Reducing Total Plate Count of Bacteria through Dark Sealed Storage for Plant Factory Nutrient Solution Sterilization

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KEYWORDS. **bacterial carrying capacity, colony reconstruction, organic carbon, ultraviolet LED, 16S rRNA**

ABSTRACT. **Plant factories are efficient systems for vegetable production under a clean and controlled environment. To improve the sterilization efficiency of nutrient solutions (NS) in plant factories, this study examined the changes in the number and composition of colonies from in-use and post-use stored NS. The sterilization effect under ultraviolet (UV) light-emitting diode light was also investigated. The results indicated that the total plate count of bacteria (TPC) of newly prepared NS was 3.99 lg CFU/mL (9870 CFU/mL); within 7 days of cultivation, this number sharply increased ~7.8 times to 4.88 lg CFU/mL (76,000 CFU/mL), then remained stable. After dark sealed storage (DSS) of post-use NS, TPC dramatically decreased by 82.8% and reached its lowest point at 4.29 lg CFU/mL (19,566 CFU/mL) on day 3, equivalent to a log survival ratio of −0.76, then rapidly rebounded by 235.6% to 4.82 lg CFU/mL (65,666 CFU/mL) on day 4. During storage, bacterial community composition was also remarkably changed, and the relative abundance of heterotrophic bacteria such as *Variovorax paradoxus* and *Pararhizobium giardinii* and photosynthetic bacteria such as *Pseudo-Nitzschia multiseries* were greatly decreased. The log survival ratio for in-use and post-use stored NS under UV sterilization were −1.29 and −1.30, respectively, and was not significantly different. Coupled with the dramatic decrease in TPC during DSS, the overall log survival ratio of post-use NS UV sterilization was −2.06 and was the most effectively form of sterilization in our study. In short, TPC of in-use NS remained high for a long period, and DSS may serve as a low-cost NS sterilization method in plant factories.**

As the most susceptible component to infection, nutrient solutions (NSs) breeds high concentrations of microorganisms (Strayer 1994) due to their abundant nutrient elements. Total plate count of bacteria (TPC) in NS can reach 5.0 to 6.0 lg CFU/mL after 20-h greenhouse tomato cultivation, nearly 100 times greater than unused NS (Berkelmann et al. 1993). Bacteria in NS will compete with plants for nutrients. More serious, this high bacteria level will last for long periods during cultivation (Li 2019), significantly increasing the risk of plant disease outbreaks (Jia et al. 2023). The closed and controlled production environment in plant factories ensures clean and high-quality vegetables. However, bacterial contamination risks remain nonnegligible. Plant cultivation in all plant factories is based on hydroponics (Kim et al. 2019), and once a system is infected, pathogens can spread widely via NS circulation (Chung et al. 2013; Liu and Huang 2019), leading to substantial economic losses. Consequently, it is imperative to sterilize NS in plant factories.

Liquid with higher TPC requires more resource input for sterilization. Three times the ultraviolet (UV) dose (An 2016) or 28% more ozone (Xu et al. 2002) was needed to achieve the same sterilization effect when TPC in samples to be tasted increased 3- to 4-fold. Therefore, reducing TPC has significant benefits in saving sterilization costs.

TPC is closely associated with environmental factors such as light and organic carbon. Light has a bidirectional regulation on bacterial reproduction. UV light can damage bacterial DNA (Chevrefils et al. 2006), and visible light is able to generate reactive oxygen species that injure cell structures (Hessling et al. 2017), leading to growth inhibition. On the other hand, light also significantly promotes increase of TPC by breaking down the macromolecular organics into smaller units that are more favorable for bacteria (Bertilsson and Stefan 1998). Lindell et al. (1995) irradiated lake water with simulated sunlight, which resulted in a 65% increase in TPC. NS exposed to light can lead to algae pollution (Schwarz and Gross 2004). As the primary carbon skeleton and energy source for heterotrophic bacterial growth (Bridson and Brecker 1970; Escobar et al. 2001), available organic carbon (AOC) is positively correlated with TPC (Escobar et al. 2001; LeChevallier et al. 1991). AOC less than 10 μg acetate-C/L in water was reported to maintain TPC at a low level below 100 CFU/mL (Kooij 1992). For NS, AOC such as organic acids, sugars, amino acids, and so on (Biate et al. 2015; Tang et al. 2018; Weisskopf et al. 2008) continuously released into NS in the form of root exudates (Nguyen 2003), which plays an important role in supporting bacterial growth (Scheuerman et al. 1988; Strayer et al. 1994).

In this study, post-use NS was preserved to investigate the impact of dark and lack of AOC on TPC. Furthermore, we analyzed bacterial community composition (BCC) before and after storage by sequencing the V3–V4 hypervariable region of the bacterial 16S rRNA gene to explain relationship between TPC and BCC.

Materials and methods

Plant materials and growing conditions

Lettuce (*Lactuca sativa* cv. Tiberius RZ) seeds were sown on a deionized

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Table 1. Modified Cornell lettuce nutrient solution recipe.

Compound	Concn ($mg \cdot L^{-1}$)
$Ca(NO_3)_2 \cdot 4H_2O$	524.20
KNO_3	485.60
Fe-EDTA	14.40
KH_2PO_4	72.60
$NH_4H_2PO_4$	53.40
$MgSO_4 \cdot 7H_2O$	245.00
$Na_2B_4O_7 \cdot 10H_2O$	1.44
$MnSO_4 \cdot H_2O$	0.76
$ZnSO_4 \cdot 7H_2O$	0.57
$CuSO_4 \cdot 5H_2O$	0.09
$Na_2MoO_4 \cdot 2H_2O$	0.06

water-soaked seedling sponge sheet (225 mm × 175 mm × 25 mm) and placed in seedling trays (330 mm × 225 mm × 40 mm) for germination under a temperature of 22 ± 0.5 °C for 48 h in the dark. Subsequently, seedlings were illuminated by the light-emitting diode (LED) at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a red-to-blue ratio (R:B) of 4:1, 16-h photoperiod (0600 to 2200 HR). Temperature, relative humidity and CO_2 concentrations were maintained at 22 ± 0.5 °C, 65 ± 10%, and 400 ± 50 μmol/mol, respectively. After 14 d of growth, uniform young plants were selected and transplanted into three cultivation beds (1500 mm × 670 mm × 60 mm) for deep flow hydroponic with a planting density of 37 plants/m^2 in a fully closed plant factory located at the Chinese Academy of Agricultural Science, Beijing, China (lat. 39°57′40.2″N, long. 116°19′34.6″E). The environmental conditions remained the same as those in seedling period.

Modified Cornell lettuce NS (Table 1) with a pH of 5.6 ± 0.1, EC of 1.6 ± 0.1 mS/cm was used for seedling and cultivation. A total of 310 L NS was added to the NS tank (482 mm × 482 mm × 800 mm) and three cultivation beds to operation liquid level (40 mm). The NS was circulated four times daily (0300 to 0500, 0900 to 1100, 1500 to 1700, and 2100 to 2300 HR).

To avoid contamination from residual bacteria, circulation system including NS tanks and three cultivation beds were soaked by sodium hypochlorite (NaClO) solution (effective chlorine 4%) for 12 h, then flush washing 3 times by deionized water until no NaClO remained before use.

Sterilization device

A UV LED nutrient solution sterilization (UV-NSS) device (Fig. 1) was developed in our previous study (Ke et al. 2023). Its main sterilization function component was eight UV-LED light bars fixed in parallel outside a quartz tube to irradiate NS in it at a close range, and NS was pumped through the UV-NSS module for sterilization.

Measurements

TPC MEASUREMENT. Luria–Bertani (LB) medium was prepared for colonies growth by fully mixed 19.2 g LB nutrient agar powder (Cat#L8290; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) and 1 L sterile water.

To measure TPC in NS that could be at extremely high levels, dilution with a gradient of 10 times was used to count colonies in plates. One hundred microliters of NS sample were diluted in 900 μL of sterile water to obtain 10-fold dilutions. In addition, 10^2-fold, 10^3-fold, and 10^4-fold dilutions were obtained by repeating this operation. Each bacterial dilution (100 μL) was then cultured on the LB plates and sterile water as the control. After culturing at 37 °C for 48 h in a sterile incubator (Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China), the plates with colonies ranging from 30 to 300 were selected to calculate TPC (CFU/mL) by using the following formula:

$$\mathrm{TPC} = \frac{BD}{V} \qquad [1]$$

In this formula, B stands for the average number of colonies across three repeats in the same dilution (CFU), D stands for the dilution multiple of the selected plate, and V stands for the sample volume added at the first dilution (10-fold dilution, mL). V was 0.1 mL in this study.

As an efficient and brief method to represent a lengthy and complex TPC number, lg CFU/mL is usually used in microorganism counting procedure. The conversion is done by following formulas:

$$N = 10^{x} \qquad [2]$$

$$\lg N\,\mathrm{CFU/mL} = x \lg \mathrm{CFU/mL} \qquad [3]$$

In these formulas, N stands for TPC in CFU/mL, and x is the value of lg CFU/mL.

To prevent contamination from miscellaneous bacteria, all containers were wrapped in tinfoil and sterilized

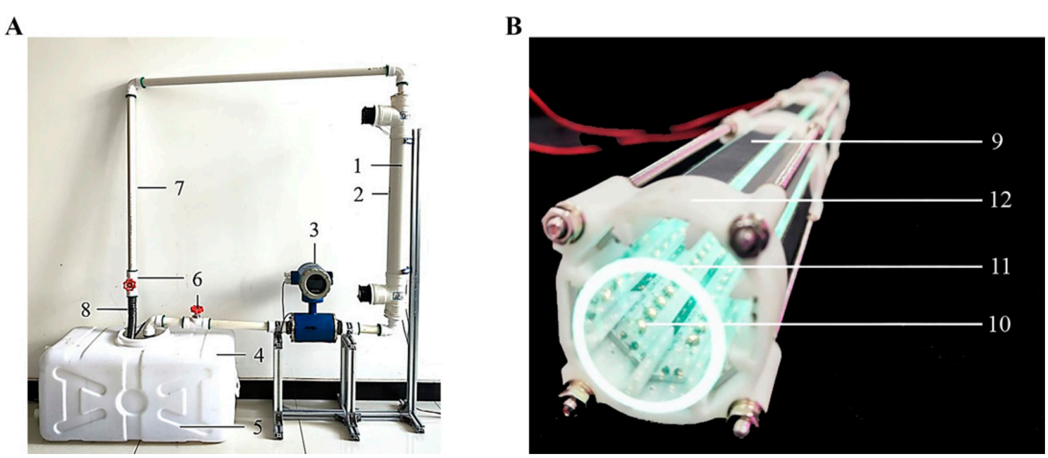

Fig. 1. Ultraviolet light-emitting diode (UV-LED) nutrient solution sterilization (UV-NSS) device. (A) Device structure where 1 = UV-NSS Module; 2 = encapsulated heat sink shell; 3 = flowmeter; 4 = test liquid tank; 5 = water pump; 6 = valve; 7 = pipeline; and 8 = water outlet. (B) Sterilization module where 9 = UV-LED bar; 10 = UV-LED beads; 11 = quartz tube; and 12 = fixtures.

at 121 °C for 20 min in a vertical pressure steam sterilizer (Shanghai Boxun Industrial Co., Ltd. Medical Equipment Factory, Shanghai, China).

Log survival ratio. The log survival ratio quantifies the extent of microbial survival after sterilization treatment in this study. The formula is as follows:

$$\text{log survival ratio} = \lg N_t/N_0 \qquad [4]$$

In this formula, N_t stands for TPC after sterilization treatment (CFU/mL), and N_0 stands for TPC before sterilization treatment (CFU/mL).

DNA extraction and sequencing. DNA was extracted by using E.Z.N.A. Soil DNA Kit (Omega Bio-tek, Inc., Norcross, GA, USA) following the instructions, then V3–V4 region of the 16S rRNA gene were amplified with the universal primer 338F (5′-ACTCC TACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′). The amplicons were sequenced with Illumina Miseq/Nextseq 2000/ Novaseq 6000 (Illumina, Inc., San Diego, CA, USA) platform at Beijing Allwegene Gene Technology Co., Ltd. (Beijing, China).

Qualified sequences were clustered into operational taxonomic units (OTUs) based on 97% similarity threshold using the Uparse (Edgar 2013) algorithm of Vsearch (v2.7.1) (Rognes et al. 2016) software. To minimize the effect of sequencing depth to the intersample variation, samples were rarefied to 56,583 sequences per sample. BCC was performed at the phylum and species levels. Bacterial α diversity analysis (Shannon, Simpson, Chao1, and observable species) and principal component analysis (PCA) were calculated based on the OTU information using QIIME (v1.8.0) (Rognes et al. 2016) and R (v3.6.0), respectively.

Statistical analysis

The data represent the means for three replicate samples of each experiment. SAS statistical software (version 9.2; SAS Institute, Cary, NC, USA) was used to analyze significant differences ($P \leq 0.05$), and standard error (*SE*) is indicated error bars.

Experimental design

In-use NS sampling. Two batches (total of 40 d) of lettuce were grown hydroponically from Sep to Nov 2021, following the procedures in described earlier. Between the two batches, the circulation system was replenished with fresh NS (Table 1) to the initial volume (310 L).

NS was collected once every 3 d throughout the cultivation process; a 1-mL sample 20 mm below the surface of three cultivation beds was taken with a sterile centrifuge tube at 1700 HR, when NS was fully circulated and mixed. NS after 40 d of cultivation (CK) was used for sterilization.

Post-use storage NS sampling. One batch (total of 20 d) of lettuce was hydroponic grown from Oct 2022 to Nov 2022 following the procedures described earlier. A total of 140 L (70 L × 2) of used NS was sealed in two nontransparent tanks (700 mm × 340 mm × 340 mm, L × W × H) and stored at room temperature (25 °C) for 6 d as the dark sealed storage (DSS) treatment.

To identify the effect of DSS on TPC decrease, NS (1 mL) 200 mm below the surface of the nontransparent tank was obtained with a sterile centrifuge tube at 1700 HR daily, and the NS with the lowest TPC (LT-DSS) was used for sterilization. Another 50 mL of NS from the same location was collected for BCC determination on days 0 and 5.

Comparison of sterilization effect. Data including TPC after sterilization and log survival ratio of CK and LT-DSS under same UV dose (current 1.6A, NS flow rate 1.05 m^3/h) were compared.

Sterilized NS samples (1 mL) were collected from the water outlet of the UV-NSS device for TPC and log survival ratio determination.

Results

TPC changes during hydroponics

As shown in Fig. 2, during the first batch of lettuce cultivation (day 0 to 20), in-use NS exhibited an increase at first and later a stable trend in TPC. More specifically, TPC in newly prepared NS (day 0) was 3.99 lg CFU/mL (9870 CFU/mL), then rapidly increased by 88% after only 1 d of use, reached 4.27 lg CFU/mL (18,600 CFU/mL). On days 4 and 7, the number reached 4.66 lg CFU/mL (45,700 CFU/mL) and 4.88 lg CFU/mL (76,000 CFU/mL), an increase of 146% and 67% compared with days 1 and 4, respectively. The growth trend then slowed until reaching the highest TPC of 5.01 lg CFU/mL (103,000 CFU/mL) on day 13.

After harvesting the first batch of lettuce, newly prepared NS was replenished to the circulation system as described

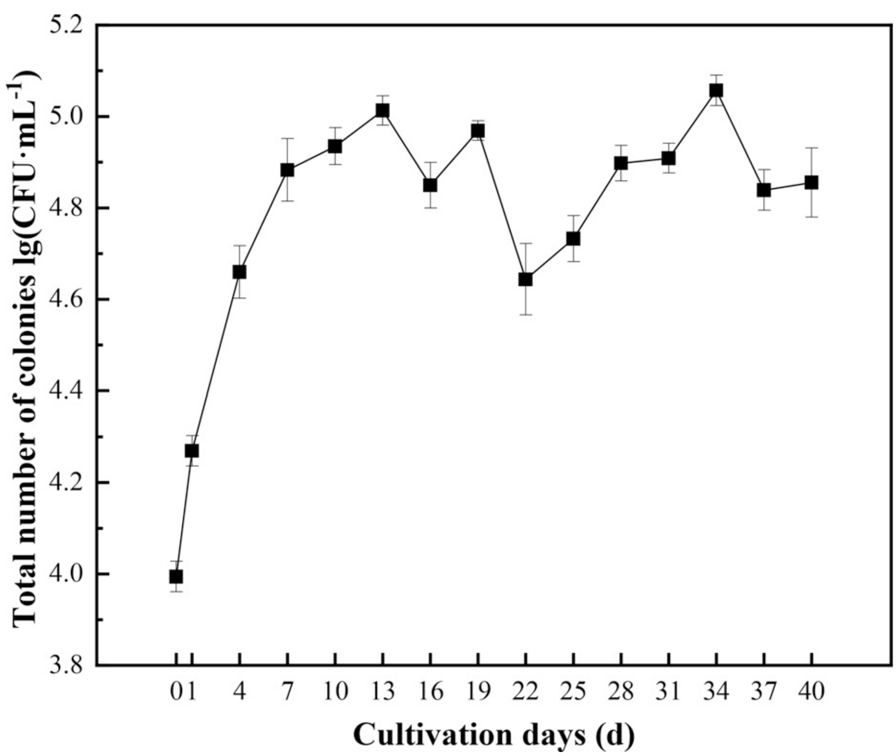

Fig. 2. Change of total plate count of bacteria on days 0 to 40. Error bars indicate standard deviation (n = 3).

earlier for the second batch of cultivation (days 21 to 40). This NS refilling procedure significantly decreased TPC by 53% to 4.64 lg CFU/mL (44,100 CFU/mL) on day 22 compared with day 19. After 14 d of growth, TPC reached its highest point of 5.06 lg CFU/mL (114,000 CFU/mL) on day 34, showing no significant difference from the highest point in the first batch ($P > 0.05$). The growth rate after adding new NS (days 22 to 28) was significantly lower than the same stage at the first batch (days 0 to 7). Under our experimental condition, stable-period TPC consistently fluctuated ~4.90 lg CFU/mL (79,000 CFU/mL), reaching bacterial carrying capacity of the NS.

Notably, during the two batches of cultivation, there was a decrease of more than 30% on days 16 and 37 ($P \leq 0.05$), respectively, 3 d after TPC reached its highest point.

TPC changes under DSS

As shown in Fig. 3, TPC showed a drastic decline and recovery in a short period (4 d). The number on day 3 (4.29 lg CFU/mL, 19,566 CFU/mL) sharply dropped by 82.8% compared with day 0 (5.06 lg CFU/mL, 113,667 CFU/mL), equivalent to a log survival ratio of −0.76, then quickly rebounded 235.6% to 4.82 lg CFU/mL (65,666 CFU/mL) on day 4. Nevertheless, the bacterial-carrying capacity seemed to be inhibited after this major turning point and was 21.3% lower than before (day 0).

Effects of DSS on bacterial diversity and community

Diversity analysis. The rarefaction curve, which tends to be flat, gradually revealed that there was sufficient OTU coverage to describe the bacterial composition, and sequence depth was enough to reflect the majority of OTUs in stored days 0 and 5 (Fig. 4); 529 and 552 number of OTUs were detected in both groups, respectively.

The α diversity index for stored days 0 and 5 are presented in Table 2. The Chao1 index and observable species were unaffected by DSS ($P > 0.05$), and community abundance showed no significant change. Shannon index and Simpson index (1-D values) were significantly increased by DSS ($P \leq 0.05$), indicating that community diversity significantly improved.

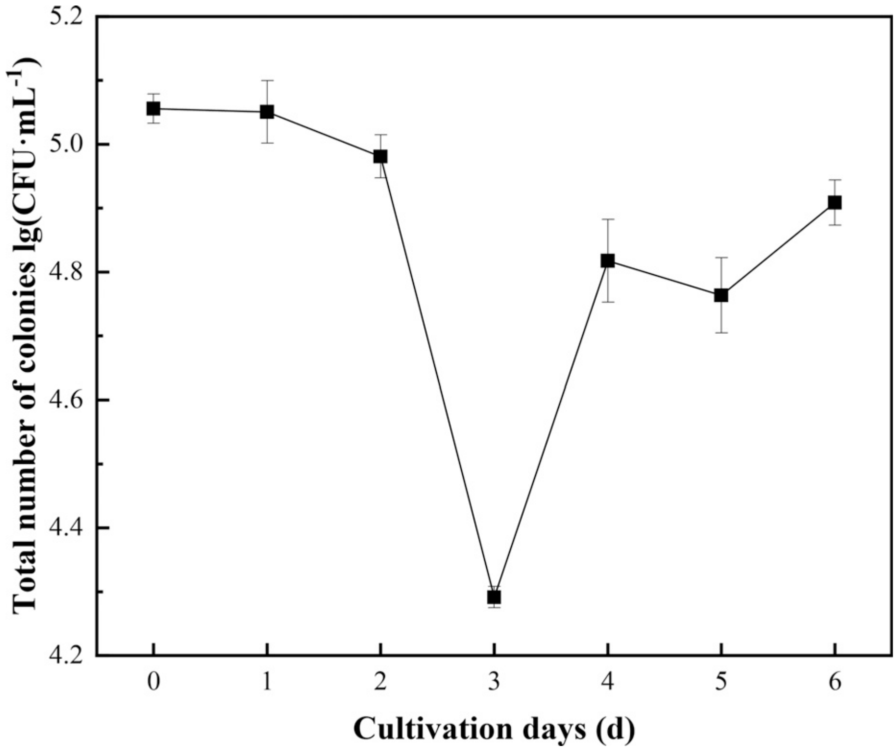

Fig. 3. Change of total plate count of bacteria during dark sealed storage. Error bars indicate standard deviation (n = 3).

DSS-induced BCC change. Figure 5 shows the 16S rRNA amplicon-based BCC before and after 5 d of DSS. At phylum level, Proteobacteria was dominant (relative abundance of 64.3%) on day 0, followed by Patescibacteria (15.7%), Actinobacteriota (11.0%), Cyanobacteria (5.0%), and Bacteroidota (2.0%); the sum of the relative abundance of the other phyla was less than 2%

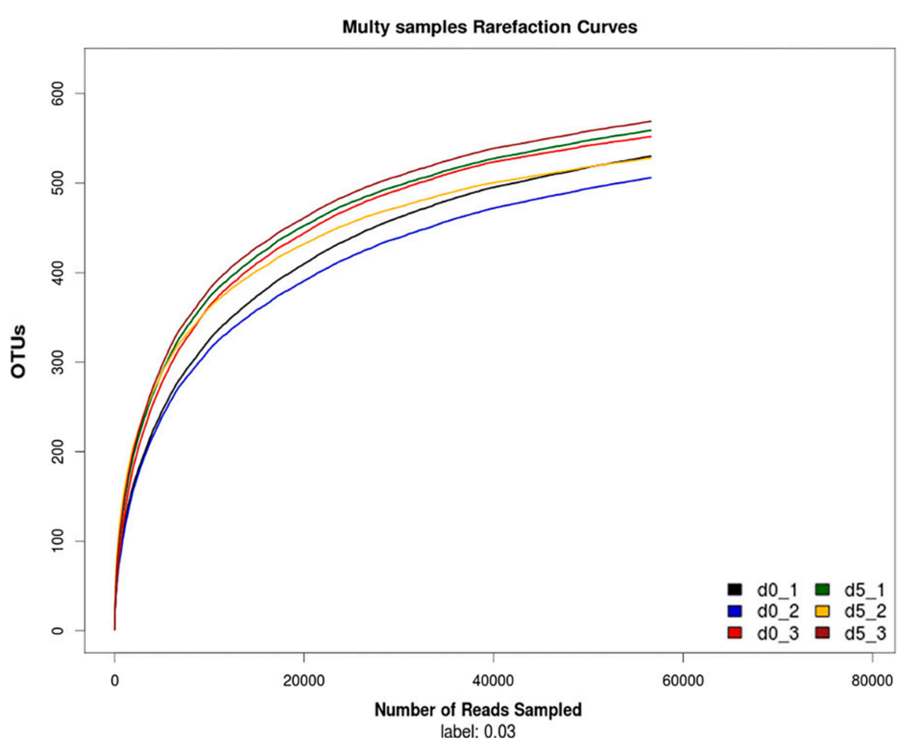

Fig. 4. Rarefaction curves of number of operational taxonomic units (OTUs). d0_1,2,3 and d5_1,2,3 are three replicates of day 0 and 5, respectively.

Table 2. α diversity index of stored on day 0 and 5.

	α diversity index			
Day	Chao1	Observable species	Shannon	Simpson
0	576.2 ± 16.5 a[i]	529.3 ± 23.0 a	4.8 ± 0.1 a	0.9 ± 0.0 a
5	599.5 ± 25.5 a	552.0 ± 21.4 a	5.7 ± 0.3 b	1.0 ± 0.0 b

[i] Values are means (n = 3) with standard deviation. The data within a column followed by different letters indicate significant difference at the 5% level.

(Fig. 6A). After 5 d of storage, the relative abundance of Proteobacteria, Actinobacteria, Bacteroidota, Myxococcota, and Armatimonadota increased to 69.3%, 11.3%, 6.0%, 5.0%, and 2.3%, respectively (Fig. 5A). The relative abundance of Patescibacteria and Cyanobacteria sharply decreased to 2.0% and 0.2%, respectively (Fig. 5A). Proteobacteria consistently maintained the most dominant bacteria during DSS.

At the species level, the most dominant bacteria on day 0 was *Variovorax paradoxus* (20.7%), followed by *Pararhizobium giardinii* (5.3%) and *Gamma proteobacterium* (4.0%). Their relative abundance decreased to 12.0%, 3.0%, and 2.7% after 5 d of storage, respectively (Fig. 5B). *V. paradoxus* exhibited the most significant decrease. Additionally, the relative abundance of *Nocardioides fonticola* increased from less than 1% to 4.3%, the *Pseudo-Nitzschia multiseries* had a relative abundance of 4.3% on day 0 and had disappeared on day 5 (Fig. 5B).

Principal component analysis. PCA was employed to better reveal the dissimilarity among multiple samples on days 0 and 5 of DSS based on OTU information (Fig. 6). Principal component 1 (PC1) explained 72.7% in this analysis, and PC2 explained 20.5%. The distance between sample points on days 0 and 5 was longer, illustrating that there were differences in BCC during 5-d storage. Furthermore, intragroup distances were shorter on day 0 and longer on day 5, and differences in the BCC group were more significant on day 5 than day 0.

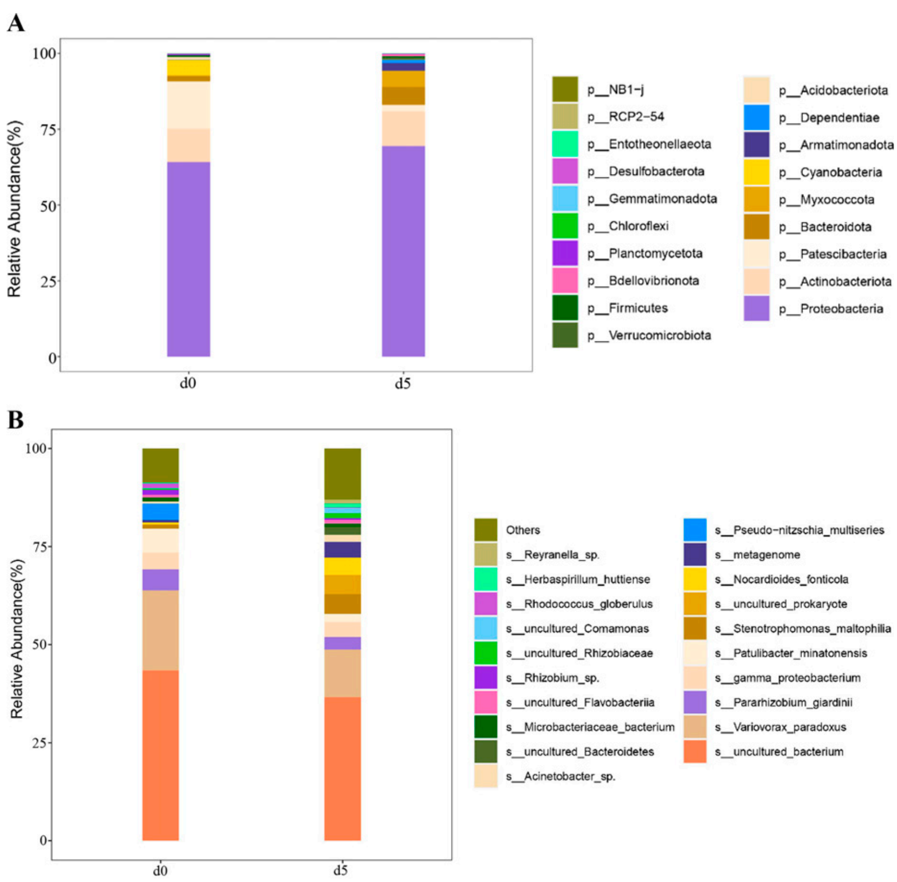

Fig. 5. Bacterial community composition (BCC) before and after 5 d of dark sealed storage (DSS). (A) Phylum level of day 0 and 5 DSS. (B) Species level of day 0 and 5 DSS.

Effect of DSS on sterilization

Our results indicate that NS after 3 d of DSS had the lowest TPC and was used for sterilization. Together with the NS from CK, they were sterilized by following the methods described earlier. As mentioned previously, the initial TPC of CK and LT-DSS were 72,000 CFU/mL (Fig. 2) and 19,566 CFU/mL (Fig. 3), respectively. As shown in Table 3, under sterilization by UV-NSS, TPC of the two treatments dropped to 3700 CFU/mL and 990 CFU/mL, respectively. Despite the 82.8% TPC reduction caused by DSS (Fig. 3), there was no significant difference in log survival ratio between the two treatments. Nevertheless, by considering the sterilization effect of DSS, the overall log survival ratio dropped to −2.06, significantly enhancing the sterilization effect compared with CK (−1.29) ($P \leq 0.05$).

Discussion

TPC changes during hydroponic and DSS

As the crucial nutrient source for bacteria (Butler et al. 2003), organic carbon, including organic acids, sugars, and amino acids (Tang et al. 2018), were directly released into NS (Biate et al. 2015) as root exudates. At the beginning of hydroponic planting, high nutrient concentration and low TPC created enormous potential and space for bacterial proliferation. Together with the promoting effect of organic carbon on bacterial growth (Lu et al. 2023), TPC quickly increased (Fig. 2). Yet with increasingly intensified competition between bacterial species and continuous plant consumption of nutrients, the organic carbon supplied could not fully support the rapid increase of bacteria, and the overall colony growth rate was inhibited (Fig. 2).

Addition of low organic carbon concentration in the newly prepared

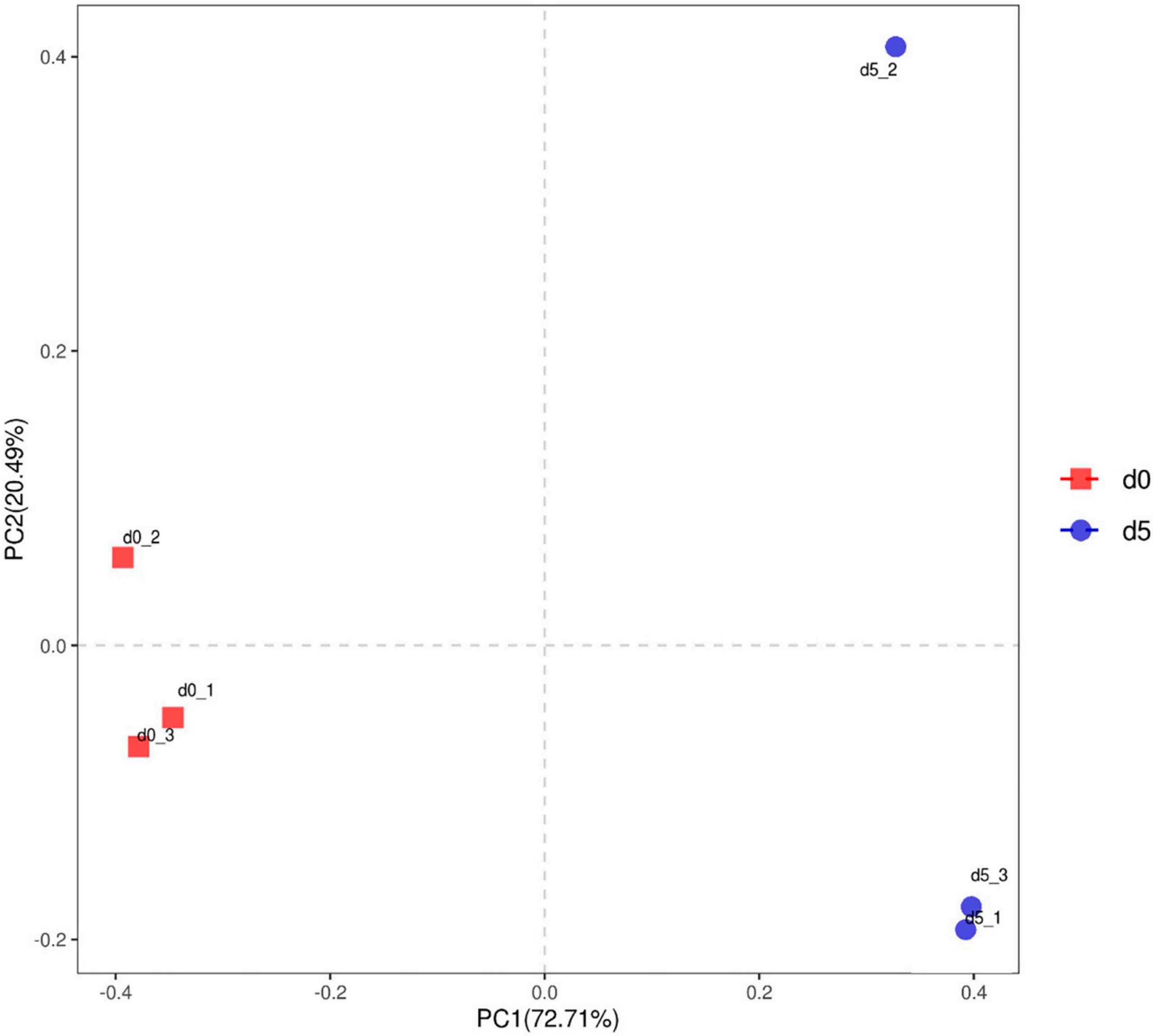

Fig. 6. Principal component (PC) analysis of days 0 and 5 storage.

NS between the two batches exerted a dilution effect and directly resulted in a significant decline in TPC by day 22 compared with day 19 (Fig. 2). However, newly prepared NS also provided potential and space for bacterial proliferation.

The relatively lower TPC growth rate during the second batch of cultivation may explain the lower organic carbon content of newly added NS and lesser root exudate of newly transplanted lettuce seedlings (Badri and Vivanco 2009; Santangeli et al. 2024).

Table 3. Sterilization effect of nutrient solution (NS) after two batches of hydroponic grown (CK) and NS with the lowest total plate count of bacteria (TPC) (LT-DSS) under ultraviolet light-emitting diode nutrient solution sterilization device.

Treatments	Initial TPC (CFU/mL)	TPC after sterilization (CFU/mL)	Log survival ratio
CK	72,000.0 ± 12,342.0 a[i]	3,700.0 ± 702.4 a	−1.29 ± 0.1 a
LT-DSS	19,566.7 ± 751.0 b	990.0 ± 58.9 b	−1.30 ± 0.0 a

[i] Values are means (n = 3) with standard deviation. The data within a column followed by different letters indicate significant difference at the 5% level.

Furthermore, limited nutrient, space, organic carbon in NS, and competition prevented TPC from growing indefinitely, which eventually stabilizes TPC ~4.90 lg CFU/mL (79,000 CFU/mL) during hydroponic under experimental conditions (Fig. 2).

When organic carbon from plants was interrupted, the remaining organic carbon was depleted by existing bacteria during DSS and caused a sudden decrease in TPC on day 3 (Fig. 3). The dead heterotrophic became the new organic carbon source for surviving bacteria (Bridson and Brecker 1970) and proliferated rapidly on day 4 (Fig. 3). After this large-scale evolution of the community structure, a new balance between bacteria and organic carbon was reestablished. Additionally, zero input of exogenous organic carbon might result in a decrease of bacterial carrying capacity at this time.

BCC changes during DSS

Bacterial species in NS were determined by amplicon sequencing in this experiment. Consistent with previous studies (Li 2019; Lin et al. 2021), Proteobacteria consistently emerged as the dominant bacteria throughout our experiment (Fig. 5A). This may be because Proteobacteria is the largest phylum of bacteria widely present in nature and plant roots (Liu et al. 2024), and therefore it is more likely to colonize in NS.

The extensive mortality of Cyanobacteria could be attributed to the dark storage conditions (Fig. 5A). As predominantly photosynthetic organisms living in light-rich environments (Whitton and Potts 2012), Cyanobacteria rely on solar energy and CO_2 exclusively for their autotrophic growth, but a dark storage environment prevents them from performing photosynthesis, leading to substantial mortality. At the species level, disappearance of *Pseudo-Nitzschia multiseries*, members of the Cyanobacteria could also be attributed to the same reason (Fig. 5B). In addition, Zheng et al. (2023) reported that with the death of Cyanobacteria, mass nutrients were released and the relative abundance of bacteria associated with organic matter degradation, such as Bacteroidetes and Actinobacteria, was simultaneously enhanced, which is in line with the results of this study.

As a bacteria found in soil, *V. paradoxus* is closely associated with natural degradation processes (Jamieson et al. 2009). *P. giardinii* and *Rhizobium* sp. are root-nodule bacteria (Debnath et al. 2023), potentially existing as saprotrophs in NS after being separated from plants (Chen et al. 2004) and can decompose a broad range of compounds (McLeod et al. 2006). The relative decreasing abundance of these dominant bacteria further indicated that the lack of organic carbon in DSS limited heterotrophic bacteria growth (Fig. 5B). A vacant ecological niche also offers opportunities for other nondominant bacterial species to thrive; bacteria such as *N. fonticola* could be more adaptable to the DSS environment and increased in relative abundance from less than 1% on day 0 to 4.3% on day 5 (Fig. 5B).

The sealing of NS also played a role in isolating air; a low-oxygen environment could be created with the process of aerobic respiration. However, aerobic bacteria, such as *V. paradoxus* (Jamieson et al. 2009) and *N. fonticola* (Prauser 1976), still grew well in present study. This may be because aerobic bacteria's oxygen demand drops. Under nutrient-limited conditions, the oxygen consumption of aerobic bacteria can be decreased by at least two orders of magnitude (Riedel et al. 2013). On the other hand, a shortage of oxygen also reduced the nutrient consumption of bacteria, ensuring their survival in low-oxygen environments (Berney et al. 2014).

Effect of DSS on sterilization

Consistent with existing research (Chuang et al. 2013; Yang et al. 2022), the TPC of LT-DSS after sterilization was sharply reduced due to its low initial value (Table 3). Although the initial TPC in CK was significantly higher than LT-DSS, there remained much spare space between the bacteria, which means that UV could completely irradiate and penetrate each bacterium, leading to a similar log survival ratio of CK and LT-DSS.

Conclusions

1. Under experimental conditions, a relatively low TPC level of 3.99 lg CFU/mL (9870 CFU/mL) was detected in newly prepared NS.
2. Once newly prepared NS was used for cultivation, the organic carbon provided by the roots supported a rapid increase in TPC. In present study, TPC in NS sharply increased 7.8 times from 3.99 lg CFU/mL (9870 CFU/mL) to its maximum capacity (4.88 lg CFU/mL, 76,000 CFU/mL) in the first week. This count fluctuated during the remaining days of the study.
3. Darkness and AOC deficiency significantly inhibited the growth of photosynthetic and heterotrophic bacteria. In present study, this led to a decrease in TPC by more than 80% on day 3 of DSS when it reached it lowest level (4.29 lg CFU/mL, 19,566 CFU/mL).
4. The DSS-induced TPC decrease could serve as a sterilization method. Its minimum log survival ratio is −0.76 under experimental conditions.
5. Because of the rapid conversion of previously dead bacteria into new organic carbon source for colony reconstruction, the DSS-induced decrease in TPC lasts only for a short time. In this study, TPC in NS rebounded 235.6% within 24 h after reaching the lowest point. It is important to confirm when to carry out subsequent sterilization operations according to actual TPC decrease.
6. DSS could save energy required for sterilization. After UV irradiation, TPC of LT-DSS was 82.8% lower than CK, which indicated that the UV LED number or energy input could be reduced while get the same sterilization effect.

References cited

An R. 2016. Analysis of the influencing factors of UV disinfection for effluent from urban wastewater treatment plants. Water Wastewater Eng. 52(S1):135–140. https://doi.org/10.13789/j.cnki.wwe1964.2016.0359.

Badri DV, Vivanco JM. 2009. Regulation and function of root exudates. Plant Cell Environ. 32(6):666–681. https://doi.org/10.1111/j.1365-3040.2008.01926.x.

Berkelmann B, Wohanka W, Wolf GA. 1993. Characterization of the bacterial flora in circulating nutrient solutions of a hydroponic system with rockwool. Acta Hortic. 361:372–381. https://doi.org/10.17660/ActaHortic.1994.361.37.

Berney M, Greening C, Conrad R, Jacobs WR, Cook GM. 2014. An obligately aerobic soil bacterium activates fermentative hydrogen production to survive reductive stress during hypoxia. Proc Natl Acad Sci USA. 111(31):11479–11484. https://doi.org/10.1073/pnas.1407034111.

Bertilsson S, Stefan LJ. 1998. Photochemically produced carboxylic acids as substrates for freshwater bacterioplankton. Limnol Oceanogr. 43(5):885–895. https://doi.org/10.4319/lo.1998.43.5.0885.

Biate DL, Kumari A, Annapurna K, Kumar LV, Ramadoss D, Reddy KK, Naik S. 2015. Legume Root Exudates: Their Role in Symbiotic Interactions, p 259–271. In: Arora N (eds). Plant Microbes Symbiosis: Applied Facets. Springer, New Delhi, India. https://doi.org/10.1007/978-81-322-2068-8_13.

Bridson EY, Brecker A. 1970. Design and formulation of microbial culture media. Methods Microbiol. 3:238–240. https://doi.org/10.1016/S0580-9517(08)70541-5.

Butler JL, Williams MA, Bottomley PJ, Myrold DD. 2003. Microbial community dynamics associated with rhizosphere carbon flow. Appl Environ Microbiol. 69(11): 6793–6800. https://doi.org/10.1128/AEM.69.11.6793-6800.2003.

Chen WX, Wang ET, Chen WF. 2004. The relationship between the symbiotic promiscuity of rhizobia and legumes and their geographical environments. Chin Agric Sci. 1:81–86. https://doi.org/10.3321/j.issn:0578-1752.2004.01.013.

Chevrefils G, Caron É, Wright H, Sakamoto G, Payment P, Barbeau B, Cairns B. 2006. UV dose required to achieve incremental log inactivation of bacteria, protozoa and viruses. IUVA News. 8(1):38–45.

Chung SW, Ha YS, Lee KY, Kim JS, Park JM, Kwon SG, Choi WS, Kwon SH, Mitsuoka M, Inoue E, Okayasu T. 2013. Sterilization in hydroponic recycling system using visible light-reactive titanium dioxide photocatalysts. Faculty of Agriculture, Kyushu University. https://doi.org/10.5109/26166.

Debnath S, Das A, Maheshwari DK, Pandey P. 2023. Treatment with atypical rhizobia, *Pararhizobium giardinii* and *Ochrobactrum* sp. modulate the rhizospheric bacterial community, and enhances *Lens culinaris* growth in fallow soil. Microbiol Res. 267:127255. https://doi.org/10.1016/j.micres.2022.127255.

Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods. 10(10):996–998. https://doi.org/10.1038/NMETH.2604.

Escobar IC, Randall AA, Taylor JS. 2001. Bacterial growth in distribution systems: Effect of assimilable organic carbon and biodegradable dissolved organic carbon. Environ Sci Technol. 35(17):3442–3447. https://doi.org/10.1021/es0106669.

Hessling M, Spellerberg B, Hoenes K. 2017. Photoinactivation of bacteria by endogenous photosensitizers and exposure to visible light of different wavelengths—A review on existing data. FEMS Microbiol Lett. 364(2):fnw270. https://doi.org/10.1093/femsle/fnw270.

Jamieson WD, Pehl MJ, Gregory GA, Orwin PM. 2009. Coordinated surface activities in *Variovorax paradoxus* EPS. BMC Microbiol. 9(1):1–18. https://doi.org/10.1186/1471-2180-9-124.

Jia ZW, Wang LP, Ji YH, Wang XJ, Wu ZH, Wang S, Wang X, Liang H. 2023. Effect of nutrient solution ozone disinfection frequency on growth and microbial environment of hydroponic fast-growing cabbage. Chin Vegetables. 2:51–56. https://doi.org/10.19928/j.cnki.1000-6346.2023.0005.

Ke HC, Li K, Cheng RF. 2023. Simulation and optimization on ultraviolet led nutrient solution sterilization module based on response surface method. J Agric Sci Technol. 25(4):132–146. https://doi.org/10.13304/j.nykjdb.2022.1068.

Kim DG, Lee C, Yun YS, Hong CH, Choi YE. 2019. Recycling waste nutrient solution originating from the plant factory with the cultivation of newly isolated *Acutodesmus* species. J Biotechnol. 289: 15–25. https://doi.org/10.1016/j.jbiotec.2018.10.010.

Kooij D. 1992. Assimilable organic carbon as an indicator of bacterial regrowth. J Am Water Works Assoc. 84(2):57–65. https://doi.org/10.1002/j.1551-8833.1992.tb07305.x.

LeChevallier MW, Schulz W, Lee RG. 1991. Bacterial nutrients in drinking water. Appl Environ Microbiol. 57(3):857–862. https://doi.org/10.1002/bit.260370511.

Li Q. 2019. Analysis of bacterial and fungal community structure in the recirculating nutrient solution of tomato plug seedlings under ebb-and-flow irrigate. Chinese Academy of Agricultural Sciences.

Lin YP, Lin CM, Mukhtar H, Lo HF, Ko MC, Wang SJ. 2021. Temporal variability in the rhizosphere bacterial and fungal community structure in the melon crop grown in a sealed hydroponic system. Agronomy. 11(4):719. https://doi.org/10.3390/agronomy11040719.

Lindell MJ, Graneli HW, Tranvik LJ. 1995. Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter. Limnol Oceanogr. 40(1):195–199. https://doi.org/10.4319/lo.1995.40.1.0195.

Liu J, Ji RQ, Li GL, Gao TT, Si YJ, Li BQ. 2024. Diversity of Proteobacteria in the ectomycorrhizosphere of *Pinus koraiensis* and *Quercus mongolica*. J Jilin Agric Univ. 1–9. https://doi.org/10.13327/j.jjlau.2023.0087.

Liu YW, Huang CK. 2019. Effects of the circulation pump type and ultraviolet sterilization on nutrient solutions and plant growth in plant factories. HortTechnology. 29(2):189–198. https://doi.org/10.21273/HORTTECH04244-18.

Lu PL, Yang H, Ding AQ, Li CY, Quan L. 2023. Metabolic regulation of bacteria with limited carbon and nitrogen sources. Acta Microbiol Sin. 63(3):946–962. https://doi.org/10.13343/j.cnki.wsxb.20220549.

McLeod MP, Warren RL, Hsiao WWL, Araki N, Myhre M, Fernandes C, Miyazawa D, Wong W, Lillquist AL, Wang D, Dosanjh M, Hara H, Petrescu A, Morin RD, Yang G, Stott JM, Schein JE, Shin H, Smailus D, Siddiqui AS, Marra MA, Jones SJM, Holt R, Brinkman FSL, Miyauchi K, Fukuda M, Davies JE, Mohn WW, Eltis LD. 2006. The complete genome of *Rhodococcus* sp. RHA1 provides insights into a catabolic powerhouse. Proc Natl Acad Sci USA. 103(42):15582–15587. https://doi.org/10.1073/pnas.0607048103.

Nguyen C. 2003. Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie. 23(5–6):375–396. https://doi.org/10.1051/agro:2003011.

Prauser H. 1976. *Nocardioides*, a new genus of the order *Actinomycetales*. Int J Syst Bacteriol. 26(1):58–65. https://doi.org/10.1099/00207713-26-1-58.

Riedel TE, Berelson WM, Nealson KH, Finkel SE. 2013. Oxygen consumption rates of bacteria under nutrient-limited conditions. Appl Environ Microbiol. 79(16): 4921–4931. https://doi.org/10.1128/AEM.00756-13.

Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 4:e2584. https://doi.org/10.7717/peerj.2584.

Santangeli M, Steininger-Mairinger T, Vetterlein D, Hann S, Oburger E. 2024. Maize (*Zea mays* L.) root exudation profiles change in quality and quantity during plant development—A field study. Plant Sci. 338:111896. https://doi.org/10.1016/j.plantsci.2023.111896.

Scheuerman PR, Schmidt JP, Alexander M. 1988. Factors affecting the survival and growth of bacteria introduced into lake water. Arch Microbiol. 150(4): 320–325. https://doi.org/10.1007/BF00408301.

Schwarz D, Gross W. 2004. Algae affecting lettuce growth in hydroponic systems. J Hortic Sci Biotechnol. 79(4):554–559. https://doi.org/10.1080/14620316.2004.11511804.

Strayer RF. 1994. Dynamics of microorganism populations in recirculating nutrient solutions. Adv Space Res. 14(11): 357–366. https://doi.org/10.1016/0273-1177(94)90322-0.

Tang XW, Tong YA, He X. 2018. Reactions of lettuce root exudation to cadmium stress. North Hortic. 19:16–22. https://doi.org/10.11937/bfyy.20180755.

Weisskopf L, Le Bayon RC, Kohler F, Page V, Jossi M, Gobat JM, Martinoia E, Aragno M. 2008. Spatiotemporal dynamics of bacterial communities associated with two plant species differing in organic acid secretion: A one-year microcosm study on lupin and wheat. Soil Biol Biochem. 40(7):1772–1780. https://doi.org/10.1016/j.soilbio.2008.02.018.

Whitton BA, Potts M. 2012. Introduction to the Cyanobacteria, p 1–13. In: Whitton B (eds). Ecology of Cyanobacteria II. Springer, Dordrecht, the Netherlands. https://doi.org/10.1007/978-94-007-3855-3_1.

Xu P, Janex ML, Savoye P, Cockx A, Lazarova V. 2002. Wastewater sterilization by ozone: Main parameters for process design. Water Res. 36(4):1043–1055. https://doi.org/10.1016/s0043-1354(01)00298-6.

Yang M, Shang W, Li PF, You J, Gu M, Sun YL, Zhang WA. 2022. Influencing factors of sodium hypochlorite disinfection and its optimization in municipal wastewater treatment plants. Chin Water Wastewater. 38(9):76–81. https://doi.org/10.19853/j.zgjsps.1000-4602.2022.09.012.

Zheng X, Wang WJ, Sheng YQ. 2023. Influence of nitrate on algal and bacterial community structures in reservoir waters. J Lake Sci. 35(6):1917–1926. https://doi.org/10.18307/2023.0616.