Supplementary material

Profiling the microbial contamination in aviation fuel from an airport

Dong Hu [a,b], Wenfang Lin [a,*], Jie Zeng [a,b], Peng Wu [a], Menglu Zhang [a,b], Lizheng Guo [ab], Chengsong Ye [a], Kun Wan [a,b], Xin Yu [a,*]

[a] *Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, People's Republic of China*

[b] *University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China*

* Corresponding author:

Wenfang Lin, Email address: wflin@iue.ac.cn;

Xin Yu, Email address: xyu@iue.ac.cn.

Fig. S1. Rarefaction curves generated for (A) 16S rRNA and (B) ITS gene based on high-throughput sequencing from samples collected in the fuel tanks.

Fig. S2. Standard curves for primers 341F/534R and FF1/FR390, constructed by analyzing serial decimal dilutions of plasmids containing specific genes by qPCR.（A) and (B) were used to quantify the microbial contamination, (C) and (D) were used to test the sensitivity of PMA-qPCR assay.

Fig. S3. The growth ability of the isolated microorganisms after being incubated in mineral solution for 30 days. (A) Blank, (B) Bacteria, (C) M17-20, (D) M19-3, (E) M19-15, (F) M19-17, (G) M19-22, (H) M17-16. Oil droplets existed at the fuel-water interface, indicated by red arrows. The attached biofilm was indicated by yellow arrow.

Fig. S4. GC-MS profile of fuel after being degraded by the tested organisms (after 30 days). (A) M17-16, (B) M17-20, (C) M19-13, (D) M19-15, (E) M19-17, (F) M19-22, (G) Blank, incubated with no microorganism. n-Tetracosane-d50 was used as an internal standard.

Fig. S5. The emulsifying activity of the tested isolates in supplementary experiment. The emulsifying activity was calculated as the percentage of the height of the emulsified layer to the total liquid height.

Table S1. The description of four aviation fuel samples used in this study.

Table S3. The emulsifying activity, surface tension lowering activity and pH lowing activity of the tested strains.

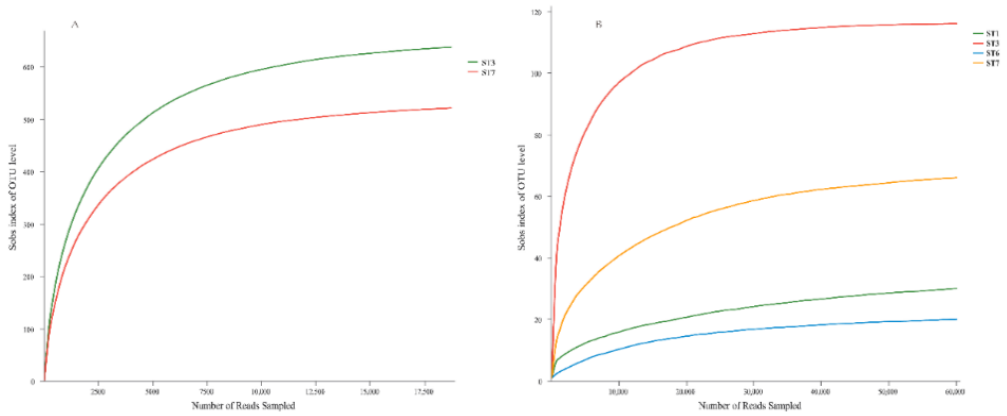
Table S4. The emulsifying activity and surface tension lowering activity of the tested strains with organic liquid medium in supplementary experiment.

Sensitivity of PMA-qPCR assay

The sensitivity of the PMA-qPCR method was investigated using the isolated strains CY1TSA7 (*Bacillus marisflavi*) and M17-20 (*Amorphotheca resinae*) as reference microorganisms. Briefly, the isolated CY1TSA7 was cultured in trypticase soy broth to the exponential growth phase, and M17-20 was cultured on MEA for 7-10 days to sporulation phase. The viable counts of CY1TSA7 were determined by plating on TSA plates. For M17-20, the hyphae and spores were scraped into malt extract broth, vortexed, and then filtered by sterile multiple gauze to remove large granular hyphae. The viable counts were determined by the same operation as bacteria. All plates were then incubated at 28 ℃ for 48 h to count the number of viable cells, and the remaining medium was stored at 4 °C. According to the CFU counts, the medium with different volumes of different species was filtered on a membrane to obtain a total bacterial or fungal biomass with 10^7, 10^6, 10^5, 10^4, 10^3 and 10^2 cells in triplicate. Next, 200 μL aviation fuel was added to the surface of membranes to simulate the fuel environment. The membranes were then treated with PMA. In addition, the viable counts of bacteria and fungi after storage at 4 °C for 48 h were determined. Finally, genomic DNA extraction and PMA-qPCR analysis were performed.

Additional experiment for testing the emulsifying activity and surface tension lowering activity

Another group of the isolates with organic liquid medium, trypticase soy broth (containing per liter: 17.0 g tryptone, 3.0 g soy peptone, 5.0 g NaCl, 2.5 g K_2HPO_4, 2.5 g glucose; pH=7.0+/-0.2) for bacteria and malt extract broth (containing per liter: 30.0 g malt extract, 3.0 g soy peptone; pH=5.6+/-0.2) for fungi, was further used to test the emulsifying activity and surface tension lowering activity. Nearly 100 mL of modified sterile liquid medium was dispensed into a sterile 250 mL conical flask. Two milliliters filter sterilized aviation fuel were added to each flask. Cells were scraped from the plates into 1 mL NaCl solution (0.9%). After being vortexed, 20 μL supernatant was transferred into the flasks in duplicate. The flasks were then incubated at 28 ℃ in a shaker (SKY-211B, Sukun, China) at 120 rpm/min in the dark for 3 days. The uninoculated flasks were used as controls. The test results were shown in Table S4.



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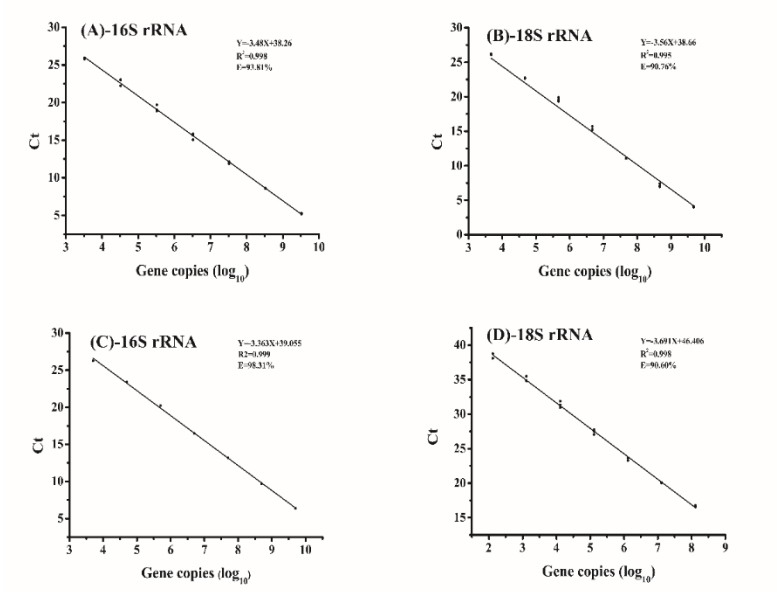

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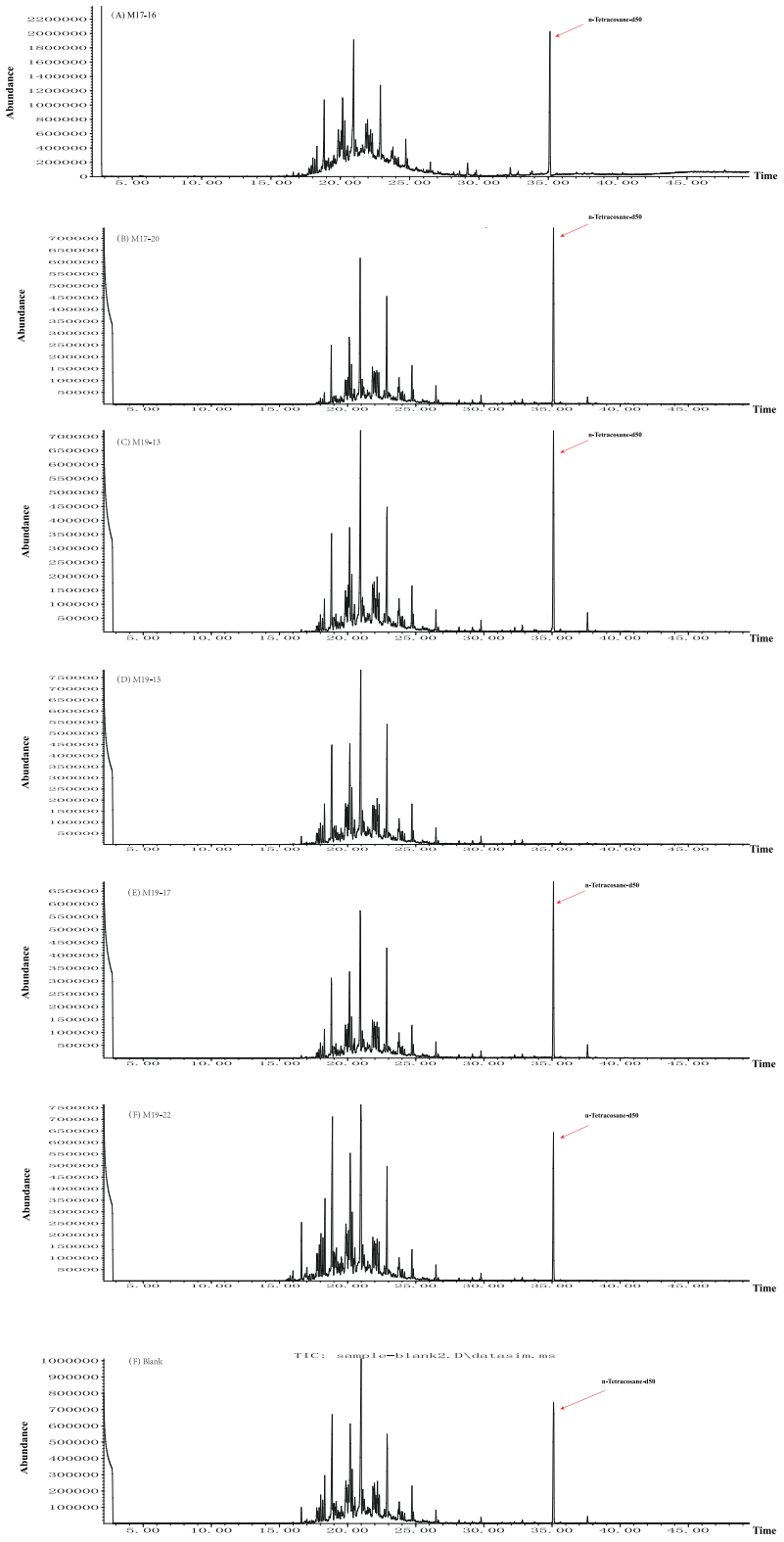

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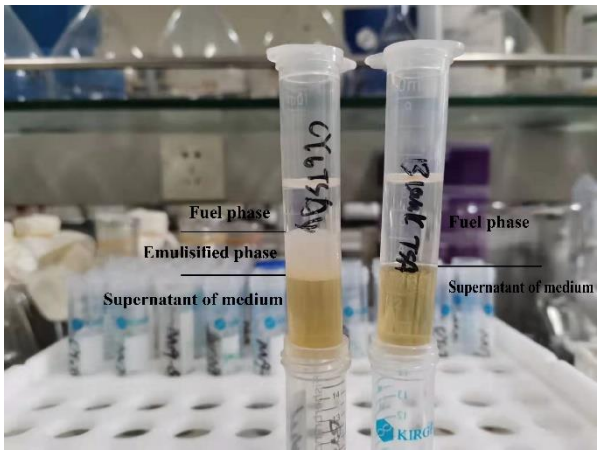

Fig. S5. The emulsifying activity of the tested isolates in supplementary experiment. The emulsifying activity was calculated as the percentage of the height of the emulsified layer to the total liquid height.

Table S1. The description of four aviation fuel samples used in this study

Sample [a]	Sampling date	Source	Description
ST1	24th May, 2018	Aviation fuel tank	10 L volume clear fuel, stored for 5 years
ST3	24th May, 2018	Aviation fuel tank	10 L volume clear fuel, continual use
ST6	24th May, 2018	Aviation fuel tank	10 L volume clear fuel, stored for 5 years
ST7	24th May, 2018	Aviation fuel tank	10 L volume clear fuel, stored for 4 years

[a] ST means the fuel storage tank.

Table S3. The emulsifying activity, surface tension lowering activity and pH lowing activity of the tested strains

Strains [a]	Emulsifying activity (%) [b]	Surface tension lowering activity (mN/m)	pH
Blank	0	68.335	6.61
M17-20	0	62.111	6.05
M17-16	0	60.540	6.30
M19-13	0	61.531	6.14
M19-15	0	61.531	6.44
M19-17	0	62.315	6.44
M19-22	0	61.533	6.20

a All of the isolated bacteria presented no biomass in the tested mineral solution.

[b] The emulsifying activity was calculated as the percentage of the height of the emulsified layer to the total liquid height.

Table S4. The emulsifying activity and surface tension lowering activity of the tested strains with organic liquid medium in supplementary experiment

Strains	Emulsifying activity (%)	Surface tension lowering activity (mN/m)
Blank-TSB	0	61.499
CY1TSA6	0	61.564
CY1TSA7	5%	56.540
CY3TSA8	10%	57.599
CY3TSA9	0	62.403
CY6TSA3	0	61.103
CY6TSA4	30%	49.687
CY6M19	5%	58.466
CY7TSA5	0	60.832
BLANK-MEB	0	61.444
M17-20	0	61.480
M17-16	0	61.977
M19-13	0	61.497
M19-15	0	61.750
M19-17	0	60.699
M19-22	10%	60.557