the group represented by the color.

Supplementary Table 1 | Sequencing statistics of gut microbiota from rice- or maize-feeding Cnaphalocrocis medinalis for three generations.

Chung, S. H., Rosa, C., Scully, E. D., Peiffer, M., Tooker, J. F., Hoover, K., et al. (2013). Herbivore exploits orally secreted bacteria to suppress plant defenses. Proc. Natl. Acad. Sci. U S A. 110, 15728–15733. doi: 10.1073/pnas.1308867110

Colman, D. R., Toolson, E. C., and Takacs-Vesbach, C. D. (2012). Do diet and
taxonomy influence insect gut bacterial communities? Mol. Ecol. 21, 5124–5137. doi: 10.1111/1365-294X.2012.05752.x

Dillon, R. J., and Dillon, V. M. (2004). The gut bacteria of insects: non-pathogenic
interactions. Annu. Rev. Entomol. 49, 71–92. doi: 10.1146/annurev.ento.49.061802.123416

Douglas, A. E. (2015). Multiorganismal insects: diversity and function of resident
microorganisms. Annu. Rev. Entomol. 60, 17–34. doi: 10.1146/annurev-ento-010814-020822

Elkmann, B. J., and Blombach, B. (2014). The pyruvate dehydrogenase complex
of Corynebacterium glutamicum: an attractive target for metabolic engineering. J. Biotechnol. 192, 339–345. doi: 10.1016/j.jbiotec.2013.12.019

Engel, P., and Moran, N. A. (2013). The gut microbiota of insects – diversity in structure and function. FEMS Microbiol. Rev. 37, 699–735. doi: 10.1111/1574-6976.12025

Frago, E., Dicke, M., and Godfray, H. C. J. (2012). Insect symbionts as hidden
players in insect-plant interactions. Trends Ecol. Evol. 27, 705–711. doi: 10.1016/j.tree.2012.08.013

Hammer, T. J., and Bowers, M. D. (2015). Gut microbes may facilitate insect
herbivory of chemically defended plants. Oecologia 179, 1–14. doi: 10.1007/s00442-015-3327-1

Hammer, T. J., Janzen, D. H., Hallwachs, W., Jaffe, S. P., and Fierer, N. (2017). Caterpillars lack a resident gut microbiome. Proc. Natl. Acad. Sci. U S A. 114, 9641–9646. doi: 10.1073/pnas.1707186114

Hammer, T. J., McLellan, W. O., and Fierer, N. (2014). Metamorphosis of a butterfly-associated bacterial community. PLoS One 9:e86995. doi: 10.1371/journal.pone.0086995

Hansen, A. K., and Moran, N. A. (2014). The impact of microbial symbionts on
host plant utilization by herbivorous insects. Mol. Ecol. 23, 1473–1496. doi: 10.1111/mec.12421

Jang, A., and Kikuchi, Y. (2020). Impact of the insect gut microbiota on ecology, evolution, and industry. Curr. Opin. Insect Sci. 41, 33–39. doi: 10.1016/j.cois.2020.06.004

Jing, T. Z., Qi, F. H., and Wang, Z. Y. (2020). Most dominant roles of insect gut
bacteria: digestion, detoxification, or essential nutrient provision? Microbiome 8:38. doi: 10.1186/s40168-020-00823-y

Johnston, P. R., and Rolff, J. (2015). Host and symbiont jointly control gut
microbiota during complete metamorphosis. PLoS Pathog. 11:e1005246. doi: 10.1371/journal.ppat.1005246

Jones, A. G., Mason, C. J., Felton, G. W., and Hoover, K. (2019). Host plant and
population source drive diversity of microbial gut communities in two
polyphagous insects. Sci. Rep. 9:2792. doi: 10.1038/s41598-019-39163-9

Karley, A. J., Douglas, A. E., and Parker, W. E. (2002). Amino acid composition and
nutritional quality of potato leaf phloem sap for aphids. J. Exp. Biol. 205, 3009–3018. doi: 10.1242/jeb.05.19.3009

Koch, H., and Schmid-Hempel, P. (2011). Socially transmitted gut microbiota
protect bumble bees against an intestinal parasite. Proc. Natl. Acad. Sci. U S A. 108, 19288–19292. doi: 10.1073/pnas.1110474108

Kyrissis, G. A., Augustinos, A. A., Caceras, C., and Bourzitz, K. (2017). Medfly
gut microbiota and enhancement of the sterile insect technique: similarities and
differences of Klebsiella oxytoca and Enterobacter sp. AAZ2 probiotics during the larval and adult stages of the VIENNA 8(D53) + genetic sexing strain. Front. Microbiol. 8:2064. doi: 10.3389/fmicb.2017.02064

THE Barsalou, A. T., Litsinger, J. A., Medina, E. B., Aguda, R. M., Bandong, J. P., Pantua, P. C. Jr., et al. (1991). The rice Cnaphalocrocis and Marasmius (lepидoptera: Pyralidae) leaffolder complex in the Philippines: taxonomy, biometrics, and control. Phil. Entomol. 8, 987–1074.

Behar, A., Jurkevitch, E., and Yuval, B. (2008). Bringing back the fruit into fruit fly–

Álvarez-Lagazzi, A. P., Cabrera, N., Francis, F., and Ramírez, C. C. (2021). Bringing back the fruit into fruit fly–
Qubaiová, J., Jakubec, P., Montoya-Molina, S., Novák, M., and Šuláklová, H. (2021). Influence of diet on development and survival of ThFanathothophilus rugosus (Coleoptera: Silphidae). J. Med. Entomol. 58, 2124–2129. doi: 10.1093/jme/tjab141

Raza, M. F., Yao, Z., Bai, S., Cai, Z., and Zhang, H. (2020). Tephritidae fruit fly gut microbiome diversity, function and potential for applications. Bull. Entomol. Res. 110, 432–437. doi: 10.1017/S0007485319000853

Shao, Y., Spitteler, D., Tang, X., Ping, L., Colies, C., Munchberg, U., et al. (2011). Crystallization of alpha- and beta-carotene in the foregut of Spodoptera larvae feeding on a toxic food plant. Insect Biochem. Mol. Biol. 41, 273–281. doi: 10.1016/j.ibmb.2011.01.004

Shao, Y. Q., Chen, B. S., Sun, C., Ishida, K., Hertweck, C., and Boland, W. (2017). Symbiont-derived antimicrobials contribute to the control of the lepidopteran gut microbiota. Cell Chem. Biol. 24, 66–75. doi: 10.1016/j.chembiol.2016.11.015

Shi, Z., Wang, L., and Zhang, H. (2012). Low diversity bacterial community and the trapping activity of metabolites from cultivable bacteria species in the female reproductive system of the Oriental fruit fly, Bactrocerza dorsalis Hendel (Diptera: Tephritidae). Int. J. Mol. Sci. 13, 6266–6278. doi: 10.3390/ijms13056266

Staudacher, H., Kaltenpeth, M., Breuewer, J., Menken, S., and Groot, A. T. (2016). Variability of bacterial communities in the moth Heliothis virescens indicates transient association with the host. PLoS One 11:e0154514. doi: 10.1371/journal. pone.0154514

Strano, C. P., Malacarino, A., Campolo, O., and Palmieri, V. (2018). Influence of host plant on Thunnosimetes pityocampa gut bacterial community. Microb. Ecol. 75, 487–494. doi: 10.1007/s00248-017-1019-6

Vilanova, C., Baixeras, J., Latorre, A., and Porcar, M. (2016). The generalist inside the specialist: gut bacterial communities of two insect species feeding on toxic plants are dominated by Enterococcus sp. Front. Microbiol. 7:1005. doi: 10.3389/fmicb.2016.01005

Voiroi, L. R. P., Frago, E., Kaltenpeth, M., Hilker, M., and Fatouros, N. E. (2018). Bacterial symbionts in Lepidoptera: their diversity, transmission, and impact on the host. Front. Microbiol. 9:356. doi: 10.3389/fmicb.2018.00356

Wagner, D. L. (2013). “Moths,” in Encyclopedia of Biodiversity 2nd, ed. S. A. Levin (Cambridge: Academic Press), 384–403.

Wang, H. X., Jin, L., Peng, T., Zhang, H. Y., Chen, Q. L., and Hua, Y. J. (2014). Identification of cultivable bacteria in the intestinal tract of Bactrocerza dorsalis from three different populations and determination of their attractive potential. Pest Manag. Sci. 70, 80–87. doi: 10.1002/pms.3528

Wang, J., Peiffer, M., Rosa, C., Zeng, R., and Felton, G. W. (2017). Helicoverpa zea gut-associated bacteria indirectly induce defenses in tomato through mediating salivary elicitor(s). New Phytol. 214, 1294–1306. doi: 10.1111/nph.14429

Wang, J., Yang, M., Song, Y., Acevedo, F. E., Hoover, K., Zeng, R., et al. (2018a). Gut-associated bacteria of Helicoverpa zea indirectly trigger plant defenses in maize. J. Chem. Ecol. 44, 690–699. doi: 10.1007/s10886-018-0970-0

Wang, X., Liu, T., Wu, Y., Zhong, D., Zhou, G., Su, X., et al. (2018b). Bacterial microbiota assemble in Aedes albopictus mosquitoes and its impacts on larvae development. Mol. Ecol. Evol. 27, 2972–2985. doi: 10.1111/mec.14732

Wang, X., Sun, S., Yang, X., Cheng, J., Wei, H., Li, Z., et al. (2020a). Variability of gut microbiota across the life cycle of Grapholitha molesta (Lepidoptera: Tortricidae). Front. Microbiol. 11:3366. doi: 10.3389/fmicb.2020.03166

Wang, Q. Y., Yuan, E. L., Ling, X. Y., Zhu-Salzman, K., Guo, H. J., Ge, F., et al. (2014). Effects of maize (Zea mays) genotypes and microbial sources in shaping fall armyworm (Spodoptera frugiperda) gut bacterial communities. Sci. Rep. 4:11429. doi: 10.1038/srep11429

Wagner, D. L. (2013). “Moths,” in Encyclopedia of Biodiversity 2nd, ed. S. A. Levin (Cambridge: Academic Press), 384–403.
Yang, Y., Liu, X., Xu, H., Liu, Y., Ali, P., Bodlah, M. A., et al. (2020a). The abundance and diversity of gut bacteria of rice leaffolder Cnaphalocrocis medinalis (Guenée) across life stages. J. Asia Pacif. Entomol. 23, 430–438. doi: 10.1016/j.aspen.2020.03.006
Yang, F. Y., Saqib, H. S. A., Chen, J. H., Ruan, Q. Q., Vasseur, L., He, W. Y., et al. (2020b). Differential profiles of gut microbiota and metabolites associated with host shift of Plutella xylostella. Int. J. Mol. Sci. 21:6283. doi: 10.3390/ijms21176283
Yang, H. (2012). Diversity of gut Bacteria in Larval of four Lepidopteran Insect Species. Master’s Thesis. Nanjing: Nanjing Agricultural University.
Yang, Y. J., Xu, H. X., Zheng, X. S., Tian, J. C., Lu, Y. H., and Lu, Z. X. (2015). Progresses in management technology of rice leaffolders in China. J. Plant Prot. 42, 691–701.
Yuan, X. Q., Zhang, X., Liu, X. Y., Dong, Y. L., Yan, Z. Z., Lv, D. B., et al. (2021). Comparison of gut bacterial communities of Grapholita molesta (Lepidoptera: Tortricidae) reared on different host plants. Int. J. Mol. Sci. 22:6843. doi: 10.3390/ijms22136843
Yun, J. H., Jung, M. J., Kim, P. S., and Bae, J. W. (2018). Social status shapes the bacterial and fungal gut communities of the honey bee. Sci. Rep. 8:2019. doi: 10.1038/s41598-018-19860-7
Yun, J. H., Roh, S. W., Whon, T. W., Jung, M. J., Kim, M. S., Park, D. S., et al. (2014). Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. Appl. Environ. Microbiol. 80, 5254–5264. doi: 10.1128/AEM.01226-14
Zhou, X., Ling, X., Guo, H., Zhu-Salzman, K., Ge, F., and Sun, Y. (2021). Serratia symbiotica enhances fatty acid metabolism of pea aphid to promote host development. Int. J. Mol. Sci. 22:5951. doi: 10.3390/ijms22115951

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Yang, Liu, Xu, Liu and Lu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.