**Supplementary material**

**Gut microbiota composition and gene expression changes induced in the *Apis cerana* exposed to acetamiprid and difenoconazole at environmentally realistic concentrations alone or combined**

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**Table S1** Sequences of primer pairs used in the real-time quantitative PCR reactions

|  |  |  |  |
| --- | --- | --- | --- |
| Target gene | Primer sequence (5' - 3') | Gene classification | Source/Reference |
| *Secapin* | F: TTCGACCCGCCAACTTGGATTTAC | Antimicrobial peptide | Self-designed |
| R: TGGGAGGACATCTGACAGGTACTG |
| *abaecin* | F: ATCTTCGCACTACTCGCCAC | Antimicrobial peptide | Wu et al., 2020 |
| R: CCTGACCAGGAAACGTTGGA |
| *defensin1* | F: AGCCACTTGAGCATCCTGAG | Antimicrobial peptide | Wu et al., 2020 |
| R: CCGTTCTTGCAATGACCTCC |
| *hymenoptaecin* | F: CGTGTTGGTTGTCTTCTGCG | Antimicrobial peptide | Wu et al., 2020 |
| R: CACCATAGGCATCTCCCGTC |
| *apidaecin* | F: CTCCTCAACCTGCGCGTATTCG | Antimicrobial peptide | Self-designed |
| R: CGGTTTAGCTTCACGGCGTAGAC |
| *Accβ1* | F: CCTATGCGTGCTCGTGTTCTACC | Acetylcholine receptor | Self-designed |
| R: GGCAGGATCTTGCTGACCAGTAAC |
| *Accβ2* | F: AGCGTAACAAGTGTCGGCATCG | Acetylcholine receptor | Self-designed |
| R: CCGTAAGGCCACCAAGTGTGATC |
| *CYP4G11* | F: CGCAAAGAGAATGGGAAGG | Detoxifying enzyme  Cytochrome P450 | Shi et al., 2013 |
| R: CTTTTGTGTGACGGAGGTGC |
| *CYP9E2* | F: CTTTTTGCAGCCGTGAACGA | Detoxifying enzyme  Cytochrome P450 | Jiang et al., 2016 |
| R: AATCTGTCGTGCTTCTGGGG |
| *CYP336A1* | F: TGTTCGGTTATTTGCCATTCC | Detoxifying enzyme  Cytochrome P450 | Zhu et al., 2016 |
| R: GGTCTGCCAGTAAACTCTTCC |
| *AcceFE4* | F: CATGGTGGCGCATTTGTAGTAGGC | Detoxifying enzyme  carboxylesterase | Ma et al., 2018 |
| R: GGCCCAAGAACATCGTATCAATCC |
| *CAT* | F: GTCTTGGCCCGAACAATTTG | Antioxidant enzyme  catalase | Chao et al., 2019 |
| R: CATTCTCTAGGCCCACCAAA |
| *SOD2* | F: TTGCCATTCAAGGTTCTGGTT | Antioxidant enzyme  Superoxide dismutase | Chao et al., 2019 |
| R: GCATGTTCCCAAACATCAATACC |
| *hbg-3* | F: CAGTTTCTGCTGGATTTTCCTC | Hormone system | Self-designed |
| R: CGAGTTCTTGTCCTTCTTTTCA |
| *vitellogenin* | F: ACCAACGACTTCATGGGACC | Immune marker | Self-designed |
| R: CGCTGTCGCTGATCACATTG |
| *β-Action* | F: GTTTTCCCATCTATCGTCGG | Housekeeping gene | Self-designed |
| R: TTTTCTCCATATCATCCCAG |

**Table S2** Statistics of sample sequencing data processing result of gut bacterial microbiota

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample ID | Raw Reads | Clean Reads | Effective Reads | AvgLen (bp) | GC (%) | Q20 (%) | Q30 (%) | Effective (%) |
| C1 | 79813 | 78895 | 67121 | 426 | 52.84 | 97.25 | 94.64 | 84.1 |
| C2 | 80032 | 79295 | 69400 | 422 | 52.03 | 97.43 | 95.02 | 86.72 |
| C3 | 80083 | 79275 | 70597 | 425 | 52.21 | 97.41 | 94.91 | 88.15 |
| TA1 | 80013 | 79054 | 70692 | 422 | 52.86 | 97.3 | 94.74 | 88.35 |
| TA2 | 61034 | 60254 | 51267 | 423 | 53.37 | 97.31 | 94.74 | 84 |
| TA3 | 72420 | 71685 | 61175 | 420 | 52.72 | 97.49 | 95.1 | 84.47 |
| TD1 | 80136 | 79216 | 72954 | 423 | 51.91 | 97.35 | 94.83 | 91.04 |
| TD2 | 80113 | 79220 | 72591 | 424 | 52.11 | 97.34 | 94.81 | 90.61 |
| TD3 | 79940 | 79055 | 72405 | 423 | 51.48 | 97.35 | 94.84 | 90.57 |
| TDA1 | 79893 | 79148 | 71959 | 423 | 52 | 97.47 | 95.05 | 90.07 |
| TDA2 | 80113 | 79220 | 72591 | 424 | 52.11 | 97.34 | 94.81 | 90.61 |
| TDA3 | 79940 | 79055 | 72405 | 423 | 51.48 | 97.35 | 94.84 | 90.57 |
| Total | 930302 | 920091 | 821985 | — | — | — | — | — |

**Table S3** Statistics of sample sequencing data processing result of gut fungal microbiota

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample ID | Raw Reads | Clean Reads | Effective Reads | AvgLen (bp) | GC (%) | Q20 (%) | Q30 (%) | Effective (%) |
| C1 | 80274 | 77365 | 76904 | 237 | 46.05 | 99.76 | 99.27 | 95.8 |
| C2 | 43218 | 40769 | 40234 | 243 | 49.7 | 99.67 | 99.02 | 93.1 |
| C3 | 80461 | 79201 | 79017 | 226 | 37.71 | 99.85 | 99.47 | 98.21 |
| TA1 | 80137 | 77528 | 77223 | 241 | 47.13 | 99.78 | 99.32 | 96.36 |
| TA2 | 80086 | 77275 | 76571 | 233 | 46.07 | 99.79 | 99.36 | 95.61 |
| TA3 | 79839 | 77043 | 76802 | 244 | 45.89 | 99.79 | 99.37 | 96.2 |
| TD1 | 79855 | 77368 | 76952 | 244 | 48.65 | 99.76 | 99.28 | 96.36 |
| TD2 | 79921 | 76369 | 76102 | 242 | 46.46 | 99.79 | 99.37 | 95.22 |
| TD3 | 80052 | 76450 | 76282 | 210 | 47.67 | 99.84 | 99.47 | 95.29 |
| TDA1 | 80014 | 78482 | 78263 | 289 | 41.69 | 99.43 | 98.64 | 97.81 |
| TDA2 | 79950 | 72050 | 71853 | 204 | 47.26 | 99.8 | 99.37 | 89.87 |
| TDA3 | 65741 | 63361 | 63010 | 193 | 42.01 | 99.77 | 99.27 | 95.85 |
| Total | 909548 | 873261 | 869213 | — | — | — | — | — |

**Table S4** Metastats statistical result at genus taxonomy level of gut bacterial microbiota

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| genus | Mean  (C) | Variance  (C) | Std.err  (C) | Mean  (TDA) | Variance  (TDA) | Std.err  (TDA) | P value | Q value |
| Lactobacillus | 1.59E-01 | 5.34E-05 | 4.22E-03 | 2.45E-01 | 4.51E-04 | 1.23E-02 | ﹤0.001 | ﹤0.001 |
| Gilliamella | 1.13E-01 | 2.45E-03 | 2.86E-02 | 2.08E-01 | 1.30E-06 | 6.57E-04 | 0.0267 | 0.277 |
| Klebsiella | 6.33E-03 | 2.12E-05 | 2.66E-03 | 1.25E-04 | 1.71E-09 | 2.39E-05 | 0.0436 | 0.277 |
| Snodgrassella | 8.25E-02 | 1.97E-04 | 8.10E-03 | 5.41E-02 | 3.69E-04 | 1.11E-02 | 0.0527 | 0.277 |
| Fructobacillus | 2.01E-02 | 2.72E-04 | 9.53E-03 | 5.84E-04 | 8.26E-07 | 5.25E-04 | 0.0576 | 0.277 |
| Escherichia-Shigella | 4.59E-02 | 2.51E-03 | 2.89E-02 | 1.60E-04 | 2.22E-08 | 8.60E-05 | 0.129 | 0.399 |
| Atopobium | 6.52E-03 | 2.44E-06 | 9.03E-04 | 3.77E-03 | 7.43E-06 | 1.57E-03 | 0.156 | 0.399 |
| Kosakonia | 1.26E-03 | 2.22E-06 | 8.60E-04 | 5.23E-06 | 8.20E-11 | 5.23E-06 | 0.182 | 0.399 |
| Aeromonas | 8.35E-03 | 1.66E-04 | 7.44E-03 | 4.08E-02 | 1.32E-03 | 2.10E-02 | 0.187 | 0.399 |
| Bartonella | 3.10E-02 | 1.47E-03 | 2.21E-02 | 1.23E-03 | 2.28E-06 | 8.72E-04 | 0.204 | 0.399 |
| Tatumella | 2.17E-05 | 3.54E-10 | 1.09E-05 | 5.40E-06 | 8.74E-11 | 5.40E-06 | 0.209 | 0.399 |
| Pectinatus | 2.23E-01 | 6.33E-03 | 4.59E-02 | 1.14E-01 | 1.42E-02 | 6.89E-02 | 0.236 | 0.399 |
| Sebaldella | 1.09E-05 | 8.84E-11 | 5.43E-06 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.237 | 0.399 |
| Pseudomonas | 5.15E-03 | 7.91E-05 | 5.14E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.324 | 0.464 |
| Enterobacter | 2.59E-03 | 2.00E-05 | 2.58E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.329 | 0.464 |
| Salmonella | 1.24E-04 | 4.60E-08 | 1.24E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.372 | 0.496 |
| Bombella | 1.20E-01 | 9.30E-03 | 5.57E-02 | 1.91E-01 | 8.38E-03 | 5.28E-02 | 0.487 | 0.591 |
| Apibacter | 1.21E-01 | 2.67E-03 | 2.98E-02 | 8.06E-02 | 3.25E-03 | 3.29E-02 | 0.492 | 0.591 |
| Bifidobacterium | 4.39E-02 | 4.67E-04 | 1.25E-02 | 5.43E-02 | 2.37E-04 | 8.89E-03 | 0.602 | 0.662 |
| Serratia | 2.46E-04 | 1.11E-07 | 1.92E-04 | 1.04E-04 | 2.37E-08 | 8.89E-05 | 0.607 | 0.662 |
| Dysgonomonas | 6.17E-03 | 6.81E-05 | 4.77E-03 | 3.30E-03 | 8.35E-06 | 1.67E-03 | 0.678 | 0.707 |
| Candidatus  \_Schmidhempelia | 1.58E-03 | 2.66E-07 | 2.98E-04 | 3.43E-03 | 6.91E-06 | 1.52E-03 | 0.250 | 0.399 |

Note: P value less than 0.05 means significant difference. C (Control) VS TDA (difenoconazole + acetamiprid treated).

**Table S5** Metastats statistical result at genus taxonomy level of gut bacterial microbiota

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| genus | Mean  (C) | Variance(C) | Std.err  (C) | Mean  (TD) | Variance  (TD) | Std.err  (TD) | P value | Q value |
| Lactobacillus | 1.59E-01 | 5.34E-05 | 4.22E-03 | 2.52E-01 | 1.23E-03 | 2.02E-02 | 0.008 | 0.152 |
| Candidatus  \_Schmidhempelia | 1.58E-03 | 2.66E-07 | 2.98E-04 | 9.32E-03 | 1.58E-05 | 2.29E-03 | 0.021 | 0.17 |
| Gilliamella | 1.13E-01 | 2.45E-03 | 2.86E-02 | 2.03E-01 | 6.52E-04 | 1.47E-02 | 0.038 | 0.205 |
| Pectinatus | 2.23E-01 | 6.33E-03 | 4.59E-02 | 7.38E-02 | 4.31E-03 | 3.79E-02 | 0.047 | 0.205 |
| Klebsiella | 6.33E-03 | 2.12E-05 | 2.66E-03 | 1.26E-04 | 4.27E-10 | 1.19E-05 | 0.051 | 0.205 |
| Fructobacillus | 2.01E-02 | 2.72E-04 | 9.53E-03 | 1.48E-04 | 3.70E-08 | 1.11E-04 | 0.061 | 0.207 |
| Escherichia-Shigella | 4.59E-02 | 2.51E-03 | 2.89E-02 | 7.02E-05 | 1.19E-09 | 1.99E-05 | 0.127 | 0.381 |
| Kosakonia | 1.26E-03 | 2.22E-06 | 8.60E-04 | 1.00E-05 | 7.53E-11 | 5.01E-06 | 0.168 | 0.407 |
| Snodgrassella | 8.25E-02 | 1.97E-04 | 8.10E-03 | 6.83E-02 | 1.15E-04 | 6.20E-03 | 0.182 | 0.407 |
| Tatumella | 2.17E-05 | 3.54E-10 | 1.09E-05 | 5.08E-06 | 7.74E-11 | 5.08E-06 | 0.191 | 0.407 |
| Bartonella | 3.10E-02 | 1.47E-03 | 2.21E-02 | 4.47E-04 | 1.69E-07 | 2.37E-04 | 0.204 | 0.407 |
| Serratia | 2.46E-04 | 1.11E-07 | 1.92E-04 | 4.53E-05 | 9.38E-10 | 1.77E-05 | 0.31 | 0.486 |
| Bombella | 1.20E-01 | 9.30E-03 | 5.57E-02 | 1.78E-01 | 1.58E-04 | 7.25E-03 | 0.315 | 0.486 |
| Pseudomonas | 5.15E-03 | 7.91E-05 | 5.14E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.34 | 0.486 |
| Enterobacter | 2.59E-03 | 2.00E-05 | 2.58E-03 | 5.08E-06 | 7.74E-11 | 5.08E-06 | 0.344 | 0.486 |
| Salmonella | 1.24E-04 | 4.60E-08 | 1.24E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.394 | 0.525 |
| Bifidobacterium | 4.39E-02 | 4.67E-04 | 1.25E-02 | 5.68E-02 | 5.09E-04 | 1.30E-02 | 0.597 | 0.731 |
| Sebaldella | 1.09E-05 | 8.84E-11 | 5.43E-06 | 4.92E-06 | 7.26E-11 | 4.92E-06 | 0.609 | 0.731 |
| Dysgonomonas | 6.17E-03 | 6.81E-05 | 4.77E-03 | 3.27E-03 | 4.68E-07 | 3.95E-04 | 0.648 | 0.74 |
| Aeromonas | 8.35E-03 | 1.66E-04 | 7.44E-03 | 1.47E-02 | 3.32E-04 | 1.05E-02 | 0.706 | 0.759 |
| Atopobium | 6.52E-03 | 2.44E-06 | 9.03E-04 | 8.76E-03 | 7.21E-05 | 4.90E-03 | 0.727 | 0.759 |
| Apibacter | 1.21E-01 | 2.67E-03 | 2.98E-02 | 1.32E-01 | 8.71E-04 | 1.70E-02 | 0.785 | 0.785 |

Note: P value less than 0.05 means significant difference. C (Control) VS TD (difenoconazole treated).

**Table S6** Metastats statistical result at genus taxonomy level of gut bacterial microbiota

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| genus | Mean  (C) | Variance(C) | Std.err  (C) | Mean  (TA) | Variance  (TA) | Std.err  (TA) | P value | Q value |
| Lactobacillus | 1.59E-01 | 5.34E-05 | 4.22E-03 | 1.00E-01 | 2.19E-04 | 8.54E-03 | 0.00724 | 0.174 |
| Apibacter | 1.21E-01 | 2.67E-03 | 2.98E-02 | 5.91E-02 | 1.54E-05 | 2.26E-03 | 0.049 | 0.588 |
| Candidatus\_  Schmidhempelia | 1.58E-03 | 2.66E-07 | 2.98E-04 | 3.27E-03 | 3.51E-06 | 1.08E-03 | 0.122 | 0.693 |
| Kosakonia | 1.26E-03 | 2.22E-06 | 8.60E-04 | 4.39E-05 | 5.70E-10 | 1.38E-05 | 0.142 | 0.693 |
| Bombella | 1.20E-01 | 9.30E-03 | 5.57E-02 | 2.29E-01 | 8.79E-03 | 5.41E-02 | 0.146 | 0.693 |
| Serratia | 2.46E-04 | 1.11E-07 | 1.92E-04 | 1.57E-05 | 7.38E-10 | 1.57E-05 | 0.173 | 0.693 |
| Bifidobacterium | 4.39E-02 | 4.67E-04 | 1.25E-02 | 2.86E-02 | 8.54E-05 | 5.34E-03 | 0.222 | 0.760 |
| Tatumella | 2.17E-05 | 3.54E-10 | 1.09E-05 | 2.01E-03 | 1.02E-05 | 1.85E-03 | 0.281 | 0.843 |
| Sebaldella | 1.09E-05 | 8.84E-11 | 5.43E-06 | 1.77E-02 | 9.42E-04 | 1.77E-02 | 0.376 | 0.895 |
| Pseudomonas | 5.15E-03 | 7.91E-05 | 5.14E-03 | 2.99E-05 | 1.53E-09 | 2.26E-05 | 0.389 | 0.895 |
| Klebsiella | 6.33E-03 | 2.12E-05 | 2.66E-03 | 6.64E-02 | 1.12E-02 | 6.10E-02 | 0.436 | 0.895 |
| Enterobacter | 2.59E-03 | 2.00E-05 | 2.58E-03 | 6.91E-05 | 8.81E-09 | 5.42E-05 | 0.447 | 0.895 |
| Salmonella | 1.24E-04 | 4.60E-08 | 1.24E-04 | 7.33E-06 | 1.61E-10 | 7.33E-06 | 0.526 | 0.909 |
| Snodgrassella | 8.25E-02 | 1.97E-04 | 8.10E-03 | 6.77E-02 | 5.82E-04 | 1.39E-02 | 0.553 | 0.909 |
| Dysgonomonas | 6.17E-03 | 6.81E-05 | 4.77E-03 | 1.10E-02 | 3.43E-05 | 3.38E-03 | 0.606 | 0.909 |
| Panax\_ginseng | 5.40E-06 | 8.75E-11 | 5.40E-06 | 1.57E-05 | 7.38E-10 | 1.57E-05 | 0.705 | 0.943 |
| Atopobium | 6.52E-03 | 2.44E-06 | 9.03E-04 | 5.85E-03 | 1.53E-06 | 7.15E-04 | 0.747 | 0.943 |
| Pectinatus | 2.23E-01 | 6.33E-03 | 4.59E-02 | 1.91E-01 | 3.55E-03 | 3.44E-02 | 0.758 | 0.943 |
| Aeromonas | 8.35E-03 | 1.66E-04 | 7.44E-03 | 4.85E-03 | 3.45E-06 | 1.07E-03 | 0.798 | 0.943 |
| Gilliamella | 1.13E-01 | 2.45E-03 | 2.86E-02 | 1.03E-01 | 2.51E-03 | 2.89E-02 | 0.877 | 0.943 |
| Bartonella | 3.10E-02 | 1.47E-03 | 2.21E-02 | 4.03E-02 | 2.72E-03 | 3.01E-02 | 0.881 | 0.943 |
| Escherichia-Shigella | 4.59E-02 | 2.51E-03 | 2.89E-02 | 3.91E-02 | 4.15E-03 | 3.72E-02 | 0.927 | 0.943 |
| Fructobacillus | 2.01E-02 | 2.72E-04 | 9.53E-03 | 2.15E-02 | 4.67E-04 | 1.25E-02 | 0.943 | 0.943 |

Note: P value less than 0.05 means significant difference. C (Control) VS TA (acetamiprid treated).

Table S7 Metastats statistical result at class taxonomy level of gut fungal microbiota

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| class | Mean(C) | Variance  (C) | Std.err  (C) | Mean  (TA) | Variance  (TA) | Std.err  (TA) | P value | Q value |
| Saccharomycetes | 1.46E-01 | 3.18E-02 | 1.03E-01 | 3.90E-01 | 3.94E-03 | 3.62E-02 | 0.021 | 0.325 |
| Eurotiomycetes | 4.48E-02 | 1.03E-03 | 1.85E-02 | 1.19E-01 | 2.41E-03 | 2.83E-02 | 0.026 | 0.325 |
| Chytridiomycetes | 2.34E-04 | 4.89E-08 | 1.28E-04 | 5.34E-05 | 2.29E-09 | 2.76E-05 | 0.119 | 0.864 |
| Tremellomycetes | 2.49E-02 | 2.20E-04 | 8.56E-03 | 1.43E-02 | 6.20E-06 | 1.44E-03 | 0.173 | 0.864 |
| Olpidiomycetes | 3.31E-04 | 2.88E-07 | 3.10E-04 | 8.92E-06 | 5.97E-11 | 4.46E-06 | 0.268 | 0.864 |
| Mucoromycetes | 0.00E+00 | 0.00E+00 | 0.00E+00 | 1.32E-03 | 5.25E-06 | 1.32E-03 | 0.345 | 0.864 |
| Orbiliomycetes | 2.56E-04 | 1.97E-07 | 2.56E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.345 | 0.864 |
| Ustilaginomycetes | 0.00E+00 | 0.00E+00 | 0.00E+00 | 3.68E-04 | 4.07E-07 | 3.68E-04 | 0.345 | 0.864 |
| Wallemiomycetes | 0.00E+00 | 0.00E+00 | 0.00E+00 | 1.64E-03 | 8.05E-06 | 1.64E-03 | 0.345 | 0.864 |
| Agaricostilbomycetes | 7.63E-04 | 1.74E-06 | 7.63E-04 | 4.50E-06 | 6.08E-11 | 4.50E-06 | 0.525 | 0.974 |
| Archaeosporomycetes | 4.59E-06 | 6.33E-11 | 4.59E-06 | 6.44E-04 | 1.24E-06 | 6.44E-04 | 0.550 | 0.974 |
| Pezizomycetes | 9.56E-06 | 2.74E-10 | 9.56E-06 | 6.30E-04 | 1.19E-06 | 6.30E-04 | 0.582 | 0.974 |
| GS35 | 1.32E-03 | 2.73E-06 | 9.54E-04 | 3.80E-04 | 1.11E-08 | 6.08E-05 | 0.608 | 0.974 |
| Malasseziomycetes | 4.69E-03 | 6.24E-05 | 4.56E-03 | 1.13E-03 | 2.06E-07 | 2.62E-04 | 0.697 | 0.974 |
| Agaricomycetes | 1.48E-01 | 1.49E-02 | 7.05E-02 | 9.80E-02 | 2.06E-06 | 8.28E-04 | 0.719 | 0.974 |
| Mortierellomycetes | 1.34E-02 | 9.20E-05 | 5.54E-03 | 2.07E-02 | 2.74E-04 | 9.56E-03 | 0.728 | 0.974 |
| Microbotryomycetes | 4.05E-03 | 2.90E-05 | 3.11E-03 | 6.60E-03 | 2.30E-05 | 2.77E-03 | 0.745 | 0.974 |
| Leotiomycetes | 3.14E-02 | 5.55E-04 | 1.36E-02 | 2.38E-02 | 9.55E-06 | 1.78E-03 | 0.774 | 0.974 |
| Sordariomycetes | 1.98E-01 | 2.24E-02 | 8.64E-02 | 1.51E-01 | 1.17E-04 | 6.24E-03 | 0.779 | 0.974 |
| Dothideomycetes | 2.93E-02 | 1.07E-03 | 1.89E-02 | 3.80E-02 | 1.92E-03 | 2.53E-02 | 0.865 | 0.987 |
| Rhizophydiomycetes | 2.65E-03 | 4.27E-06 | 1.19E-03 | 2.19E-03 | 9.18E-06 | 1.75E-03 | 0.883 | 0.987 |
| Cystobasidiomycetes | 6.98E-04 | 1.46E-06 | 6.98E-04 | 8.55E-04 | 2.19E-06 | 8.55E-04 | 0.908 | 0.987 |

Note: P value less than 0.05 means significant difference. C (Control) VS TA (acetamiprid treated).

Table S8 Metastats statistical result at class taxonomy level of gut fungal microbiota

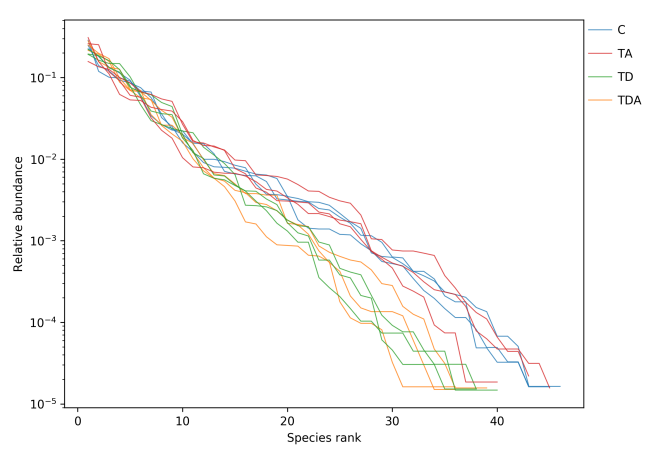
|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| class | Mean  (TA) | Variance  (TA) | Std.err  (TA) | Mean  (TDA) | Variance  (TDA) | Std.err  (TDA) | P value | Q value |
| Malasseziomycetes | 1.13E-03 | 2.06E-07 | 2.62E-04 | 6.96E-05 | 3.02E-09 | 3.17E-05 | 0.005 | 0.119 |
| Chytridiomycetes | 5.34E-05 | 2.29E-09 | 2.76E-05 | 1.90E-04 | 1.52E-09 | 2.25E-05 | 0.010 | 0.119 |
| Leotiomycetes | 2.38E-02 | 9.55E-06 | 1.78E-03 | 1.44E-02 | 1.34E-05 | 2.11E-03 | 0.014 | 0.119 |
| Eurotiomycetes | 1.19E-01 | 2.41E-03 | 2.83E-02 | 2.72E-02 | 5.68E-05 | 4.35E-03 | 0.024 | 0.119 |
| Microbotryomycetes | 6.60E-03 | 2.30E-05 | 2.77E-03 | 1.42E-04 | 1.79E-08 | 7.73E-05 | 0.045 | 0.178 |
| Sordariomycetes | 1.51E-01 | 1.17E-04 | 6.24E-03 | 1.18E-01 | 5.94E-04 | 1.41E-02 | 0.050 | 0.178 |
| Archaeorhizomycetes | 0.00E+00 | 0.00E+00 | 0.00E+00 | 2.87E-04 | 6.23E-08 | 1.44E-04 | 0.058 | 0.182 |
| Agaricostilbomycetes | 4.50E-06 | 6.08E-11 | 4.50E-06 | 3.34E-04 | 9.65E-08 | 1.79E-04 | 0.085 | 0.236 |
| Mortierellomycetes | 2.07E-02 | 2.74E-04 | 9.56E-03 | 5.88E-03 | 1.96E-06 | 8.08E-04 | 0.117 | 0.292 |
| GS35 | 3.80E-04 | 1.11E-08 | 6.08E-05 | 5.72E-04 | 5.76E-08 | 1.39E-04 | 0.156 | 0.354 |
| Dothideomycetes | 3.80E-02 | 1.92E-03 | 2.53E-02 | 8.48E-03 | 1.43E-05 | 2.18E-03 | 0.199 | 0.393 |
| Tremellomycetes | 1.43E-02 | 6.20E-06 | 1.44E-03 | 1.10E-02 | 1.84E-05 | 2.48E-03 | 0.204 | 0.393 |
| Archaeosporomycetes | 6.44E-04 | 1.24E-06 | 6.44E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.306 | 0.403 |
| Cystobasidiomycetes | 8.55E-04 | 2.19E-06 | 8.55E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.306 | 0.403 |
| Mucoromycetes | 1.32E-03 | 5.25E-06 | 1.32E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.306 | 0.403 |
| Pezizomycetes | 6.30E-04 | 1.19E-06 | 6.30E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.306 | 0.403 |
| Ustilaginomycetes | 3.68E-04 | 4.07E-07 | 3.68E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.306 | 0.403 |
| Wallemiomycetes | 1.64E-03 | 8.05E-06 | 1.64E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.306 | 0.403 |
| Agaricomycetes | 9.80E-02 | 2.06E-06 | 8.28E-04 | 3.02E-01 | 1.52E-01 | 2.25E-01 | 0.595 | 0.723 |
| Rhizophydiomycetes | 2.19E-03 | 9.18E-06 | 1.75E-03 | 6.31E-04 | 1.33E-07 | 2.11E-04 | 0.607 | 0.723 |
| Olpidiomycetes | 8.92E-06 | 5.97E-11 | 4.46E-06 | 6.20E-05 | 1.15E-08 | 6.20E-05 | 0.644 | 0.732 |
| Saccharomycetes | 3.90E-01 | 3.94E-03 | 3.62E-02 | 4.56E-01 | 1.37E-01 | 2.14E-01 | 0.802 | 0.872 |

Note: P value less than 0.05 means significant difference. TA (acetamiprid treated) VS TDA (difenoconazole + acetamiprid treated).

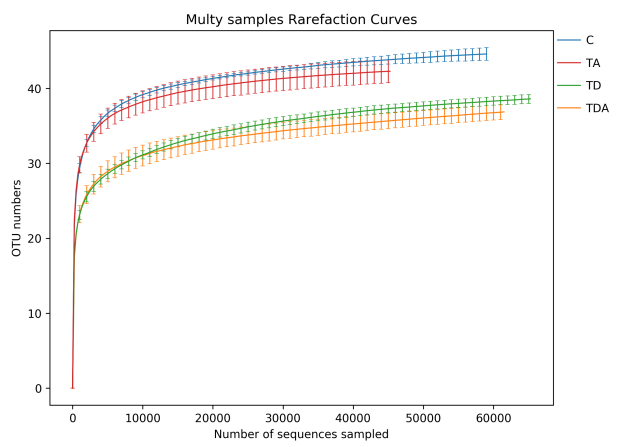
Table S9 Metastats statistical result at class taxonomy level of gut fungal microbiota

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| class | Mean  (TD) | Variance  (TD) | Std.err  (TD) | Mean  (TDA) | Variance  (TDA) | Std.err  (TDA) | P value | Q value |
| Sordariomycetes | 1.65E-01 | 3.58E-04 | 1.09E-02 | 1.18E-01 | 5.94E-04 | 1.41E-02 | 0.020 | 0.264 |
| Dothideomycetes | 1.14E-01 | 6.10E-03 | 4.51E-02 | 8.48E-03 | 1.43E-05 | 2.18E-03 | 0.034 | 0.264 |
| Malasseziomycetes | 1.33E-03 | 9.38E-07 | 5.59E-04 | 6.96E-05 | 3.02E-09 | 3.17E-05 | 0.039 | 0.264 |
| Eurotiomycetes | 1.12E-01 | 5.16E-03 | 4.15E-02 | 2.72E-02 | 5.68E-05 | 4.35E-03 | 0.053 | 0.264 |
| Mortierellomycetes | 8.34E-03 | 2.87E-06 | 9.79E-04 | 5.88E-03 | 1.96E-06 | 8.08E-04 | 0.076 | 0.317 |
| Leotiomycetes | 1.94E-02 | 8.50E-06 | 1.68E-03 | 1.44E-02 | 1.34E-05 | 2.11E-03 | 0.091 | 0.326 |
| Tremellomycetes | 1.62E-02 | 8.28E-06 | 1.66E-03 | 1.10E-02 | 1.84E-05 | 2.48E-03 | 0.106 | 0.330 |
| Cystobasidiomycetes | 1.73E-03 | 4.55E-06 | 1.23E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.146 | 0.405 |
| Microbotryomycetes | 3.77E-03 | 2.55E-05 | 2.92E-03 | 1.42E-04 | 1.79E-08 | 7.73E-05 | 0.199 | 0.496 |
| Agaricostilbomycetes | 1.29E-02 | 4.43E-04 | 1.22E-02 | 3.34E-04 | 9.65E-08 | 1.79E-04 | 0.278 | 0.631 |
| Agaricomycetes | 7.30E-02 | 3.57E-04 | 1.09E-02 | 3.02E-01 | 1.52E-01 | 2.25E-01 | 0.303 | 0.631 |
| Wallemiomycetes | 2.44E-03 | 1.77E-05 | 2.43E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.354 | 0.681 |
| Exobasidiomycetes | 3.93E-04 | 4.63E-07 | 3.93E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.387 | 0.691 |
| Chytridiomycetes | 8.81E-03 | 2.28E-04 | 8.72E-03 | 1.90E-04 | 1.52E-09 | 2.25E-05 | 0.487 | 0.793 |
| Olpidiomycetes | 4.52E-06 | 6.12E-11 | 4.52E-06 | 6.20E-05 | 1.15E-08 | 6.20E-05 | 0.511 | 0.793 |
| Archaeorhizomycetes | 3.70E-03 | 4.12E-05 | 3.70E-03 | 2.87E-04 | 6.23E-08 | 1.44E-04 | 0.539 | 0.793 |
| Rhizophydiomycetes | 4.85E-04 | 2.67E-08 | 9.43E-05 | 6.31E-04 | 1.33E-07 | 2.11E-04 | 0.717 | 0.995 |
| Saccharomycetes | 3.78E-01 | 2.66E-02 | 9.42E-02 | 4.56E-01 | 1.37E-01 | 2.14E-01 | 0.806 | 1.000 |
| GS35 | 5.49E-04 | 6.01E-08 | 1.41E-04 | 5.72E-04 | 5.76E-08 | 1.39E-04 | 0.905 | 1.000 |
| Archaeosporomycetes | 4.52E-06 | 6.12E-11 | 4.52E-06 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 1.000 | 1.000 |

Note: P value less than 0.05 means significant difference. TD (difenoconazole treated) VS TDA (difenoconazole + acetamiprid treated)



B



A



Fig.S1 Effects of acetamiprid or/and difenoconazole on gut bacterial (A and B) and fungal (C and D) microbiota. OUT rank curves (A and C) of gut microbiota for each group and rarefaction curves (B and D) of gut microbiota for each samples, respectively.

C



Fig.S2 Alpha diversity of gut bacteria measured by the Simpson (A), ACE (B) and Chao1 (C) indices. Data (mean ± SEM) were analyzed by Student’s t-test (\* *P* < 0.05).



Fig.S3 Alpha diversity of gut fungi measured by the Simpson (A) and ACE (B) indices. Data (mean ± SEM) were analyzed by Student’s t-test (\* *P* < 0.05).

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